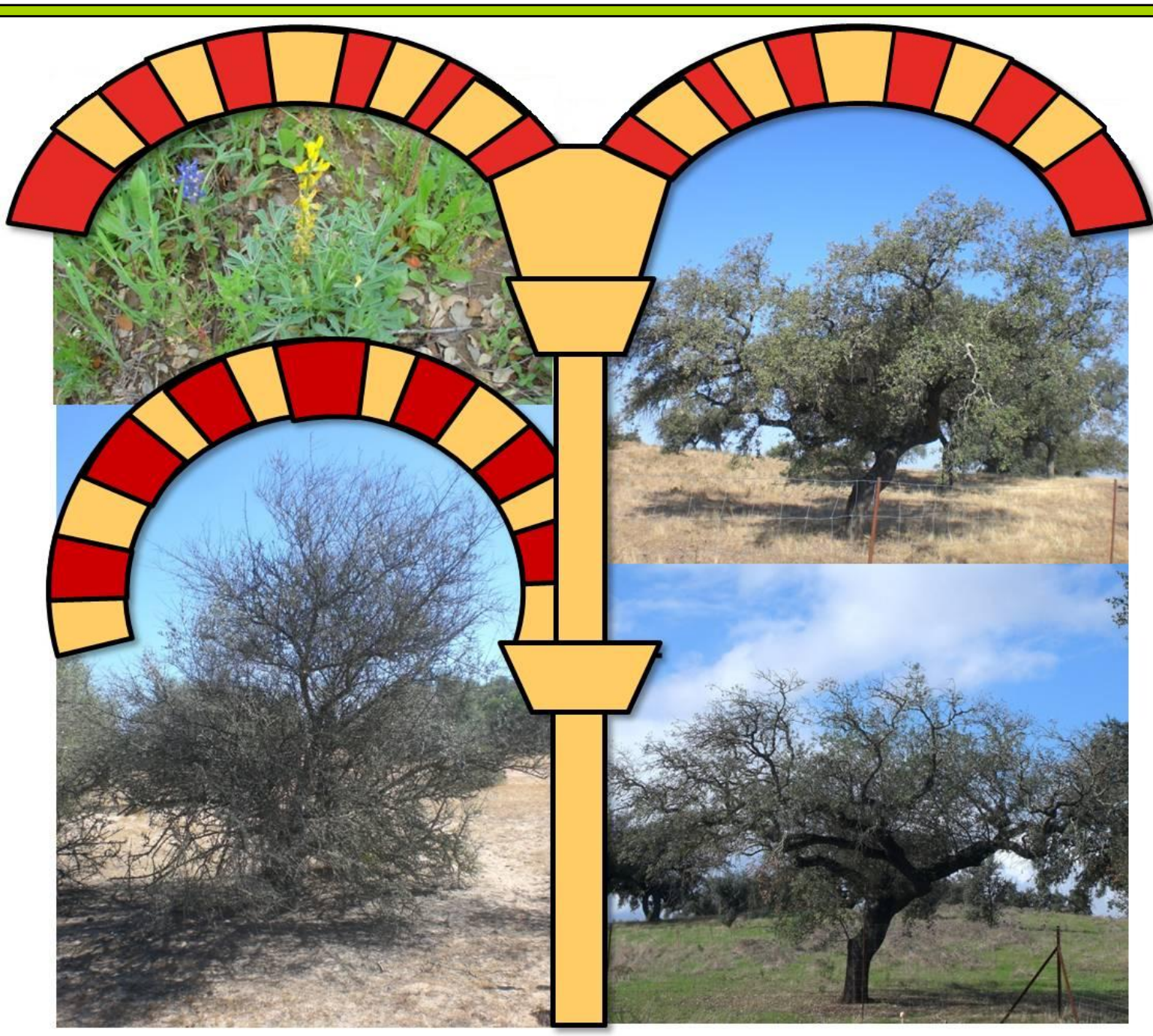
Phytophthoras in Forests and Natural Ecosystems

Proceedings of the 6th Meeting of the International Union of Forest Research Organizations (IUFRO) Working Party S07-02-09

September 9-14, 2012, Còrdoba, Spain



Proceedings of the Sixth Meeting of the

International Union of Forest Research Organizations

(IUFRO) Working Party S07-02-09

Phytophthora in Forests and Natural Ecosystems

Córdoba (Spain)
9th - 14th September
2012

Editors

Thomas Jung is a plant pathologist, Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, Faculty of Science and Technology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal (email adress: trjung@ualg.pt)

Clive M. Brasier is an emeritus mycologist and plant pathologist, Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK (email adress: clive.brasier@forestry.gsi.gov.uk)

Maria E. Sánchez is a plant pathologist, Agronomy Department, University of Córdoba, Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain (email adress: ag1sahem@uco.es)

Ana Pérez-Sierra is a plant pathologist, Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK (email adress: ana.perez-sierra@forestry.gsi.gov.uk)

Abstract

Jung T., Brasier C.M., Sánchez M.E., Pérez-Sierra A. (editors) 2014. Proceedings of the sixth meeting of the International Union of Forest Research Organizations (IUFRO) Working Party S07.02.09: Phytophthoras in forests and natural ecosystems.
202 pp.

The sixth meeting of the International Union of Forest Research Organizations (IUFRO) Working Party S07.02.09, Phytophthoras in Forests and Natural Ecosystems provided a forum for current research on Phytophthora species worldwide. One-hundred-and-fourty submissions describing papers and posters on recent developments in Phytophthora diseases of trees and natural ecosystems in Europe, Australasia, and the Americas are included. Research topics covered are Phytophthora adaptation and evolution, climate change, diversity, ecology, ecophysiology, epidemiology, experimental taxonomy, geographic origins, invasion and spread, management, the nursery pathway, pathogenesis and resistance, and population biology.

Keywords: Phytophthora species, forest tree diseases.

COMMITTEE MEMBERS

Scientific Committee	Organising Committee	Local Organising Committee
Paloma Abad-Campos	Paloma Abad-Campos	Mónica Berbegal
Clive Brasier	Ana Cristina Coelho	Paolo De Vita
Ana Cristina Coelho	Pilar Fernández Rebollo	Santiago Català
Everett Hansen	Luis Ventura García	Alma García
Giles Hardy	Thomas Jung	Marilia Horta Jung
Thomas Jung	Ana Pérez Sierra	Ramón Leal
Ana Pérez Sierra	Cristina Ramo	Maela León
María Esperanza Sánchez	María Esperanza Sánchez	Jonatan Sánchez
Andrea Vannini	Andrea Vannini	María Serrano

SPONSORS

Secretaría de Estado de Investigación, Desarrollo e Innovación. Ministerio de Economía y Competitividad. Gobierno de España

Excelentísima Diputación Provincial de Córdoba

Universidad de Córdoba

Universitat Politècnica de València

CEMACAM-Centro Educativo del Medio Ambiente Font Roja-Alcoi

Ajuntament d'Alcoi (Alicante)

Consorcio de Turismo de Córdoba

Fertinyect SL

COLLABORATORS

Universidade do Algarve

Consejería de Agricultura y Medio Ambiente, Junta de Andalucía

D. Javier Crusells. Dehesa La Jarosa (Córdoba)

D. José Rosa. Dehesa Los Bueyes (Huelva)

Campo Baldío (Huelva)

Ayuntamiento de la Puebla del Río (Sevilla)

List of Contents

15 Surveys, Diversity, Invasion and Spread

infected with *Phytophthora cinnamomi*
A. Acedo, E. Cardillo, I. Núñez

106 Pythiaceous and fungal species isolated from coniferous and deciduous seedlings in some Turkish nurseries
A.G. Aday, A. Lehtijärvi, H.T. Doğmuş-Lehtijärvi

107 Characterisation of the two informally designated ITS Clade 6 taxa *Phytophthora* taxon Forestsoil and P. sp. hungarica.
J. Bakonyi, Z. Á. Nag, T. Burgess, A. Szigethy, J. Nechwatal, A. Koltay, S. Woodward, L. Belbahri, T. Jung

108 Population fluctuation of *Phytophthora* spp in streams and their possible role as early colonizers
Y. Balci

109 *Phytophthora* species associated with disease in peri-urban woodland and forest ecosystems
P. Barber, T. Burgess, T. Paap, G. Hardy

110 Diversity of oomycetes detected in the laurel forest in Tenerife (Canary Islands)
A. Beltrán, M. León, P. Abad-Campos, F. Siverio, L. Gallo, A. Pérez-Sierra

111 Diversity and distribution of *Phytophthora* species in association with water quality and the health of trees in fragmented riparian ecosystems
T. Burgess, M. Kunzinger, D. White, A. Lymbery, G. Hardy

112 Next Generation sequencing shows *Phytophthora* species diversity in soil samples of Macaronesian laurel forests from the Canary Islands
S. Català, A. Beltrán, A. Pérez-Sierra, P. Abad-Campos

113 Airborne infections of *Phytophthora citrophthora* on citrus in Sicily
A*. De Patrizio, R. Faedda, A. Pane, S.O. Cacciola*

114 ITS-based identification and phylogeny of *Phytophthora* spp. detected in UK gardens
G. Denton, J. Denton, I. Waghorn, S. Archer, B. Henricot

115 Pathogenicity of *Phytophthora* species on *Liquidambar orientalis* and *Castanea sativa* seedlings
H.T. Doğmuş-Lehtijärvi, A.G. Aday, A. Lehtijärvi, T. Jung

118 *Phytophthora ipomoeae* causing blight on *Ipomoeae orizabensis* in Michoacan, Mexico
S. Fernández-Pavía, G. Rodríguez-Alvarado, N. Gómez-Dorantes, H. Silva-Rojas

119 Research driving management and policy-making on Sudden Oak Death in California
M. Garbelotto, S. Mascheretti, D. Schmidt

120 Reducing the spread of *Phytophthora ramorum* on the Redwood Nature Trail,

155 Evaluation of fungal secondary metabolites against *Phytophthora* spp.
B. Scanu, B. Linaldeddu, A. Franceschini, A. Evidente, L. Maddau

156 Evidence of lipophilic phytotoxic metabolites produced by *Phytophthora* spp. involved in chestnut ink disease
B. Scanu, B. Linaldeddu, A. Franceschini, L. Maddau

157 *Phytophthora* species occurring in declining oak ecosystems in Sardinia (Italy)
B. Scanu, B. Linaldeddu, T. Jung, L. Maddau A. Franceschini

158 Development, comparison and validation of a Real-Time PCR tool for the detection of *Phytophthora lateralis*.
N. Schenck, C. Fourrier, C. Robin, R. Ioos

160 Differential susceptibility of the commonest Andalusian morphotypes of Holm oak to *Phytophthora cinnamomi*
M. S. Serrano, P. De Vita, P. Fernández, M. E. Sánchez

161 Effectiveness of calcium and potassium fertilizers for control of *Quercus ilex* root rot caused by *Phytophthora cinnamomi*
M.S. Serrano, P. De Vita, P. Fernández, M.E. Sánchez

162 Proteomics analysis of responses to *Phytophthora cinnamomi* in Holm oak
B. Sghaier–Hammami, J. Valero–Galvàn, M. C. Romero–Rodríguez, R. M. Navarro–Cerrillo, J. Jorrín–Novo

163 An overview of research activities on Sudden Oak Death (*Phytophthora ramorum*) at the Canadian Forest Service: results and progress
S. F. Shamoun, D. Rioux

164 Foliar infection of *Rhododendron* by zoospores and cysts of *Phytophthora pini* Leonian
S. Shay, J. Eberhart, J. Parke

165 Infection of oak (*Quercus robur*) and beech (*Fagus sylvatica*) seedlings growing in elevated CO_2 conditions with pathogenic *Phytophthora* species
K. Sikora, M. Siebyła, J.Nowakowska, T. Oszako

166 An ecological role for *Phytophthora* taxon Oaksoil in western Oregon riparian ecosystems
L. Sims, E. Hansen

167 Biological pollution: *Phytophthora* species threatening forest nurseries and natural ecosystems in Portugal
M. Horta Jung, A. Pèrez-Sierra, A. Solla, A. Cravador, T. Jung

169 A simple, rapid and inexpensive chemical method for the detection phosphite in plant tissue
P. Stasikowski, D. Clark, J. McComb, B. Shearer, P. O'Brien, G. Hardy

170 Calcium supplementation of soil augments the control of *Phytophthora cinnamomi* by phosphite
P. Stasikowski, J. McComb, B. Shearer, P. O'Brien, G. Hardy

171 *Phytophthora*-baiting in Norwegian waterways
V. Talgø, M. L. Herrero, M. B. Brurberg, A. Stensvand

172 *Phytophthora* on trees in Norway
V. Talgø, M. L. Herrero, M. B. Brurberg, A. Stensvand

173 Subspecies identification of *Phytophthora alni* in alder riparian stands in the Czech Republic
M. Tomšovský, P. Štěpánková, K.Černý

174 The presence of *Phytophthora ramorum* in Greece: The risk of spread into forest ecosystems
P. Tsopelas, N. Soulioti, S.E. Tjamos, E.J. Paplomatas, E.K. Kulakiotu

178 *Phytophthora* species from native forests of Patagonia
M.L. Vélez, P.V. Silva, A.G. Greslebin

179 Pyrosequencing as a tool for detection of Phytophthoras: error rate and risk of false MOTU's
A.M. Vettraino, P. Bonants, A. Tomassini, N. Bruni, A. Vannini

180 Biodiversity of *Phytophthora* community in a coastal oak ecosystem in Italy
A.M. Vettraino, G. Natili, A. Vannini

181 *Phytophthora obscura* and the widespread bleeding canker of *Aesculus* in Europe
N. J. Grünwald, S. Werres, E. M. Goss, C. R. Taylor, V. J. Fieland

183 Specificity of a Lab-on-a-Chip system for *Phytophthora* diagnosis
S. König, S. Wagner, S. Werres, L. Schwenkbier, K. Weber, J. Weber

185 Variability in response to infection of *Phytophthora cinnamomi* in different families of *Quercus ilex*
I. León-Sánchez, J. J. García-Martínez, M. Fernández-Martínez, J. Vázquez-Piqué, A. Cravador, R. Tapias-Martín

186 Characterising the distribution of *Phytophthora* taxon Agathis (PTA) in the bark, cambium, and wood of diseased New Zealand kauri (*Agathis australis*)
M.P. Wheat, S.E. Bellgard

187 *Phytophthora cinnamomi* infection results in changed patterns of fine root production in Scots pine
E. Siasou, H. Braganca, S. Woodward

188 *Phytophthora plurivora* and other Oomycota in an Aberdeenshire watershed
D. Belo, D. Nianiaka, P. van West, L. Belbahri, I. Baccelli, E. Siasou, S. Woodward

Sporangium of *Phytophthora cinnamomi* from a bark canker of *Banksia litoralis* in Western Australia showing nested proliferation and constriction of the sporangiophore enabling aerial spread (photo by Thomas Jung)

EVOLUTION, POPULATION BIOLOGY, EXPERIMENTAL TAXONOMY

Determining the origin of the emerging pathogen, *Phytophthora multivora*

A. Rea[1], G.E.St.J. Hardy[1], M. Stukely[2], T.I. Burgess[1]

[1]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.
[2]Science Division, Department of Environment and Conservation, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.

Corresponding author: tburgess@murdoch.edu.au

Abstract

Phytophthora multivora is widespread in Western Australia (WA); it has a wide host range and considerably variability in the sequence of the mitochondrial gene *cox*1 led to the hypothesis that it may be endemic to the region. To test this hypothesis, four nuclear (ITS, enolase, HSP90 and r*as*) and three mitochondrial (*cox*I, c*ox*IGS and *nadh*1) loci were sequenced for 60 isolates of *P. multivora* isolated from Australia, South Africa (RSA)and Europe and the data were subjected to phylogenetic, coalescent-based and population genetic analyses. Isolates from RSA possess greater nucleotide diversity and a greater number of alleles at three of the nuclear loci and at all three mitochondrial loci than those from WA. In addition, the RSA population had more unique multilocus genotypes than the WA population. While *P. multivora* is widely distributed in natural ecosystems in WA and RSA, it is usually isolated from nurseries or horticulture elsewhere in the world. Additionally, *P. multivora* is consistently isolated from cankers and dead and dying plants of numerous endemic hosts in WA, but is predominantly isolated from soil associated with asymptomatic plants in RSA. Based on this evidence it is proposed that *P. multivora* is endemic to RSA and has been introduced to Western Australia.

Four phenotypically distinct lineages identified within *Phytophthora lateralis*

C.M. Brasier[1], S. Franceschini[1], A.M. Vettraino[2], E. Hansen[3], S. Green[4], C. Robin[5], J. Webber [1], A. Vannini[2]

[1]*Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK.*
[2]*DIBAF, University of Tuscia, Via S. Camillo de Lellis, Viterbo 01100, Italy.*
[3]*Department of Botany and Plant Pathology, Oregon State University, Corvallis OR 97331, USA.*
[4]*Forest Research, Northern Research Station, Roslin, Scotland, EH259SY, UK.*
[5]*UMR 1202 BIOGECO, INRA, 69 Route d'Arcachon, 33612, Cestas Cedex, France.*

Corresponding author: clive.brasier@forestry.gsi.gov.uk

Abstract

Until recently *Phytophthora lateralis* was known only as the cause of dieback and mortality of *Chamaecyparis lawsoniana* in its native range in the Pacific Northwest [1]. Since the 1990s however disease outbreaks have occurred increasingly on ornamental *C. lawsoniana* in Europe; and in 2007 the pathogen was discovered in soil around old growth *C. obtusa* in Taiwan, where it may be endemic [2]. When the phenotypes of over 150 isolates of *Phytophthora lateralis* from Taiwan; across the Pacific Northwest (British Columbia to California); and from France, the Netherlands and the UK were compared three well separated growth rate groups were resolved: one from Taiwan, one from the Pacific Northwest and Europe and one from a small area of the UK. Among these groups nine distinct types were identified based on colony patterns and spore metrics and discriminated in a multivariate analysis. The assumption that the three main growth rate groups represented distinct phylogenetic units was tested by comparative sequencing of two mitochondrial and three nuclear genes. This assumption was confirmed. In addition two phenotype clusters within the Taiwan growth group were also shown to be separate lineages. The characteristics and distribution of the four *P. lateralis* lineages will be presented and their evolutionary, taxonomic and plant health significance discussed.

Literature Cited

Hansen E.M., Goheen D.J., Jules E.S., Ullian B., 2000. Managing Port-Orford-Cedar and the introduced pathogen *Phytophthora lateralis*. *Plant Disease* **84:** 4-14.

Brasier C.M., Vettraino A.M., Chang T.T., Vannini A., 2010. *Phytophthora lateralis* discovered in an old growth *Chamaecyparis* forest in Taiwan. *Plant Pathology* **59:** 595-603.

Population dynamics of aerial and terrestrial populations of *Phytophthora ramorum* in a California watershed under different climatic conditions

M. Garbelotto, M. Kozanitas, C. Eyre

Forest Pathology and Mycology Laboratory, University of California Berkeley, 54 Mulford Hall, Berkeley CA 94720

Corresponding author: matteog@berkeley.edu

Abstract

In the attempt to understand the epidemiology of Sudden Oak Death in California, we present the first combined genetic analysis of *P. ramorum* from soil and leaves. Successful isolations from leaves of the transmissive host California bay laurel increased from 15 to 39% between dry and wet conditions. Symptoms caused by other foliar pathogens were highest (69%) in dry conditions, suggesting that *P. ramorum* and other pathogens are favored by different climatic conditions. Some foliar genotypes of *P. ramorum* were more abundant in wet than in dry conditions and persistent through time. Soil and foliar populations were genetically distinct, but were not segregated in different portions of a minimum spanning network, suggesting intermixing of the two. We surmise that the genetic structure between substrates is not due to the presence of two distinct populations, but to the different ability of genotypes to adapt to different substrates. To support this hypothesis, we show that ranking of genotypes based on abundance are clearly different between soil and leaf populations. We provide evidence that in climatic conditions unfavorable to the pathogen, genetic diversity increases both within and between sites, while in favorable conditions diversity decreases due to greater migration levels of some genotypes. Finally, we show that foliar genotypes can spread further than soil genotypes in wet years, and that soil appears to be re-inoculated on a yearly basis. Cumulatively, results indicate leaves act as a relatively persistent source of inoculum, while soil is potentially inconsequential for the natural spread of the disease.

Phenotypic diversification is associated with host-induced transposon derepression and repression of crinkler genes in the Sudden Oak Death pathogen

M. Garbelotto[1], D.M. Rizzo[2], T. Kasuga[3]

[1]*Forest Pathology and Mycology Laboratory, University of California, Berkeley, CA.*
[2]*Department of Plant Pathology, University of California, Davis, CA.*
[3]*USDA-ARS Crop Pathology & Genetics Research, Davis, CA.*

Corresponding author: matteog@berkeley.edu

Abstract

Phytophthora ramorum is responsible for sudden oak death in California. *P. ramorum* is a generalist pathogen with over 100 known host species. Three or four closely related genotypes of *P. ramorum* (from a single lineage) were originally introduced in California forests and the pathogen reproduces clonally. Because of this, the genetic diversity of *P. ramorum* is extremely low in Californian forests. However, *P. ramorum* shows diverse phenotypic variation in colony morphology, colony senescence, and virulence. In this study, we show that phenotypic variation among isolates is associated with the host species from which the microbe was originally cultured. Microarray global mRNA profiling detected derepression of transposable elements (TEs) and down-regulation of crinkler effector homologs (CRNs) in the majority of isolates originating from coast live oak (*Quercus agrifolia*), but this expression pattern was not observed in isolates from California bay laurel (*Umbellularia californica*). In some instances, oak and bay laurel isolates originating from the same geographic location had identical genotypes based on multilocus simples sequence repeat (SSR) marker analysis but had different phenotypes. Expression levels of the two marker genes analyzed by quantitative reverse transcription PCR were correlated with originating host species, but not with multilocus genotypes. Because oak is a non-transmissive dead-end host for *P.ramorum*, our observations are congruent with an epi-transposon hypothesis; i.e., physiological stress is triggered on *P. ramorum* while colonizing oak stems and disrupts epigenetic silencing of TEs. We propose the *P. ramorum*-oak host system in California forests as an ad hoc model for epi-transposon mediated diversification.

EU2, a fourth evolutionary lineage of *Phytophthora ramorum*

K. Van Poucke[1], S. Franceschini[2], J. Webber[2], K. Heungens[1], C.M. Brasier[2]

[1]*ILVO Plant Sciences Unit, Burg. Van Gansberghelaan 96 box 2, 9820 Merelbeke, Belgium.*
[2]Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK.

Corresponding author: clive.brasier@forestry.gsi.gov.uk

Abstract

Studies in North America and Europe over the past decade have demonstrated the occurrence of three lineages of *Phytophthora ramorum* informally designated the NA1, NA2 and EU1 lineages. Each lineage appears to represent a reproductively isolated population, but whether they have come from different geographic regions is unknown. Only the EU1 lineage had been found in Europe until recently. EU1 is believed to have been introduced into Europe around 1990. Since then it has spread widely and rapidly across the continent, including the UK and Ireland, via the plant trade. In 2011 *P. ramorum* isolates from Northern Ireland and a closely adjacent area of western Scotland, mostly from *Larix* but also from *Quercus, Rhododendron and Vaccinium*, were found to have molecular profiles not matching those of any known lineage. Following a phylogenetic study based on eleven polymorphic loci and an SSR analysis they were assigned to a new lineage, informally designated EU2. This analysis indicates the EU2 lineage may be ancestral to the other lineages. No SSR-based intra-EU2 lineage genotypic diversity was detected. All EU2 isolates examined to date have all been of A1 mating type. As this is the same mating type as that of EU1 in Europe, sexual recombination with EU1 lineage genotypes already resident in the UK is unlikely. The earliest isolation dates to 2007. Present evidence points to a recent introduction of EU2 in the context of ongoing phytosanitary emergency measures. The arrival of EU2 highlights an urgent need to identify the geographic origins of *P. ramorum* in order to understand the organism's natural ecology, the processes that have produced the lineages, and whether further lineages exist. Presently, studying the organism in the context of introduction and invasion, we may only be looking at half the picture.

Characterization of *Phytophthora* hybrids from ITS clade 6 associated with riparian ecosystems in South Africa and Australia

T.I. Burgess[1,2], J. Nagel[2], D. Hüberli[3], G.E.St.J. Hardy[1], M. Stukely[4], M. Wingfield[2]

[1]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.
[2]Forestry and Agriculture Biotechnology Institute, University of Pretoria, Pretoria, 0002, South Africa.
[3]Department of Agriculture and Food, Western Australia, 3 Baron-Hay CourtSouth Perth, WA 6151.
[4]Science Division, Department of Environment and Conservation, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.

Corresponding author: tburgess@murdoch.edu.au

Abstract

Recent surveys of Australian and South African rivers have revealed numerous Clade 6 Phytophthoras, which either have ITS gene regions that were highly polymorphic or could not be sequenced. These isolates were suspected to be hybrids. In order to establish the hybrid nature of these isolates, three nuclear loci and one mitochondrial locus were amplifiied and, in the case of the nuclear gene regions cloned, and sequenced. Abundant recombination within the ITS region was observed and this combined with phylogenetic comparison of other three loci confirmed the presence of four distinct hybrids involving three known parental species: *P. amnicola*, *P. thermophila* and *P.* taxon PgChlamydo. In each case the hybrid is between two parental species. For the single copy nuclear genes (ASF and GPA) examined, two alleles were obtained, one of which corresponded to each of the parental species. In all cases, only a single *cox*I allele was obtained indicating that mitochondria were always uniparentally inherited from one of the nuclear parents. This pattern of nuclear and mitochondrial inheritance suggests that each hybrid is a result of an independent hybridization event involving two parental species. The hybrid species are sterile and have physiological traits similar to those of the maternal parental. The pathogenicity of these hybrids is unknown, but several isolates from Western Australia were obtained from the rhizosphere soil of dying plants. The serendipitous and simultaneous discovery of the same hybrid complex on two continents is intriguing. However, the wide geographic distribution, frequent isolation and presence of all four hybrids and all parental species suggest that their origin lies in Australia. The association of the sampled riverways with botanical gardens in South Africa containg Australian plants may be a clue to the pathway of introduction.

Promiscuity, fertility and survival of ITS clade 6 hybrids associated with riparian ecosystems in Western Australia

T.I. Burgess[1], D. Hüberli[2], D. White[1], M. Stukely[3], G.E.St.J. Hardy[1]

[1]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia; [2]Department of Agriculture and Food, Western Australia, 3 Baron-Hay CourtSouth Perth, WA 6151; [3]Science Division, Department of Environment and Conservation, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.

Corresponding author: tburgess@murdoch.edu.au

Abstract

Over the past few years several large scale *Phytophthora* surveys have been undertaken in Western Australia. In all cases, the ITS region of numerous isolates obtained from water or riparian soil have been unsequencable. These isolates are hybrids, all involving parental species from ITS clade 6, sub-clade II. Parental species are *P. thermophila* (T), *P. fluvialis* (F), *P. litoralis* (L), *P. amnicola* (A) and *P.* taxon stagnum (S). In most cases, two alleles were found for the nuclear genes and a single allele for the mitochondria gene, suggesting that each hybrid is a result of an independent sexual hybridization event involving two parental species with the mitochondria inherited from the maternal parent. To date the following hybrids have been characterised (maternal parent first); A-F, A-S, F-S, L-S, T-A, T-S and S-F. The hybrid isolates all appear to be sterile and readily produce sporangia on soil extract, however, some of them produced unusual or aborted sporangia. The growth and colony pattern produced by the hybrids on three different agar media is similar to that of the maternal parent. These hybrids have predominantly been isolated from natural waterways but some have been isolated from the rhizosphere soil of dying plants. There is no evidence of subsequent hybridization events (back crossing or hybrids crossing with hybrids), but this cannot be ruled out at this stage. The two parental ITS alleles are combined and subsequently recombined through mitotic recombination events creating significant variation between the rDNA subunits. The fact that this occurs suggests that the hybrids are relatively stable and are able to survive without resting structures, probably through continual sporulation within riparian ecosystems. Their role in the environment remains a mystery.

Analysis of the global population structure of *Phythophtora plurivora* using newly developed, polymorphic SSR markers

C.N. Schoebel, S. Prospero

Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland.

Corresponding author: corine.schoebel@wsl.ch

Abstract

Phytophthora species are well known to cause devastating diseases on numerous crops, ornamentals, and native plants. In recent years, their spread has been accelerated by the increasing international trade of woody plants. However, with a few exceptions (e.g. *P. ramorum*, the causal agent of Sudden Oak Death), detailed knowledge on the global population structure and the pathways of spread of forest *Phytophthora* species is still missing. This lack of knowledge is mainly due to the absence of appropriate species-specific molecular markers.
P. plurivora, a member of the *P. citricola* species complex, is involved in widespread beech (*Fagus sylvatica*) and oak (*Quercus* sp.) declines in natural and semi-natural forest ecosystems in Europe (1). Moreover, this species is frequently found in European ornamental nurseries and has been reported in North American nurseries and plantations not long ago.
We recently developed polymorphic SSR markers for *P. plurivora* (2) and used them to analyze the worldwide population structure, genotyping ~500 isolates from 18 countries of this cosmopolitan plant pathogen. Here, we will present the results of this study which aimed (a) to determine whether Europe can be considered as the center of origin of *P. plurivora*, and (b) to characterize the pathways of spread of *P. plurivora* within Europe and between Europe and US.

Literature Cited

Jung T., Burgess T.I., 2009. Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* **22:** 95–110.
Schoebel C.N., Jung E., Prospero S., 2013. Development of new polymorphic microsatellite markers for three closely related plant-pathogenic *Phytophthora* species using 454-Pyrosequencing and their potential applications. *Phytopathology* **103:** 1020-1027.

AFLP analysis reveals low genetic diversity of *Phytophthora austrocedrae* in Patagonia, Argentina

M.L. Vélez[1], M.P.A. Coetzee[2], M.J. Wingfield[2], M. Rajchenberg[1], A.G. Greslebin[1]

[1]*Centro de Investigación y Extensión Forestal Andino Patagónico (CIEFAP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de la Patagonia San Juan Bosco (UNPSJB), Esquel, Argentina.*
[2]*Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.*

Corresponding author: mvelez@ciefap.org.ar

Introduction

Austrocedrus chilensis (D. Don) Pic. Serm & Bizarri (ciprés de la cordillera, cypress) is an endemic tree in the Cupressaceae, found in southern Argentina and Chile (Patagonia). The species is found across 140,000 ha in a wide variety of ecological niches. *Austrocedrus chilensis* is important not only because of its ecological functions but because of the high quality of its wood and its aesthetic value.

High levels of *A. chilensis* mortality were first detected in 1948 on Victoria Island in Neuquén Province. Since then, mortality has extended to almost all of the growth range of *A. chilensis* in Argentina where the disease was referred to as "Mal del Ciprés" (MDC) (Greslebin & Hansen, 2010).

Phytophthora austrocedrae Gresl. & E.M. Hansen (2007) is believed to be the primary cause of *A. chilensis* mortality (Greslebin & Hansen, 2010). *P. austrocedrae* is present across the native growth range of the trees in Argentina. Major symptoms of MDC include chlorosis, wilting of the foliage, root rot, and presence of necrotic lesions at the phloem in roots and stems (Greslebin & Hansen, 2010). Inoculation studies on 2-year-old saplings with *P. austrocedrae* showed a progressive reduction in photosynthesis, stomatal conductance and stem-specific hydraulic conductivity. The dramatic impact on plant physiology was mainly attributed to disruption of phloem and xylem transports caused by extensive death of the bark and cambium tissue, as well as hyphal colonization and presence of resinous plugs in xylem, and death of xylem ray parenchyma (Vélez et al., 2012).

The geographic origin of *P. austrocedrae* is unknown although the impact of the pathogen suggests that it has been introduced into Argentina. However, the population genetic structure of the pathogen has not been established. The aim of this study was to assess the genetic diversity of *P. austrocedrae* in Argentina.

Materials and Methods

Isolates of *P. austrocedrae* were collected during several surveys of declining *A. chilensis* stands in Patagonia, Argentina, following protocols of Greslebin et al. (2007) (Table 1). Forty-eight isolates from 19 stands were selected for analysis, reflecting the geographical range of *A. chilensis* distribution (550 km between the two most distant locations, Corcovado in the south and San Martin de los Andes in the north).

To produce mycelium for DNA extraction, tomato juice broth (Greslebin et al., 2007) was inoculated with agar plugs cut from the actively growing margins of fresh cultures. After 30 days of growth at 16 °C, the
mycelium was harvested and extracted using the procedure described by Möller et al. (1992).

AFLP analysis was performed following the protocol of Vos et al. (1995) with modifications (De Vos et al., 2007). A screening test with 16 *Eco*RI and *Mse*I primers with two-base-additions combinations was done. The *Eco*RI primer was labeled with infrared dyes. AFLP fragment analysis was performed on a model 4200 LI-COR® automated DNA sequencer. Based on the

clarity and reproducibility of the resulting fingerprinting profiles 4 primer combinations (E-AC/M-AC, E-AA/M-TG, E-AC/M-CC, E-CC/M-CC) were selected. The AFLP procedure was replicated from the initial restriction digest step for all study strains. The presence (1) or absence (0) of bands matrices were analyzed using Popgene version 1.31 to assess the genetic diversity. To analyze the degree of genetic similarity between isolates, a pairwise distance matrix was generated using the Jaccard, Dice and Simple Matching coefficients of similarity (S) (Infostat version 2011). Since isolate grouping was shown to be highly similar among the different coefficients, only results from the Dice index are presented in the results section. Distance dendrograms were constructed (Infostat). PAUP version 4.0b10 was used to obtain bootstrap values at the nodes for a tree generated from Nei and Li genetic distances. To test possible grouping of the isolates by provenance a principal coordinate analysis was done (Infostat).

Results

Of the 332 bands that were scored, only 40 (12%) were polymorphic. The total number of bands scored per primer combination ranged from 65 to 97. The lowest percentage of polymorphic loci was 8.3% while the highest percentage obtained was 16.2%.

Genetic analyses of the bands yielded gene diversities (*h*) that ranged from 0.0050 to 0.0258. The Shannon index (*I*) ranged from 0.0180 to 0.0432. A high degree of genetic similarity was found for the *P. austrocedrae* isolates. The pairwise similarity (S) values ranged from 0.958-1 (0.993 ± 0.009; mean ± S.D.). The largest distances were obtained between isolate 8-Phy-203 and isolates 16-Phy-270 and 15-Phy-271, all originating from the Chubut Province. Isolates obtained from the most distant locations (Corcovado and San Martín de los Andes) yielded high similarity values (0.969-0.998). Isolates originated from the same stand yielded similarity values that were comparable with those from different stands.

Data were concatenated to generate a dendrogram (fig. 1). The isolates resided in one large group that was supported by a 100% bootstrap value (fig. 1). Within this group, 45 isolates grouped in a single cluster (92% bootstrap support), while three isolates grouped separately but with no bootstrap support (54%) (fig. 1). Among the 45 isolates that grouped together, a subgroup of 5 isolates from 2 different stands located approximately 90 km from each other could be distinguished (89%) (fig. 1). This subgroup was, in turn, subdivided into two according to isolate provenance (90%). However, other isolates from the same sites grouped apart and mixed with isolates obtained in other stands and sites (fig. 1). Thus, there was no overall clear partitioning of genetic diversity that corresponded to geographic origin. In principal coordinate analyses, three-dimensional plots did not resolve any clear grouping of isolates on the basis of geographical origin (data not shown).

Discussion

This study revealed high levels of within-species genetic similarity and no evidence of partitioning of genetic diversity among the collection sites. This suggests that the pathogen represents a single population with low heterogeneity and that it was introduced into Argentina.

The first detection of mortality of *A. chilensis* trees occurred during 1948 on Victoria Island (Greslebin & Hansen, 2010). The subsequent development of the disease is characteristic of an introduced pathogen that has encountered a highly susceptible host grown over an extended area. Victoria Island is known for the introduction of many exotic woody plants from different continents during the 1920s and 1930s. This, together with the fact that the first appearance of *A. chilensis* mortality occurred in this area, lead us to believe that *P. austrocedrae* was introduced to the island on infected plants. The results of this study support this view.

The recent discovery that *P. austrocedrae* is causing mortality of *Chamaecyparis nootkatensis* in Scotland and of *Juniperus communis* in England (Anonymus 2011, 2012) is relevant to the present study. The source of *P. austrocedrae* in these areas has not been determined, but it has been suggested that the pathogen was also introduced. Clearly, it is impossible to determine the origin of *P. austrocedrae* without having isolates from a hypothetical natural host or area of origin. The same situation is true for many *Phytophthora* spp. that have unexpectedly appeared in new areas but for which the likely areas of origin are unknown. In this regard, global surveys for *Phytophthora* spp. must continue and, in the case of *P. austrocedrae* that

appears to be a conifer-specific pathogen, such surveys should include areas where related conifers occur naturally.

Table1: Origin and isolation dates of *Phytophthora austrocedrae* cultures used in this study

Site	Isolate N°	Province	Location	Description	Date of isolation
1	Phy-255 Phy-263	Neuquén	S 40º 9' 45.79'' W 71º 20' 44.88''	Lanin National Park, Cte. Díaz hill, San Martín de los Andes city	May 2008
2	Phy-308	Neuquén	S 40º 29' 58.4'' W 71º 21' 6.39''	Lanin National Park, Filo-Huaum lake	May 2009
3	Phy-256 Phy-257 Phy-258	Neuquén	S 40º 40' 34.95 W 71º 18' 41.62''	Lanin National Park, road from Confluencia to Traful village	May 2008
4	Phy-298 Phy-304 Phy-299 Phy-300 Phy-305 Phy-312 Phy-314	Neuquén	S 40º 39' 48.024'' W 71º 22' 20.71'' S 40º 40' 9.66'' W 71º 21' 6.39'' 40º 40' 0.007'' 71º 21' 36.12''	Lanin National Park, various stands of *A. chilensis* in the surroundings o Traful Village	May 2009
5	Phy-290 Phy-292	Neuquén	40º 58' 52.41'' 71º 31' 0.73''	Nahuel Huapi National Park, 2 different sites (Puerto Totora and Bella Vista Hill), in Victoria Island	Jan 2009
6	Phy-286	Río Negro	S 41º 13' 44.32'' 71º 25' 11.12''	Nahuel Huapi National Park, Gutierrez Lake	Oct 2008
7	Phy-276 Phy-278 Phy-279 Phy-281 Phy-318	Chubut	S 42º 0' 26.98'' W 71º 32' 11.27''	Epuyén, Golondrinas, Reserva Forestal del INTA	Sep 2008 May 2009
8	Phy-203 Phy-205 Phy-209 Phy-211 Phy-213 Phy-215 Phy-338	Chubut	S 42º 46' 29.97'' W 71º 32' 11.27''	Los Alerces National Park, Braese stream	Oct 2005 Jan 2010
9	Phy-219 Phy-221 Phy-223 Phy-225 Phy-232	Chubut	S 42º 48' 26.8'' W 71º 38' 58.9''	Los Alerces National Park, Quebrada del León stream	Oct 2005
10	Phy-243 Phy-244	Chubut	S 42º 53' 5.21'' W 71º 35' 52.58''	Los Alerces National Park, Las Rocas camping area	Apr 2006
11	Phy-294	Chubut	S 43º 9' 55.8'' W 71º 42' 18.4''	Los Alerces National Park, seccional Río Grande	Nov 2008
12	Phy-234 Phy-337	Chubut	S 43º 7' 34.36'' W 71º 33' 46.10''	Trevelin, Aldea Escolar, Estación Experimental INTA Trevelin	Jan 2006
13	Phy-191 Phy-195 Phy-201 Phy-309	Chubut	S 43º 12' 55.5'' W 71º 32' 50.9''	Futaleufú, Trevelin, Rio Grande Valley, "La 106" property	Sep 2005 Jun 2009
14	Phy-238 Phy-239	Chubut	S 43º 11' 39.7'' W 71º 28' 23.2''	Futaleufú, Trevelin, Río Grande valley, Nant y Fall falls	Jan 2006
15	Phy-271 Phy-273	Chubut	S 43º 34' 31.11'' W 71º 41' 10.64''	Corcovado, Momberg property	Aug 2008
16	Phy-267 Phy-270	Chubut	S 43º 33' 51.97'' W 71º 38' 54.83''	Corcovado, Underwood property	Aug 2008

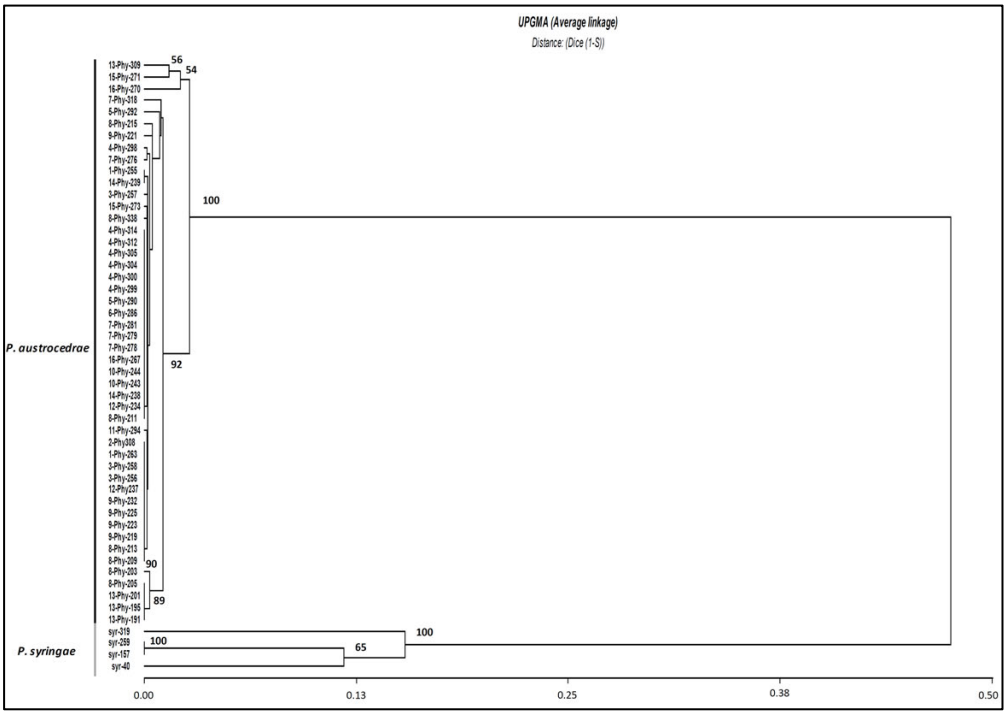


Figure 1: Dendrogram generated from AFLP distance matrix (1-Sj) for *Phytophthora austrocedrae*. Groups of isolates with zero branch length are comprised of samples with identical AFLP genotypes. Values at the branches are bootstrap values. *Phytophthora syringae* was used as outgroup.

Acknowledgements

This work was supported through an International Cooperation Program between Argentina and South Africa (MINCYT_DST SA/10/02), Agencia Nacional de Promoción Científica y Técnica (ANPCyT) of Argentina (PICTO 36776, PICT 08-0579), the Tree Protection Co-operative Programme (TPCP) and the NRF/DST Centre of Excellence in Tree Health Biotechnology (CTHB) of South Africa**.**

Literature Cited

Anonymus, 2011. Further findings of *Phytophthora lateralis* and first record of *Phytophthora austrocedrae* in Scotland (GB). Eppo Reporting Service 06-01: 135.

Anonymus, 2012. The Pathology Bulletin Issue 24: News release N°15292. www.forestry.gov.uk.

De Vos L, Myburg AA, Wingfield MJ, Desjardins AE, Gordon TR, Wingfield BD, 2007. Complete genetic linkage maps from an interspecific cross between *Fusarium circinatum* and *Fusarium subglutinans*. Fungal Genetics and Biology **44:** 701-714.

Greslebin AG, Hansen EM, Sutton W, 2007. *Phytophthora austrocedrae sp. nov.*, a new species associated with *Austrocedrus chilensis* mortality in Patagonia (Argentina). Mycological Research **111:** 308-316.

Greslebin AG, Hansen EM, 2010. Pathogenicity of *Phytophthora austrocedrae* on *Austrocedrus chilensis* and its relation with *mal del ciprés* in Patagonia. Plant Pathology **59:** 604-612.

Möller EM, Bahnweg G, Sandermann H, Geiger HH, 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. Nucleic Acids Research **20:** 6115-6116.

Vélez ML, Silva PV, Troncoso OA, Greslebin AG, 2012. Alteration of physiological parameters of *Austrocedrus chilensis* by the pathogen *Phytophthora austrocedrae.* Plant Pathology **61:** 877-888.

Vos P, Hogers R, Bleeker M, Reijans M, Van der Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M, 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research **23:** 4407-4414.

Isolation of *Phytophthora quercina* from rhizosphere soil of a declining *Quercus ilex* tree in the Natural Park 'Carrascar de la Font Roja' in Eastern Spain using young leaves of *Quercus robur* as baits (photo by Thomas Jung)

SURVEYS, DIVERSITY, INVASION AND SPREAD

Role of *Phytophthora pseudosyringae* in widespread dieback of *Nothofagus* plantations in Britain

B. Scanu[1,2], J.F. Webber[1]

[1]*Centre for Forestry and Climate Change, Forest Research, Farnham, Surrey GU10 4LH, UK.*
[2]*Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università di Sassari, Via E. De Nicola 1, 07100 Sassari, Italy.*

Corresponding author: bscanu@uniss.it

Abstract

Since 2009 extensive dieback and mortality of *Nothofagus obliqua* associated with bleeding cankers on stem and branches has been observed in Britain. The casual agent of this disease was identified as *Phytophthora pseudosyringae*, based on morphological and ITS analysis. In 2011 a survey was undertaken to assess the frequency and type of *P. pseudosyringae* infections, the comparative susceptibility of *N. obliqua* and other different woody hosts, and the sporulation potential of *P. pseudosyringae* on *Nothofagus* foliage. Infections of *P. pseudosyringae* on *Nothofagus* appeared to be widespread in Britain with infected trees being found on at least three sites in England, two in Scotland and one in Wales. Additional symptoms such as twig blight and leaf necrosis suggested that aerial infection was occurring. Besides *N. obliqua*, also *Nothofagus alpina*, *Fagus sylvatica* and *Vaccinium myrtillus* were found to be infected. In pathogenicity tests *P. pseudosyringae* was shown to be an effective bark pathogen of *Nothofagus*, but with significant differences between the different woody hosts assayed. Susceptibility of foliage showed marked differences between the host species tested, with *N. obliqua* leaves proving to be highly susceptible. The high levels of sporulation observed on infected *N. obliqua* leaves suggests that *P. pseudosyringae* has the potential to sporulate heavily on foliage and spread from there to shoots, branches and stems. *P. pseudosyringae* on *Nothofagus* represents the third *Phytophthora* species causing aerial infection on forest trees in the UK and could have the potential to pose a serious threat to *Nothofagus* in its native southern hemisphere.

More new *Phytophthora* species from natural ecosystems in Western Australia

A. Simamora[1], G.E.St.J. Hardy[1], M. Stukely[2], T.I. Burgess[1]

[1]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.
[2]Science Division, Department of Environment and Conservation, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.

Corresponding author: tburgess@murdoch.edu.au

Abstract

In 2009 we reported 11 apparently new *Phytophthora* species, designated P.sp.1-11, from natural ecosystems in Western Australia WA). Since then many of these species have been described: *P. multivora* (P.sp.4), *P. elongata* (P.sp.2), *P. constricta* (P.sp.9), *P. arenaria* (P.sp.1), *P. thermophila* (P.sp.3), *P. litoralis* (P.sp.11), *P. gregata* (P.sp.7), *P. fluvialis* (P.sp.8). P.sp.5 falls in the *P. cryptogea* species complex and P.sp.6 has been identified as *P.* taxon personii. Additionally we have described *P. gibbosa* and *P. amnicola.* Further sampling and continued molecular re-evaluation of the culture collection at the Department of Environment and Conservation's Vegetation Health Service (VHS) has uncovered more new species tentatively named P. aff. humicola, P. aff. rosacearum, P. aff. elongata, P. aff. arenaria, P. aff. captiosa, P. taxon kwongan (=P.sp.10), P. taxon casuarina. A large number of the new species are from ITS clade 6, sub-clade I and they have been isolated in remote natural vegetation. All known species and taxa in sub-clade I, with the exception of *P. humicola,* have been isolated in WA, perhaps illuminating a WA origin for this clade. Studies are currently underway to formally describe the new species in conjunction with large scale pathogenicity trials of these and the other newly described species.

Literature Cited

Burgess T.I., Webster J.L., Ciampini J.A., White D.W., Hardy G.E.St.J., Stukely M.J.C., 2009. Re-evaluation of *Phytophthora* species isolated during 30 years of vegetation health surveys in Western Australia using molecular techniques. *Plant Disease* **93:** 215-223.

Landscape-scale epidemic of *Phytophthora ramorum* on larch in the UK

J. Webber, C.M. Brasier

Forest Research (FR), Alice Holt Lodge, Farnham, Surrey GU10 4LH, United Kingdom

Corresponding author: joan.webber@forestry.gsi.gov.uk

Extended Abstract

Phytophthora ramorum has been reported from most European Union (EU) Member States, mainly affecting ornamental plants in nurseries (Anon. 2011). The most epidemiologically important hosts are those that support abundant sporulation and until recently in Europe this applied primarily to rhododendron and *Vaccinium myrtillus* (bilberry). In contrast to the USA, the impact of *P. ramorum* on trees in the UK was limited and only around a hundred trees were affected, comprising mainly beech (*Fagus sylvatica*) and non-native species of oak (Webber 2008). However, the first findings of *P. ramorum* affecting Japanese larch (*Larix kaempferi*) in south west Britain in August 2009 signalled the start of a marked change in the scale of damage caused by the disease (Brasier & Webber 2010).

Although the initial findings were of Japanese larch with *P. ramorum* infected foliage, many trees also showed extensive dieback leading to mortality. All ages of Japanese larch were affected, ranging from mature (25-30 m tall) to juvenile 5 to 6 year old trees, and the dieback symptoms were always associated with copious resin bleeding on trunks and branches. Isolation and re-inoculation confirmed that the symptoms were caused by *P. ramorum* invasion of the phloem tissue of mature bark. It also soon became clear that infected needles of *L. kaempferi* could produce huge numbers of sporangia (Webber *et al.* 2010). This was demonstrated not only in the laboratory but was also observed on naturally infected trees in autumn just before needle fall. The number of sporangia counted on individual larch needles infected with *P. ramorum* sometimes exceeded hundreds, even thousands, suggesting Japanese larch had a sporulation potential comparable to California bay laurel (*Umbellularia californica*) (Davidson *et al.* 2008). As with tanoak (*Notholithocarpus densiflorus*), larch proved to be both a canker and a foliar host, such that sporulation from infected foliage then provided inoculum for bark infection, resulting in multiple branch and stem infections on individual larch trees. The heavy sporulation on infected needles also led to infection and the formation of bark lesions on the stems of other trees adjacent to the larch. Affected species have included Douglas fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), fir (Abies spp) and birch (*Betula pendula*) as well as known canker hosts such as European beech (*Fagus sylvatica*) and sweet chestnut (*Castanea sativa*). All affected trees tended to be at a distance of a 100 m or less from the sporulating larch foliage. When infected larch were felled to remove the *P. ramorum* inoculum source, pathogen impact on the adjacent tree species was usually arrested.

In the three years since the first findings in south west England, 'ramorum disease' of larch has spread throughout the western side of the UK where the climate is apparently especially conducive to infection and sporulation. Findings on rhododendron suggest that *P. ramorum* was already present throughout much of this geographical range (Fera 2012); this probably facilitated the spread to larch. However, once established in larch plantations, intense sporulation events have allowed *P. ramorum* to spread further and infect many more trees compared to the pre-2009 conditions when rhododendron was the main sporulating host driving the epidemic in the UK. To control disease and comply with EU regulations millions of larch trees have now been felled and aerial surveillance is a vital part of early disease detection. All

three larch species that are grown commercially in the UK (*L. kaempferi*, *L. decidua* and *L.* x *eurolepis*) have been found naturally infected by *P. ramorum* although they differ in their bark and foliar susceptibility, and in the sporulation potential of infected needles. Overall, when the three larch species are compared, Japanese larch tends to sustain the highest levels of sporulation and have highly susceptible bark when challenged under laboratory conditions with *P. ramorum*, but susceptibility and sporulation levels vary significantly with the time of year (Anna Harris and Joan Webber, unpublished data).

The spread to larch raises many challenges to our understanding of the disease and pathogen including how *P. ramorum* may be changing in this new environment and the need for improved diagnostic methods when detecting *Phytophthora* spp in conifer material. For example, isolation success with larch bark samples tends to be low (less than 25%) necessitating PCR based diagnosis to confirm the presence of *P. ramorum* on many affected sites. The presence of inhibitory extractives such as tannins and resin acids in larch bark (Aaron 1982) appears to affect PCR detection of *P. ramorum*, requiring modified methods for sample preparation and DNA extraction prior to PCR. Use of PCR-based diagnosis to confirm the presence of *P. ramorum* in larch has also revealed that other *Phytophthora* species cause bark cankers on larch but these have previously gone undetected, possibly because of cryptic symptom development and isolation difficulties.

The changing disease dynamic on larch has become even more complex with the finding of a new lineage of *P. ramorum* in two locations in the UK. This was revealed in 2011 when preliminary screening of seven larch isolates with SSR markers and *Cox* II sequencing led to evidence of a novel genotype on larch in Northern Ireland and western Scotland, potentially distinct from the three known lineages (EU1, NA1, NA2: Grünwald *et al*.2012). The isolates were assigned to a new lineage: EU2 (Van Poucke *et al.* 2012), and an assessment of any adaptive differences between the newly arrived EU2 lineage and the widely established EU1 lineage is underway, including comparisons of pathogenicity to larch bark and sporulation potential of larch needles.

Literature Cited

Aaron J.R., 1982. Conifer bark: its properties and uses. HMSO. Second Edition: pp 23.

Anonymus, 2011. Scientific Opinion on the Pest Risk Analysis on *Phytophthora ramorum* prepared by the FP6 project RAPRA. *EFSA Journal* **9:** 2186-2294.

Brasier C.M., Webber J., 2010. Sudden larch death. *Nature* **466:** 824-825.

Davidson J.M., Patterson H., Rizzo D.M., 2008. Sources of inoculum for *Phytophthora ramorum* in a redwood forest. *Phytopathology* **98:** 860–866.

Fera, 2012. *Phytophthora ramorum* distribution map. http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/phytophthora/pRamorum/ [webpage accessed 2 February 2013].

Grünwald N.J., Garbelotto M., Goss E.M., Heungens K., Prospero S., 2012. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. *Trends in Microbiology* **20:** 131-138.

Van Poucke K., Franceschini S., Webber J.F., Vercauteren A., Turner J., McCracken A.R., Heungens K., Brasier C.M., 2012. Discovery of a fourth evolutionary lineage of *Phytophthora ramorum*: EU2. *Fungal Biology* **116:**1178-1191.

Webber J.F., 2008. Status of *Phytophthora ramorum* and *P. kernoviae* in Europe. In: Frankel SJ, Kliejunas JT, Palmieri, KM (eds), *Proceedings of the Sudden Oak Death Third Science Symposium*. USDA Forest Service Pacific South West General Technical Report 214. Albany, CA, 195-198.

Webber J.F., Mullett M., Brasier C.M., 2010. Dieback and mortality of plantation Japanese larch (*Larix kaempferi*) associated with infection by *Phytophthora ramorum. Plant Pathology New Disease Reports* **22:** 19.

Evaluating the effect of *Phytophthora* spp. on tree health in exotic plantations in New Zealand

M. Dick, N. Williams, I. Hood, J. Gardner

Scion (New Zealand Forest Research Institute), Private Bag 3020, Rotorua 3046, New Zealand.

Corresponding author: margaret.dick@scionresearch.com

Abstract

Historically the exotic forest plantations in New Zealand have been largely free of diseases caused by *Phytophthora* spp. in spite of the presence of some capable pathogens such as *P. cinnamomi* and *P. kernoviae*. However because of the increasing number of *Phytophthora* species causing novel diseases world-wide a re- evaluation of those isolated from New Zealand forestry species and their association with symptoms of disease was undertaken.
In a survey programme covering four years, stands aged from 2-28 years in plantation forests across the North Island were assessed for wilt, dieback, stem and branch cankers and needle disease that could not be attributed to known biotic and abiotic causal agents. Where non-attributable conditions were identified a potential association with an oomycete(s) was sought through isolation, by ELISA test (followed by PCR confirmation), and through microscopic examination of tissues.
Some novel host/*Phytophthora* associations of undetermined significance were encountered and these will be discussed.

Surveys of soil and water from asymptomatic natural ecosystems in South Africa reveal a goldmine of *Phytophthora* diversity

E. Oh[1,2], M. Wingfield[1], M. Gryzenhout[1,3], B. Wingfield[1], T.I. Burgess[1,4]

[1]*Forestry and Agriculture Biotechnology Institute, University of Pretoria, Pretoria, 0002, South Africa.*
[2]*Division of Forest Insects Pest and Diseases, Korea Forest Research Institute, 57 Hoegiro, Dondaemun-gu, Seoul 130-712, South Korea.*
[3]*Department of Plant Sciences, University of the Free State, Bloemfontein 9301, South Africa;*
[4]*Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.*

Corresponding author: tburgess@murdoch.edu.au

Abstract

Phytophthora species are well known as destructive plant pathogens, especially in natural ecosystems. However, little is known regarding the *Phytophthora* diversity in forests of South Africa. In this study, *Phytophthora* species were isolated using standard baiting techniques from 150 soil and water samples and these were identified based on ITS and *cox*I sequence data. The 162 resulting *Phytophthora* isolates resided in 11 taxa including four known species (*P. multivora, P. capensis*, *P. frigida, P. cinnamomi*) the known but as yet unnamed, *Phytophthora* taxon PgChlamydo and *P.* taxon emzansi and five new taxa. The most commonly isolated species from soil was *P. multivora* (75%), a species recently described from Western Australia where it has been extensively associated with dead and dying trees. *Phytophthora capensis* and *P.* taxon emzansi have recently been described from the Cape region of South Africa and *P. multivora* was also reported from this region. The extensive isolation of *P. multivora* from asymptomatic natural vegetation suggests that South Africa may be the origin of this species. *Phytophthora frigida* was isolated for the first time from stream water and *P.* taxon PgChlamydo was isolated for the first time in Africa. The new species were isolated from water and not surprisingly belong to ITS Clades 6 and 9. With the exception of *P. cinnamomi,* very little is known regarding the biology, epidemiology or origin of these species.

Species of *Phytophthora* associated with *Quercus* decline in the Mediterranean Park 'Carrascar de la Font Roja' (Spain)

A. Pérez-Sierra[1], C. López-García[1], M. León[1], J. García-Jiménez[1], P. Abad-Campos[1], T. Jung[2,3]

[1]Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.
[2]Phytophthora Research and Consultancy, Thomastrasse 75, 83098 Brannenburg, Germany; [3]
[3]Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Corresponding author: aperesi@eaf.upv.es

Abstract

The Natural Park Carrascar de la Font Roja is one of the best preserved nature areas (2298 ha) in Comunidad Valenciana (Eastern Spain) that includes the Menejador mountain range, with the highest altitude of 1356 m. The forest is dominated by Mediterranean *Quercus* spp., ie. *Quercus ilex*, *Q. faginea* and *Q. coccifera*. During the last decade a severe decline of oaks has been observed. The symptoms include dieback of branches and parts of the crown, increased transparency of the crown, withering of leaves and death of trees. A significant decrease in the production of acorns and saplings affecting natural regeneration has also been observed. No previous studies on a possible involvement of *Phytophthora* and other oomycetes in the decline have been carried out. Therefore, during 2010-2011 soil samples from affected trees were collected and their roots examined. An extensive loss of both lateral small woody roots and fine roots and callussing or open cankers were observed. Soil samples containing fine roots from declining trees were baited using both *Q. robur* leaves and apple fruits as baits. Six *Phytophthora* species were detected: *P. cryptogea, P. gonapodyides, P. megasperma, P. quercina, P. psychrophila* and *P. syringae.* Pathogenicity test with representative isolates of these species were conducted for six months under control conditions with one-year old seedlings of *Q. ilex* and *Q. faginea. P. cinnamomi* was included in the pathogenicity test for comparison. The results showed that *Q. ilex* seedlings were more susceptible to infection than *Q. faginea* with *P. cinnamomi* and *P. megasperma* causing severe reduction in root biomass. The role of all *Phytophthora* species detected in the decline of Mediterranean *Quercus* spp. is discussed.

First approach into the knowledge of the *Phytophthora* species diversity in Mediterranean Holm oak forests based on 454 parallel amplicon pyrosequencing of soil samples

S. Català, A. Pérez-Sierra, M. Berbegal, P. Abad-Campos

Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain.

Corresponding author: pabadcami@eaf.upv.es

Abstract

The evergreen holm oak (*Quercus ilex* L.) is the most representative tree species in the Iberian Peninsula and the main tree in oak-rangeland ecosystems (dehesas). Among the most important problems that threaten the persistence of oak groves and dehesas are the absence of natural regeneration and root rot due to *Phytophthora cinnamomi*. Recently, new species of *Phytophthora* other than *P. cinnamomi* were detected in holm oak forest using traditional baiting methods and isolation. Since it is not easy to detect, identify or quantify *Phytophthora* species, the need arises to find a rigorous technique, which is rapid, reliable and highly reproducible to evaluate their diversity in living in holm oak forests. In this study, the polymorphic ITS1 region is used to evaluate the presence of *Phytophthora* species in soil samples. Tagged amplicons were obtained with *Phytophthora* template-specific primers which excluded other oomycetes and fungi. Similarity of the barcoded reads obtained by 454-pyrosequencing after BLAST against GenBank database and comparison of abundance was assessed from three different soil samples: from trees showing decline symptoms, rainfall runoff areas from the same forest, and soil samples from an asymptomatic forest. The results show the reads distribution, the species abundance from each soil and the phylogenetic analyses. This study provides important insights into the *Phytophthora* species diversity in Mediterranean holm oak forests.

Introduction of *Phytophthora ramorum* in Fagaceae forests in Italy

A.M. Vettraino, A. Tomassini, N. Bruni, S. Franceschini, AndreaVannini

DIBAF, University of Tuscia, Via S. Camillo de Lellis, Viterbo 01100, Italy.

Corresponding author: vannini@unitus.it

Abstract

Since it become a quarantine pathogen for Europe, *Phytophthora ramorum* has been detected officially twice in Italian nursery stocks. Due to the low level of attention put in detection activities in the last years, presence of *P. ramorum* in Italian nurseries might be underestimated. Differently Italian *Fagaceae* forests have been largely inspected for *Phytophthora* presence especially in soil by means of classical baiting methods followed by morphological and molecular identification. Results of these activities never recorded the presence of *P. ramorum* in *Fagaceae* forests in Italy. Recent utilization of mass sequence techniques provided the possibility to analyse in one step, and high sensitivity, *Phytophthora* population in forest soils. Pyrosequencing analysis of chestnut soils has been carried out in two sites in Italy to evaluate the diversity of resident *Phytophthora* community. Sequence data have been analysed with dedicated database and resulted in a range of Phytophthoras including species known to be common in chestnut and beech forest soils in Italy. In addition some new species resulted to be present and represented by a discrete number of reads. Among these, *P. ramorum* was commonly detected in chestnut soils. To confirm the detection, DNA's utilised for pyrosequencing was amplified with species specific primers sets for *P. ramorum* and the amplicons obtained were sequenced. Sequences obtained matched with 100% identity with *P. ramorum* sequences on database. Cryptic presence of *P. ramorum* in forest soils would represent an important improvement of knowledge on epidemiology and invasion mechanisms of this species in Mediterranean climate.

Phytophthora bilorbang prov. nom., a new species associated with declining *Rubus anglocandicans* (blackberry) in Western Australia

S. Aghighi[1], G.E.St.J. Hardy[1], J.K. Scott2, T.I. Burgess[1]

[1]*Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.*
[2]*CSIRO Ecosystem Science and Climate Adaptation Flagship, WA, Australia.*

Corresponding author: S.Aghighi@murdoch.edu.au

Abstract

Rubus anglocandicans is the most widespread and invasive species in the *Rubus fruticosus* aggregate (European blackberry) found in Australia [1]. Blackberry has been targeted by biological control since the 1980s and most of this effort has focused on introducing exotic strains of the host-specific leaf rust, *Phragmidium violaceum.* During surveys established to assess the releases of the rust fungus in 2005, dead and diseased blackberry plants were found at two locations along the Warren and Donnelly Rivers in the Manjimup region of Western Australia (P. Yeoh and L. Fontanini personal communication). The disease symptoms could not be attributed to the rust fungus and the phenomenon has been referred to as 'blackberry decline'. The disease appears to be due to root pathogen(s) and during initial sampling several *Phytophthora* species were isolated. In order to investigate the cause(s) of disease and the potential role of *Phytophthora* species in the decline, field surveys were carried out over 2010 and 2011 in the decline and non-decline sites along the Warren and Donnelly Rivers. During these surveys, *P.* taxon oaksoil was recovered from decline sites. Several isolates of this taxon have been isolated from Europe [2], and given a provisional name (oaksoil) until formal description. This taxon is described here as *Phytophthora bilorbang* prov. nom.; a new taxon within the ITS Clade 6, sub-clade II of *Phytophthora*. This is the first report of this new *Phytophthora* species in association with declining *R. anglocandicans.*

Literature Cited

Evans K.J., Weber H. E., 2003. *Rubus anglocandicans* (Rosaceae) is the most widespread taxon of European blackberry in Australia. *Australian Systematic Botany* **16:** 527–537.
Brasier C.M., Cooke D.E.L., Duncan J.M., Hansen E.M. 2003. Multiple phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides–P. megasperma* ITS Clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile. *Mycological Research* **107:** 277–290.

A species concept for *Phytophthora* taxon Agathis (PTA) — causal agent of root and collar rot of *Agathis australis* in New Zealand

S.E. Bellgard, B.S. Weir, P.R. Johnston, D. Park, D.J. Than, N. Anand, S.R. Pennycook

Landcare Research Private Bag 92170, Auckland Mail Centre, Auckland, 1142, New Zealand.

Corresponding author: WeirB@LandcareResearch.co.nz

Abstract

Kauri Dieback has been identified as an increasing problem affecting kauri (*Agathis australis*) across the Auckland and Northland regions. *Phytophthora* taxon Agathis (PTA) has been identified as a causal agent of a root and collar rot of kauri [1]. 'PTA' shares a place in *Phytophthora* ITS Clade 5 [2] with *P. heveae* and *P. katsurae*. PTA was originally misidentified as the morphologically similar *P. heveae.* It has been established that PTA has a different oogonial morphology to both *P. heveae* and *P. katsurae.* The sequencing of eight loci from both the nuclear and mitochondrial genomes has been used to resolve the species boundaries within Clade 5. Bayesian inference phylogenies reveal PTA is a discrete taxonomic entity, separate from either *P. katsurae* or *P. heveae*. Further, because of its unique colony morphology, oogonial characters, persistent sporangia and pathogenicity to *Agathis australis* we recognise PTA indet. as a new species in ITS Clade 5 of the genus *Phytophthora.*

Acknowledgements

Dr Nicholas Waipara Biosecurity Advisor, Auckland Council, Professor Andrea Vannini University of Tuscia, Dr Andre Drenth University of Queensland, Dr Peter Buchanan, Elsa Paderes, Karyn Hoksbergen, and Chris Winks Landcare Research Tamaki.

Literature Cited

Beever R.E., Waipara N.W., Ramsfield T.D., Dick M.A., Horner I.J., 2009. Kauri (*Agathis australis*) under threat from *Phytophthora?* Proceedings 4th IUFRO Working Party 7-02-09 "*Phytophthora* in Forests and Natural Ecosystems", 26-31st August 2007, Monterey, California, USA: 74-85.

Cooke D.E.L., Drenth A., Duncan J.M., Wagels G., Brasier C.M., 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* **30:** 17-32.

Phytophthora ITS Clade 3 expands to include a new species, *P. pluvialis*.

P.W. Reeser, W. Sutton, E.M. Hansen

[1]*Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331 USA.*

Corresponding author: reeserp@science.oregonstate.edu

Abstract

Phytophthora ilicis was described in 1957 as a pathogen of holly (*Ilex aquifolium*). In 2000 it was one of two founding members of ITS Clade 3. In 2002–2003 three new species were added. We propose to add a another new species to be named *P. pluvialis*. While exhibiting unique features, *P. pluvialis* shares some morphological features with other members of Clade 3. It has ovoid, partly caducous, semi-papillate sporangia with variable length pedicels borne on unbranched or simple sympodial sporangiophores. Antheridia are predominately amphigynous. Oogonia measure around 32 µm diameter. Distinctive hyphal swellings are produced in agar and in water. All isolates studied have identical nuclear rDNA-ITS sequence, with at least seven different haplotypes recognized by mitochondrial *cox* spacer sequences. Compared with other species in Clade 3, DNA sequences form a unique phylogenetic taxon. *P. pluvialis* has been recovered mostly from canopy drip, soil, and streams in the mixed tanoak (*Notholithocarpus densiflorus*)-Douglas-fir (*Pseudotsuga menziesii*) forest in Curry County, Oregon, USA. It has been found only rarely associated with twig and stem cankers on tanoak. It is also found infrequently in streams in other areas of western Oregon. Like the other Clade 3 species, *P. pseudosyringae* and *P. psychrophila*, *P. pluvialis* is not strongly host-associated, and may be endemic. This contrasts with *P. ilicis*, which has been found only causing disease on holly, and with *P. nemorosa*, which is strongly associated with disease on tanoak and Oregon myrtlewood (*Umbellularia californica*). A manuscript for formal description of the new species is being submitted for publication.

Acknowledgments

We thank the Oregon Department of Forestry and the USDA Forest Service, Pacific Southwest Experiment Station and Southwest Oregon Forest Insect and Disease Center for participation in, and support of, this work.

Phytophthora acerina sp. nov., a new species from the *P. citricola* complex causing aerial cankers on *Acer pseudoplatanus* in Italy

B. Ginetti[1], S. Moricca[1], A. Ragazzi[1], T. Jung[2,3]

[1]*University of Florence, Agricultural and Biotechnology Department, Section of Plant Protection, Cascine Square 28 -50144, Florence, Italy.*
[2]*Phytophthora Research and Consultancy, Thomastrasse 75, D-83098 Brannenburg, Germany.*
[3]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*

Corresponding author: beatrice.ginetti@unifi.it

Abstract

A new homothallic *Phytophthora* species with paragynous antheridia, semipapillate, persistent and highly variable sporangia, and optimum and maximum temperatures of 25 and 32 °C, respectively, was consistently isolated from aerial bleeding cankers and rhizosphere soil of declining *Acer pseudoplatanus* trees in planted forests in northern Italy. In underbark inoculation tests all isolates were highly aggressive to *A. pseudoplatanus* and *Fagus sylvatica* indicating that this pathogen might pose a serious threat to maple and beech forests in Europe. All isolates share identical ITS and *cox*1 sequences and represent a distinct subclade of the *P. citricola* complex. Interestingly, all isolates showed a high abortion rate of the oospores distinguishing this taxon from all other known species and taxa of the *P. citricola* complex. Most likely, this species evolved under conditions that did not require oospores as long-term resting structures so that selection did not weed out deleterious mutations in the breeding system. A gradual loss of fertility in favour of a continuous asexual reproduction by zoospores is well-known from *Phytophthora* species in wet or aquatic habitats. Due to its unique combination of morphological, physiological and molecular characters this new taxon is currently being described as a new species, *P. acerina* sp. nov.

Phytophthora acerina nom. prov., a new species from the *P. citricola* complex causing aerial cankers on *Acer pseudoplatanus* in Italy

Beatrice Ginetti[1], Salvatore Moricca[1], Alessandro Ragazzi[1] and Thomas Jung[2,3]

[1]University of Florence, Agricultural and Biotechnology Department, Section of Plant Protection, Cascine Square 28 -50144, Florence, Italy. [2]Phytophthora Research and Consultancy, Thomastrasse 75, D-83098 Brannenburg, Germany. [3]Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, (IBB/CGB), Plant and Animal Genomic Group, Laboratório de Biotecnologia Molecular e Fitopatologia,University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

INTRODUCTION

A new homothallic *Phytophthora* species was isolated from aerial bleeding cankers and rhizosphere soil of declining *Acer pseudoplatanus* trees in planted forests in northern Italy.

Trees showed symptoms such as bleeding bark cankers along the stem, stunted growth and general crown dieback.

Fig. 1. Crown dieback and bleeding bark cankers with xylem discoloration on *Acer pseudoplatanus* trees.

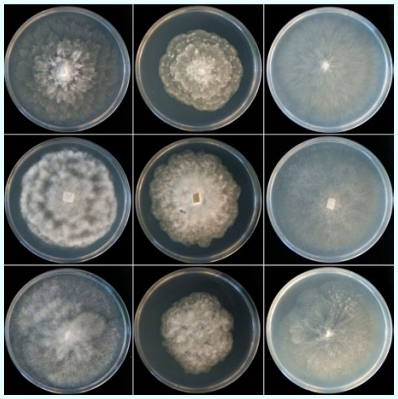


Fig. 2. Colony morphology of isolates B063, B077 and B080 (from top to bottom) after 7 d growth at 20°C on V8A, PDA and MEA (from left to right).

MORPHOLOGICAL FEATURES

Colony growth patterns were chrysantemum to faintly petaloid or petaloid on V8A, rosaceous to petaloid on PDA and uniform to faintly stellate on MEA (Fig. 2).

Phytophthora acerina prov. nom. is homothallic with paragynous antheridia. Oogonial diameter was 32.0 ± 4.4 µm. Oospores were globose averaging 28.4 ± 3.9 µm (Fig. 3a-b). All isolates showed high abortion rates of the oospores (38.5%; 31-90%; Fig. 3c-d) distinguishing this taxon from all other taxa of the *P. citricola* complex. Oospores were mostly aplerotic (69.6%; 40–96%). Oospores were thick-walled with a mean wall diameter of 2.00 ± 0,38 µm and a mean oospore wall index of 0.38 ± 0.09. The antheridia were paragynous and measured 12.8 ± 3,4 x 9.5 ± 1,6.

Non-caducous semipapillate sporangia were terminally produced on unbranched sporangiophores and ranged in shape from ovoid or limoniform to obpyriform, ellipsoid, obovoid, mouse-shaped, broad-ovoid or distorted shapes (Fig. 4). Unusual features such as curved apices, lateral attachment of the sporangiophore, intercalary insertion, hyphal swellings on sporangiophore, short hyphal projections, widening of the sporangiophore towards the base of the sporangium or direct germination were common in all isolates. Sporangia averaged 52.0 ± 13 x 32.8 ± 7.7 µm with a length/breadth ratio of 1.6 ± 0.3 µm. Exit pores averaged 8.1 ± 1.8 µm.

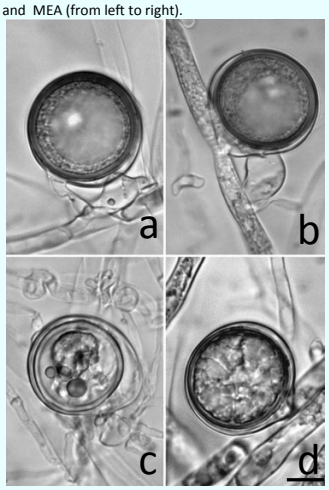


Fig 3. a-b: viable oogonia with slighly aplerotic oospores and paragynous antheridia; c-d: aborted oogonia. Bar = 10 µm

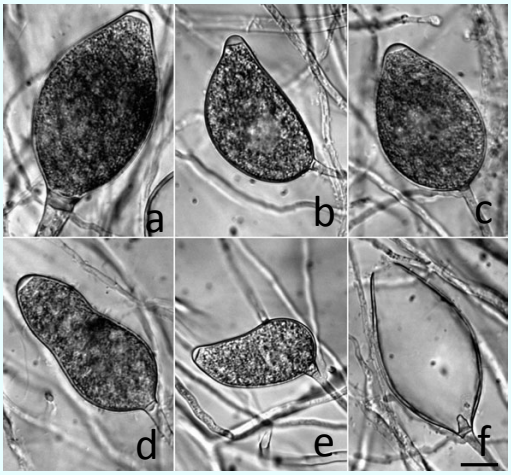


Fig. 4. Non-caducous and semipapillate sporangia of *P. acerina*; a: limoniform with conspicuous basal plug; b: mouse-shaped with pointed apex; c: ovoid; d: obpyriform; e: intercalary with curved apex; f: empty sporangium. Bar = 10 µm

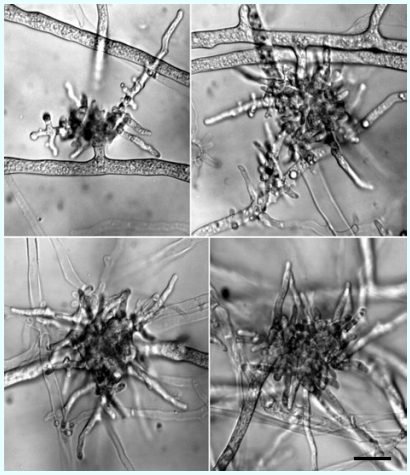


Fig. 5. Stromata-like hyphal aggregations of *P. acerina*. Bar = 10 µm

All isolates of *P. acerina* produced globose to subglobose hyphal swellings and stromata-like hyphal aggregations (Fig. 5).

In an underbark inoculation test *P. acerina* caused lesion lengths of 9.9 ± 1.3 cm on *A. pseudoplatanus* and 4.6 ± 1.3 cm on *Fagus sylvatica* (Fig. 6).

All isolates shared identical ITS and *cox*1 sequences and clustered as a distinct subclade within the *P. citricola* complex (Fig. 7).

Fig. 6. a-b. Inoculated stems showing distinct lesions caused by *P. acerina* after three weeks of incubation at 20°C in a wet chamber; *a:* on *A. pseudoplatanus;* b: on *Fagus sylvatica*. All inoculated isolates could be re-isolated.

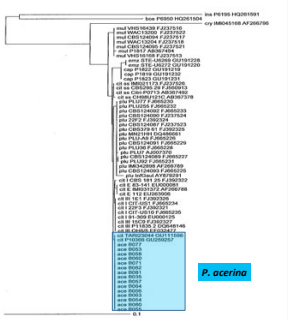


Fig. 7. Phylogenetic position of *P. acerina* within the *P. citricola* complex.

Table 1. List of the most important morphological and physiological characters of *P. acerina*.

Sporangia lxb mean	52.0 ± 13.0 x 32.8 ± 7.7
l/b ratio	1.6 ± 0.26
Oogonia mean diam	32.0 ± 4.4
Oospores mean diam	28.4 ± 3.9
Wall diam	2.0 ± 0.4
Oospores wall index	0.38 ± 0.09
Aplerotic oospores	69.6 % (40 - 96 %)
Abortation rate	38.5 % (10 - 99 %)
Antheridia lxb mean	12.8 ± 3.4 x 9.48 ± 1.6
Hyphal aggregations	+
Maximum temperature (°C)	32
Optimum temperature (°C)	25
Growth rate on V8A at optimum (mm/d)	7.75 ± 0.19
Growth rate at 20 °C (mm/d) on V8A	6.46 ± 0.22
Growth rate at 20 °C (mm/d) on PDA	4.28 ± 0.16
Lesions lenght on *Acer pseudoplatanus*	4.6 ± 0.22
Lesions lenght on *Fagus sylvatica*	9.9 ± 0.3

CONCLUSIONS

Due to its unique combination of morphological, physiological and molecular characters this new taxon is currently being described as a new species, *P. acerina* sp. nov. This new species is causing a lethal canker disease of *Acer pseudoplatanus* trees and is potentially posing a serious risk to *Fagus sylvatica*, too.

LITERATURE

Jung, T. & Burgess T.I. (2009), *Persoonia* 22, 95-110.

Rain water ponding at the surface of a *Phytophthora cinnamomi* infested dehesa of *Quercus ilex* in Extremadura, Spain due to soil compaction caused by grazing cattles (photo by Thomas Jung)

ECOLOGY, EPIDEMIOLOGY AND CLIMATE CHANGE

Ectomycorrhizae and *Phytophthora cinnamomi* relations within the rhizosphere of *Quercus ilex*

T. Corcobado, A. Pérez, M. Vivas, G. Moreno, A. Solla

Ingeniería Forestal y del Medio Natural. Universidad de Extremadura. Avenida Virgen del Puerto 2, 10600 Plasencia, Spain.

Corresponding author: asolla@unex.es

Abstract

Oak decline is a major forest problem in Iberia and is principally associated with the loss of fine roots caused by *Phytophthora cinnamomi*. Ectomycorrhizae (ECM) are known to be beneficial for trees promoting plant growth and protecting roots from pathogen infections [1]. This study aimed to analyse the ECM community structure and its relation with the presence of *P. cinnamomi*. In 96 declining stands in Extremadura, Spain, three non-declining and three declining trees (≤ 5% and 21-40% of crown transparency, respectively) per stand were selected. For ECM and *Phytophthora* assessment monoliths of rhizosphere soil were collected from each tree. Each ECM tip was counted and categorized into morphotypes and the ECM abundance, species richness and diversity were estimated. Concerning physical and chemical soil factors, A soil horizon depth, soil bulk density, soil texture and pH, soil redox status and contents of ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3$) were measured. Preliminary results revealed a higher percentage of mycorrhizal root tips in non-declining trees than in declining trees (62.3 ± 17.8% and 57.6 ± 15.8 %, respectively; $p<0.05$). The declining status of trees had also an impact on species richness and Shannon diversity index, which were higher in non-declining trees ($p<0.05$). The presence of *P. cinnamomi* was associated with an increase of fine root mortality ($p<0.001$). Significant correlations were found between physical and chemical soil factors and ECM abundance. These results showed that crown transparency had a negative effect on ECM structure as demonstrated in other studies [2].

Literature Cited

Branzanti M.B., Rocca E., Pisi, A., 1999. Effect of ectomycorrhizal fungi on chestnut ink disease. *Mycorrhiza* **9:** 103-109.

Kuikka K., Härmä E., Markkola A. M., Rautio P., Roitto M., Saikkonen K., Ahonen-Jonnarth U., Finlay R., Tuomi, J., 2003. Severe defoliation of Scots pine reduces reproductive investment by ectomycorrhizal symbionts. *Ecology* **84:** 2051–2061.

Wildfire influences forest disease dynamics through selective host mortality and pathogen suppression: sudden oak death in Big Sur, CA

M.R. Metz[1], M.M. Beh[1], K. Frangioso[1], R.K. Meentemeyer[2], D.M. Rizzo[1]

[1]Department of Plant Pathology, University of California, Davis, CA 95616 USA.
[2]Department of Geography and Earth Sciences, University of North Carolina, Charlotte, NC, 28223 USA.

Corresponding author: dmrizzo@ucdavis.edu

Abstract

Most disease ecology in forests has focused on shifts to community composition due to disease without consideration of the role of interacting disturbances. Sudden oak death (SOD), caused by *Phytophthora ramorum*, is associated with extensive tree mortality in coastal California forests. Wildfire is an important disturbance in these forests influencing community composition in the absence of SOD [1]. Fire may impact SOD directly through suppression of *P. ramorum* or indirectly through mortality of epidemiologically important hosts. Through surveys of burn severity, tree mortality, and regeneration following wildfires in SOD-impacted forests, we asked (i) how wildfire affected *P. ramorum* survival; and (ii) how forest recovery differs under the separate or joint influences of SOD and wildfire. Both disturbances cause selective mortality because the dominant tree species in these forests differ in their susceptibility to mortality from SOD and fire. In two habitat types, the dominant hosts for pathogen sporulation suffered greater fire-caused mortality than other species, which should lead to disease suppression in burned, infested areas relative to unburned, infested areas. We observed such suppression because only 20% of sampled, previously infested burned areas were found to contain *P. ramorum* immediately following the fire. In non-burned areas, forest composition has been shifting to dominance by sporulating species that do not die from pathogen infection, leading to positive feedbacks on disease prevalence in these areas and continued mortality of canker hosts. The trajectory of post-disturbance recovery thus differs greatly among sites depending on the separate or joint influences of SOD and fire.

Literature Cited

Metz M. R., Frangioso K., Meentemeyer R.K., D.M. Rizzo, 2011. Interacting disturbances: wildfire severity affected by stage of forest disease invasion. *Ecological Applications* **21:** 313-320.

Susceptibility to *Phytophthora cinnamomi* of the main crops in dehesas and their influence in the epidemiology of the oak root disease

M.S. Serrano[1], P. De Vita[1], P. Fernández[2], M.E. Sánchez[1]

[1]Agronomy Department, University of Córdoba, Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain.
[2]Forestry Department, University of Córdoba, Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain.

Corresponding author*:* ag1sahem@uco.es

Abstract

Phytophthora cinnamomi, the main cause of *Quercus* root rot in south-western Spain, is an aggressive pathogen on *Lupinus luteus* (yellow lupin), causing root rot, wilting and death of this crop [1], but not on other crops (*Triticum aestivum, Avena sativa* or *Vicia sativa*) common in oak-rangeland ecosystems (*dehesas*) in the region. The pathogen was isolated from roots of wilted lupins in the field. Artificial inoculations on four cultivars of *L. luteus* reproduced the symptoms of the disease, both in pre- and post-emergence stages, recovering the pathogen from necrotic roots. Under controlled conditions and also in field samples, it was observed that *Lupinus luteus* increased the inoculum levels in the soil. For the rest of crops, by means of artificial inoculations with *P. cinnamomi*, positive isolations from infected roots of yellow lupin (symptomatic) and vetch (asymptomatic) were obtained, but never form wheat and oat (asymptomatic). Through *in vitro* infection experiments, it was demonstrated that yellow lupin highly stimulated the production of zoospores of *P. cinnamomi*. Vetch, wheat and oat did not stimulate zoospore production. In addition and opposite to lupin, vetch did not influence the viability of chlamydospores in the soil. We concluded that the culture of wheat, oat and even vetch in rangelands did not influence the epidemiology of *Quercus* root disease and they could be a good alternative to yellow lupin in rangeland ecosystems affected by root rot.

Literature Cited

Serrano M.S., Fernández-Rebollo P., De Vita P., Carbonero M.D., Sánchez M.E., 2010. *Lupinus luteus,* a new host of *Phytophthora cinnamomi* in Spanish oak-rangeland ecosystems. *European Journal of Plant Pathology* **128:** 149-152.

Sudden oak death impacts to communities and ecosystems in California forests

R.C. Cobb, D.M. Rizzo

University of California Davis, Department of Plant Pathology, One Shields Ave, Davis CA, USA.

Corresponding author: rccobb@ucdavis.edu

Abstract

Recognition of *Phytophthora* importance has increased as more of these pathogens are introduced to naive host populations. We demonstrate epidemiological drivers of *Phytophthora* impacts on community composition, distribution of forest biomass, and ecosystem processes including litterfall, decomposition, and nitrogen cycling in California forests impacted by *Phytophthora ramorum* and the resulting disease sudden oak death. For *P. ramorum*, patterns of host mortality, pathogen spread, and accumulation of large woody debris are greatly determined by the prevalence of sporulation supporting species especially tanoak (*Notholithocarpus densiflorus*) which is rapidly killed following infection and California bay laurel (*Umbellularia californica*) which does not suffer deleterious impacts from infection. This leads to apparent competition (increased dominance of species not directly impacted by outbreak organisms) between the two species and positive feedbacks on pathogen populations in many *P. ramorum* impacted forests. *P. ramorum* caused mortality leads to modest changes in litterfall chemistry and soil N availability but the long-term consequences of species shifts are much larger and long-lasting consequences to ecosystem processes. Control and eradication of *P. ramorum* is very difficult because of the pathogen's broad host range, survival in the environment, and prolific basal sprouting from disease-killed trees. The long-term consequence of this disease is removal of tanoak from the overstory in many forests, an impact similar to chestnut blight in the Northeastern USA. However, we found thresholds for pathogen persistence in epidemiological models suggesting a conservation strategy that combines identification of host resistance and management to reduce sporulation can retain biodiversity associated with tanoak.

Could climate warming be one of the causes of *Phytophthora alni* emergence in Europe?

J. Aguayo, C. Husson, B. Marçais

INRA, Nancy-Université, UMR 1136 Interactions Arbres-Microorganismes, 54280 Champenoux, France.

Corresponding author: marcais@nancy.inra.fr

Abstract

Emerging infectious diseases have become a major threat to the forest ecosystem conservation. Several factors may cause these emergences, among which evolution of sylviculture practices, environnemental changes, or introduction of alien pathogen by international trade. Those causes often can occur together and precisely assessing their importance is an important scientific question. In France, *Alnus glutinosa* is threatened by the development of the epidemic caused by *Phytophthora alni*. This pathogen is known to have been invasive in part of Europe and is the result of an interspecific hybridation event between *Phytophthora alni* subsp. *uniformis* and *Phytophthora alni* subsp *multiformis*. The aim of this study was to assess whether the climate warming of the last decades might have participated determine in the disease emergence. For that, *P. alni* soil inoculum and incidence of the crown decline and of canker were monitored on 16 sites located along a altitudinal gradient in NE France used as an proxi for a temperature gradient. The results show that the disease incidence, i.e. the likelihood of new disease case in the sites, was positively correlated with the mean temperature of the winter. Evolution of past temperature in the last 40 years suggests that climate warming could by one of the cause explaining the emergence of *P. alni* alder decline.

Influence of bird faeces in the behaviour of the root rot of *Quercus suber* caused by *Phytophthora cinnamomi* at Doñana Biological Reserve (SW Spain)

P. De Vita[1], M.S. Serrano[1], L.V. García[2], C. Ramo[3], M.E. Sánchez[1]

[1]*Dpto. Agronomía. ETSIAM, Universidad de Córdoba, Apdo. 3048, 14080-Córdoba, Spain.*
[2]*IRNAS (CSIC), Avda Reina Mercedes 10, 41012-Sevilla, Spain.*
[3]*Estación Biológica de Doñana (CSIC). Avda Américo Vespucio s/n, 41092-Sevilla, Spain.*

Corresponding author: er2vivip@uco.es

Abstract

Centennial cork oaks are considered keystone structures in the ecosystem of the stabilised sands of the Doñana Biological Reserve. These remnant big trees are currently threatened by nesting of colonial waterbirds, whose debris induced deep soil chemical changes. Since 2008 *P. cinnamomi* is also being isolated from roots and rhizosphere of declining trees [1]. *Phytophthora cinnamomi* has experienced a large spread in the Park over the last years, taking advantage of the extremely wet 2010 spring and winter. The objective of this work was to analyze the ability of the pathogen for oak root infection at various concentrations of natural and commercial (guano) bird dejections:
a) *in vitro*, by testing the influence of three concentrations of bird faeces on clamydiospore viability, sporangial production and zoospore release, and
b) *in planta*, adding bird faeces to infested soil at different concentrations and analyzing plant response at the synergy between pathogen and dejections on infection of seedling roots.
The results obtained in the *in vitro* experiments showed that high concentrations of faeces inhibit crucial steps in the life cycle of the pathogen and consequently, could affect its infection ability. Results to be obtained in plant experiments will be show at the congress.

Literature Cited

De Vita P., Serrano M.S., Callier P., Ramo C., García L.V., Sánchez M.E., 2012. *Phytophthora* root disease: a new threat for cork oaks at Doñana National Park (South-Western Spain). *IOBC/WPRS Bulletin* **76:** 93-96.

Spatial patterns of *Phytophthora cinnamomi* in declining Mediterranean forests: implications for tree species regeneration

L. Gómez-Aparicio[1], B. Ibáñez[1], M.S. Serrano[2], P. De Vita[2], J.M. Ávila[1], I.M. Pérez-Ramos[1], L.V. García[1], M.E. Sánchez[2], T. Marañón[1]

[1]*Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, PO Box 1052, Sevilla E-41080, Spain.*
[2]*Dpto. Agronomía, ETSIAM, Universidad de Córdoba, PO Box 3048, Córdoba E-14080, Spain*

Corresponding author: lorenag@irnase.csic.es

Abstract

Soil-borne pathogens are a key component of the belowground community due to the significance of their ecological and socio-economic impacts. However, very little is known about the complexity of their distribution patterns in natural systems. Here we explored the patterns, causes and ecological consequences of spatial variability in the abundance of the soil-borne pathogen *Phytophthora cinnamomi* in Mediterranean forests, where this species represents a major driver of oak decline. We used spatially-explicit neighborhood models to predict *Phytophthora* abundance as a function of local abiotic conditions (soil texture) and the characteristics of the tree and shrub neighborhoods (species composition, size and health status). The implications of *Phytophthora* abundance for tree seedling performance were explored by conducting a sowing experiment in the same locations where pathogen abundance was quantified. *Phytophthora* abundance in the forest soil was not randomly distributed, but exhibited spatially predictable patterns influenced by both abiotic and particularly biotic factors (tree and shrub species). Soil texture seemed to affect *Phytophthora* abundance indirectly through its effects on soil water content, whereas woody species affected *Phytophthora* mostly directly by providing living host tissue with different susceptibility to pathogen attack. *Phytophthora* abundance reduced seedling emergence and survival, but not in all sites or tree species. Our findings suggest that heterogeneous spatial patterns of *Phytophthora* abundance at fine spatial scale can have relevant implications for the dynamics and restoration of declining Mediterranean forests.

Roads and Streams Are Not Significant Pathways for SOD Spread in Tanoak Forests

E.K. Peterson[1], J.M. Hulbert[2], E.M. Hansen[2]

[1]*Oregon State University Dept. Forest Engineering, Resources and Management, Corvallis, OR 97331.*
[2]*Oregon State University Dept. Botany and Plant Pathology, Corvallis, OR 97331.*

Corresponding author: petersoe@science.oregonstate.edu

Introduction

The phytopathogen *Phytophthora ramorum*, causal agent of sudden oak death (SOD) in oaks and tanoaks, continues to spread within Oregon tanoak forests. Soils and streams have been presumed to be sources of primary inoculum for this pathogen in natural ecosystems. As in California, *P. ramorum* can be recovered from soils at Oregon's SOD sites and from waterways downstream of both pre- and post-eradicated areas (Davidson et al. 2005, Goheen et al. 2008, Sutton et al. 2009, Reeser et al. 2011). The importance of this inoculum in the initial establishment of SOD in new areas is thus far inconclusive, however.

Methods

In our GIS analysis we performed two spatial joins to calculate the distance between each SOD site and the nearest road or stream. We tested for a spatial relationship between *P. ramorum* and roads or streams with a restricted randomization test, which compares the observed median distance to that resulting from a repeated sampling from a dataset of random points.

In our understory road and stream surveys, we sought to systematically assess the risk of roadside and stream infection. While the presence of *P. ramorum* in streams has been long established, no prior attempt has been made to isolate *P. ramorum* from soils along roads traversing infested areas in Oregon. If *P. ramorum* is dispersing along roads we presumed that it should be recovered in roadside water or in roadside vegetation. If *P. ramorum* is dispersing out of streams, it should be recovered more frequently from stream-side vegetation than in vegetation away from the streams, provided hosts are present.

To sample roads, we collected soils and water samples from the road surfaces (when dirt or gravel), or directly adjacent to the roads within SOD infested areas. We additionally surveyed roadside vegetation in 100 m transects running adjacent to the roadways.

For our streamside surveys, we established transects adjacent to streams known to harbor inoculum. Along each transect the presence of major foliar hosts was noted; symptomatic foliage was gathered and plated in selective media to discern the presence or absence of *P. ramorum* or other *Phytophthora* spp. We compared host and pathogen diversity in streamside vegetation (within the splash and flood zone) to that collected from hosts located up to 5 m away from the stream bank.

Results

In our landscape GIS analysis, SOD sites were no closer to roads than expected by chance. Approximately 50% of the randomizations had a median distance to road further than our observed median distance. The remaining 50% had a median distance to road closer than observed. In contrast, SOD sites were significantly closer to streams than expected by chance.

P. ramorum was rarely recovered from roadside soils or vegetation, and only when associated with overstory infection. Of the 108 soil and water samples, only 2 were positive for *P. ramorum*; of the 92 vegetation samples, only 7 were positive.

Despite the abundance of understory hosts and other *Phytophthora* spp., *P. ramorum* was not recovered from foliage along streams bearing inoculum except when associated with overstory mortality. California bay laurel was the most common host at all locations, although understory and overstory tanoak were present at all sites. *P. nemorosa* was the most common *Phytophthora* spp. recovered, and was equally as abundant directly adjacent to streams as away from them. *P. ramorum* was isolated from only 4 sites. We preferentially recovered *P. ramorum* from tanoak growing beyond the splash and flood line. All samples positive for *P. ramorum* were directly below overstory mortality; immediately downstream of overstory mortality we failed to recover *P. ramorum* from either of our transect sets.

Discussion

We found no evidence that soil or stream-borne inoculum is causing significant infection in understory vegetation, a pre-requisite for the dispersal of this pathogen along roads or streams. Dissimilar to other known soil-borne *Phytophthora* spp., for example *P. lateralis* (Jules et al. 2002), we were unable to confirm a strong landscape relationship between SOD and the road network. Additionally, with few exceptions we were unable to isolate *P. ramorum* from the road surface or from roadside vegetation, even in areas of widespread SOD.

We were able to establish that *P. ramorum* is preferentially located closer to waterways. We cannot, however, attribute this to significant dispersal of inoculum out of the waterway into streamside vegetation. Despite inoculum being present in some of our sampled waterways for a decade and the abundance of easily infected hosts and other *Phytophthora spp.* along the streamways, *P. ramorum* was primarily isolated from tanoak foliage beyond the splash and flood line. As with the road surveys, *P. ramorum* was preferentially recovered directly downhill (not downstream) of overstory infection. We expect that some streamside and roadside vegetation may be occasionally infected, but neither roads nor streams appear to be a major means by which *P. ramorum* has been introduced into new areas.
We hypothesize the lack of soil-mediated spread may be attributed to any of the three reasons: Oregon's quarantine, eradication, and sanitation practices may be maintaining an amount of *P. ramorum*-inoculum in soils and streams below thresholds required for establishment; *P. ramorum* may be unable to survive or spread from the habitat adjacent to roads and streams; or, soil and stream infestation is, under natural conditions, a dead end for *P. ramorum*. In light of this information other long distance dispersal methods must be considered, of which aerial dispersal of sporangia remains the most plausible. Stream valleys are also significant pathways for air movement, which could account for the strong stream-association we observed. Most likely infection is establishing in overstory tanoak via aerial means, and is dispersing locally in rain splash. Neither water in streams nor mud on roads are important pathways for the continued expansion of SOD in Oregon.

Acknowledgements

Thanks to the USDA Forest Service, Pacific Northwest Region (Region 6) Forest Health Monitoring Program and the Pacific Southwest Experiment Station for funding.

Literature Cited

Davidson JM, Wickland AC, Patterson HA, Falk KR, Rizzo DM, 2005. Transmission of *Phytophthora ramorum* in Mixed-Evergreen Forest in California. *Phytopathology* **95:** 587-596.

Fichtner EJ, Lynch SC, Rizzo DM, 2009. Survival, dispersal, and soil-mediated suppression of *Phytophthora ramorum* in a California redwood-tanoak forest. *Phytopathology* **99:** 608-619.

Goheen EM, Hansen E, Kanaskie A, Sutton W, Reeser P, 2008. Vegetation response following *Phytophthora ramorum* eradication treatments in Southwest Oregon Forests. In: Frankel, S.J., Kliejunas, J.T. and Palmieri, K.M. (Eds). Proceedings of the sudden oak death third science symposium. Gen Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 301-303.

Jules ES, Kauffman MJ, Ritts WD, Carroll AL, 2002. Spread of an invasive pathogen over a variable landscape: A nonnative root rot on Port Orford cedar. *Ecology* **83:** 3167-3181

Reeser PW, Sutton WS, Hansen EM Remigi P, Adams GC, 2011. *Phytophthora* species in forest streams in Oregon and Alaska. *Mycologia* **103:** 22-35.

Sutton W, Hansen EM, Reeser PW, 2009. Stream monitoring for detection of *Phytophthora ramorum* in Oregon tanoak forests. *Plant Disease* **93:**1182-1186.

Nature of Phytophthora inoculum in flowing surface waters

K. Aram, D.M. Rizzo

Department of Plant Pathology, University of California, Davis, CA 95616 USA.

Corresponding author: karam@ucdavis.edu

Abstract

Phytophthora species are regularly recovered from surface waters, often in the absence of apparent sources of inoculum [1, 2]. The persistence of such plant pathogens in water bodies has significant implications for their spread and management in agricultural as well as more natural contexts. To determine if *Phytophthora* spp. can complete their life cycle in aquatic environments, we have undertaken experiments focused on *P. ramorum* and *P. gonapodyides* in California coastal streams. Exposing fresh rhododendron leaves along with those killed by drying or freezing to inoculum in naturally infested streams and in controlled environment experiments indicated that *P. ramorum* has a limited ability to colonize degraded leaf litter in aquatic environments. In contrast, *P. gonapodyides* more readily colonized dead leaves. The potential of aquatic and riparian plants as sources of *Phytophthora* inoculum in aquatic environments will be addressed by surveys for cryptic infections. Colonies of *P. ramorum* and *P. gonapodyides*-like spp. were recovered from glass slides exposed in streams, indicating that propagules passively adhere to substrates in streams. Colonies were also recovered from leaves and glass slides suspended in tubes in stream flow, indicating that propagules adhere without being trapped between surfaces. To determine the presence of sporangia, zoospores and cysts in flowing stream water, selective isolation of propagule types will be based on differences in size and lack of a cell wall in zoospores.

Literature Cited

Hong C.X., Moorman,G.W., 2005. Plant pathogens in irrigation water: Challenges and opportunities. *Critical Reviews in Plant Sciences* **24: 1**89-208.
Reeser P.W., Sutton W., Hansen E.M., Remigi P., Adams G.C. 2011. *Phytophthora* species in forest streams in Oregon and Alaska. *Mycologia* **10:** 22-35.

Seasonal variation of inoculum density and species composition of soilborne Phytophthoras in an infected black walnut stand in Hungary

J. Kovács, F. Lakatos, I. Szabó

University of West-Hungary, Institute of Sylviculture and Forest Protection. H-9400 Sopron, Bajcsy-Zsilinszky E. Str. 4., Hungary.

Corresponding author: kovacsj@emk.nyme.hu

Abstract

Previous studies showed the presence of *Phytophthora plurivora* and *Phytophthora cactorum* in the soil of declining black walnut stands in Hungary, and the pathogenicity of these species was proved by inoculation of seedlings [1].
The health condition of a 73 years black walnut stand was examined in June and September 2011 in West-Hungary.. The stand is situated on a drained floodplain. The trees showed declining symptoms: sparse crown, drying branches, small leaves with yellowish discolouration. Twenty trees were selected for monitoring survey. The health condition of the trees was evaluated on a 4-pointed scale. Soil samples were collected from the rhizosphere of each of examined trees for isolation of Phytophthoras. *Phytophthora* species were isolated on selective agar media, using the leaf baiting method (*Rhododendron* and *Prunus laurocerasus* leaves as baits). The spots on the baits were counted to estimate the inoculum density in the soil sample. The isolates were identified by morphological and molecular methods. The morphological features of the isolates were examined on cultures grown on carrot agar at 20 °C. The molecular identification was performed by sequencing the ITS 1 and ITS 2 region of the rDNA of selected isolates.
The healthy state of 30% of the investigated trees got worse during the summer. However, the inoculum density and the isolation success were lower in September in almost every soil samples. There were changes also in the species composition:In June 59.26% of the isolates were *Phytophthora cactorum* and 18.52% *Phytophthora plurivora,* however, 100% of the isolates were *Phytophthora plurivora* in the collection of September.

Literature Cited

Szabó I., Lakatos F. 2008. *Phytophthora* species isolated from declining forests in Hungary. *Növényvédelem* **44:** 607-613.

Epidemiology of *Phytophthora ramorum* and *Phytophthora kernoviae* on *Vaccinium* in the natural environment in the UK

G.L. Thorp, J.A. Turner, P. Jennings

Fera – The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK.

Corresponding author: gilli.thorp@fera.gsi.gov.uk

Abstract

Phytophthora kernoviae was first detected causing stem blackening and leaf necrosis on *Vaccinium myrtillus* in late 2007 in woodland in the South West of England. Further UK wide surveys have since detected the pathogen in 12 additional locations, mainly in the South West of England. *Phytophthora ramorum* was confirmed infecting vaccinium in late 2008, causing similar symptoms to those seen by *P. kernoviae*, and to date has been recorded at 10 sites, predominantly in the West Midlands and Wales. Differences can be seen in the habitats favoured by the two pathogens with *P. kernoviae* mainly infecting vaccinium in heathland, whilst *P. ramorum* infections mainly occur in vaccinium growing in a woodland environment. Although the total number of sites infected with either pathogen is small, the number of confirmed new sites has been increasing year on year.

Laboratory experiments have been carried out in order to determine the relative susceptibility of *V. myrtillus* to varying spore concentrations of both *P. kernoviae* and *P. ramorum* and how this compares to other host species including rhododendron and viburnum. In order to establish if there are periods of high host susceptibility bait plants were positioned in *P. kernoviae* diseased areas in both heathland and woodland along with a datalogger recording temperature and humidity. Spore washes were also carried out to determine timing of sporulation. Monitoring over 2 years has shown vaccinium is susceptible all year with peaks in infection and sporulation coinciding with periods of high humidty/rainfall.

Phytophthora species isolated from *Alnus rubra* in western Oregon riparian ecosystems

L. Sims, S. Navarro, E.M. Hansen

Department of Botany and Plant Pathology, Oregon State University, 1085 Cordley Hall, Corvallis Oregon 97331, USA.

Corresponding author: simsla@science.oregonstate.edu

Abstract

A survey of alder health was conducted from 2010 - 2012 in western Oregon USA with particular attention to *Phytophthora* species associated with decline. In 2010 - 2011 efforts were focused on sampling from around alders: from stream water, baited unwashed roots and baited soil. In 2011- 2012 bark, and root samples were collected systematically. Root samples were washed and surface sterilized. Specifically, necrotic margins, cankered tissue, and water soaked regions of above ground bark and below ground roots were cultured onto *Phytophthora* selective media. *P. siskiyouensis* was the primary *Phytophthora* species recovered from above ground alder bark. During the course of the survey, it was recovered from symptomatic bark with bleeding lesions throughout the study area. However, collection from bleeding lesions was unsuccessful during the summer. *P. alni* subsp. *uniformis, P. siskiyouensis,* and other *Phytophthora* species from ITS clades 2, 6 and 7 were recovered from symptomatic root tissue. Performance of Koch' s postulates on alder saplings in a green house with isolates obtained from diseased alder tissue from western Oregon suggests that both *P. alni* subsp. *uniformis* and *P. siskiyouensis* are able to cause significantly larger cankers when compared to cankers caused by *P*. taxon oaksoil collected from stream water. *P. alni* subsp. *uniformis* was only recovered from diseased root tissue of alders and not from anywhere else in the ecosystem. *P. alni* subsp. *uniformis* was only collected from two counties so its role in western Oregon is unclear. Observation suggests that where *P. siskiyouensis* is infecting alder trees in a stand, more trees within a stand have canopy dieback, more insect damage, and more pathogen damage. Large scale canopy dieback did not occur anywhere in western Oregon and even in stands with the most symptomatic trees, healthy trees were also present.

Acknowledgments

Thank you to the Oregon Department of Forestry and the USDA Forest Service SW Oregon Forest Health Service Center for planning, assistance and field support. Also, thank you to the Forest Health Monitoring Program Pacific Northwest Region USDA Forest Service.

Phytophthora ramorum and *P. lateralis* in Northern Ireland

L. Quinn, A. McCracken, B. Moreland

Applied Plant Science & Biometrics Division, Agri-Food & Biosciences Institute, 18A Newforge Lane, Belfast, Northern Ireland, UK.

Corresponding author alistair.mccracken@afbini.gov.uk

Abstract

Phytophthora ramorum was first detected in Northern Ireland on plants in trade in 2004. The first outbreak on plants in the wild was in 2006 on rhododendron in private gardens and unmanaged woodlands on private estates. The first outbreak of *P. ramorum* on larch was in 2010. A strategy of eradication and containment has resulted in the felling of 300+ ha of larch. Aerial surveys of N. Ireland have given no evidence that the pathogen has spread to the west. Significant improvements have been made on both the extraction of *P. ramorum* DNA from wood and its amplification which has very significantly improved detection sensitivity. Isolates of *P. ramorum* obtained from rhododendron, larch, oak and Vaccinium have been shown to belong to a new lineage of *P. ramorum* [1]. The epidemiological significance of this new lineage is currently being investigated.

In August 2011 *P. lateralis* was first diagnosed on specimen arboretum trees of Lawson cypress (*Chamaecyparis lawsoniana*) growing in a large public forest park in the southern part of Northern Ireland. It was subsequently found in another public park in the north as well as in two large Lawson cypress forest plantations. A number of individual trees or groups of trees, mainly in private gardens have been found to be infected. Studies are currently being undertaken to determine the factors influencing the survival and spread of the pathogen which will in turn inform the most appropriate disease control and biosecurity measures to be taken.

Literature Cited

Poucke K., Franceschini S., Webber J. F., Vercauteren A., Turner J., McCracken A.., Heungens K., Brasier C.M. 2012. Discovery of a fourth evolutionary lineage of *Phytophthora ramorum*: EU2. *Fungal Biology* **116:** 1178-1191.

Spatial patterns of holm and cork oak decline in Extremadura, Spain

E. Cardillo, A. Acedo, C. Perez

Institute of Cork, Wood and Charcoal (IPROCOR), Extremadura Government, Calle Pamplona s/n. Apdo 437 - 06800 Merida Spain.

Corresponding author: enrique@iprocor.org

Abstract

Nowadays Phytophthora epidemic, which causes oak decline, represents one of the major threats for Iberian forest putting at risk its environmental, economical and cultural wealth. In order to develop rational strategies to prevent and control this forest disease, knowledge of coarse-scale disease dynamics in space, time and severity dimensions is needed. The aims of this study are 1) to know where and how much oak decline disease there is in Extremadura, 2) to establish a current baseline to measure control effort efficiency and 3) to identify risk factors. A 10% of regional area was sampled randomly pìcking quadrangles of aerial infrared ortho-photography. IR digital imagery was interpreted by trained operators to find out and delineate oak decline symptomatic foci. Preliminary results point out that more than 1% of holm and cork oak forests of Extremadura exhibit symptoms of decline. These foci were completely spread over regional oak forest area with a geographic pattern that matches with the isolation record of *Phytophthora cinnamomi*. The number of disease plots increased from 470 reported circa 2000 [1] to more than 4000 symptomatic points estimated in this work, a ten fold increase in a decade. Secondary infections originated recently could be explaining the observed pattern consisting on clusters of small size foci. Finally, disease foci were found more frequently in areas with signs of livestock heavy trampling or in zones near water.

Literature Cited

Del Pozo J.L., 2006. Prospección de la seca en Extremadura. Análisis de los resultados. In: Gestión Ambiental y Económica del Ecosistema Dehesa en la Península Ibérica (M. Espejo, M. Martín, C. Matos, S.J. Mesías, eds.), Junta de Extremadura, Mérida: 131-143.

Phytophthora species in Serbia

I. Milenkovic[1], N. Keca[1], J. Nowakowska[2], K. Sikora[2], M. Borys[2], T. Oszako[2], T. Jung[3,4]

[1]University of Belgrade, Faculty of Forestry, 1, Kneza Viseslava street, 11030, Belgrade, Serbia.
[2]Forest Research Institute – IBL, ul. Braci Lesnej 3, Sekocin Stary, 0509 – Raszyn, Poland.
[3]Phytophthora Research and Consultancy, Thomastrasse 75., Brannenburg, Germany.
[4]Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Corresponding author: ivan.milenkovic@sfb.bg.ac.rs

Abstract

Since there is a great economic and ecological threat posed by *Phytophthora* species, a study has been performed during the years 2009-2012, aiming at determining the presence and diversity of *Phytophthora* species in both natural ecosystems and amenity trees in Serbia.
Sampled trees showed symptoms typical of *Phytophthora* infections, such as presence of collar rots or stem cankers with dark exudates, chlorosis and wilting of leaves, increased crown transparency, dieback, dying of shoots, branches or parts of the crown, root lesions, and decay and loss of fine roots. Sampling and isolation methods were according to Jung (2009) and Jung *et al.* (1996). Tissue samples were taken from necrotic parts and plated directly onto selective agar medium (V8A-PARPNH). Soil containing fine roots was sampled in the form of soil monoliths, measuring ~ 25x25x25 cm, and isolation tests were performed using oak, beech and cherry laurel leaves as baits. Both symptomatic and healthy trees were sampled. In total 167 samples were taken from 26 different host species including *Quercus robur, Q. petraea, Q. cerris, Fagus sylvatica, Fraxinus angustifolia, Acer pseudoplatanus, A. platanoides, A. heldreichii, Populus* spp., *Juglans regia* and *Betula pendula,* in both forest ecosystems and amenity tree stands. *Phytophthora* species were isolated from about 68% of samples. Also, many isolates of *Pythium* spp. were obtained.
After a detailed morphological and molecular identification of all isolates, nine different *Phytophthora* species have been confirmed, i.e. *P. europaea, P. cambivora, P. citricola, P. cactorum, P. plurivora, P. polonica, P. quercina*, *P*. taxon ´Pg chlamydo‘, and *P. lacustris* (previously known as *P*. taxon ‘Salixsoil’), and some of them were recorded for the first time in different ecosystems in Serbia.

Acknowledgements

We are grateful to COST FP0801-Short Term Scientific Missions and to project TR 37008, Ministry of Education and Science, Republic of Serbia for material support during these studies.

Literature Cited

Jung T., 2009. Beech decline in Central Europe driven by the interactions between Phytophthora infections and climatic extremes. *Forest Pathology* **38** 73-94 (2009).
Jung T., Blaschke H., Neumann P., 1996. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. *European Journal of Forest Pathology* **26:** 253-272.

Two mature *Quercus suber* trees in Portugal with different field susceptibility to *Phytophthora cinnamomi* (photo by Marilia Horta Jung)

RESISTANCE, PATHOGENESIS, ECOPHYSIOLOGY

Blocking of α-plurivorin compromises *Phytophthora plurivora* pathogenicity towards *Fagus sylvatica* seedlings

R.J.D. Dalio[1], F. Fleischmann[1], A. Chambery[2], R. Eichmann[3], N. Massola[4], S.F. Pascholati[4], W. Osswald[1]

[1]Section Pathology of Trees, Technische Universität München, Hanz-Carl-von-Carlowitz-Platz 2, 85354, Freising-Weihenstephan, Germany.
[2]Department of Life Sciences, Second University of Naples, Caserta, Via Vivaldi 43, I-81100, Italy.
[3]Lehrstuhl für Phytopathologie, Technische Universität München, Emil-Ramann-Straße 2, 85350 Freising-Weihenstephan, Germany.
[4]Department of Plant Pathology and Nematology, University of Sao Paulo, Av. Padua Dias 11, 13418-900, Piracicaba, SP, Brazil.

Corresponding author: osswald@wzw.tum.de

Abstract

To manipulate host metabolism during infection, *Phytophthora* species secrete many effectors, including high amounts of elicitins, a small protein family first described to elicit defence responses in tobacco plants. Elicitins trigger a variety of defence responses, including programmed cell death (PCD) in several plants, sharing many features of pathogen-associated molecular patterns (PAMPs). However, the precise role of elicitins as a virulence factor has yet to be clarified.
Here, we show that α-plurivorin, an elicitin secreted from *Phythophthora plurivora*, is essential for virulence and correlated with pathogen penetration in the host root tissues and defence suppression in beeches.
The blocking of α-plurivorin by incubation with a specific antibody during infection drastically impaired its internalization in host tissues and *P. plurivora* penetration, disabling the pathogen's disease promotion in beech seedlings. Furthermore, the lack of α-plurivorin inside the host tissues led to an up-regulation of defence-related genes, suggesting that α-plurivorin acts as a defence suppressor during infection. All of the infected plants treated with the anti-α-plurivorin antibody survived whereas most of the other infected plants died by the end of the experiment. Remarkably, given the potential of hundreds of effector genes in the *P. plurivora* genome, inhibition of α-plurivorin compromises *P. plurivora* pathogenicity, suggesting that α-plurivorin is essential for virulence.
Because elicitins are ubiquitously secreted by *Phytophthora* species, it is very likely that these molecules can also act as virulence factors in other *Phytophthora*-susceptible plant interactions. Therefore, the selective blocking of elicitins function might be a specific target for protecting plants against *Phytophthora*.

Identification of *Phytophthora cinnamomi* gene transcripts in infected cork oak roots

M. Horta Jung[1,2], A.C. Coelho[1,3], A. Cravador[1,2]

[1]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*
[2]*University of Algarve, Campus de Gambelas, 8005-139 FARO Portugal.*
[3]*Center for Research in Chemistry of Algarve (CIQA), University of Algarve, Campus de Gambelas, 8005-139 FARO, Portugal.*

Corresponding author: mhorta@ualg.pt

Abstract

Phytophthora cinnamomi is associated with the severe decline that is threatening typical agroforestry ecosystems with an overstorey of cork (*Quercus suber*) and holm (*Q. ilex*) oaks, resulting in significant economic and ecologic losses. The transcriptome analysis of *Q. suber* directed to genes related to biotic stress caused by *P. cinnamomi* infection is presently in progress in our laboratories as part of the Cork Oak ESTs Consortium programme (http://coec.fc.ul.pt/).
Cork oak roots were immersed in a *P. cinnamomi* zoospore suspension and incubated for 8, 20, and 36 h. RNA was extracted and pooled and cDNA was synthesised. cDNA was fragmented, the sequencing adaptors ligated and pyrosequenced using 454 GS FLX Titanium (Roche-454 Life Sciences) technology. Reliable reads were assembled and the resulting fasta files were run in the Blast2GO application (http://www.blast2go.org). Blast2GO is an all in one tool for Functional Annotation (FA) of sequences and the analysis of annotation data. The FA was run in 3 steps: BLAST to find homologous sequences [queries against the NCBI Databases using BLASTx], MAPPING to retrieve Gene Ontology terms and ANNOTATION to select reliable functions. Different annotation databases were used: GO, Enzyme Codes, InterPro and KEGG.
More than 580 *Phytophthora* contigs were identified and their Functional categories will be presented. Transcripts putatively involved in pathogenicity will be disclosed. The project sponsored by the DOE Joint Genome Institute for the sequencing of the whole genome of *P. cinnamomi* was completed in March 2012. Upon its public release, it will be possible to access information on the complete sequences of the *P. cinnamomi* genes expressed following infection of cork oak roots.

Transcriptome analysis of *Quercus suber* roots in response to *Phytophthora cinnamomi*

M. Horta Jung[1,2], A. Cravador[1,2], A.C. Coelho[2,3]

[1]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*
[2]*University of Algarve, Campus de Gambelas, 8005-139 FARO Portugal.*
[3]*Center for Research in Chemistry of Algarve (CIQA), University of Algarve, Campus de Gambelas, 8005-139 FARO, Portugal.*

Corresponding author: acoelho@ualg.pt

Abstract

The oomycete *Phytophthora cinnamomi* is widely distributed in Iberian soils and causes root rot on *Q. suber* and *Q. rotundifolia*. The EST analysis of *Q. suber* directed to genes responsive to *P. cinnamomi* infection is presently in progress in our laboratories as part of the Cork Oak ESTs Consortium programme (http://coec.fc.ul.pt/).
Cork oak roots were immersed in a *P. cinnamomi* zoospore suspension and incubated for 8, 20, and 36 h. Non treated roots were used as controls. RNA was extracted from healthy (RC) and infected (RIZ) roots and cDNA was pyrosequenced using 454 GS FLX Titanium (Roche-454 Life Sciences) technology. Adapter and polyA clipped reads were submitted to *de novo* assembly.
Only contig sequences where at least 10 reads were clustered together were considered for quantification purposes. Expression values of contigs in the RIZ and RC samples were obtained in RPKM (Reads Per Kb exon (contig) per Million mapped reads) and were directly comparable to each other. A ten-fold difference in the RPKM expression values was used as a clear cutoff for differential expression. Contigs showing over/under expression or presence/absence were selected for further analysis with the Blast2GO application (http://www.blast2go.org).
195 contigs were found to be over-expressed in RIZ and 85 under-expressed; 1771 were only present in RIZ and 1606 were only present in RC.
Blast2GO allowed to assign ontology classes to genes over expressed or only present in infected roots. In the biological process category, genes function categories associated with biosynthetic, catabolic, primary and secondary metabolic processes, response to stress and to biotic stimulus are represented in the transcriptome. In the molecular function category the most highly represented category includes genes involved in kinase and transferase activities and in the cellular component category, genes function categories associated with cell wall, cytoplasm and plasma membrane are the most represented.
Overall, we conclude that *Q. suber* respond to *P. cinnamomi* infection by activating resistance responses.

Quantitative trait loci for resistance to *Phytophthora cinnamomi* in two host species

C. Robin[1], J.M. Gion[1,2], T. Barreneche[3]

[1] *INRA, UMR 1202 BIOGECO, 69 Route d'Arcachon, 33612 Cestas Cedex, France.*
[2]*CIRAD, Department of Biological System, Research unit", Campus International de Baillarguet, 34398 Montpellier Cedex, France.*
[3]*INRA, UREF, 71 Avenue Edouard-Bourlaux, B.P.81- 33883 Villenave d'Ornon Cédex, Cedex.*

Corresponding author: robin@bordeaux.inra.fr

Abstract

Castanea sativa and *Quercus robur* are two ecological and economical important European species. Both are host for *Phytophthora cinnamomi*, the causal agent of oak or chestnut ink disease. Sources of resistance to ink disease were identified in *C. mollissima* and *C. crenata* and susceptibility to *P. cinnamomi* is varying in *C. sativa* and *Q. robur* [1]. However, very little is known about the genetic and the evolution of this adaptive trait in Fagaceae species.
Knowing the macrosynteny and macrocolinearity occurring between the two genura [2] our objective was to compare the genetic architecture of resistance to *P. cinnamomi* trait in oak and chestnut. We will then test the hypothesis that the synteny can allow us to use the *Quercus* candidate genes involved in resistance to pathogens as putative candidate genes in *Castanea*.
Components of genetic resistance to *P. cinnamomi* were investigated in a full-sib family of *Q. robur*. For chestnut, two progenies from two controlled crosses (*C. sativa* x *C. crenata* and *C. sativa* x *C. mollissima*) were used. The three progenies were vegetatively propagated by cuttings. Resistance to ink disease was estimated in glasshouse by inoculating *P. cinnamomi* on the cuttings stems and by measuring the length of the induced lesion. The experiments were repeated two successive years.
Nine quantitative trait loci (QTL) involved in *P. cinnamomi* resistance mechanisms were located on eight linkage groups of the oak parental genetic maps, explaining 4 to 9 % of the phenotypic variation. For chestnut, QTL are under investigation (ongoing project).

Literature Cited

Robin C., Morel O., Vettraino A.M., Perlerou C., Diamandis S., Vannini, A. 2006. Genetic variation in susceptibility to *Phytophthora cambivora* in European chestnut (*Castanea sativa*). *Forest Ecology and Management* **226:**199-207.
Barreneche T., Casasoli M., Russell K., Akkak A., Meddour H., Plomion C., Villani F., Kremer, A., 2004. Comparative mapping between *Quercus* and *Castanea* using simple-sequence repeats (SSRs). *Theoretical and Applied Genetics* **108:** 558–566.

Interactions between *Phytophthora cinnamomi* and *Phlomis purpurea,* a plant resistant to the pathogen

D. Neves[1], C. Maia[1], S. Duraes[1], M. Horta Jung[1,2], S. Campos[1], M.C. Mateus[1,3], F.G. Breijo[4], J.R. Arminana[5], A. Cravador[2]

[1]Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, 8005-139 Faro, Portugal.
[2]Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.
[3]CIQA – Centro de Investigação em Química do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, 8005-139 Faro, Portugal.
[4]Departamento de Ecosistemas Agroforestales. Universidad Politécnica de Valencia. Camino de Vera s/n. 46022-Valencia, Spain.
[5]Laboratorio de Anatomía Vegetal "Julio Iranzo". Jardín Botánico de la Universidad de Valencia. c/ Quart, 81. 46008-Valencia, Spain.

Corresponding author: neves.dina@gmail.com

Abstract

Phlomis purpurea is spontaneous in *Quercus suber* and *Q. ilex* forest habitats in southern Portugal growing in areas affected by the decline disease. As previously described, *in vitro* inhibition of the oomycete life cycle structures by *P. purpurea* crude root extracts (PRE) at 10 mg ml^{-1}, ranged from 85% to 100% [1]. Recently, we have shown *in planta* that, at the same concentration, PRE significantly inhibited the infection of *Q. suber* roots by *P. cinnamomi* Moreover, PRE appear to elicit a defence response: radicles of two-week-old *Q. suber* exposed to PRE at 10 $mgml^{-1}$, 24 h prior to zoospore challenge were significantly protected from infection.

Cyto-histological evaluations are being made to elucidate:

1. How *P. purpurea* manages to avoid infection by *P. cinnamomi* zoospores;
2. How PRE inhibits the infection of *Q. suber* by zoospores;
3. Whether PRE are elicitors.

Control and inoculated roots at 0, 6, 24, 48 and 72 hours post inoculation (hpi) for *P. purpurea* and at 0 and 48 hpi for *Quercus suber* are being analysed, by light microscopy. Preliminary results showed the pathogen does not penetrate the plant rhizodermis, suggesting a type I resistance (the first time, to our knowledge it is observed in respect to *P. cinnamomi*). These features make this plant interesting to study its interactions with the oomycete and to explore it in a biocontrol perspective. A fraction isolated from PRE, showed a 100% *P. cinnamomi* inhibition at 0.5 mg ml^{-1}. Its major compound, m/z 473, was separated from contaminants. Its structure determined by MS and NMR will be presented.

Literature Cited

Neves D., 2007. *In vitro* activity of plant crude extracts and in situ protective effect of *Phlomis purpurea* against *Phytophthora cinnamomi*. MSc dissertation. Cranfield University.

How do *Phytophthora* spp. harm woody plants?

W. Oßwald[1], F. Fleischmann[1], T. Cech[2], A. Chambery[3], A. Cravador[4], S. Diamandis[5], J. Díez Casero[6], A.C. Coelho[7], E. Hansen[8], M. Horta Jung[4], T. Jung[4,9], N.S. Massola[10], L.B. Orlikowski[11], S. Prospero[12], M. Ptaszek[11], D. Rigling[12], C. Robin[13], A. Solla[14], G. Sipos[12], A. Trzewik[11], S. Werres[15]

[1]*Section Pathology of Woody Plants, Technische Universität München, Freising, Germany.*
[2]*Federal Research Centre for Forests, Natural Hazards and Landscape, Vienna, Austria.*
[3]*Dept. of Life Sciences, Second University of Naples, Casterta, Italy.*
[4]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*
[5]*Laboratory of Forest Pathology & Mycology, Forest Research Institute, Thessaloniki, Greece.*
[6]*Sustainable Forest Managment Research Institute, University of Valladolid-INIA, Palencia, Spain.*
[7]*Centro de Investigação em Química do Algarve (CIQA*), University of Algarve, Portugal.
[8]*Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA.*
[9]*Phytophthora Research and Consultancy, Brannenburg, Germany.*
[10]*Plant Pathology and Nematogy, University of Sao Paulo, Piracicaba, Brazil.*
[11]*Institute of Horticulture, Skierniewice, Poland.*
[12]*WSL Swiss Federal Research Institute, Birmensdorf, Switzerland.*
[13]*UMR 1202 BIOGECO, INRA, Cestas Cedex, France.*
[14]*Ingenieria Forestal y del Medio Natural, Universidad de Extremadura, Plasencia, Spain.*
[15]*Julius Kühn Institut – Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Foreststt, Braunschweig, Germany.*

Corresponding author: osswald@wzw.tum.de

Abstract

One of the objectives of working group 2 “Host-pathogen- interactions” within the FPC Cost action FP0801 (“Established and Emerging *Phytophthora*: Increasing Threats to Woodland and Forest Ecosystems in Europe”) was to analyze susceptible and resistant *Phytophthora* host interactions and shed light on the question “how do *Phytophthora* ssp. may harm woody plants”. Our evaluation comprised thirteen worldwide distributed species of the genus *Phytophthora* with the potential to invade at least nineteen different woody plant species.
Different conceptual models describing the primary infection of roots, trunks or of leaves were developed, based on extensive literature review and group discussions. We aimed to figure out which plant organs are infected first and in which tissues the mycelium grows during the early and late infection stages.
The significance of host-root exudates and their specific components to attract *Phytophthora* zoospores is of great interest. In order to understand local and systemic responses, different physiological and biochemical reactions of host plants triggered by pathogen attack were evaluated. Molecular studies completed the synopsis, focusing especially on virulence and avirulence genes of the pathogens as well as on the components of *Phytophthora* secretomes such as PAMPS and effector molecules. In this context the significance of *Phytophthora* elicitins to establish a susceptible host-pathogen interaction or to act as elicitor is discussed.

Multitrophic interactions between *Quercus robur*, *Phytophthora quercina* and *Piloderma croceum* (on a joint experimental platform)

O. Angay[1,2], F. Fleischmann[2], S. Recht[3], S. Hermann[3], T. Grams[1]

[1]*Ecophysiology of Plants, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising, Germany.*
[2]*Pathology of Woody Plants, Technische Universität München, Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising, Germany.*
[3]*Soil Ecology, UFZ-Helmholtz Centre of Environmental Research, Theodor-Lieser-Str. 4, 06120 Halle (Saale), Germany.*

Corresponding author: angay@wzw.tum.de

Abstract

Within the joint research project "TrophinOak", we analyze multitrophic interactions of *Quercus robur* micro-cuttings DF159 with respect of the rhythmic growth of oak. Our research team focuses on interactions with the root pathogen *Phytophthora quercina.* In addition, we compare the effects of the ectomycorrhizal (EM) fungus *Piloderma croceum F 1598,* as an additional interacting partner.
Earlier conceptual models described the effects of alternating root and shoot-flushes of oaks on the susceptibility against *P. quercina* [1]. It was postulated, that ongoing shifts of resource availability towards flushing shoots should render the non-flushing roots less capable of repair and defense and therefore more susceptible against *P. quercina*.
Research on *P. croceum* revealed a protective effect of EM against root pathogens, possibly due to chemical and/or mechanical shielding. Further studies demonstrated the prevalent role of alternating root/shoot-growth of oaks for this EM formation [2].
The oaks were cultured in axinic soil-systems either single- or co-inoculated with interaction partners. ^{15}N-/^{13}C stable-isotope labeling was applied and the oaks, expressing root- or shoot-flush, were harvested. Subsequently, we quantified *Phytophthora*-infection, analyzed resource allocation by isotopic-tracing as well as by analysis of soluble sugar and starch, and measured gene-expression patterns in oaks using Illumina high throughput sequencing.
Our data revealed significant differences between treatments and growth-stages concerning resource-allocation and susceptibility against *P. quercina*. Although investigations are still ongoing, the above hypothesis that higher C availability in roots reduces susceptibility to *P. quercina* has to be rejected for our system.

Literature Cited

Jönsson U., 2006. A conceptual model for the development of *Phytophthora* disease in *Quercus robur*. *New Phytologist* **171:** 55–68.
Branzanti M.B., Rocca E., Pisi A. 1999. Effect of ectomycorrhizal fungi on chestnut ink disease. *Mycorrhiza* **9:** 103-109.

Drought, fire and flood: approaches to the renewed challenge of *Phytophthora cinnamomi* in south-eastern Australia

D. Cahill[1], J. Rookes[1], J. Cullum[1], H. Bramwells[2], D. Phillips[1], P. Beech[1]

[1]*School of Life and Environmental Sciences, Deakin University, Victoria, Australia.*
[2]*Department of Sustainability and Environment, Melbourne, Victoria, Australia.*

Corresponding author: david.cahill@deakin.edu.au

Abstract

For the years 2000–2010 south-eastern Australia experienced the driest climatic period on record. In 2006 devastating bushfires raged across the state of Victoria and again in 2009 resulting in Australia's greatest loss of life from bushfire. Since late 2010 there have been episodic and catastrophic floods. *Phytophthora cinnamomi* and the disease that it causes in native vegetation have not been abated by these increasingly harsh environmental conditions. We now have evidence of renewed, widespread and severe impacts of the pathogen on natural systems. Many of the areas in Victoria for which *P. cinnamomi* has been recorded [1] have been affected by drought and fire and recent high rainfall events have stimulated large disease outbreaks in many national parks and reserves. In collaboration with government agencies we are undertaking an extensive surveillance and monitoring program in key biodiverse regions in the state with the aim of prioritising management. Work is continuing at the molecular level to unravel the basis of resistance [2] in a range of host species, for example, we have good evidence for the involvement of specific resistance-related signalling pathways in roots of the model, *Zea mays.* This finding may give us leads into ways in which we can modify or engineer resistance to *P. cinnamomi* in susceptible plants. We have also identified a unique signalling phospholipase from *Phytophthora* that may explain the lack of phospholipase C in the genomes of the three sequenced species. We are currently investigating this protein as a possible target for antibiotics.

Literature Cited

Cahill D.M., Rookes J.E., Wilson B.A., Gibson L., McDougall K.L., 2008. *Phytophthora cinnamomi* and Australia's biodiversity: impacts, predictions and progress towards control. *Australian Journal of Botany* **56:** 279-310.
Allardyce J.A., Rookes J.E., Cahill D.M., 2012. Defining plant resistance to *Phytophthora cinnamomi*: A standardised approach to assessment. *Journal of Phytopathology* **160:** 269-276.

A new method to quantify zoospore chemo-attraction

N.S. Massola Jr.[1], R. Dalio[2], F. Fleischmann[2] and W. Osswald[2]

[1]*Department of Plant Pathology and Nematology, University of Sao Paulo, Av. Padua Dias 11, 13418-900, Piracicaba, SP, Brazil.*
[2]*Section Pathology of Woody Plants, Technische Universität München, Hanz-Carl-von-Carlowitz-Platz 2, 85354, Freising-Weihenstephan, Germany.*

Corresponding author: fleischmann@wzw.tum.de

Abstract

Phytophthora species release zoospores which are, in case of soil born species, attracted by root exudates, before they encyst and infect root tissue. To test the activity of zoospores to several root exudates and to quantify zoospore attraction we developed a simple, cheap and easy-to-built new device. It consists of three cylindrical plastic containers A, B and C (length 11.5 cm, diameter 3 cm), that are connected to each other at the bottom with two tubes (length 1cm, diameter 0.5 cm) holding a dialysis membrane in the middle position. Reservoir A can be filled with the control (water, buffer) and C with the test solution (e.g. root exudate). Flask B in the middle holds the zoospore suspension. The zoospores swim towards the attracting solution and are finally trapped at the dialysis membrane where they encyst. The activity of different zoospore attractants can be compared and quantified by counting the zoospores cists on the membrane under the microscope or by quantitative real-time PCR in combination with *Phytophthora* primers. Using this zoospore trap, we tested the attraction of zoospores of *P. plurivora* and *P. nicotianae* towards root exudates of their hosts. qRT-PCR data showed that *P. plurivora* zoospores were attracted 20.000 times more to root exudates of *F. sylvatica* as compared to distilled water. Citrus Sunki (susceptible) root exudates attracted 6 times more zoospores of *P. nicotianae* than did Swingle (resistant).
This new trap can easily be built with more than two containers connected to the zoospores reservoir (B), so that it is possible to compare differential attraction of several root exudates as well as of single compounds regarding their activity on zoospore attraction. This would contribute with important information about the early interactions in the rhizosphere.

Fluorescent *in situ* hybridization (FISH) assay as a tool to microscopically view *Phytophthora cinnamomi* growth within plant tissues

A. Li[1], M. Crone[2], P. Adams[1], S. Fenwick[1], N. Williams[2,3], G.E.St.J. Hardy[2]

[1]*School of Veterinary and Biomedical Sciences, Division of Health Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia.*
[2]*Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, Western Australia, 6150, Australia.*
[3]*Scion, New Zealand Research Institute Ltd., 49 Sala St., Private Bag 3020, Rotorua 3046, New Zealand.*

Corresponding author: g.hardy@murdoch.edu.au

Abstract

The microscopic examination of naturally infected plant material for the presence of *Phytophthora cinnamomi* can be problematic as structures such as hyphae, hyphal swellings, chlamydospores, and oospores are often indistinguishable from those of other oomycetes or fungi. Frequently, it would be useful to be able to clearly differentiate *P. cinnamomi* from other micro-organisms, especially when trying to determine how the pathogen is surviving in plant material particularly in harsh environments. Consequently, the lack of stains that can clearly and definitively localise hyphae and reproductive structures of *P. cinnamomi* within plant material is a limitation in increasing our understanding of the biology of the pathogen in susceptible and tolerant plant species in different ecosystems. This study demonstrates that a *P. cinnamomi* specific, fluorescently labelled DNA probe can be used to specifically detect and visualise *P. cinnamomi* in plant material using fluorescent in situ hybridization (FISH) without damage to plant or pathogen cell integrity. The method will allow us to more accurately study plant-*P. cinnamomi* interactions in plants, and to be particularly useful in naturally infected material.

Histological changes of *Quercus ilex* seedlings infected by *Phytophthora cinnamomi*

M.A. Redondo[1], A. Pérez-Sierra[1], P. Abad-Campos [1], J. García-Jiménez[1], A. Solla[2], J. Reig[3], F. García-Breijo[1,3]

[1]*Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain.*
[2]*Ingeniería Forestal y del Medio Natural. Universidad de Extremadura. Avenida Virgen del Puerto 2, 10600 Plasencia, Spain.*
[3]*Jardí Botànic de la Universitat de València, C/ Quart 80, 46008 Valencia, Spain.*

Corresponding author: aperesi@eaf.upv.es

Abstract

Since the early 1980s, severe decline and occasional sudden death of extensive areas of holm oak (*Quercus ilex*) and cork oak (*Q. suber*) woodlands in the southwest of the Iberian Peninsula have been associated to the damage caused by *Phytophthora cinnamomi*, an alien and invasive pathogen infecting roots of many woody species. The objective of this work was to perform histological studies of holm oak infected by *P. cinnamomi*. To achieve this, *Q. ilex* subsp. *ballota* acorns were collected in Extremadura (Spain) and germinated in sterile vermiculite. Five-week-old seedlings were inoculated by immersing them in flasks with *P. cinnamomi* colonised agar and soil extract solution. Root sections were obtained every 12 hours, during 7 days. Root tissue was fixed with FAA and Karnovsky fixative and processed afterwards using different microscopic techniques. Low temperature scanning electron microscopy (LTSEM) showed hyphae and encysted zoospores 24 h after inoculation. Root sections (10-15 µm) treated with calcofluor white were observed under epifluorescence microscopy and *P. cinnamomi* hyphae covering root surface and going progressively through cortex tissue were also observed. Roots sections (8-10 mm) stained with safranin-fast green and observed under light microscopy showed hyphae on the external root tissue 24 h after inoculation. Light microscopy was also used with 2 µm root sections stained with toluidine blue and allowed the detection of *P. cinnamomi* hyphae penetrating the parenchyma tissue. Finally, 80 nm root slices were obtained and examined through transmission electron microscopy (TEM). *P. cinnamomi* penetrated the cell walls forming haustorial-like structures and also grew through intercellular spaces.

Quick dissemination of *Phytophthora cinnamomi* threatens biodiversity in a World Heritage Site (Doñana Biological Reserve, SW Spain)

P. De Vita[1], L.V. García[2], C. Ramo[3], M.S. Serrano[1], C. Aponte[2], M.E. Sánchez[1]

[1]*Dpto. Agronomía, ETSIAM, Universidad de Córdoba, PO Box 3048, Córdoba E-14080, Spain.*
[2]*Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, PO Box 1052, Sevilla E-41080, Spain.*
[3]*Estación Biológica de Doñana (EBD), CSIC,* P.O. Box *1056, 41080, Sevilla, Spain.*

Corresponding author: ventura@cica.es

Abstract

Scattered big trees play a main role in maintaining biodiversity in savannah-like ecosytems. In this study we analyze the case of a remnant centenarian Cork oak (*Quercus suber* L.) population in the Doñana Biological Reserve (DBR, SW Spain), a Biosphere Reserve. Previous studies demonstrated that it was threatened by herbivorous and nesting wading birds proliferation [1]. Several sudden dieback events not related to any of previously reported causes lead us to investigate the potential role of pathogenic oomycetes.
Along four years of study period (2008-2011) tree rizosphere was increasingly being colonized by *Phytophthora cinnamomi.* We found that 2010 late winter/ early spring rainfall values exceeded all previous (32 yr) records and significantly extended the period with flooding/high soil moisture towards warmer months. These outstanding climatic conditions seem to have favoured the massive spread of this invasive water-dependent pathogen.
On the other hand, we found a significant correlation between the occurrence of *P. cinnamomi* in 2008/9 and tree crown health status in late 2010, suggesting a delay between pathogen arrival and the appearance of crown symptoms.
We analyzed future perspectives for pathogen spreading and discussed the feasibility of implementing currently available control measures in the context of a highly protected biodiversity reserve. We conclude that only individual treatments could allow for simultaneously save infected trees, prevent infections on healthy trees and avoid any chemical release to the environment.

Literature Cited

García L.V., Ramo C., Aponte C. Moreno A., Dominguez M.T., Gómez-Aparicio L., Redondo R., Marañón T., 2011. Protected wading bird species threaten relict centennial cork oaks in a Mediterranean Biosphere Reserve: a conservation management conflict . *Biological Conservation* **144:** 764–771.

Aerial application of potassium phosphite in a declining forest of *Fagus sylvatica*, *Quercus petraea* and *Quercus robur* in Germany infested by *Phytophthora plurivora* and *Phytophthora quercina* (photo by Thomas Jung)

MANAGEMENT AND CONTROL

Sudden Oak Death: Reactions to a new forest threat

S.J. Frankel

United States Department of Agriculture, Forest Service, Pacific Southwest Research Station, Urban Ecosystems and Social Dynamics Program, 800 Buchanan Street, Albany, CA, 94710, USA.

Corresponding author: sfrankel@fs.fed.us

Abstract

Sudden oak death, caused by *Phytophthora ramorum,* was first recognized in the mid-1990s in central coastal California. Over the past 20 years, the exotic plant pathogen has killed over 3 million trees, predominantly tanoak (*Notholithocarpus densiforus*) and coast live oak (*Quercus agrifolia*). The mortality pattern and appearance can be striking. Typically, patches of trees are killed and each tree's crown quickly turns from green to brown, as if freeze dried. New to science and an emerging threat to urban and wildland landscapes, *P. ramorum* captivated the interest of homeowners, landowners, hikers, and bikers as it drew significant media attention. The pathogen's impacts in forests, presence in nurseries, limited distribution, and threat to Eastern US oak forests triggered institutions to implement quarantine regulations, craft legislative bills, and issue best management practices. For individuals, it prompted management action, hazard tree removal, fuels-reduction treatments, and preemptive pesticide applications, as well as volunteer work, such as tree plantings and citizen monitoring.

The emotional response to sudden oak death is harder to decipher, but is reflected in music, photography, paintings, and other works of art that use sudden oak death as a theme. Sudden oak death has appeared on CD covers, in adult fiction, children's literature, and performance pieces. In these works, besides despair, the poignant response to the threatened and actual loss of trees has often prompted people to affirm their attachment to, and affection for, trees. Through art and commentary, sudden oak death is being incorporated into American culture. Forest pathologists can assist society in defining the sudden oak death phenomenon through scientific documentation, predictions of the extent of tree mortality, and reassurance that there are limits to the pathogen's host range, virulence, and survival

Monitoring the effectiveness of *Phytophthora ramorum* eradication treatments in Oregon tanoak forests

E.M. Goheen[1], A. Kanaskie[2], E. Hansen[3], W. Sutton[3], P. Reeser[3], N. Osterbauer[4]

[1]USDA Forest Service, Pacific Northwest Region FHP, Southwest Oregon Forest Insect and Disease Service Center, 2606 Old Stage Road, Central Point, OR 97502 USA.
[2]Oregon Department of Forestry, Salem, OR USA.
[3]Oregon State University, Corvallis, OR USA.
[4]Oregon Department of Agriculture, Salem OR USA.

Corresponding author: egoheen@fs.fed.us

Abstract

Phytophthora ramorum, the cause of sudden oak death, was first discovered in Oregon forests in July 2001. An aggressive eradication treatment program consisting of cutting and burning infected and exposed host plants, and where possible, injecting herbicide, was immediately put into place on all lands where it was found. To monitor the effectiveness of treatments we are revisiting treated sites and sampling soil and vegetation on fixed-area plots centered on stumps of infected trees. We established 145 plots in 2008-2009 and 143 plots in 2010. 109 of these plots were visited in both time periods.
Phytophthora ramorum was not recovered from soil or vegetation on 74 plots sampled in 2008-2009. Forty-seven plots yielded *P. ramorum* from soils only. The pathogen was present in soil and vegetation on 18 plots, and was recovered from vegetation only on six plots. In 2010, *P. ramorum* was not recovered from soil or vegetation on 90 of the plots sampled. Thirty-six plots yielded *P. ramorum* from soil only, on ten plots the pathogen was present in soil and vegetation, and on seven plots, *P. ramorum* was recovered from vegetation only. All positive vegetation samples were from tanoak in 2008-2009. Two *P. ramorum*-positive samples of Oregon myrtle were collected in 2010 samples along with infected tanoak sprouts.
Analysis continues on these data. Of particular interest is how different components of the treatment prescriptions and/or abundance and composition of post-treatment vegetation affect pathogen survival and disease development. These data are also being used to inform 2012 sampling.

Detection and Eradication of *Phytophthora ramorum* from Oregon Forests, 2001-2012

A. Kanaskie[1], E. Hansen[2], E.M. Goheen[3] Nancy Osterbauer[4], M. McWilliams1, J. Laine[1], M. Thompson1, S. Savona[1], H. Timeus[1], B. Woosley[1], R. Wiese[1], W. Sutton[2], P. Reeser[2], Joe Hulbert[2], R. Shultz[5], D. Hilburn[4]

[1]Oregon Dept. of Forestry, 2600 State Street, Salem, OR 97310, USA.
[1] Dept. Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.
[1] SW OR Forest Insect and Disease Service Center, Central Point, OR 97502, USA.
[5] USDI Bureau of Land Management, North Bend, OR 97459, USA.
[6] Oregon Dept. of Agriculture, 635 Capitol St NE, Salem, OR 97301, USA.

Corresponding author: akanaskie@odf.state.or.us

Extended Abstract

Sudden Oak Death was first discovered in Oregon forests in July 2001 near the coastal city of Brookings, 8 km (5 mi) north of the California border. Archival aerial photographs suggest that disease probably was present there since 1998 or 1999. At the time of discovery in Oregon there were 5 infested sites encompassing a total of 14.6 ha (36 ac) distributed within an 8 km^2 (3 mi^2) area. Soon after the initial detection, personnel from the Oregon Department of Agriculture (ODA), Oregon Department of Forestry (ODF), Oregon State University (OSU), and the USDA-Forest Service decided to attempt eradication of the pathogen by cutting and burning all infected and symptomatic host plants in the infested sites. At the same time the Oregon Department of Agriculture established an emergency quarantine area of 22.5 km^2 (9 mi^2) from which movement of all host material was prohibited.

Ecology of *P. ramorum* in Oregon Forests. In Oregon the primary host of *Phytophthora ramorum* is tanoak (*Notholithocarpus densiflorus*) which is killed by the pathogen and acts as a source of inoculum throughout the year (Hansen et al., 2008). The disease spreads locally by rain-splash and over long distances (several miles) by wind and wind-driven rain from the canopy of infected tanoaks trees (Reeser et al., 2009). Many other forest plant species also are susceptible to the pathogen when growing close to tanoak, but they do not appear important for disease spread in Oregon forests. Humans can spread disease by transporting infected plants or infested materials, but this has not been documented in Oregon forests.

The time between initial infection of tanoak and the development of disease symptoms is not clearly understood in natural forest conditions. Infections on leaves and fine twigs can be seen within weeks of infection as leaf blotches and small lesions, but these are difficult or impossible to detect in standing tanoak trees until infection is abundant. The time between initial infection in the crown of tanoaks and the development of trunk cankers and tree death appears to range from several months to several years (McPherson et al., 2010). This latent period, when the pathogen is present but not readily detectable, is extremely important to the early detection/ eradication program because spore production can occur throughout this period.

Early Detection Surveys. The early detection program consists of: 1) four aerial surveys per year with ground-checking of all dead or symptomatic trees; 2) intensive ground –based surveys by field crews looking for pre-mortality symptoms, and; 3) stream baiting in 50 to 60 drainages. If symptoms are present, two samples of symptomatic plant tissue are collected; one is plated in

the field onto *Phytophthora*-selective agar and the other is taken to the laboratory for plating and polymerase chain reaction analysis (PCR).

Eradication Treatments. Mandatory eradication began in the autumn of 2001 under the statutory authority of the Oregon Department of Agriculture. In 2001 and 2002, the treatment area boundary was 15 to 30 m (50 to 100 ft) from infected or symptomatic plants. In subsequent years it was increased to 100 m (300 ft). On private and USDA-Forest Service land, eradication treatments consist of felling and burning all host plants within the treatment area as soon as possible after detection. Since 2004, all tanoaks in treated areas (except those on some federal land) have been injected with herbicide (imazapyr or glyphosate) prior to felling to prevent sprouting from stumps. Eradication treatments on private lands were delayed for several months many times between 2008 and 2011. When funds were available, priority was given to treating outlying sites and sites considered most important in terms of spread outside the quarantine area. Since 2001, eradication treatments have been completed on approximately 1330 ha (3185 ac) of land, at a cumulative cost of $8.2 million. An additional 607 ha (1500 ac) of tanoak forest have been felled or killed with herbicide in advance of the disease in areas of probable disease spread, mostly in the northern part of the quarantine area.

Treatment Effectiveness Monitoring. Eradication of *P. ramorum* from individual infested forests sites is difficult but not impossible. The disease usually does not persist on infested sites following cutting and burning, but the pathogen frequently can be recovered from soil several years after treatment (Goheen et al., 2010). Details of post-treatment monitoring results are described by Goheen et al. (this proceedings).

Disease Spread, 2001 to 2012. The number of new infested sites discovered each year is shown in Figure 1. Most new infestations occurred close to previously know infestations but apparent long-distance spread was observed several times with distances of 3 to 5 km (2 to 3 mi) between infested sites and occasionally as far as 20 km from the nearest known infested site (Figure 2).

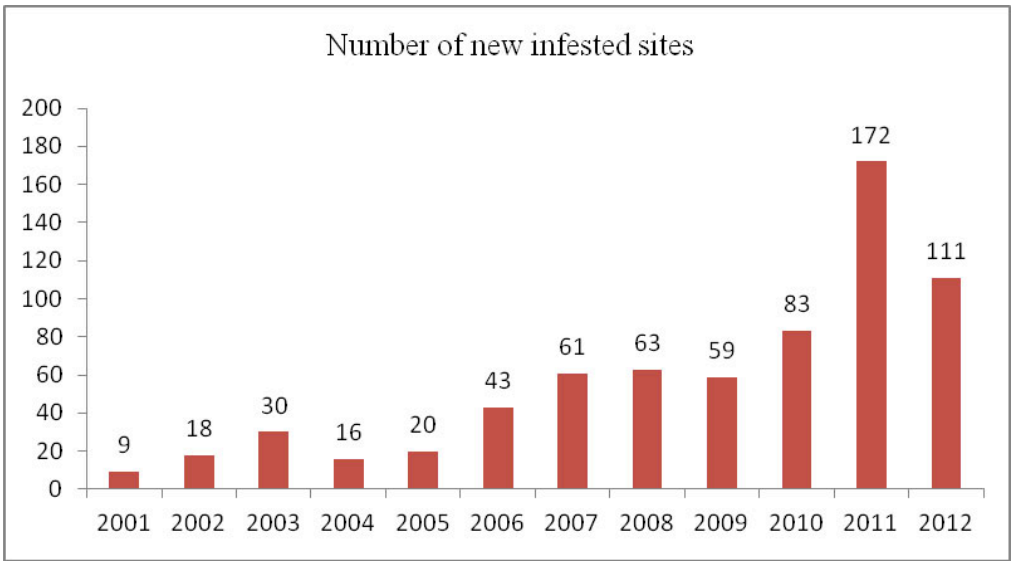


Figure 1. Number of new sites infested with *Phytophthora ramorum* discovered annually between 2001 and 2012 in Curry County, Oregon forests. Data for 2012 understates the amount of disease because of decreased survey effort within the generally infested area.

On some sites eradication treatments apparently eliminated disease and stopped spread into the adjacent forest. In at least three instances where disease was in early stages (one or two infected trees) and the treatment area was large (10 to 16 ha (25 to 40 ac)), the disease has not been detected in the adjacent forest four years post-treatment. Treatment area buffers of 200

m (600 ft) or more from infected or symptomatic plants probably are necessary to capture most nearby (but not readily detectible) infected plants.

Disease spread during the 11-year period has been predominantly northward, following the prevailing wind direction during storms and wet weather. The disease has spread from the initial infestations southward 1.9 km (1.2 miles), and northward and eastward 28 km and 12 km (17.3 mi and 7.4 mi), respectively. The area under quarantine has expanded five times: from 22 km^2 (9 mi^2) in 2001 to 505 km^2 (202 mi^2) in early 2012. The 2012 quarantine area and distribution of the disease are shown in figure 2. Continued spread of sudden oak death is attributed to the slow development of symptoms in infected trees which hinders early detection, and to delays in completing eradication treatments which allow disease spread from known infestations.

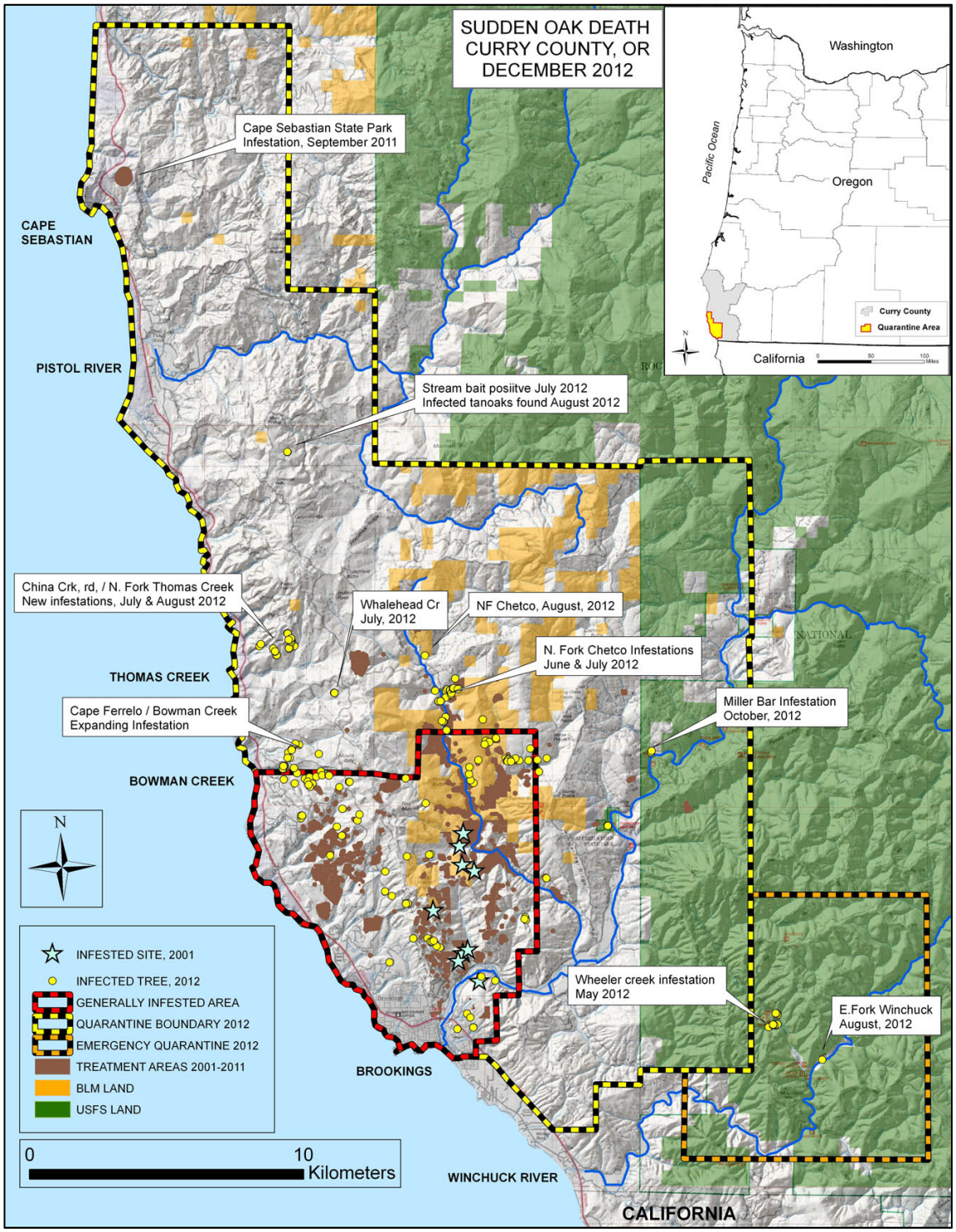


Figure 2. Location of trees infected with *Phytophthora ramorum* in southwest Oregon that were discovered in 2012 (enlarged for visibility). Brown polygons indicate eradication treatment areas. Curry County, Oregon, USA

Changes to the Sudden Oak Death Program. Changes to the Oregon quarantine regulations became effective in March, 2012. The revised quarantine: 1) establishes a "generally-infested area" within the quarantine boundary where *P. ramorum* has persisted or intensified and treatment is no longer required by the State; 2) defines high-priority sites where eradication treatments are required, and; 3) allows increased utilization of tanoak within the quarantine area.

Although *P. ramorum* will not be eradicated from Oregon forests, an ongoing well-funded disease management program will slow its progress, prevent or delay environmental and economic damage, and reduce the probability of spread to other forests. Key elements of the program include: 1) early detection and rapid eradication of new infestations that are epidemiologically important; 2) reducing inoculum levels wherever practical through cost-share projects and best management practices, and; 3) improved education and outreach to prevent spread by humans. The current planned annual budget for the program is approximately $1 to $1.2 million.

Acknowledgements

Funding for detection, monitoring, and eradication was provided by: USDA Forest Service Region 6 Forest Health Protection; USDA Forest Service Region 5 Forest Health Protection; USDA Forest Service Forest Health Management; USDA Forest Service Pacific Southwest Research Station; USDI Bureau of Land Management; USDA Animal and Plant health Inspection Service; Oregon State University; Oregon Parks and Recreation Department, Oregon Department of Agriculture, and Oregon Department of Forestry. Finally, we extend appreciation to landowners in Curry County, especially the South Coast Lumber Co., for their cooperation and tolerance.

Literature Cited

Goheen E.M., Kanaskie A., Hansen E., Sutton W., Osterbauer N., Reeser P., Osterbauer, N., 2010. Eradication effectiveness monitoring in Oregon tanoak forests: Proceedings of the Sudden Oak Death Fourth Science Symposium. Gen. Tech. Rep. PSW-GTR-229. pp. 233-235. U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. Albany, California.

Hansen E.M., Kanaskie A., Prospero S., McWilliams M., Goheen E.M., Osterbauer N., Reeser P., Sutton W., 2008. Epidemiology of *Phytophthora ramorum* in Oregon tanoak forests. Canadian Journal of Forest Research 38:1133-1143.

McPherson B. A., Wood D. L., Mori S., Kelly M., Storer A. J., Svihra P., Standiford R. B., 2010. Survival of oaks and tanoaks after eight years of sudden oak death monitoring in coastal California. *Forest Ecology and Management* **259:** 2248-2255.

Reeser P., Hansen E., Sutton W., Kanaskie A., Laine J., Thompson M., Goheen, E.M., 2009. Using rain bucket spore traps to monitor spore release during SOD eradication treatments in Oregon tanoak forests. Proceedings of the Sudden Oak Death Fourth Science Symposium, June 15-19, 2009, Santa Cruz, CA. Gen. Tech. Rep. PSW-GTR-229. p. 358. U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. Albany, CA.

Mitigating against dispersal of *Phytophthora* spp. on forest produce

I. Hood[1], M. Dick[1], N. Arhipova[2], J. Gardner[1], P. Taylor[1]

[1]Scion (New Zealand Forest Research Institute), Private Bag 3020, Rotorua 3046, New Zealand.
[2]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, 750 07 Uppsala, Sweden.

Corresponding author: ian.hood@scionresearch.com

Abstract

In the past two decades a disturbing number of serious new Phytophthora diseases have been recognised around the world. These have generally been the result of a new association between a species of *Phytophthora*, and a host that has not previously been encountered. Both described *Phytophthora* species, and those that were unrecognised prior to the emergence of the new disease, have been implicated. Often the origin of an incursion is not known, however the movement of tree products pose a potential risk for such introductions through the international trade of raw forest products. With increasing globalisation of trade there is concomitant concern that many more diseases caused by *Phytophthora* species will be discovered as these organisms are dispersed both nationally and internationally.

The potential for elimination of propagules of *Phytophthora* spp. from forest produce has been examined. Spores of *Phytophthora* species were applied in aqueous suspension to the bark on wood segments at densities of 350-550/cm2, incubated at five temperatures likely to be encountered in transit. Material was tested for survival of the inoculum at successive intervals thereafter. The efficacy of the fumigants methyl bromide, phosphine and sulfuryl fluoride on the elimination of surface contamination was also tested.

The extent of possible natural contamination of forest produce remains to be determined but it appears likely that *Phytophthora* spp. surface-contaminating products leaving the forest will survive for only a short period of time.

Survival and eradication of *Phytophthora cinnamomi* from black gravel graveyard sites in the *Eucalyptus marginata* (jarrah) forest

M. Crone[1], J. McComb[1], P. O'Brien[1], V. Stokes[2], I. Colquhoun[2], G.E.St.J. Hardy[1]

[1]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, 90 South Street, Murdoch, WA 6150, Australia.
[2]Alcoa of Australia, Ltd, Hun Location, Huntley Mine Site, Pinjarrah Refinery Access Road, Pinjarrah, WA 6208, Australia.

Corresponding author: m.crone@murdoch.edu.au

Abstract

Phytophthora cinnamomi is known to survive more than 50 years on impacted sites in the *Eucalyptus marginata* forest. One of the most severely impacted landscapes within this area are the 'black gravel' sites and persistence of the pathogen has made these areas extremely difficult to rehabilitate. Previous research has shown that *P. cinnamomi* is a poor competitive saprophyte so it was postulated that complete removal of the vegetation will kill the pathogen. Eradication experiments on black gravel sites investigated the length of time *P. cinnamomi* can survive in the soil without living plant tissue. Results encourage the view that the pathogen can be eliminated from infested sites as recoveries decreased significantly two years after removal of living plants. Annual and herbaceous perennials play an unexpectedly important role in the disease cycle and must be eliminated if eradication is to be successful.

Investigations on control measures for *Phytophthora ramorum* and *Phytophthora kernoviae* in heritage gardens and parks

E.F. Wedgwood[1], D. Lockley[1], J. Turner[2], G. Thorp[2], B. Henricot[3]

[1]*ADAS UK Ltd., ADAS Boxworth, Boxworth, Cambridge, CB23 4NN, UK.*
[2]*The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK.*
[3]*The Royal Horticultural Society, Wisley, Woking, Surrey, GU23 6QB, UK.*

Corresponding author: Erika.Wedgwood@adas.co.uk

Abstract

In Britain, *Phytophthora ramorum* and, to a lesser extent, *Phytophthora kernoviae*, have caused die-back of invasive *Rhododendron ponticum* in woodlands, *Vaccinium myrtillus* (bilberry) in heathland and ornamental plants in nurseries and gardens. Plantations of *Larix kaempferi* (Japanese larch) have been severely affected by *P. ramorum* and have been cut down prematurely to reduce the risk of pathogen spread. The aim of this project [1] is to develop practical approaches for managing both pathogens in heritage gardens and parks.
Approaches being tested include:

1) Tree injection: The fungicides mandipropamid, mefenoxam and potassium phosphite were injected into *Magnolia* and forestry larch trees at infected locations in South West England. Leaves were assayed for residues and are being monitored for the presence of both pathogens.
2) Soil / leaf litter disinfestation: Products containing dazomet, mustard meal, *Trichoderma harzianum* T-22 or *Gliocladium catenulatum* J1446 have been incorporated into soil before re-planting. In other plots, mulches of woven ground-cover material, bark chips, wool waste mats, copper hydroxide treated mats and heat-treated wood shavings have been placed around rhododendron plants to prevent the splash-up of infested soil.
3) Fungicide applications: Based on detached leaf assays, several fungicides were selected for efficacy and tested on new plantings of rhododendron and pieris. Applications with products containing ametoctradin + dimethomorph, benthiavalicarb-isopropyl + mancozeb, fluopicolide + propamocarb hydrochloride or metalaxyl-M + mancozeb were made in 2011 under conditions of natural infection by *P. ramorum* and *P. kernoviae*.
4) Susceptibility periods and susceptible hosts: Field observations and a desk study are being carried out to aid planting decisions.

All the experiments are currently being assessed and results will be presented.

Literature Cited

Key Research Findings from research commissioned from 1st April 2009 in support of the 5-year Defra "Phytophthora Disease Management Programme". Project Code PH0604. www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/phytophthora/documents/prPkResearchJan12.pdf.

The state of *Phytophthora* science and management in natural ecosystems in Australasia

G.E.St.J. Hardy[1], N. Williams[1,2]

[1]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.
[2]Scion, New Zealand Research Institute Ltd., 49 Sala St., Private Bag 3020, Rotorua 3046, New Zealand.

Corresponding author: g.hardy@murdoch.edu.au

Abstract

Since the last IUFRO held in New Zealand 2010 there has been significant activity with regards to research and management of *Phytophthora* species in natural and peri-urban forest, woodland and heathland ecosystems in Australasia. For example, numerous new *Phytophthora* species have been described with a number of others in progress. There are numerous stable hybrid *Phytophthora* species being found and questions are being asked about their role in natural ecosystems. Nurseries continue to play a role in the dissemination of *Phytophthora* species. Containment and eradication of *P. cinnamomi* at a management level has occurred for the first time in a range of diverse plant communities and varying soil types in Western Australia. This clearly shows that eradication is a viable option, although good hygiene measures still remain extremely important in reducing spread into disease-free areas. The sequencing of the *P. cinnamomi* genome has been completed. Research is on-going on determining how phosphite induces defence mechanisms in plants at a molecular and biochemical level. The interaction between drought and *P. cinnamomi* is being studied, particularly with regards to projected warming and drying in the next few decades by all climate models for southern Australia. Community engagement and participation remains high, although on-going funding remains an issue and will be one of the challenges for the future. These activities clearly indicate that *Phytophthora* diseases still remain a key concern in the Australasian context. These and other activities will be addressed in more detail at the meeting in Spain.

A comparison between liquid phosphite injections and novel soluble phosphite and nutrient implants to control *Phytophthora cinnamomi* in *Banksia grandis* and *Eucalyptus marginata*

P. Scott[1], P. Barber[1,2], G.E.St.J. Hardy[1]

[1]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.
[2]Arborcarbon PO Box 1065 Willagee Central, WA, Australia.

Corresponding author: g.hardy@murdoch.edu.au

Abstract

Stem injections with phosphite liquid protect *B. grandis* and *E. marginata* from *P. cinnamomi* for at least four years [1]. However, stem injection of phosphite is labour intensive and requires training, specialised equipment, and the mixing of chemicals. The recent development of soluble phosphite implants which can be quickly inserted into stems, overcomes the need for training and the use of specialised equipment. Systemic nutrient implants and injections have been effectively used to correct nutrient deficiencies in ornamental and horticultural plants and can help increase tree vigour to pests and pathogen attack. However, soluble implants of phosphite and nutrients have never been trialled for the control of *Phytophthora.* This study aimed to determine if liquid phosphite, soluble implants of phosphite alone, or combinations of macro and micro nutrients within implants inserted into the trunks of the trees could control lesion development caused by *P. cinnamomi.* In *B. grandis* and *E. marginata*, phosphite liquid and soluble phosphite implants significantly reduced lesion length compared to the control and application of nutrient implants. In *B. grandis* and *E. marginata*, nutrient implants reduced significantly the average lesion length compared to the control. Results show that both phosphite liquid and implants are effective at controlling lesion extension in *B. grandis* and *E. marginata*, caused by *P. cinnamomi*. Stem treatment with soluble phosphite implants will facilitate the rapid treatment of trees, and control of *P. cinnamomi*, in diseased areas. In addition, the uptake is passive, there is likely less damage to internal stem tissues, and less risk from phytotoxicity due to slow release of the phosphite compared to the liquid treatments.

Literature Cited

Shearer B.L., Fairman R.G., Grant M.J., 2006. Effective concentration of phosphite in controlling *Phytophthora cinnamomi* following stem injection of *Banksia species* and *Eucalyptus marginata*. *Forest Pathology* **36:** 119-135.

Community involvement in Phytophthora dieback management - looking back and forward

I. Colquhoun[1], C. Dunne[1], J. Young[2]
I.

[1]*Dieback Working Group, WA, Australia (http://www.dwg.org.au).*
[2]*Environmental consultant, WA, Australia.*

Corresponding author: iancolquhoun@iinet.net.au

Abstract

The south west corner of Western Australia is internationally recognised as a biodiversity hot spot. The federal and state governments recognise *Phytophthora cinnamomi* as one of the biggest threats to this biodiversity. The community has being playing an important role in the management of Phytophthora dieback since 1993. By the early 1990s the State Government environment department and large mining companies were routinely implementing prescriptions to minimise the spread and impact of *P. cinnamomi*. These prescriptions were based on nearly 30 years of research that had discovered that this introduced pathogen spreads during soil movement and in the surface runoff of water from infested sites. The successful use of phosphite to protect susceptible plants from *P. cinnamomi* had also been demonstrated. Meanwhile, no management of Phytophthora dieback was taking place in natural ecosystems being managed by other state government departments, local governments and private landowners. The spread of disease in these ecosystems was termed 'inadvertent' - 'inadvertent' because the land managers did not know about the disease or how to minimise its spread and impact. It was the community that mobilised to address this knowledge gap. In 1993 and 1994 the education of two local governments was completed. In 1995 the community-based Dieback Working Group formed and the program to facilitate the adoption of Phytophthora management policies and prescriptions commenced in earnest. Nineteen years later no management plan for a local government bushland reserve would be submitted without Phytophthora dieback management being addressed. Project Dieback was launched in 2004 to ensure integrated management of threats to biodiversity at the regional scale regardless of land tenure and to address gaps in strategic, regional, dieback planning. Community has guided and embarked on protecting native vegetation from Phytophthora infestation in a number of regional priority areas. The talk will focus on the list of successes, the reasons for the successes but also highlight the current gaps that still need to be plugged – our work is not done!

Successful containment and eradication of *Phytophthora cinnamomi* at a management level from diverse natural ecosystems in Western Australia

C. Dunne[1], R. Hartley, W. Dunstan[2], P. Scott[2], T. Paap[2], G.E.St.J. Hardy[2]

[1]*Science Division, Department of Environment and Conservation, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.*
[2]*Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.*

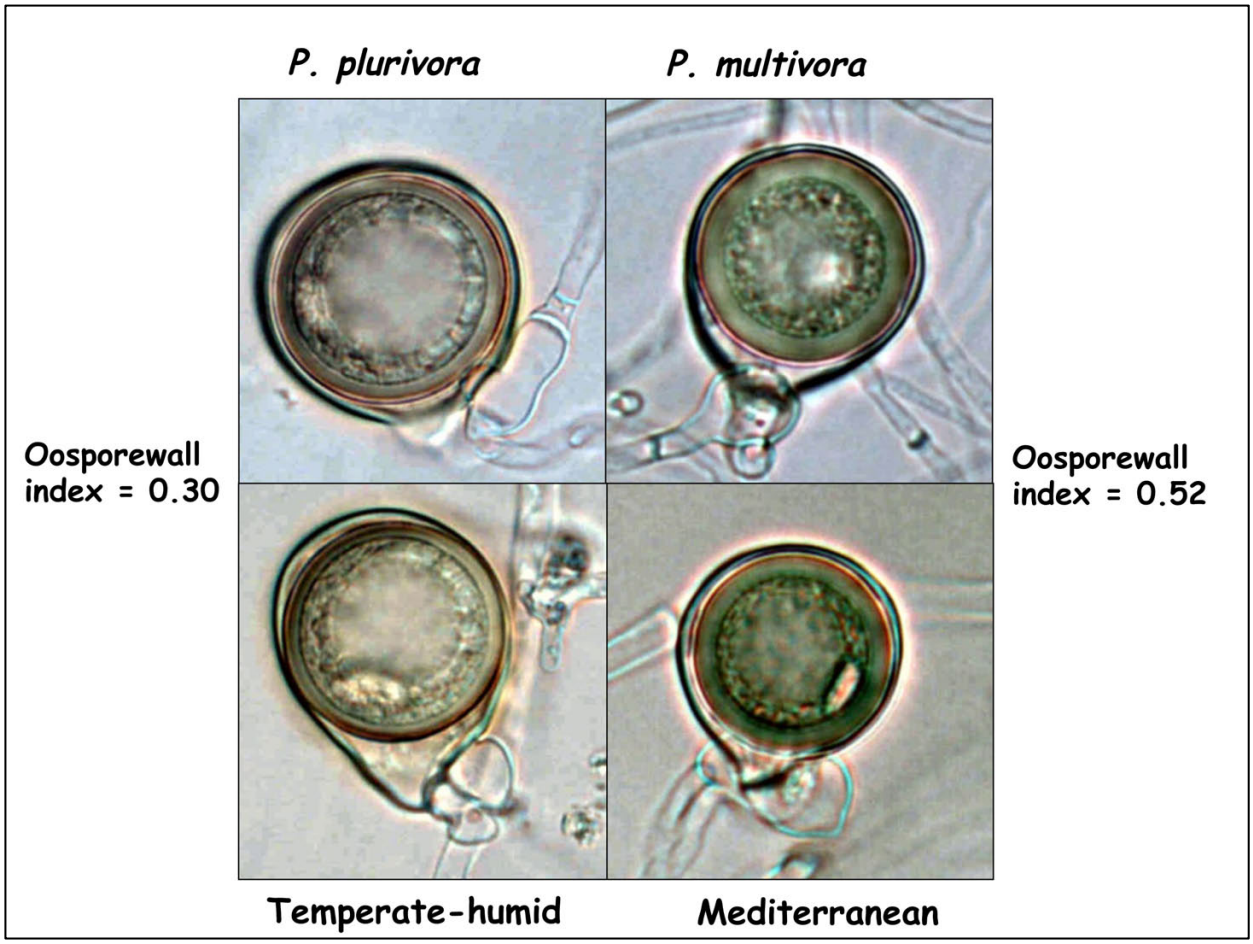
Corresponding author: g.hardy@murdoch.edu.au

Abstract

Here we demonstrate that management scale containment and eradication can be achieved for *Phytophthora* dieback infestations in three different native plant communities in Western Australia. The communities include: (1) a Kwongan vegetation type on a soil varying from sandy to exposed rocky subsurface to a clay above a rock subsurface, (2) a Proteaceous heathland on a deep sand profile, and (3) a Kwongan Banksia woodland on a deep sand over a sandy loam-clay. All of these communities are highly impacted by *P. cinnamomi* resulting in substantial loss of biodiversity assets. The successful approach taken involved the following tasks: risk assessment of the project goals and proposed techniques, implementation of hygiene plans, extensive and intensive soil and plant sampling and *in situ* baiting to accurately map the occurrence of the pathogen, detailed hydrological characterisation using remote sensing techniques, 2D hydraulic modelling, development of hydrological engineering options, catchment modelling, installation of fences to reduce animal vectoring, herbicide applications to remove living host support for the pathogen, phosphite foliar sprays, fumigation with metham sodium and on-going monitoring of the sites to demonstrate the success of the approach taken. Prevention of further spread through these high priority natural ecosystems is now of high priority. This project has involved partnerships between government and non-government agencies, industry, researchers and community groups. These partnerships have included the construction of hygiene infrastructure around priority National Parks and the engagement of key stakeholders in the management of Phytophthora Dieback. The approach described has huge potential for the eradication and containment of other soil-borne *Phytophthora* species around the world.

Literature Cited

Dunstan W.A., Rudman T., Shearer B.L., Moore N.A., Paap T., Calver M.C., Dell B., Hardy G.E.St.J., 2010. Containment and spot eradication of a highly destructive, invasive plant pathogen (*Phytophthora cinnamomi*) in natural ecosystems. *Biological Invasions* **12:** 913-925.



Climatic adaptation of the oospore wall thickness in two species from the *Phytophthora citricola* complex (photos by Thomas Jung and Peter Scott)

DISCUSSION SESSION: ADAPTATION AND EVOLUTION

Morphological and physiological adaptability of the genus *Phytophthora*
Thomas Jung

Fitness, selection and evolutionary divergence in *Phytophthora*
Clive Brasier

What is a *Phytophthora* species?
Everett Hansen

Morphological and physiological adaptability of the genus *Phytophthora*

T. Jung[1,2]

[1]*Phytophthora Research and Consultancy, Thomastrasse 75, D-83098 Brannenburg, Germany.*
[2]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*

E-mail: trjung@ualg.pt

Abstract

The genus *Phytophthora* shows a remarkable adaptive flexibility unrivalled by any other oomycete or fungal genus: being outcrossing, inbreeding or sterile; having a partially saprophytic, necrotrophic or biotrophic lifestyle with narrow to very wide host ranges; forming various types of long- and shortterm resting structures; producing sporangia that can be either caducuous or persistent, enabling airborne and/or soilborne spread, and that germinate either directly or indirectly; forming zoospores that following encystment either germinate directly or continue spreading via a secondary zoospore or a microsporangium; and having highly different cardinal temperatures.

Several well-studied forest and aquatic *Phytophthora* species will be used as case studies to demonstrate the fine-tuned and ongoing morphological, physiological and breeding strategy adaptations of Phytophthoras to the ecological conditions driving their evolution, and to correct some popular misapprehensions about survival and ecology of *Phytophthora*.

Mainly driven by the prevailing funding policies and the pressure to publish in scientific journals with high impact factors the interests in molecular detection tools and phylogenetic studies on the one hand and morphological, physiological and ecological studies on the other hand have diverged diametrically. Meanwhile, most Phytophthora research groups are lacking scientists able to recognise the specific morphological and physiological features of different Phytophthora species in order to understand what the phenotype can tell us about their ecology and pathogenicity and get to a more complete picture of the organisms we are dealing with. Not before long the Phytophthora community will run out of experts able to teach the required skills to younger scientists with profound negative consequences to Phytophthora research in general. This presentation aims to stimulate an intense and possibly quite controversial discussion.

Literature Cited

Brasier C.M., 1992. Evolutionary biology of *Phytophthora*. Part I: Genetic system, sexuality and the generation of variation. *Annual Review of Phytopathology* **30:** 153-171.
Brasier C.M., Cooke D.E.L., Duncan J.M., Hansen E.M., 2003. Multiple new phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides–P. megasperma* ITS Clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile. Mycological Research 107: 277-290.
Jung T., Colquhoun I.J., Hardy, G.E.S.t.J., 2013. New insights into the survival strategy of the invasive soilborne pathogen *Phytophthora cinnamomi* in different natural ecosystems in Western Australia. *Forest Pathology* 43*:* 266-288.
Jung T., Stukely M.J.C., Hardy G.E.St.J., White D., Paap T., Dunstan W.A., Burgess T.I., 2011. Multiple new *Phytophthora* species from ITS Clade 6 associated with natural ecosystems in Australia: evolutionary and ecological implications. *Persoonia* **26:** 13-39.

Fitness, selection and evolutionary divergence in *Phytophthora*

C.M. Brasier

Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK.

E-mail: clive.brasier@forestry.gsi.gov.uk

Abstract

Today we put a lot of effort into demonstrating where *Phytophthora* taxa lie at the ends of the clades in our molecular phylogenetic trees. But to truly understand *Phytophthora* phylogeny we need to evaluate the evolutionary processes that caused related taxa to diverge from each other in the first place: the processes that occurred at the nodes of the trees. Similarly, we need to evaluate the microevolutionary processes that are leading to alterations in genetic structure, to reproductive isolation and perhaps even to new taxa in modern *Phytophthora* populations. Such processes tend to be genetically complex and quantitative and can therefore be more difficult to measure than DNA polymorphisms. Often they fall more within the realm of ecological genetics than population genetics. They can involve studying the *Phytophthora* genetic system: the role of heterozygosity, chromosomal arrangements and patterns of outcrossing and inbreeding. They can involve understanding the components of adaptation and fitness; the balance of sexual versus asexual growth and reproduction; the role of gene flow between the organism's pathogenic and saprotrophic phases; and the role of host specialization. Particularly significant today are the influence of episodic selection events, such as sudden exposure of a *Phytophthora* to crop monoculture or to the nursery environment, or its sudden introduction into a new biogeographic zone. Such events can bring about rapid changes in structure and adaptation in *Phytophthora* populations, including emergence of fitted clones and a potential for rapid genetic modification via interspecific hybridization. These issues will be discussed.

Literature Cited

Brasier C.M., 2009. *Phytophthora* biodiversity: How many *Phytophthora* species are there? In *Phytophthoras in Forests and Natural Ecosystems*. Proc. 4th IUFRO Working Party 7.02.09. General Technical Report PSW-GTR-221. Albany, CA: USDA Forest Service, Pacific Southwest Research Station: 101-115.

Brasier C.M., 1998. Fitness, continuous variation and selection in fungal populations: an ecological perspective. In '*The structure of fungal populations*' (J. Worrall, ed.): 289-318. Kluwer Academic Publishers, Boston & New York.

Brasier C.M., 1983. Problems and prospects in *Phytophthora* research. In 'Phytophthora, Its *Biology, Ecology and Pathology*' (D.C. Erwin, P.H. Tsao, and S. Bartnicki-Garcia, Eds.): 351-364. American Phytopathological Society, St Paul, Minnesota.

Brasier C.M., 1995. Episodic selection as a force in fungal microevolution with special reference to clonal speciation and hybrid introgression. *Canadian Journal of Botany* **73:** 1213-1221.

What is a *Phytophthora* species?

E.M. Hansen

Department of Botany and Plant Pathology, Oregon State University, 1085 Cordley Hall, Corvallis Oregon 97331, USA.

Email: hansene@science.oregonstate.edu

Abstract

What is a species? Why do we care? *Phytophthora* species have traditionally been defined by morphology and pathogenicity but we are all familiar with the challenges that morphological descriptions place on identification. The biological species is the operational unit of evolution and any modern discussion of speciation and phylogenetic relationships must be based on this concept. Mayr defined biological species as groups of interbreeding populations reproductively isolated from other such populations. How should such zoologically based ideas be applied to uniparental and asexual organisms? The key features of this definition are its evolutionary orientation and its population basis. *Phytophthora* species can be defined as groups of populations that share a common evolutionary lineage and have maintained genetic similarity in morphology, physiology, and ecological behavior. Such a definition is impractical, however, without tools to measure gene flow. Molecular analytical methods have provided the tools, and increasingly sophisticated population genetics and statistical analysis now allow hypothesis testing. A *Phytophthora* phylogeny based on biological species would seem to be within our grasp. Today's reality, however, is still short of that goal. We now define "phylogenetic species" based solely on DNA sequence similarity and ask how many base pair differences does it take to make a new species? Even when time and resources are available for a thorough population study, we may be limited by gaps in our understanding of speciation processes in populations of clonal or inbreeding organisms and the seeming fluidity of hybridization in some groups.

Sudden wilting and mortality of planted *Quercus suber* in Extremadura, Spain caused by *Phytophthora quercina* and *Phytophthora psychrophila* (photo by Thomas Jung)

DISCUSSION SESSION: THE NURSERY PATHWAY

Importance of the nursery pathway for the spread of invasive *Phytophthora* across Europe
Thomas Jung

Patterns of repeated introductions and migrations of *P. ramorum* in North America
Nik Grünwald

Ubiquitious *Phytophthora* infestations of nurseries and plantings in Europe demonstrate major failure of plant biosecurity

T. Jung[123*], L. Orlikowski[4], B. Henricot[5], P. Abad-Campos[6], A.G. Aday[7], O. Aguín Casal[8], J. Bakonyi[9], S.O. Cacciola[10], T. Cech[11], T. Corcobado[2], A. Cravador[3], G. Denton[5], S. Diamandis[12], H.T. Doğmuş-Lehtijärvi[7], B. Ginetti[13], J. Hantula[14], G. Hartmann[15], M. Herrero[16], A. Lilja[14], M. Horta[3], N. Keca[17], V. Kramarets[18], A. Lyubenova[19], H. Machado[20], G. Magnano di San Lio[10], P.J. Mansilla Vázquez[8], B. Marçais[21], I. Matsiakh[18], I. Milenkovic[17], S. Moricca[13], J. Nechwatal[22], T. Oszako[23], A. Pane[10], E.J. Paplomatas[24], C. Pintos Varela[8], C. Rial Martínez[8], C. Robin[25], A. Rytkönen[14], M.E. Sánchez[26], B. Scanu[27], A. Schlenzig[28], J. Schumacher[29], A. Solla[2], E. Sousa[20], V. Talgø[16], P. Tsopelas[30], A. Vannini[31], A.M. Vettraino[31], M. Wenneker[32], A. Peréz-Sierra[6]

[1]Phytophthora Research and Consultancy, Thomastrasse 75, D-83098 Brannenburg, Germany.
[2]Ingeniería Técnica Forestal, Universidad de Extremadura, Avenida Virgen del Puerto 2, 10600 Plasencia, Spain.
[3]Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.
[4]Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland.
[5]Royal Horticultural Society, Wisley, Woking, Surrey GU23 6QB, UK.
[6]Grupo de Investigación en Hongos Fitopatógenos, Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n,46022 Valencia, Spain.
[7]Süleyman Demirel University, Faculty of Forestry, 32260 Isparta, Turkey.
[8]EXCMA, Diputación Provincial de Pontevedra, Servicio Agrario, Estación Fitopatológia "do Areeiro", Subida a la Robleda S/N, 36153 Pontevedera, Spain.
[9]Plant Protection Institute, Hungarian Academy of Sciences, Herman Ottó út 15, 1022 Budapest, Hungary.
[10]Department of Agri-Food and Environmental Systems Management, Plant Pathology Section, University of Catania, 95123 Catania, Italy.
[11]Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW), Seckendorff-Gudent-Weg 8, A-1131 Vienna, Austria.
[12]NAGREF-Forest Research Institute, 570 06 Vassilika-Thessaloniki, Greece.
[13]Department of Agricultural Biotechnology, Section of Plant Protection, University of Florence, Piazzale delle Cascine 28, I-50144 Florence, Italy.
[14]Finnish Forest Research Institute, Vantaa Research Centre, P.O. Box 18, 01301 Vantaa, Finland.
[15]Lower Saxony Forest Reseach Station, Grätzelstrasse 2, D-37079 Göttingen, Germany.
[16]Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway.
[17]Faculty of Forestry, University of Belgrade, Kneaza Viseslava 1, 11030 Belgrade, Serbia.
[18]National Forestry University of Ukraine, Forestry Department, General Chuprynki Str. 103, 79057 Lviv, Ukraine.
[19]Agrobioinstitute, Centre of Excellence in Plant Biotechnology , 8 Dragan Tsankov Blvd.,1164 Sofia, Bulgaria.
[20]Instituto Nacional de Recursos Biológicos, Unidade de Silvicultura e Produtos Florestais, Quinta do Marquês, 2780-159 Oeira, Portugal.
[21]INRA, Nancy Universite´, UMR 1136 Interactions Arbres/Microorganismes, IFR 110, F-54280 Champenoux, France.

[22]*Bavarian State Institute of Agriculture (LfL), D-85354 Freising, Germany.*
[23]*Department of Forest Phytopathology, Forest Research Institute, ul. Braci Lesnej 3, 05-090 Raszyn, Poland.*
[24]*Laboratory of Plant Pathology, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece.*
[25]*INRA, UMR BioGeCo, 69 route d'Arcachon, 33612 Cestas Cedex, France.*
[26]*Departamento Agronomía, ETSIAM, Universidad de Córdoba. Apdo. 3048, 14080-Córdoba, Spain.*
[27]*University of Sassari, Department of Plant Protection, Via Enrico De Nicola, 9, 07100 – Sassari, Italy.*
[28]*Scottish Government-SASA, Plant Health, Roddinglaw Rd, Edinburgh, EH12 9FJ, UK.*
[29]*Forest Research Institute Baden-Wuerttemberg, Department of Forest Protection, Wonnhaldestrasse 4, D-79100 Freiburg, Germany.*
[30]*NAGREF-Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 11528 Athens, Greece.*
[31]*Department for Innovation in Biological, Agro-Food and Forest Systems, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy.*
[32]*Wageningen UR/Applied Plant Research, Sector Flower bulbs, Tree nursery & Fruit, Lingewal 1, 6668 LA Randwijk, 6670 AE Zetten, the Netherlands.*

Corresponding author: trjung@ualg.pt

Abstract

Large-scale *Phytophthora* surveys in (1) forest nurseries, advanced tree nurseries, horticultural nurseries and ornamental nurseries and (2) forest, riparian, amenity, landscape and ornamental plantings and horticultural plantations were conducted by 32 research groups in 21 European countries between 1977 and 2012 with most surveys dating from after 2000.

Over all countries and nursery types, 1283 out of 1620 nursery fields and container stands (79.2%) in 563 out of 601 nurseries (93.7%) were found infested by a total of 40 different species and designated taxa of *Phytophthora*.

In most nurseries highly deleterious host-*Phytophthora* combinations were found, eg. *Alnus* spp. and *P. alni*; *Quercus* spp. and *P. cambivora*, *P. cinnamomi*, *P. quercina* and/or *P. plurivora*; *Castanea sativa* and *P. cambivora* and/or *P. cinnamomi*; *Fagus sylvatica* and *P. cactorum*, *P. cambivora* and/or *P. plurivora*; *Citrus* spp. and *P. citrophthora* and/or *P. nicotianae*; *Rhododendron* and *P. cinnamomi*, *P. plurivora* and/or *P. ramorum*.

In contrast to many southern European nurseries where wilting and dieback symptoms were quite common, most of the infested plants in intensely managed nurseries in Central and Western Europe with regular applications of several fungicides and fungistatic chemicals appeared visually healthy underpinning the uselessness of international plant health protocols that are primarily based on visual inspections.

In the planting surveys a total of 48 *Phytophthora* taxa were recovered from 1498 of the 2353 tested plantings (63.6%) As with the nursery fields, plants were often infected by the most aggressive pathogens towards the respective host species. Infected plants often showed symptoms such as thinning, chlorosis and dieback of the crown, extensive fine root losses and collar rot.

The average numbers of *Phytophthora* species/taxa per infested nursery and planting were 1.8 and 1.4, respectively.

Thirty-two of the *Phytophthora* species/taxa detected are considered exotic invasive species. Amongst them *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. plurivora* and *P. quercina* are widespread in Europe and must be considered as well established in both nurseries/plantations and mature stands.

Several *Phytophthora* species/taxa have been found for the first time in Europe, ie. *P. austrocedrae*, *P. gregata*, *P. humicola*, *P. quercetorum*, *P. rosacearum*, *P.* taxon citricola 5 and 6, *P.* taxon organica and P. taxon gregata-like; while others have never or only rarely or regionally been recorded from mature stands, ie. *P. kernoviae*, *P. lateralis*, *P. multivora*, *P. pini* and *P. ramorum*. These apparently recent introductions demonstrate that alongside the exponentially increasing volume of imports of living plants from overseas to Europe the unintended introductions of *Phytophthora s*pecies are also increasing dramatically.

According to a conservative calculation 770000 infested forest plantings with a total area of 5.4 milllion hectares have been established in Europe between 1990 and 2010. Millions of infested landscape plantings and ornamental plantings in the urban-forest interface and tens of thousands of kilometers of roadside and riparian plantings of infested advanced trees and shrubs are completing the dense network of *Phytophthora* infestations across Europe.

The findings of this and previous studies demonstrate major failure of plant biosecurity in Europe which will be discussed.

Literature Cited

Brasier C.M., 2008. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology* **57:** 792–808.

Drew J., Anderson N., Andow D., 2010. Conundrums of a complex vector for invasive species control: a detailed examination of the horticultural industry. *Biological Invasions* **12:** 2837–2851.

Jung T., 2009. Beech decline in Central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. *Forest Pathology* **39:** 73-94.

Jung T., Blaschke M., 2004. *Phytophthora* root and collar rot of alders in Bavaria: distribution, modes of spread and possible management strategies. *Plant Pathology* **53:** 197–208.

Jung T., Vettraino A.M., Cech T.L., Vannini A., 2013. The impact of invasive *Phytophthora* species on European forests. In: Lamour, K., (ed), *Phytophthora*: A global perspective. CABI, Wallingford, UK: 146–158.

Liebhold A.M., Brockerhoff E.G., Garrett L.J., Parke J.L., Britton K.O., 2012. Live plant imports: the major pathway for forest insect and pathogen invasions of the US. *Frontiers in Ecology and The Environment* **10:** 135–143.

MacDonald J.D., Ali-Shtayeh M.S., Kabashima J., Stites J., 1994. Occurrence of *Phytophthora* species in recirculated nursery irrigation effluents. *Plant Disease* **78:** 607-611.

Moralejo E., Pérez-Sierra A., Álvarez L.A., Belbahri L., Lefort F., Descals, E., 2009. Multiple alien *Phytophthora* taxa discovered on diseased ornamental plants in Spain. *Plant Pathology* **58:** 100–110.

Pérez-Sierra A., Jung T., 2013. *Phytophthora* in woody ornamental nurseries. In: *Phytophthora*: A global perspective. (Lamour, K, ed), CABI, Wallingford, UK: 166-177.

Patterns of repeated introductions and migrations of *P. ramorum* in North America

N.J. Grünwald

Horticultural Crops Research Lab, USDA Agricultural Research Service, Corvallis, OR 97330, USA.

Email: grunwaln@science.oregonstate.edu

Abstract

P. ramorum has emerged repeatedly in North America and Europe despite concerted efforts to eradicate the pathogen [1]. Here, I explore the repeated emergence of *P. ramorum* in the US reconstructing the pattern and process of emergence since discovery of the pathogen in North America based on research published to date. The NA1 clonal lineage was first introduced into California, most likely on nursery crops [2]. The pathogen subsequently migrated to Oregon, Washington and British Columbia via nursery shipments [3]. Furthermore, several shipments from the West coast also moved the pathogen East to a range of States [3]. The EU1 clonal lineage was moved from Europe into the Pacific Northwest [4]. From its initial introduction to either British Columbia or Washington, this lineage was further distributed to Oregon and California. The NA2 clone was first introduced into British Columbia or Washington and has to date only spread to California. Thus, the EU1 and NA1 clonal lineages remain restricted to the West coast states and provinces, while the NA1 lineage is now found in many states in the East and West. While both mating types have been found in nurseries, to date sexual reproduction has not been detected. It is apparent that the nursery shipments and imports are the cause for repeated introductions and movement of the pathogen across continental North America.

Literature Cited

Grünwald N.J., Garbelotto M., Goss E.M., Heungens K., Prospero S., 2012. Emergence of the sudden oak death pathogen *Phytophthora ramorum*.*Trends in Microbiology* **20:** 131-138.

Mascheretti S., Croucher P.J.P., Vettraino A., Prospero S., Garbelotto M., 2008. Reconstruction of the Sudden Oak Death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. *Molecular Ecology* **17:** 2755-2768.

Goss E.M., Larsen M., Chastagner G.A., Givens D.R., Grünwald N.J., 2009. Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. *PLoSPathog* 5: e1000583.

Goss E.M., Larsen M., Vercauteren A., Werres S., Heungens K., Grünwald N.J., 2011. *Phytophthora ramorum* in Canada: Evidence for migration within North America and from Europe. *Phytopathology* **101:** 166-171.

Subtropical lowland rainforest in Taiwan dominated by species of the genera *Castanopsis*, *Lithocarpus*, *Cinnamomum* and *Machilus* which is healthy despite of high inoculum of *Phytophthora cinnamomi* (photo by Thomas Jung)

DISCUSSION SESSION: GEOGRAPHIC ORIGINS OF SPECIES

Variation in *Phytophthora*: a key to historical pathways?
Frans Arentz

Are tropical forests *Phytophthora* hot spots?
Yilmaz Balci

Variation in *Phytophthora*: a key to historical pathways?

F. Arentz

Consultant Forest Pathologist, Yungaburra, Qld, Australia.

Email: arentz@activ8.net.au

Extended Abstract

A small but significant portion of the published literature on *Phytophthora* spp. has focussed on postulating the possible geographic origin of a number of species as this would inform possible control measures in areas where the pathogen is suspected of having been introduced as well as providing a potential source of genetic resistance in hosts species that have co-existed with the pathogen for long periods (Zentmyer 1985). Criteria used in formulating various hypotheses have included equal ratios of the two mating types of heterothallic species, host associations, the absence of manifest disease symptoms in the natural vegetation (implying that susceptible hosts have been eliminated) the level of genetic variation that occurs within the species (Shepherd 1975), and the location of the pathogen within the landscape (Ko, Wang & Ann 2006). On the basis of one or several of these criteria, the geographic origin of a number of *Phytophthora* spp. has been variously placed in eastern Asia and New Guinea (Table 1).

Table 1. Postulated geographic origin of selected *Phytophthora* spp.

Species	Postulated origin	Criteria	Reference
P. cinnamomi	South East Asia	Resistance of native vegetation to disease.	Crandall & Gravatt, 1967
	New Guinea/Celebes	Relative frequency of mating types, location in landscape, absence of disease.	Shepherd, 1975
	Asia, including Taiwan	Relative frequency of mating types, absence of disease, location in landscape.	Ko *et al.*, 1978
	Papua New Guinea (PNG)	Location in landscape; isolate variability, absence of disease.	Arentz & Simpson, 1986
		Genetic variability.	Dobrowolski *et al.* 2003
P. heveae	Taiwan	Location within landscape.	Ko *et al.*, 2006
P. katsurae	Taiwan	Location within landscape.	Ko *et al.*, 2006
P. palmivora	Central & South America	Occurrence of native hosts.	Zentmyer, 1988

Determining a centre of origin for *P. cinnamomi* has attracted a lot of interest among plant pathologists; a debate prompted as much by the impact of this species on the native vegetation of southern and south-western Australia, where it has caused major disease outbreaks, as well as its impact on horticultural crops such as the avocado. Crandall & Gravatt (1967) proposed a south-east Asia centre of origin for this species as much of the native vegetation where the type isolate of *P. cinnamomi* was first recovered was immune or resistant to infection by the pathogen. This hypothesis was contested by Shepherd (1975) who used criteria of relative frequencies of the two mating types of *P. cinnamomi* within Australia and elsewhere, variation within the pathogen, and the general absence of plant mortality in northern and north-eastern Australia, to propose New Guinea/Celebes as the centre of origin for this species. The Crandall & Gravatt hypothesis was supported by Ko *et al.* (1978) who used the same criteria as Shepherd (1975) to argue that Taiwan lay inside an Asian centre of origin for *P. cinnamomi*. On the other hand, some support for the Shepherd hypothesis was provided by Arentz & Simpson (1986) on the basis of the genetic variation found among PNG populations of the A1 mating type of *P. cinnamomi* (Old *et al*. 1984) and the widespread distribution of the A1 mating type in

healthy forests in remote areas of PNG. However, based on its association with exotic plants, Arentz and Simpson (1986) concluded that the A2 mating type of *P. cinnamomi* was probably a relatively recent introduction to PNG and further questioned the use of the A1:A2 mating type ratio as a criterion in determining a centre of origin for *P. cinnamomi*.

In this paper, the geographic distribution of four *Phytophthora* spp. (Table 1) found in PNG (Arentz 1986) is re-examined in conjunction with the different "centre of origin" criteria. Possible pathways for the movement of the four *Phytophthora* spp. within the region and the probable antiquity of these pathways are reviewed.

Phytophthora in Papua New Guinea

Between 1974 and 1985 a systematic survey was undertaken in PNG to determine the occurrence and distribution of *Phytophthora* species in native forests and associated agricultural land. Over 600 isolations representing nine species were made and the identity and mating type of representative isolates verified by the Commonwealth Mycological Institute, Kew (Arentz 1986).

Both *P. heveae* and *P. katsurae* were recovered from soil at a number of widely separated geographic localities in PNG at elevations from sea level to 1500 m a.s.l. (Arentz 1986). *P. heveae* was recovered from nine locations and *P. katsurae* from seven locations; both species were recovered from the same sites at two locations. Both species were isolated from remote locations, the principle criterion used by Ko *et al.* (2006) to support their hypothesis that the species were indigenous to Taiwan. On the basis of this criterion it could also be argued that the two species are indigenous to PNG.

Phytophthora palmivora is taxonomically complex (Brasier & Griffin 1979) and high variation has been shown in the *P. palmivora* population of PNG. More than 260 isolates of both A1 and A2 mating types of *P. palmivora* were recovered by baiting soil collected from forest plantations, rubber and cocoa plantation, from rainforest sites and by direct isolation from diseased cocoa pods (Arentz 1986). On the basis of criteria of genetic variation, host association and the occurrence of both mating types and "sterile" isolates in soil in close proximity to each other (Arentz, unpublished data), it could be argued that *P. palmivora* is indigenous to PNG.
During the 1974-85 systematic survey, 87 isolates of *P. cinnamomi* were obtained from 16 localities. Sixty-six of the isolates were of the A1 mating type, recovered from eleven localities, and 21 of the isolates were of the A2 mating type, recovered from six localities (Arentz & Simpson 1986). All *P. cinnamomi* isolates were recovered at elevations over 600 m above sea level (a.s.l.).

Isozyme analysis was carried out by Old *et al*. (1984) of representative *P. cinnamomi* isolates from PNG. Eighteen isolates were tested, eight A1 mating type isolates from eight separate localities (including one on the island New Britain) and 10 A2 mating type isolates from five localities, all on the mainland. The same isolates were also used by Dobrowolski *et al*. (2003) as part of their comparative genetic study of *P. cinnamomi* populations in Australia and from elsewhere in the world. Both studies confirmed that there were two clonal lineages of the A2 mating type in PNG identical to those found in Australia and elsewhere (Dobrowolski *et al*. 2003), and in accord with the conclusion that the A2 mating type was a recent introduction to PNG (Arentz & Simpson 1986). Both genetic studies also showed that seven of the eight A1 mating type isolates from PNG represented genotypes found nowhere else in the world (Old *et al*. 1984; Dobrowolski et *al*. 2003). An eigth isolate, obtained from a *Castanopsis* forest at Paiella, Southern Highlands, appeared to be genetically similar to one of the Australian A1 isolates (Old *et al*. 1984) providing evidence of a direct link between these two populations. A ninth clonal lineage was identified for isolates found in locations other than PNG (Dobrowolski *et al*. 2003). The presence of seven genotypes of *P. cinnamomi* A1 mating type unique to PNG suggests that these genotypes have been isolated from each other for a considerable period of time. Moreover, there is also a real possibility that there are additional unique genotypes in PNG, given that survey work to date has been limited largely by ease of access to sampling

localities.

Possible pathways for the movement of *Phytophthora spp.* in the New Guinea region
There is strong evidence to support a hypothesis of Asian origins for all the four *Phytophthora* spp. being reviewed in this paper. There is equally strong evidence to support the hypothesis that the four *Phytophthora* species, *P. palmivora, P. heveae, P. katsurae* and *P. cinnamomi*, are indigenous (*sensu* Shepherd 1975) to PNG and this presents a conundrum as to the origin of these species.

Indigenous implies "without benefit of man's initial interference". In order for these four species to have moved spontaneously between Taiwan and/or Asia and New Guinea requires a historical land bridge between the two regions. However, the Australian plate, of which New Guinea is an integral part, probably separated from Asia at least 135 million years ago and has been in its present location since the beginning of the Pleistocene (2.5 million years ago), isolated from Asia by deep oceanic trenches (Kirch 2000). Thus, if the four *Phytophthora* species are to be treated as indigenous to both New Guinea and Asia, they would have had to have been present in their present form at least 135 million years ago. This is highly unlikely. If we accept the thesis that the *Phytophthora* spp. have not been spontaneous migrants between Asia and New Guinea, then they must have been moved by man. This raises questions on when this could have first occurred and the mechanisms involved.

Archaeological evidence suggests that the whole of the New Guinea region was first colonised during the Pleistocene at least 40,000 years ago, with the original Papuan speakers migrating overland from Asia (Kirch 2000). Archaeological and ethno-botanical records further indicate that New Guinea and adjacent Bismarck Archipelago were centres of early plant domestication of a range of tropical crop species which originated in New Guinea (and are now widespread) and of horticultural development, the latter dating back 6,000-10,000 years in the New Guinea Highlands (Kirch 2000). By the time a second wave of colonisation of the New Guinea and the Bismarck Archipelago (and of Madagascar (Kirk 2000)) took place approximately 4,500 years ago, this time by Austronesian speakers who probably came from Taiwan (Kirch 2000), the original inhabitants were already practicing shifting agriculture. The Austronesian speakers, also agriculturists, settled mainly in the coastal areas, their descendants eventually colonising the Pacific Islands over the next 2,000 years (Kirch 2000). There is evidence that the Austronesian colonisers successfully transported up to 28 crop plants, either as seed, cuttings or rooted seedlings, as they moved through the region and into Oceania (Kirch 2000).

Although the movement of people was mainly from west to east, there would also have been voyages back in the direction of the Taiwan "homeland" to the west, particularly during the early phases of migration and colonisation 4,500 years ago. Thus it is highly probable that there would have been a translocation of the lowland *Phytophthora* spp. (i.e. *P. heveae*, *P. katsurae* and *P. palmivora*) during this time. Although from the available evidence it is not possible to ascertain where these species first evolved, there would likely have been many opportunities for a reciprocal movement of these species along the migration route between Taiwan and New Guinea. Furthermore, there is no reason why one or several of these species could not have moved further west into the Solomon Islands and into Oceania or to Australia at that time.

This postulated role of the early Austronesian speakers in translocating any of these three *Phytophthora* spp. within the Asia-New Guinea-Pacific region cannot hold for *P. cinnamomi*. As pointed out by Zentmyer (1985), *P. cinnamomi* grows best in mild temperate or subtropical regions, with no growth or survival at soil temperatures below 6°C or above 34-36°C. In PNG, *P. cinnamomi* has only been isolated at elevations above 600m a.s.l. (Arentz & Simpson 1986; Arentz, unpublished data), suggesting that environmental conditions at lower elevations do not favour the pathogen. This means that *P. cinnamomi* was probably absent in the lowlands during the period of Austronesian colonisation 4,500 years ago. Linguistic and anthropological evidence also suggests that there was little or no contact between the lowland Austronesian speakers and the Papuan speaking people living at higher elevations until modern times.

It is suggested that *P. cinnamomi* is an ancient organism of untraceable origin and that the A1 mating type alone arrived in New Guinea and New Britain at least 40,000 years ago, possibly through the agency of man, although other modes of translocation must also be considered. Moreover it is suggested that the A1 mating type was able to migrate spontaneously to northern Australia as recently as 13,000 years ago during the last ice age when conditions in the New Guinea lowlands would have been cooler and more benign for *P. cinnamomi* and the last time that there was a land bridge connecting New Guinea to Australia. Genotype determination of *P. cinnamomi* A1 isolates from Australia has only been carried out for southern populations (Dobrowolski *et al*. 2003). The hypothesis of a long term presence of the A1 mating type of *P. cinnamomi* in Australia could be tested by determining the genotypes of A1 isolates from rainforests in northern Australia and comparing them with those found elsewhere.

Literature Cited

Arentz F., 1986. A key to *Phytophthora* species found in Papua New Guinea with notes on their distribution and morphology. *Papua New Guinea Journal of Agriculture, Forestry and Fisheries* **34:** 9-18.

Arentz F., Simpson J.A., 1986. Distribution of *Phytophthora cinnamomi* in Papua New Guinea and notes on its origin. *Transactions of the British Mycological Society* **87:** 289-295.

Brasier C.M., Griffin M.J., 1979. Taxonomy of '*Phytophthora palmivora*' on cocoa. *Transactions of the British Mycological Society* **72:** 111-143.

Crandall B.S., Gravatt G.F., 1967. The distribution of *Phytophthora cinnamomi*. *Ceiba* **13:** 43-53.

Dobrowolski M.P., Tommerup I.C., Shearer B.L., O'Brien P.A., 2003. Three clonal lineages of *Phytophthora cinnamomi* in Australia revealed by microsatellites. *Phytopathology* **93:** 695-704.

Godwin R.A.M., Coffey M.D., 1994. Isozyme diversity in *Phytophthora palmivora*: evidence for a southeast Asian centre of origin. *Mycological Research* **98:** 1035-1043.

Kirch P.V., 2000. On the Road of the Winds: an archaeological history of the Pacific Islands before European contact. Univ. Calif. Press, Berkeley: 424pp.

Ko W.H., Chang H.S., Su H.J., 1978. Isolates of *Phytophthora cinnamomi* from Taiwan as evidence for an Asian origin of the species. *Transactions of the British Mycological Society* **71:** 496-499.

Ko W.H., Wang S.Y., Ann P.J., 2006. The possible origin and relation of *Phytophthora katsurae* and *P. heveae*, discovered in a protected natural forest in Taiwan. *Botanical Studies* **47:** 273-277.

Old K.M., Moran G.F., Bell J.C., 1984. Isozyme variability among isolates of *Phytophthora cinnamomi* from Australia and Papua New Guinea. *Canadian Journal of Botany* **62:** 2016-2022.

Shepherd C.J., 1975. *Phytophthora cinnamomi* – an ancient immigrant to Australia. *Search* **6:** 484-490.

Zentmyer G.A., 1985. Origin and distribution of *Phytophthora cinnamomi*. *California Avocado Society Yearbook* **69:** 89-96.

Zentmyer G.A., 1988. Origin and distribution of four species of *Phytophthora*. *Transactions of the British Mycological Society* **91:** 367-378.

Are tropical forests *Phytophthora* hot spots?

Y. Balci

University of Maryland, Department of Plant Science and Landscape Architecture, College Park, MD, USA.

Email: ybalci@umd.edu

Abstract

In an effort to discover the species present in tropical rain forests in Latin America two expeditions were conducted. First study site was in lowland Amazon in Iquitos, Peru and the second site at Grenada in West Indies. Sampling consisted of collection of forest soil, necrotic leaf samples recently dropped from the canopy and stream baiting. Samples were identified based on morphological features as well as multilocus sequencing. Species identified from the necrotic foliage from canopy included *P. heveae* and *P. tropicalis* for both sites. However, in Peru one and in Grenada, beside *P. palmivora*, two other new species were isolated. *P. heveae* and *P. tropicalis* were also found from soil samples in Peru. The greatest assemblage of species was found in aquatic environments. *P. tropicalis*, *P. heveae*, *P. palmivora*, *P. macrochlamydospora* and *P. insolita* were identified from streams in Grenada. Additionally, in Peru, three new species and in Grenada four new species were identified. In Grenada, *P. palmivora* and *P. heveae* were isolated from necrotic stem and root tissues of nutmeg (*Myristica fragrans*) trees and data collected suggested that these species are possibly associated with the disease known as nutmeg wilt.

Open woodland of *Quercus ilex* in Andalusia with severe dieback and mortality caused by *Phytophthora cinnamomi* (photo by Thomas Jung)

FIELD PRESENTATIONS in ANDALUSIAN FORESTS

Can limestone amendments and pig slurry be considered as control methods for *Quercus ilex* root rot caused by *Phytophthora cinnamomi* in dehesa? Preliminary results from field trials

M.S. Serrano[1], J.R. Leal[2], P. De Vita[1], P. Ríos[1], P. Fernández[2], M.E. Sánchez[1]

[1]*Agronomy Department, University of Córdoba, Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain.*
[2]*Forestry Department, University of Córdob, Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain.*

Corresponding author: ag1sahem@uco.es

Abstract

The root rot caused by *Phytophthora cinnamomi* is leading to high oak tree mortality in dehesa ecosystems located in southwestern Spain. Experiments performed with artificially infested soil in controlled conditions showed that some Ca^{2+} fertilizers induce a decrease of *P. cinnamomi* infections, mainly due to a significant inhibition of sporangial production. Furthermore, under controlled conditions, pig slurry significantly decreased the inoculum potential in infested soil. The aim of this work has been to evaluate in field conditions the ability of limestone amendments and pig slurry to decrease the infectivity of the pathogen. Experiments with two types of limestone amendments, calcium carbonate (1.500 kg ha^{-1}, OCa richness 55,78%), calcium sulphate (2.500 kg ha^{-1}, OCa richness 32,51%) and pig slurry (3000 l ha^{-1}), were carried out in two oak forests in the province of Huelva (Spain): *Los Bueyes*, an open Holm oak woodland (dehesa), and *Campo Baldío*, a former empty land afforested with Holm and cork oaks 15 years ago. In both farms, trees are infected by *P. cinnamomi*. Soil treatments were applied in autumn of 2010 and 2011. Periodically chemical changes on soil (spring), chlamydospore density and infectivity (spring and autumn), tree defoliation, shoot growth and foliar nutrient content (early summer) were evaluated. Although treatments do not influence the density of chlamydospores in the soil, their ability to cause infections decreased significantly in comparison with control (untreated) plots.
A parallel set of experiments were located in small plots (1 m^2) for testing a higher number of fertilizers with 12 replicates (plots) per fertilizer plus controls, distributed in three blocks. In 2010, after soil treatments, 10 Holm oak seedlings per small plot were planted. Seventeen months after plantation, there was an increment in survival in some treated plots in comparison with controls, but some differences were detected among blocks depending on their up or down slope location.

Literature Cited

Serrano M.S., De Vita P., Fernández P., Sánchez M.E., 2012. Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*. *European Journal of Plant Pathology* **132:** 271-279.
Vicente M., Sánchez M.E., Fernández P., Trapero A., 2009. Evaluation of biofumigant plants and organic amendments for supresiveness of root rot of *Quercus* spp. caused by *Phytophthora cinnamomi*. *Phytopathology* **99:** S134-135.

Holm oak regeneration in dehesas with presence of the soilborne pathogen *Phytophthora cinnamomi*: something to do?

M.S. Serrano[1], P. De Vita[1], A.M. García[2], M.D. Carbonero[2], J.R. Leal[2], M.E. Sánchez[1], P. Fernández[2]

[1]*Agronomy Department, University of Córdoba, Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain.*
[2]*Forestry Department, University of Córdoba, Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain.*

Corresponding author: ag1sahem@uco.es

Abstract

Since 1997, near 120 000 ha of former agricultural or abandoned lands have been forested in southern Spain. These afforestation programmes led to a great increase in forest nursery production, but plant quality was not well controlled. *Quercus ilex* spp. *ballota* (Holm oak) and *Q. suber* (cork oak) were the main species planted, occupying 70% of the land afforested in southern Spain in the last 15 years. The quality and health status of oak seedlings produced in the nursery greatly determines the success of afforestations, influencing growth and survival of plants. At the nursery environment, diseases spread easily and quickly and, following infection, symptoms may appear rapidly, but they may also be delayed until the seedlings have been planted out in the forest. *Phytophthora* diseases have rarely been diagnosed in Spanish oak nurseries, and death of seedlings has usually been attributed to a deficient watering or to a bad aeration of the substrates. However, *Phytophthora* species have been demonstrated to be present in Andalusian nurseries and almost certainly they can be considered not only as the main cause of seedling death, but also responsible of initiate infections that result in disease when already infected seedlings were planted out in the forest. The natural soil used as part of planting substrate seems to be the most probable source of inoculum in nurseries. This process is thought to have been an important mean of dissemination of *P. cinnamomi* through the oak forests in southern Spain.
What to do when the pathogen is already established in afforestations? How to manage its quick spread that is killing hundreds of young trees every year?
Different approaches have been addressed at the University of Córdoba: Preventive phosphite treatments or limestone amendments at nursery level. Now, successful treatments are being tested separately or combined at the Holm oak afforestation *Campo Baldío*. Preliminary results will be exposed and discussed.

Literature Cited

Sánchez M.E., Andicoberry S., Trapero A., 2005. Pathogenicity of three *Phytophthora* spp. causing late seedling rot of *Quercus ilex* ssp. *ballota*. *Forest Pathology* **35:** 115-125.
Navarro R.M., Terán A.I., Sánchez M.E., 2006. Acción preventiva y curativa del fosfonato en el control de *Phytophthora cinnamomi* Rands en encina y alcornoque. *Boletín de Sanidad Vegetal Plagas* **32:** 685-694.
Serrano M.S., De Vita P., Fernández P., Sánchez M.E., 2012. Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*. *European Journal of Plant Pathology* **132:** 271-279.
Serrano M.S., Fernández P., De Vita P., Sánchez M.E., 2012. Calcium mineral nutrition increases the

tolerance of *Quercus ilex* to *Phytophthora* root disease affecting oak rangelands ecosystems in Spain. *Agroforestry Systems* **87:** 173-179.

Phytophthora root rot in a wild olive forest (*P. megasperma* and *P. inundata*)

M.E. Sánchez[1], A. Trapero[1], C.M. Brasier[2]

[1]*Agronomy Department, University of Córdoba, Ctra. Madrid-Cádiz Km, 396, 14014 Córdoba, Spain.*
[2]*Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK.*

Corresponding author: ag1sahem@uco.es

Abstract

From the beginning of 90's, wilting and death of cultivated olive trees (*Olea europaea*) are common in southern Spain, mainly affecting new plantations. Death of trees occurs rapidly, with or without previous yellowing or defoliation. In field surveys, most of the affected plantations had waterlogged or very wet soils. In all of these plantations, tree death was associated with root rot. Fungal isolations from affected roots coming from 166 olive orchards all along Andalusia, consistently yielded *Phytophthora* spp. (94% of the orchards showing root rot). These *Phytophthora* isolates were firstly characterized on the basis of their morphological and physiological properties, and assigned to two distinct groups, A and B, representing 66% and 33% of the isolates respectively. All the group A isolates conformed to *P. megasperma sensu stricto* and its identification was confirmed based on molecular analysis of the ITS regions. Group B isolates exhibited unique characteristics, being heterothallic but with a degree of sporadic self-fertility. All these characteristics and their ITS sequences conformed closely to those of the former 'O-group' taxon described by Brasier et al. In a later study involving O-group isolates from a wide range of hosts, both A1 and A2 mating types were identified and the 'O-group' taxon designated as a new species: *P. inundata*.
In the last decade a similar root rot has been detected in afforestations of wild olives and recently also in a mature wild olive woodland (Dehesa de Abajo, Sevilla). In both cases trees were located in seasonal waterlogged soils and *P. megasperma* and *P. inundata* were consistently isolated from necrotic roots and rizosphere. It seems reasonable that susceptibility to *Phytophthora* root rot may have limited the natural ecological distribution of olive trees. It is clear that wild olive should not be planted in conditions where flooding occurs or in areas with poor drainage.
Suitable control measures to decrease infection rates and pathogen dispersal in mature olive forests will be exposed and discussed.

Literature Cited

Sánchez M.E., Ruiz A., Pérez de Algaba A., Blanco M.A., Trapero A., 1998. Occurrence and etiology of death of young olive trees in southern Spain. *European Journal of Plant Pathology* **104:** 347-357.
Sánchez M.E., Muñoz M., Brasier C.M., Trapero A., 2001. Identity and pathogenicity of two *Phytophthora* taxa associated with a new root disease of olive trees. *Plant Disease* **85:** 411-416.
Brasier C.M., Sánchez-Hernández E., Kirk S.A., 2003. *Phytophthora inundata* sp. nov., a part heterothallic pathogen of trees and shrubs in wet or flooded soils. *Mycological Research* **107:** 477-484.

POSTERS

Assessment of the presence of oomycetes in forest nurseries in Eastern Spain

P. Abad-Campos[1], A. Puértolas[1], A. Ortega[2], P. Guirao[2], M. León[1], J. García-Jiménez[1], A. Pérez-Sierra[1]

[1]Grupo de Investigación en Hongos Fitopatógenos, Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain.
[2]Dpto. Producción Vegetal, Escuela Politécnica Superior de Orihuela, Carretera de Beniel km 3.2, 03312 Orihuela, Spain.

Corresponding author: pabadcam@eaf.upv.es

Abstract

Sanitary quality of seedlings is one of the several factors involved in the survival of plants in the field. An extensive survey was conducted in forest nurseries throughout the eastern Iberian Peninsula in order to assess the presence of oomycetes and plant pathogenic fungi in plant material to be used in reforestation programs. Plants showing disease symptoms such as dieback, wilting, chlorosis, leaf spots, discoloration of needles, aborted buds, defoliation and / or stunting were collected as well as the substrate contained in their pots. Asymptomatic plants were also randomly sampled. Isolation from plant material was performed onto CMA-PARPBH and PDAS. Apple baiting method was used to recover oomycetes from soil samples. .After incubation at 25ºC in the dark, hyphal tips were transferred to PDA and V8-Agar media for further studies and conserved on OA medium. *Phytophthora* and *Pythium* isolates were identified based on morphological, physiological and molecular information among the following hosts: *Pinus halepensis, P. nigra P. pinea, P. pinaster, P. sylvestris Arbutus unedo Ceratonia siliqua Fraxinus ornus Quercus ilex* subs. *Ballota Sorbus domestica* and the shrub species *Cistus albidus, Myrtus communis Pistacia lentiscus Viburnum lantana Viburnum tinus*. The oomycetes *Phytophthora cactorum, P. citrophthora, P. cryptogea, P. nicotianae, P. plurivora,* and several isolates of *Pythium* were identified from affected roots and substrate. At the moment no link has been found between the *Phytophthora* species isolated in these surveys with those detected in the natural ecosystems studied in Eastern Spain.

Effect of three control treatments on the survival of *Quercus ilex* seedlings infected with *Phytophthora cinnamomi*

A. Acedo, E. Cardillo, I. Núñez

Institute of Cork, Wood and Charcoal (IPROCOR), Extremadura Government, Calle Pamplona s/n. Apdo. 437 Mérida Badajoz, Spain.

Corresponding author: angel@procor.org

Abstract

Phytophthora cinnamomi is an invasive root pathogen that causes decline and mortality in main evergreen oaks species in Spain. Several fungicide treatments have been tested both in vitro [1] and in vivo [2] against *P. cinnamomi* and chemical treatment could play a major role in future control strategies for Phytophthora root rot.
Holm oak (*Quercus ilex*) seedlings 15 month-old cultivated in greenhouse were treated with potassium phosphonate applied to the stem (300 µL 2.5%/plant+ surfactant), soluble silicon soil drench (75 ml 2% SiO_2/plant) and gypsum amendment (5% p/p) before being inoculated with a chlamydospore suspension of a *P. cinnamomi* isolate with proven virulence. Potting media were kept moist for optimal infection and eventually flooded with tap water during 5 days. Mortality was registered during 12 weeks. Mortality was modeled with generalized linear model and treatment effects were compared by deviance analysis. No mortality occurred before the plants were flooded. Inoculation with *P. cinnamomi* reduced survival of plants from 95% to 78%, however none of the treatments used was able to reduce the mortality and survival time in the inoculated plants.

Literature Cited

Bekker T.F., Kaiser C., Van Der Merwe R., Labugchagne N., 2006. *In-vitro* inhibition of mycelial growth of several phytopathogenic fungi by soluble silicon. *South African Journal of Plant and Soil* **26:** 169-172.
Serrano M.S., De Vita P., Fernández-Rebollo P., Sánchez M.E. 2012. Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*. *European Journal of Plant Pathology* **132**: 271-279.

Pythiaceous and fungal species isolated from coniferous and deciduous seedlings in some Turkish nurseries

A.G. Aday[2], A. Lehtijärvi[1], H.T. Doğmuş-Lehtijärvi[1]

[1]*Süleyman Demirel University, Faculty of Forestry, 32260 Isparta, Turkey.*
[2]*Süleyman Demirel University, Yenişarbademli Vocational School, 32280 Isparta, Turkey.*

Corresponding author: guldenaday@sdu.edu.tr

Abstract

Turkish private and state owned forest nurseries have been responsible for the supply of increasing numbers of seedlings for afforestation. The most important phytopathological problems in forest nurseries are mainly caused by root rot fungi and Pythiaceous species like *Phytophthora, Phytopythium* and *Pythium*, which bring about severe economical losses in seedling production.
In this study, forest nurseries of Denizli, İzmir, Adapazarı, Bursa and Muğla Provinces were investigated for the presence of root rot fungi and *Phytophthora*, *Phytopythium* and *Pythium* species. For this purpose potted and bare rooted seedlings showing collar rot, chlorosis, wilting and dieback symptoms and also soils of coniferous and deciduous trees species were sampled. Isolations from symptomatic roots were performed on selective PDA medium. Soil samples were baited with carnation, and young leaves of rhododendron and cork oak. Infected leaves were placed onto petri dishes containing selective PARPNH agar. Identification of isolates was performed both based on colony patterns, growth rates, and morphological features and molecular methods. Internal transcribed spacer sequences of ribosomal DNA, 820 bp long, were amplified using the ITS1 and ITS4 primer pair. *Abies bornmülleriana, Quercus virginiana*, *Thuja occidentalis*, *Pinus sylvestris*, *Quercus suber*, *Platanus orientalis*, *Buxus sempervirens*, *Laurus nobilis*, *Castenea sativa* seedlings were found to be infected by root rot fungi and Pythiaceous species. *Fusarium oxysporum*, *F. moniliforme*, *Fusarium* spp. *Rhizoctonia solani* Kühn., *Alternaria* spp. *Cylindrocarpon destructans* were the main fungal species commonly isolated from seedlings. Beside these fungal species *Pythium ultimum*, *Pythium irregulare*, *Phytopythium vexans*, *Phytopythium litorale, Phytophthora cactorum, P. citricola and P. plurivora* were identified so far. Morphological identifications and ITS region sequence comparisons of more Pytiaceaus species are still going on.

Literature Cited

Gardner J.F., Dick M.A., Bulman L.S., 2006. Detection of disease in forest nurseries. Commercial in Confidence Client Report No. 40490.
Lilja A., Kokkola M., Hantula J., Parikka P., 2006. Phytophthora spp. a new threat to tree seedlings and trees. In: Solheim, H. & Hietala, A.M. (eds.). Forest pathology research in the Nordic and Baltic countries 2005. Proceedings from the SNS meeting in Forest Pathology at Skogbrukets Kursinstitutt, Biri, Norway, 28-31 August 2005. Aktuelt fra Skogforskningen 1/06: 48-51.

Characterisation of the two informally designated ITS Clade 6 taxa *Phytophthora* taxon Forestsoil and *P.* sp. Hungarica

J. Bakonyi[1], Z.A. Nagy[1], T.I. Burgess[2], A. Szigethy[1], J. Nechwatal[3], A. Koltay[4], S. Woodward[5], L. Belbahri[6], T. Jung[7.8]

[1]*Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Herman Ottó str. 15, H-1022 Budapest, Hungary.*
[2]*Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, 90 South Street, Murdoch, WA 6150, Australia.*
[3]*Institute for Plant Protection, Bavarian State Research Center for Agriculture, Lange Point 10, D-85354 Freising, Germany.*
[4]*Forest Research Institute, Hegyalja str. 18., H-3232 Mátrafüred, Hungary.*
[5]*Institute of Biological and Environmental Sciences, University of Aberdeen, St. Machar Drive, Aberdeen AB24 3UU, Scotland, UK.*
[6]*Institute of Biology, Laboratory of Soil Biology, University of Neuchâtel, CH-2009 Neuchâtel, Switzerland.*
[7]*Phytophthora Research and Consultancy, Thomastrasse 75, D-83098 Brannenburg, Germany.*
[8]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*

Corresponding author: bakonyi.jozsef@agrar.mta.hu

Abstract

Phytophthora ITS Clade 6 contains numerous informally designated taxa and recently described new species. We report here on the characterisation of the two informally designated taxa *P.* taxon Forestsoil and *P.* sp. hungarica. The first culture of *P.* taxon Forestsoil was collected from soil of an oak-*Carpinus* forest in 1998 and partially characterised later. Subsequently, a few isolates from alder soil with identical ITS sequences to *P.* t. Forestsoil were reported as *P.* sp. sylvatica and *P.* sp. H-6/02 at Genbank in 2007. At the same time other isolates from alder soil, differing from *P.* t. Forestsoil and its synonyms in their colony pattern on carrot agar (CA) and at the same 4 positions in their ITS, were named *P.* sp. hungarica, *P.* sp. H-7/02 and *P.* sp. H-8/02. Additional 'hungarica' isolates were then collected in Alaska, also from alder soils or nearby waterways. In our detailed study, the only consistent morphological difference between 'Forestsoil' and 'hungarica' was still the culture pattern on CA. Both taxa showed similar growth rates and cardinal temperatures, produced similar sporangia, were homothallic with mostly paragynous antheridia and aplerotic oospores often with several ooplasts. Concatenated analyses using 4 nuclear and 2 mitochondrial gene sequences were in concordance with the morphological and physiological features supporting the grouping of 'Forestsoil' and 'hungarica' isolates into 2 very closely related but distinct clades. These results might help to unravel the taxonomic and phylogenetic position of these informally designated taxa.

Literature Cited

Brasier C.M., Cooke D.E.L., Duncan J.M., Hansen E.M. 2003. Multiple new phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides–P. megasperma* ITS Clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile. *Mycological Research* **107:** 277–290.
Adams G.C., Catal M., Trummer L., 2010. Distribution and severity of alder *Phytophthora* in Alaska. In: Frankel, S.J., Kliejunas, J.T. and Palmieri, K.M. (tech. coords.) Proceedings of the Sudden Oak Death

Population fluctuation of *Phytophthora* spp in streams and their possible role as early colonizers

Y. Balci

University of Maryland, Department of Plant Science and Landscape Architecture, College Park, MD, USA.

Corresponding author: ybalci@umd.edu

Abstract

The population fluctuation of *Phytophthora* was quantified for a one-year period in two streams. The Paint Branch creek (PB) and Comcast Center creek (CC), part of the Anacostia watershed in Maryland, were sampled. The CC creek was considerably smaller and is a tributary of PB. Streams differed in both their size and the amount of water available. Every week for two days (Monday and Wednesday) 1 L water sample was collected twice at 8 AM and 2 PM. Of the 1 L sample, two or three 200 ml subsamples were filtered through a 3 µm pore size 9 cm diameter nitrocellulose Millipore membrane filter and placed on clarified V8 juice based PARPNH growth media selective for isolation of *Phytophthora* spp. After two days of incubation in darkness at room temperature, colonies were counted. Streams differed significantly in *Phytophthora* colony numbers. The smaller CC stream had almost twice as many colonies compared to PB stream. However, there was no significant difference between the samples that were collected during the morning (8am) or afternoon (2pm). A significant decrease of population of *Phytophthora* was found with increasing temperatures in both streams. Sampling month played a significant role in *Phytophthora* colony numbers. In both streams June, October and November had the greatest number of colony counts. When live and dead leaf baits (leaves were oven dried for one week) of various plants were deployed at CC stream. Overall, more *Phytophthora* colonies were isolated from live compared to dead leaves. However, with red maple (*Acer rubrum*) and red oak (*Quercus rubra*), dead and live leaf baits were equally colonized by *Phytophthora* spp.

Phytophthora species associated with disease in peri-urban woodland and forest ecosystems

P. Barber[1,2], T.I. Burgess[2], T. Paap[2], G.E. St.J. Hardy[2]

[1]*Arborcarbon PO Box 1065 Willagee Central, WA, Australia.*
[2]*Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.*

Corresponding author: g.hardy@murdoch.edu.au

Abstract

Perth, the capital city of Western Australia, is situated on a river with numerous bays. The peri-urban environment extends along the coast for 100 km north and south of the city. Within the region is a fragmented landscape of suburbs, parks and remnant woodlands. The *Eucalyptus gomphocephala* woodland south of Perth is classified within the peri-urban environment. Numerous *Phytophthora* species have been isolated from dead and dying endemic trees. The most frequently isolated species is *P. multivora* (65%), followed by *P.* aff. arenaria (21%); *P. palmivora, P. syringae, P. inundata, P. aff. humicola, P. nicotianae* and *P.* sp. *ohioensis* have also been isolated, although rarely. *Phytophthora multivora* and *P.* aff. *arenaria* have both been isolated from dying *E. marginata* (jarrah), *E. gomphocephala* (tuart), *Corymbia calophylla* (marri), *C. ficifolia* (red flowering gum) and *Agonis flexuosa* (WA peppermint). While *P. multivora* is commonly encountered in less impacted ecosystems, the other species found in the peri-urban environment (*P. inundata* and *P. nicotianae*) are rarely or never isolated. In the riparian ecosystem, *P.* aff. *humicola* has been isolated from dying *Casuarina obesa.* The knowledge about the impact of these species on our remnant trees is lacking. Further research is required on the origin, pathogenicity and control of these species to deliver effective management strategies.

Diversity of oomycetes detected in the laurel forest in Tenerife (Canary Islands)

A. Beltrán[1], M. León[1], P. Abad-Campos[1], F. Siverio[2], L. Gallo[2], A. Pérez-Sierra[1]

[1]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain.
[2]Instituto Canario de Investigaciones Agrarias, Ctra. de El Boquerón s/n, Valle Guerra, La Laguna, 38270 Tenerife, Spain.

Corresponding author: aperesi@eaf.upv.es

Abstract

The laurel forest (laurisilva) is a unique type of vegetation found on the Canary Islands, Madeira and the Azores. It is found in areas with high humidity and relatively stable and mild temperatures. This type of forest is a unique ecosystem, centre of plant diversity, and of great importance for conservation. The objective of this study was to investigate the presence of oomycetes that could be threatening the health of the laurisilva vegetation in the Anaga Rural Park (14419 ha) at the north-eastern end of the Island of Tenerife. A survey was carried out during autumn 2011 and samples of roots and soil were collected from symptomatic and asymptomatic trees and shrubs (*Ocotea foetens*, *Arbutus canariensis*, *Viburnum rigidum* and *Persea indica*) in 22 different locations in the Anaga Rural Park. Direct isolation from roots was performed onto PARPH and avocado leaves were used as baits for soil isolation. Sixty eight oomycete isolates were obtained and grouped on the basis of the morphology of their vegetative and reproductive structures. The ITS region of the ribosomal DNA of isolates from each group was amplified and sequenced with the primers ITS4 and ITS6. Sequences obtained were compared with sequences in GenBank. *Phytophthora multivora* was the only *Phytophthora* sp. detected in this preliminary survey. Five different *Pythium* spp. were detected: *P. diclinum, P. heterotallicum, P. litorale, P. mamillatum, P. mercuriale* and *P. vexans.* Three *Pythium* spp. could not be identified on the basis of their morphology and sequences. This is the first detection of these oomycetes in the laurel forest in the Canary Islands. Further work to study the pathogenicity of these oomycetes is needed in order to evaluate the risk that they might pose to the laurel forest.

Diversity and distribution of *Phytophthora* species in association with water quality and the health of trees in fragmented riparian ecosystems

T.I. Burgess, M. Kunzinger, D. White, A. Lymbery, G.E.St.J. Hardy

Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.

Corresponding author: tburgess@murdoch.edu.au

Abstract

The riparian zone in western Australia is dominated by *Eucalyptus rudis* in a similar manner to *Alnus* spp. in Europe. For the last 20 years the health of these trees have been declining. This is attributed to an endemic leaf sucking Psyllid, however contributing factors could be an increase in the salinity of the waterways or the presence of a root pathogen, both of which would reduce the health of the trees. We sampled 25 sites along different rivers and streams in the southwest of Western Australia. At each site we recorded tree health, determined water quality and filtered water for the isolation of pythiaceous oomycetes. There was considerable variation in water quality (pH and salinity) and the health of the adjacent *E. rudus,* however the poor health was not related to low water quality. There was also considerable variation in the number of colony forming units (from 1.33 to 90 L^{-1}), the proportion of *Phytophthora* compared to *Pythium* isolates (from 0-100%) and the species biodiversity. In general, far more isolates were obtained from low quality water, except for when the pH was greater than 8.5. Water quality did not effect the proportion of *Phytophthora* isolates. *Phytophthora* species isolated included *P. thermophila, P. fluvialis, P. amnicola* and hybrids between these species. Additionally, numerous isolates of *P.* taxon salixsoil were obtained. Remnant sites closer to the urban area contained predominantly *P. thermophila* while *P.* taxon salixsoil predominated on the more southerly sites from remnants within agricultural zones. The link between tree health, water quality and associated pythiaceous populations was not established in this study.

Next Generation sequencing shows *Phytophthora* species diversity in soil samples of Macaronesian laurel forests from the Canary Islands

S. Català, A. Beltrán, A. Pérez-Sierra, P. Abad-Campos

Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain.

Corresponding author: pabadcam@eaf.upv.es

Abstract

Laurel forests (also called laurisilva) are exclusive of the Macaronesian biogeographical region, which comprises three archipielagos: Madeira, Azores and Canary Islands. The main vegetation of the laurisilva in the Canary Islands is dominated by tree and shrub species with laurel-shaped leaves, like *Ocotea foetens*, *Arbutus canariensis*, *Viburnum rigidum*, *Myrica faya* or *Persea indica*, which are developed under high humid conditions. The geographical isolation of the volcanic islands has stimulated the emergence of numerous endemic organisms. However, little is known about the biodiversity of soil microorganisms. Soil DNA was extracted from ten samples from different locations in the Anaga Rural Park (Tenerife). ITS1 amplicons were obtained through a nested PCR using *Phytophthora*-specific primers 18Ph2F and 5.8S-1R [1] in PCR1 and fusion primers based on ITS6 in PCR2, and pyrosequenced on a Roche Junior GS platform (454 Life Sciences). Six of the samples produced amplification signal. The results obtained in this work will bring light into the diversity of *Phytophthora* spp. in this unique ecosystem.

Literature Cited

Scibetta S., Schena L., Chimento A., Cacciola S. O., Cooke D.E.L., 2012. A molecular method to assess *Phytophthora* diversity in environmental samples. *Journal of Microbiological Methods* **88:** 356-360.

Airborne infections of *Phytophthora citrophthora* on citrus in Sicily

A. De Patrizio, R. Faedda, A. Pane, S.O. Cacciola

Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali, University of Catania, 95123 Catania, Italy.

Corresponding author: olgacacciola@unict.it

Abstract

Citrus species native to the far-east of Asia have been introduced into the Mediterranean area as cultivated plants since time immemorial and are grown on a large scale for their fruits, and presently they characterize the Sicilian landscape. Two exceptional climatic events, a hail storm on t 7th March, 2012 and on 9th March hurricane Athos, with blasts of wind with a speed of 80-100 km/h, hit the southern-east coastal area of Sicily. As a consequence, severe fruit brown rot infections were observed on the higher part of the canopy up to 3.4 m above soil level in citrus groves. Fruit symptoms were associated with leaf blight and severe defoliation of the trees. In the past, these kinds of aerial infections of citrus brown rot in Sicily had been attributed to *Phytophthora hybernalis* on the basis of symptomatic diagnosis. However, a survey of citrus orchards affected by these exceptional climatic events using molecular diagnostic methods demonstrated that the causal agent of this epidemic burst of brown rot was actually *P. citrophthora* (Smith & Smith) Leonian. This species is endemic in citrus orchards in Sicily and is the major causal agent of trunk gummosis, root rot and occasional epidemic outbreaks of fruit brown rot of citrus occurring from late autumn to early spring, which is the rainy period in the Mediterranean climate. *P. citrophthora* is typically a soil-borne pathogen and usually infects fruits of the lower part of the canopy near the soil up to 1 m.

ITS-based identification and phylogeny of *Phytophthora spp.* detected in UK gardens

G. Denton[1], J. Denton[1], I. Waghorn[1], S. Archer[2], B. Henricot[1]

[1]*The Royal Horticultural Society, Wisley, Woking, Surrey. GU23 6QB, UK.*
[2]*Imperial College, London, UK.*

Corresponding author: geoffdenton@rhs.org.uk

Abstract

Many studies of *Phytophthora* species have been conducted in commercial settings, such as nurseries, and wild habitats, often forgetting our own back gardens. Gardens are unique environments with plants being a mixture of natives and non-natives from around the world. The current work will detect and identify the *Phytophthora* species present in these microhabitats. Additionally, this artificial mixture of plant species enables investigation into the potential host range of *Phytophthora* species on both native and non-native plants. Environmental samples, from UK gardens, were surveyed during 2006 to 2009 for the presence of *Phytophthora* species, comparing apple baiting with a nested PCR protocol based on the ITS region. The ITS region has become increasingly used for identification of *Phytophthora* species via direct sequencing, development of arrays, real-time PCR and used in many phylogenetic studies. The ITS region from the recovered isolates was sequenced and phylogenetic analysis was undertaken of the amplicons from both cultures and nested PCR. Phylogenetic analysis, incorporating host and morphological data, will be presented. Through baiting the species *P. cactorum, P. cinnamomi, P. citrophthora*, *P. cryptogea*, *P. gonapodyides, P. plurivora* and *P. niederhauserii* were identified. With the nested PCR a wider range of *Phytophthora* species were detected, *P. alni, P. austrocedri, P. cambivora, P. hibernalis, P. megasperma, P. porri, P. quercina* and *P. syringae* were additionally identified. This will be the first study of UK gardens presenting species identification and phylogenetic analysis.

Literature Cited

Cooke D.E.L., Drenth A., Duncan J.M., Wagels G., Brasier C.M., 2000. A molecular phylogeny of *Phytophthora* and related Oomycetes*. Fungal Genetics and Biology* **30:** 17-32.

Pathogenicity of *Phytophthora* species on *Liquidambar orientalis* and *Castanea sativa* seedlings

H.T.Doğmuş-Lehtijärvi[1], A.G. Aday[2], A. Lehtijärvi[1], T. Jung[3,4]

[1]*Süleyman Demirel University, Faculty of Forestry, 32260 Isparta, Turkey.*
[2]*Süleyman Demirel University, Yenişarbademli Vocational School, 32280 Isparta, Turkey.*
[3]*Phytophthora Research and Consultancy, Brannenburg, Germany.*
[4]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*

Corresponding author: tugbadogmus@sdu.edu.tr

Extended Abstract

Introduction

Liquidambar orientalis Mill commonly known as oriental sweetgum or Turkish sweetgum, is one of the endemic decidious tree species in Turkey which is native to the eastern Mediterranean region. The total area of pure sweetgum forests in Turkey covers 1,348 hectares, all in the south western regions of the country.

Castanea sativa Mill (Anatolian chestnut) is one of Turkey's indigenous species widely growing from Black sea coasts to Marmara Region and western Anatolia. There are 200,400 ha chestnut forests in Turkey, pure and mixed with broad leaved and conifer trees. Of this area, 6,383; 52,174 and 141,841 ha are located in Aegean, Marmara and Black Sea regions, respectively. It is also locally occurring in Antalya, Aydın and Isparta province with two natural chestnuts stands in Ayazmana and the Yenice and Dere districts within Isparta city.

In this study, isolates of *Phytophthora* and *Phytopythium* species obtained from sweetgum and chestnut stand in Forest Nature Protection Area which is located in Bucak alongside Karacaören dam reservoir within the administrative borders of Isparta in 2012 were evaluated Pathogenicity test.

Material and Methods

In an earlier study in this area a total number of 160 and 60 Pythiaceous isolates were obtained from *L. orientalis* and *C. sativa*, respectively. After morphological and molecular identification 24 isolates representing the main groups from both tree species were proceeded to pathogenicity tests.

Pyhtophthora cactorum (Lebert & Cohn) J. Schröt*., Phytophthora plurivora* T. Jung and T.I. Burgess*, Phytophthora citricola* Sawada, *Phytopythium vexans Abad, de Cock, Bala, Robideau,* Lodhi & Lvesque, *Phytopythium citrinum* B. Paul and *Phytopythium litorale* Nechw. isolates obtained from sweetgum and chestnut soil samples were inoculated to *Quercus suber* and *Quercus robur* seedlings using two different methods. Inoculation trials were carried out in September 2012.

In the first method, isolates were infested to soil of *Quercus suber* L. and *Quercus robur* L. seedlings with two isolates of each *Phytophthora* and *Phytopythium* species with 10 replicates. Inocula of each isolates were grown at 20°C in 500 ml Erlenmeyer flasks on an autoclaved mixture of 250 ml of fine vermiculite and 20 ml of whole millet seeds moistened with 127 ml of carrot broth. One sterile glass tubes about 15mm were placed near each seedling. These tubes were removed at the time of the inoculation to provide cavity which is filled with inoculum at 20ml of inoculum per 1000ml of soil. Control seedlings were inoculated with carrot broth and millet mixture.

In the second inoculation method, stem inoculation test was carried out on 1-year-old seedlings of *Q. robur* and *Q. suber* seedlings in climate chamber. Seedlings were inoculated with two isolates of each species with 10 replicates. On each seedling, a 4 mm diameter bark plug was removed with a cork borer approximately 5 cm above the collar. The inoculum was applied to the exposed surface and the bark plug was replaced afterwards. The wound was covered with wet, autoclaved cotton wool and sealed with Parafilm. Inoculum consisted of plugs of carrot agar bearing mycelium from the margin of a 7-day-old culture of the test isolate. In addition, 10 control plants of each tree species were treated similarly with sterile carrot agar plugs. The inoculated seedlings were incubated at 20°C in a growth chamber for 3 months. Lesion lengths were compared and analysed using one way analysis of variance (ANOVA), and the inoculations tests were also compared with each other using SPSS 17, data analysis software.

Random reisolations were made using selective PARPNH-agar media to confirm *Phytophthora* and *Phytopythium* species from the lesions and infected roots.

Results and Conclusions

All three tested *Phytophthora* and *Phytopythium* species caused necroses on abscised roots and stem of *Q. robur* and *Q. suber*.
At the end of the incubation period, mortality observed on both *Quercus* species inoculated with *Phytopythium* isolates was lower than that for *Phytophthora* isolates.
With an average root mortality of 35% and 42%, respectively, the *P. cactorum* isolates were the most aggressive species within all *Phytophthora* species tested. All *Phytophthora* and *Phytopythium* species could be reisolated from symptomatic oak roots; nothing was recovered from roots of control plants.
In the second pathogenicity trial, browning of inoculated plant tissues was already observed after 5 days of incubation. After 10 days, *P. cactorum* had caused more symptoms on leaves.
An average mortality and infection incidence on both hosts were similarly higher when the seedlings were inoculated with *P. cactorum, P. citricola* and *P. plurivora*, respectively. Significant differences in lesion length were noted between *Phytophthora* and *Phytopythium* isolates.

Pathogenicity of *Phytophthora* species to stem and roots of *Q. robur* and *Q. suber* has previously been demonstrated in many studies. In this study, *P. cactorum*, *P. plurivora* and *P. citricola* were found to be pathogenic to roots and stems of two oak species and were successfully reisolated from diseased tissues while *Phytopythium* species were less pathogenic.

Lesion length in the inner bark and root rot may be indicators of the ability of the pathogen to penetrate the living bark tissue. The average lesion lengths were similar to those reported in several previous studies, indicating a pathogenic interaction between the pathogen and the host, especially as lesions and root rot was not formed on control seedlings.

Seedlings and 1-year-old plants of *Q. suber* proved to be more susceptible than *Q. robur* to

most of the isolates tested. On the basis of the soil infestation test which more closely mimics the natural process of root infection, *Q. suber* proved to be as susceptible as *Q. robur* in terms of mortality and lesion lengths. Interestingly, in the study of Tuset et al. showed higher susceptibility of *Q. ilex* compared to *Q. suber.*

The results from this study indicate that *Phytophthora* species are pathogenic on both investigated tree species. In contrast to soil and stem inoculations, where the pathogen is introduced directly into the living sapwood or root, natural infection in forest usually takes place via roots and is affected by several environmental factors. Therefore, to assess the significance of *Phytophthora* and *Phytopythium* species in forests, extensive field data are needed for estimates of degree of damage in individual trees and pathogenicity on sweetgum and chestnut seedlings.

Literature Cited

Jung T., Blaschke H., Neumann P. 1996. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands *European Journal of Forest Pathology* **26:** 253-272.
Doğmuş-Lehtijarvi H.T., Aday A.G,, Jung T. 2011. *Phytophthora* species from declining *Liquidambar orientalis* and *Castanea sativa* trees in south eastern part of Turkey. COST Action Meeting 21-22 November, Budapest, Book of Abstracts, 32pp.
Orlikowski L.B., Duda B., Szkuta G., 2004. *Phytophthora citricola* on European beech and silver fir in Polish forest nurseries. *Journal of Plant Protection Research* **44:** 57–64.
Tuset J.J., Hinarejos J.L., Mira J.L., Cobos J.M., 1996. Implication of Phytophthora cinnamomi Rands en la enfermedad de la seca de encinas y alcornoques, *Bol. San. Veg. Plagas* **22:** 491-499.

Phytophthora ipomoeae causing blight on Ipomoeae orizabensis in Michoacan, Mexico

S. Fernández-Pavía[1], G. Rodríguez-Alvarado[1], N. Gómez-Dorantes[1], H. Silva-Rojas[2]

[1]*Laboratorio de Patología Vegetal, Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Km. 9.5 carr. Morelia-Zinapécuaro, Tarímbaro, Michoacán, 58880, Mexico.*
[2]*Producción de Semillas, Colegio de Postgraduados Km. 36.5 Carr. México-Texcoco, Montecillo, 56230, México.*

Corresponding author: fpavia@umich.mx

Abstract

Several wild species of *Ipomoea* grow in Michoacan, Mexico forests. *Ipomoea orizabensis* plants with blighted leaves and petioles were observed in a pine-oak forest located in Charo, Michoacan. Diseased leaves were placed in a humidity chamber to induce sporulation. Isolates were obtained on rye agar selective medium, transferring sporangia from sporulating lesions with a piece of agar to rye agar selective medium. A *Phytophthora* sp. was consistently isolated from blighted leaves. Species identification was based on sporangial and gametangial characteristics of three cultures grown on rye agar. Sporangia production was achieved placing mycelial discs in sterile soil extract. Sporangia were mainly ellipsoid but occasionally ovoid, semipapillated, and deciduous with a short pedicel. The isolates were homothallic with smooth walled and aplerotic oospores. Genotypic analysis for the allozymes Peptidase and Glucose 6-phosphate isomerase indicated that the isolates belonged to one genotype 96/96 (*Pep*) and 108/108 (*Gpi*). Morphological characteristics correspond to the species *P. ipomoeae* Flier & Grünwald. This species has been reported causing blight on *Ipomoeae* only in central Mexico. To confirm the identity of the pathogen, sequences of the internal transcribed spacers (ITS) were obtained from two isolates. The ITS sequences that were obtained shared 100% similarity with strains of *P. ipomoeae* from central Mexico. Pathogenicity tests are underway.

Research driving management and policy-making on Sudden Oak Death in California

M. Garbelotto, S. Mascheretti, D. Schmidt

Forest Pathology and Mycology Laboratory, University of California Berkeley, 54 Mulford Hall, Berkeley CA 94720, USA.

Corresponding author: matteog@berkeley.edu

Abstract

We briefly present results from different studies that are justifying specific disease management options and policies to curtail the spread of Sudden Oak Death in California.
1)- Yearly treatments with the phosphonate Agrifos has reduced mortality rate of tanoaks threefold in approximately 1000 studied trees from 32 study plots across the State
2)- Selective removal of bay laurels 10 m around oaks has significantly reduced, but not eliminated, the number of instances in which oaks are subject to inoculum levels sufficient to cause their infection. Removal of bays to 20 m, however, reduces that number of instances to zero
3)- Cuts on large branches and trunks are ten times more likely to become infected than unwounded trees right after the pruning, however after four months, pruned and un-pruned large branches are comparable in susceptibility
4)- Removal of all organic matter, soil, and plant debris until tools are visually clean reduces infectivity of tools to zero, without the need for chemical treatments
5) – Composting will kill the pathogen, but some composts can be infected once they become old, if exposed to high inoculum levels
6)- Population genetics analyses show that; a)- accidental introductions of the pathogen from infected nursery plants to the landscape were occurring as recently as 2006 in multiple locations, and b)- large wild infestations are the most likely sources of further infestations in California
The implications of these findings for disease management and policy-making will be discussed.

Reducing the spread of *Phytophthora ramorum* on the Redwood Nature Trail, Curry County Oregon: A case study

E.M. Goheen[1], P. Reeser[2], W. Sutton[2]

[1]USDA Forest Service, Pacific Northwest Region FHP, Southwest Oregon Forest Insect and Disease Service Center, 2606 Old Stage Road, Central Point, OR 97502 USA.
[2]Oregon State University, Corvallis, OR USA.

Corresponding author: egoheen@fs.fed.us

Abstract

In August 2009, a tanoak adjacent to a popular hiking trail was confirmed infected by *Phytophthora ramorum*. The trail was immediately closed to the public. An eradication treatment consisting of injected herbicide and cutting, piling, and burning tanoaks and other selected hosts in a 100m radius around the infected tanoak was completed by December 2009.
Close to 490 m of trail lies within or on the boundary of the treatment area while approximately 60m of trail passes through the infested zone. To limit the number of *P. ramorum* spores in soil and the potential for spore splash dispersal, a 4-inch thick layer of *Thuja plicata* heartwood chips was placed on the trail in July 2010. The trail was then reopened to public use.
Soil samples were collected at 11 locations on the trail four times prior to chip treatment and three times after chip treatment. *Phytophthora ramorum* was recovered from at least one of the 11 samples on all sampling occasions except in July 2010 and June 2011. The number of *P. ramorum*-positive soil samples was 2/11, 5/11, and 6/11 before-chip treatment and 1/11 and 1/11 samples after-chip treatment. All *P. ramorum*-positive samples were found within approximately 8m of the infected tree.
Phytophthora ramorum's presence in trail soil appears to have been reduced in the year after chip treatment. Recently, additional *P. ramorum* infections have been detected near the trail and due to use, chip depth has also been greatly reduced. Additional treatments will be done and monitoring will continue.

Host and habitat index for Phytophthora species in Oregon forests (32 species!)

E. Hansen, P. Reeser, W. Sutton, L. Sims

Department of Botany and Plant Pathology, Oregon State University, Corvallis OR 97331.

Corresponding author: hansene@science.oregonstate.edu

Abstract

Phytophthora species are abundant in streams in healthy forests and widespread in forest soils causing cryptic diseases, in addition to their more traditional roles as aggressive pathogens. We compiled existing Oregon records from available sources of reliably identified *Phytophthora* species from forests and forest trees and summarized the results by host and habitat (Forest Phytophthoras, http://www.forestphytophthoras.org/). Details of documented isolates including locations, available cultures, Genbank acquisition numbers, and citations are in the accompanying interactive database.

Thirty-two *Phytophthora* species have been identified associated with 25 host species from Oregon forests or forest trees. This total includes 19 species recovered from forest streams and 19 from forest soils, generally in the absence of noticeable disease on associated vegetation. A total of 29 *Phytophthora* species were identified from the various environments in forests. Fourteen species came from trees or forest shrubs growing in cultivated and urban environments. Only three species were unique to the latter, however, including *P. ilicis*, from cultivated holly (*Ilex*), and *P. sansomeana* and *P*. taxon ceanothus from forest nurseries. Three species, *P. gonapodyides*, *P*. taxon oaksoil, and *P*. *lacustris* were recovered from streams in all surveyed counties. The most widespread species causing root disease or bole cankers of trees was *P. lateralis* on Port-Orford-cedar in landscape plantings throughout the state as well as on forest trees in its limited native range. *Phytophthora cambivora* and *P. cinnamomi* were widespread but uncommon on a number of forest trees.

Characterization of *Phytophthora alni* isolates from *Alnus glutinosa* in Castilla y León, Spain

M.M. Haque, E. Hidalgo, A.I. de Lucas, J.J. Diez

Sustainable Forest Management Research Institute, University of Valladolid-INIA. Avenida de Madrid, 44, 34071 Palencia, Spain.

Corresponding author: mhaque@pvs.uva.es

Abstract

Extensive dieback and mortality of alder (*Alnus* spp.) reported from several European countries has also been observed in Spain in 2009. Surveys were conducted to collect information on disease symptoms and possible causes of decline of *Alnus glutinosa* growing on river banks of *Castilla y León*, Spain, in 2010 and 2011. Isolates were obtained from necrotic bark at collar and lower stems of the diseased alders. Morphological and molecular characteristics of the selected isolates together with their physiology were studied. The isolates were homothallic and produced oogonia on V8 juice agar (V8A) with single or two-celled amphigynous and paragynous antheridia having smooth to ornamented walls and bullate protuberances with a large quantity of aborted oospores. Long sporangiophores were found bearing terminal non-papillate, ellipsoid to ovoid sporangia. Colony growth patterns developed on carrot agar (CA) and V8 juice agar showed irregular to uniform radial growth having appressed to slightly woolly aerial overgrowth. Colony growth rates to different temperature, pH and osmotic potential varied as cultured on several growth media. ITS DNA region was sequenced, and compared with GenBank showing identity with *P. alni*, and confirming the morphological and physiological identification of the pathogen isolated. Complementary molecular studies are undergoing in order to identify the pathogen at subspecies level.

Characterization of *Phytophthora alni* isolates from *Alnus glutinosa* in Castilla y León, Spain

Haque, M. M., Hidalgo, E., de Lucas, A. I. and Diez, J. J.
Sustainable Forest Management Research Institute, University of Valladolid-INIA, Avenida de Madrid 44, 34071 Palencia, Spain
E-mail: mhaque@pvs.uva.es

INTRODUCTION

Phytophthora alni a new hybrid pathogen (Brasier et al., 2004), has been described as highly destructive and host specific to alder (*Alnus* spp.) spreading all over Europe (Gibbs, 1995; Gibbs et al., 1999, 2003; Jung & Blaschke, 2004. The pathogen has also been reported to cause diseases in *Alnus glutinosa* in Spain (Solla et al., 2010; Varela et al., 2010). The present study was done to characterize the isolates of *P. alni* obtained from necrotic bark tissue at the collar of *A. glutinosa*.

MATERIALS & METHODS

In July-September 2010 and 2011, surveys were made at 19 different locations in Salamanca, Spain to study Phytophthora root and collar rot of *A. glutinosa* growing on river banks. Morphological measurements of sporangia, oogonia and antheridia of the isolates obtained were studied in soil extract and on V8A at x 400. Isolates were grown on V8A (V8 juice agar), MEA (Malt-Extract Agar) and CA (Carrot juice agar) to examine colony morphology. For temperature-growth relationships, isolates of *P. alni* were grown on HSPDA (Half strength Potato-Dextrose-Agar), MEA, V8A at 2, 6, 15, 20, 25, 30 and 32.5°C. To examine effect of pH (5, 7, 9 and 11) on the radial growth rate of the isolates, HCl or KOH 1N was added to PDA to obtain required pH. To estimate the effect of different osmotic potential on mycelial growth of *P. alni* isolates, different concentrations of KCl (250, 500, 750 and 1000mM) were added to PDA. DNA was extracted from isolates following (Vainio et al., 1998). ITS region was amplified and sequenced using ITS1 and ITS4 primers.

Fig. 2. a. Common alder with characteristics dieback symptoms. Fig. b-d. Tarry and rusty exudates at the collar of affected common alder

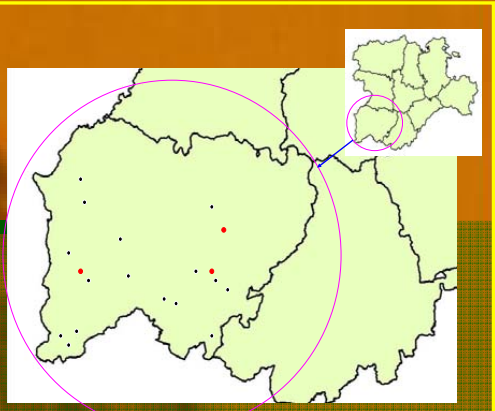


Fig. 1. Surveyed sites in Salamanca, Spain. Red circles indicate where *Phytophthora alni* was isolated; black circles denote where *P. alni* was not isolated

RESULTS & DISCUSSION

ISOLATE	Sporangia			Oogonia		Oospore		Antheridia	
	Length range	Width range	Average	Range	Average	Range	Average	Length range	Breadth range
PFSPA2008	42-62	32-45	55.6x43.3	26-46	42.4	20-39	34.7	20-28	10-18
PFSPA2010	45-60	33-44	52.3x41.4	27-48	43.7	21-40	35.2	22-27	12-20
PFSPA4017	37-55	29-39	51.9x39.2	32-51	41	25-42	32.4	18-25	8-15
PFSPA4018	38-58	27-37	50.8x40.7	28-52	50.5	22-44	40	21-26	11-17
PFSPA4020	40-59	25-38	51.1x41.8	30-49	48.4	24-41	38.5	19-24	10-14
PFSPA5024	45-65	33-47	57.3x45.2	33-53	50.1	27-45	41.3	19-26	8-15
PFSPA5029	44-58	31-48	56.5x45.4	34-49	47.7	28-40	39.7	13-22	6-13
PFSPA6034	48-59	34-42	60.3x48.9	28-44	42	21-35	36.4	14-21	7-12
PFSPA6035	52-66	33-44	61.1x49.7	30-46	48.9	25-40	42.8	16-22	8-15
PFSPA7040	34-54	28-40	55.5x43.8	25-52	43.5	21-44	38.7	22-26	10-16
PFSPA7051	36-55	29-42	57.7x45.6	26-48	44.7	22-42	33.6	18-24	9-14
PFSPA7054	34-59	28-35	56.8x44.8	27-55	47.8	23-46	43.8	21-29	12-19
MMSPA8055	42-62	26-40	58.3x45.5	35-55	43.1	29-48	31.9	19-26	11-20
MMSPA8059	44-58	29-39	59.6x48.3	32-51	38.6	25-42	32.1	14-22	8-13

Table 1. Morphological characteristics of *P. alni* isolates (Dimensions are in µm)

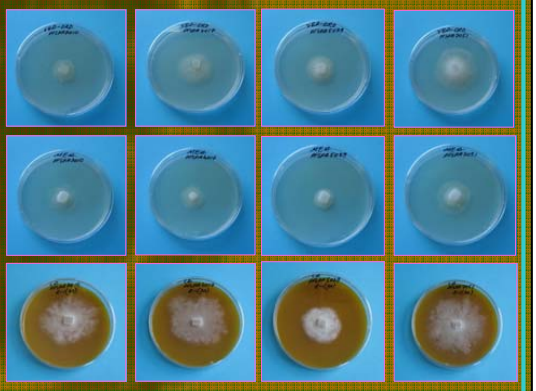


Fig. 3. Colony morphology of *P. alni* isolates at 20°C on V8A, MEA and CA (from top to bottom)

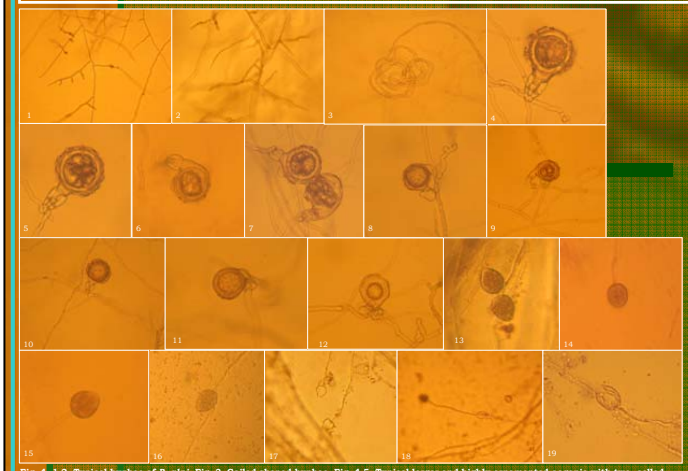


Fig. 4. 1-2. Typical hyphae of *P. alni*. Fig. 3. Coiled-shaped hyphae. Fig. 4-5. Typical larger and highly ornamented oogonia with two- celled antheridia. Fig. 6-7. Comma-shaped oogonia. Fig. 8-9. Paragynous antheridia. Fig. 10. Oogonia with single-celled antheridia. Fig. 11-12. Partially developed or smaller oogonia. Fig. 13-15. Sporangia varying in size and shape. Fig. 16. Sympodial sporangiophore with a sporangium. Fig. 17. Empty sporangia with wide exit pores. Fig. 18. A sporangium which has been proliferated to produce a second sporangium. Fig. 19. Remains from nesting and internal proliferation

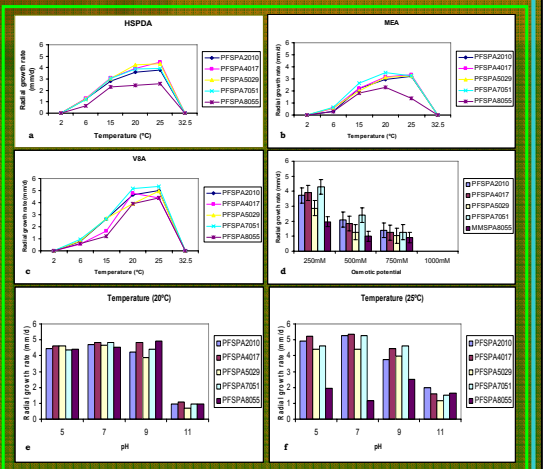


Fig. 5. a-c. Temperature and radial growth rate relationships of five isolates of *P. alni* grown on HSPDA, MEA and V8A Fig. d. Effect of osmotic potential on growth of the isolates of *P. alni* growth at 20°C on PDA. Fig. e-f. Radial growth rate and pH relationships of five isolates of *P. alni* growth at 20°C and 25°C on PDA (from left to right)

Colonies grown on V8A and CA, usually uniform and appressed-felty with sparse aerial mycelium. On CA, colonies often irregular in outline, faster or slower growing areas often failing to grow up to the edge of the Petri dishes. Oogonia formed more at the edge regions than center of the colony. Matured and aborted oospores having both one or two-celled antheridia observed on V8A. Optimum temperature for growth on V8A, MEA and CA was recorded at 25°C. No growth of the isolates observed at 2°C and 32.5°C. Radial growth rate of the isolates varied at 250mM, 500mM and 750mM. No growth observed at 1000mM. Isolates showed higher radial growth rate at pH 5, 7 and 9 but lower growth rate recorded at pH 11. Both sequences (forward and reverse) were aligned using GENIOUS and a consensus sequence was established for each isolate. Consensus sequences from all isolates were aligned showing a few positions ambiguous or diverseed a general very good alignment. Comparing with GenBank showed identity with *P. alni*, and confirmed the morphological identification of the pathogen isolated. Further study will be done in order to identify the pathogen at subspecies level.

REFERENCES

Brasier, C.M., Kirk, S.A., Delcan, J., Cooke, D., Jung, T., Man in'T Weld, W. (2004). *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycological research 108: 1172-1184.
Gibbs, J.N. (1995). *Phytophthora* root disease of alder in Britain. Bulletin OEPP/EPPO Bulletin 25, 661-4.
Gibbs, J.N., Lipscombe, M.A., Peace, A.J. (1999). The impact of *Phytophthora* disease on riparian populations of common alder (*Alnus glutinosa*) in Southern Britain. European Journal of Forest Pathology 29: 39-50.
Gibbs, J.N., Van Dijk, C., Webber, J.F. (2003). *Phytophthora* Disease of Alder in Europe. Edinburgh, UK: Forestry Commission Bulletin No. 126.
Jung, T., Blaschke, M. (2004). *Phytophthora* root and collar rot of alders in Bavaria: distribution, modes of spread and possible management strategies. Plant Pathology 53: 197-208.
Solla, A., Pérez-Sierra, A., Corcobado, T., Haque, M.M., Diez, J.J., Jung, T. (2010). *Phytophthora alni* on *Alnus glutinosa* reported for the first time in Spain. Plant Pathology 59: 798.
Vainio, E. J., Korhonen, K., Hantula, J. (1998) Genetic variation in *Phlebiopsis gigantea* as detected with random amplified microsatellite (RAMS) markers. Mycological Research 102: 187-192.
Varela, C.P., Martínez, C.R., Vázquez, J.P.M., Casal, O.A. (2010). First Report of *Phytophthora* rot on Alders caused by *Phytophthora alni* subsp. *alni* in Spain. Plant Pathology 94 (2): 273.

ACKNOWLEDMENTS

We thank Ana Belén and Juan Carlos working at Centro de Sanidad Forestal de Calabazanos, Junta de Castilla y León, Spain for their assistances and technical support during conducting the surveys.

Persistence of *Phytophthora ramorum* on infested larch sites

A.R. Harris[1,2], S. Sancisi-Frey[1], J. Webber[1]

[1]*Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK.*
[2]*Department of Biological Sciences, Imperial College, London SW7 2AZ, UK.*

Corresponding author: anna.harris@forestry.gsi.gov.uk

Abstract

Phytophthora ramorum emerged in the 1990s almost simultaneously in both Europe and North America. It was first reported in the UK in 2002, but tree infections were comparatively rare until 2009. Then, a completely unanticipated change occurred with *P. ramorum* transferring to commercially grown larch causing a landscape-scale epidemic. Larch is both a foliar host and a canker host to *P. ramorum*. A single infected needle can generate hundreds, even thousands of sporangia, and chlamydospores are also produced. As the needles fall, the litter layer under infected trees becomes a reservoir of *P. ramorum* inoculum available to infect new plantings.
This study analysed the persistence of *P. ramorum* on a site in south west England, where stands of Japanese larch (*Larix kaempferi*) and European larch (*L. decidua*) showed disease symptoms. All the trees were felled, then each stand was surveyed systematically and both needle-litter and soil samples removed and baited. A total of 180 samples were taken from the Japanese larch stand and 132 from the European larch stand. One year later, the survey was repeated on the cleared Japanese larch stand. Two clear results emerged: (1) Much higher levels of *P. ramorum* were detected under the felled Japanese larch compared with the European larch (67% and 2% respectively); (2) A year after felling, levels of *P. ramorum* had reduced moderately in the felled Japanese larch stand. The findings support field observations that disease development on European larch is less than on Japanese. Also needles of Japanese larch usually support the highest levels of sporulation, consistent with the finding that *P. ramorum* was much more prevalent in fallen needles under this host species.

Comparative sporulation of *Phytophthora ramorum* on larch, rhododendron and bay laurel

A.R. Harris[1,2], J. Webber[1]

[1]*Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK.*
[2]*Department of Biological Sciences, Imperial College, London SW7 2AZ, UK.*

Corresponding author: anna.harris@forestry.gsi.gov.uk

Abstract

In Britain, *Phytophthora ramorum* has been recorded infecting plants since 2003, but tree infections were comparatively rare until 2009 when the pathogen was found killing commercially grown Japanese larch (*Larix kaempferi*) and causing a landscape-scale epidemic in south west England. This larch species is both a foliar host and a canker host to *P. ramorum* and initial studies showed that a single infected needle of Japanese larch could generate hundreds, even thousands of sporangia, suggesting this species has considerable potential to drive epidemics. However, the sporulation capability of other larch species grown in Britain, such as European (*L. decidua*) and hybdrid larch (*L. x eurolepis*), is unknown.

To evaluate the spore producing potential of foliage of the three species of British-grown larch and compare with other known sporulating hosts (eg *Umbellularia califonica* and *Rhododendron ponticum*), laboratory tests were carried out using shoots of Japanese larch, hybrid larch and European larch challenged with zoospores suspensions of *P. ramorum* (EU1 lineage). These tests were carried out at different times of year and have shown that sporulation potential varies with larch species, pathogen genotype and also with the age of the foliage. Japanese larch generally supported the highest levels of sporulation, even exceeding that on *U. californica.* Sporulation on larch needles can also occur in the absence of any symptoms particularly early in the season and in the field; symptoms on infected needles only become visible towards the end of the season just before they are shed.

Phytophthora pini found on thuja in Norway

M.L. Herrero, V. Talgø, M.B. Brurberg, K. Ørstad, E. Fløistad, A. Stensvand

Bioforsk - Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway.

Corresponding author: maria.herrero@bioforsk.no

Abstract

In October 2011, we received a thuja (*Thuja occidentalis* 'Smaragd') with partly dead foliage at our plant clinic. It was approximately a meter high and was one of 45 plants that had been established in a hedgerow in Oslo in April 2011. By July 2011, five plants had to be replaced due to severe disease symptoms. The plants had been imported via a garden center. There was no canker symptom in the stem base of the plant we received, but the roots had typical dieback symptoms; fine roots were absent and the roots were clearly discolored below the bark. A *Phytophthora* sp. was isolated from the roots. It was identified as *P. pini* by ITS sequencing. This species was first described in 1925, isolated from roots of *Pinus resinosa* in Minnesota, USA. Later the species was merged with *P. citricola,* but has recently been segregated again from the *P. citricola* complex. It has mostly been reported from USA, but also from nurseries in Europe. Among other hosts it has been reported from *Fagus sylvatica*, *Rhododendron* sp., and *Thuja* sp. It has also been isolated from streams and irrigation water. Results of a pathogenicity test will be presented.

Literature Cited

Hong C., Gallegly M.E., Richardson, P.A., Kong P., 2011. *Phytophthora pini* Leonian resurrected to distinct species status. *Mycologia* **103:** 351-360.
Jung T., Burgess T.I., 2009. Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* **22:** 95–110.

Disease Management of *Phytophthora ramorum* in a Research Quarantine Nursery at NORS-DUC

W. Schweigkofler[1], K. Kosta[2], V. Huffman[1], T. Pastalka[1], G. Copeland[1], K. Suslow[1], A. Posadas[3], R. Bulluck[4], S. Ghosh[1]

[1]*Department of Natural Sciences and Mathematics, Dominican University of California, 50 Acacia Avenue, San Rafael, California 94901, USA.*
[2]*California Department of Food and Agriculture, 1220 N Street, Sacramento, California 95814, USA.*
[3]*National Plant Board, P.O. Box 847, Elk Grove, California 95759, USA.*
[4]*Center for Plant Health Science and Technology, USDA-APHIS-PPQ, 1730 Varsity Drive, Raleigh, NC 27606, USA.*

Corresponding author: wolfgang.schweigkofler@dominican.edu

Introduction

Sudden Oak Death, caused by *Phytophthora ramorum*, is among the most important emerging forest diseases with potentially devastating effects on temperate forests. The nursery trade is a factor in the long-range spread of *P. ramorum.* To address threats posed by plant trade and aid in developing an environmentally-sustainable nursery industry, the National Ornamentals Research Site at Dominican University of California (NORS-DUC) was developed (www.dominican.edu/norsduc). NORS-DUC is a sophisticated field nursery designed to contain quarantined pathogens for the purpose of conducting research in a safe environment that reflects an authentic nursery setting.
Nursery soil tested positive for *P. ramorum* has to be treated to eliminate the pathogen according to the federal *P. ramorum* quarantine program implemented by USDA APHIS (Anonymous 2007). Steam sterilization is a well established method for the eradication of plant pathogens from soils (Baker & Roistacher, 1957), but only limited information is available on its use to control *P. ramorum* (Linderman & Davis, 2008). Research at NORS-DUC focuses on the development of fast and efficient methods to treat infested soils and to develop sanitization guidelines for the nursery industry. Additionally, each plot used for *P. ramorum* research at NORS-DUC is required to be sanitized after completion of the study. The target soil temperature in this study is 50°C for at least 30 minutes. Here we report preliminary results on the effect of aerated steam on the survival of *P. ramorum*.

Material and Methods

For steaming research beds with a surface of 3.7 x 4.6 m (approximately 17 m^2) lined with water-proof pond liners were used. The beds were filled with a 28 cm deep layer of gravelly loam soil with a pH of 5.8 and 5.9 percent organic matter; which was digged up previously from the Dominican campus. A steamer unit SIOUX Steam-Flo SF-11 (Sioux Corp., Beresford, SD, USA) with a boiler horsepower of 3.13 W and a steaming output of approximately 170 l/hour was used with an attached soaker hose (length: 31 m) laid out on the soil surface, which then was covered with a tarp (Figure 1).
The temperature was measured using HOBO-data loggers U12 (Onset Corp., Cape Cod, MA, USA) with sensors positioned at several locations at 5, 15 and 28 cm, respectively, below surface. Temperature data were collected with a fifteen minute interval. Rhododendron leafs infected with *P. ramorum* isolate 1418886 were used as inoculum; 20 leaf plugs overgrown with mycelium and chlamydospores were added to the deepest soil layer in a sachet before steaming. After steaming, the leaf plugs were plated on PARPH-V8 plates, incubated in the dark at 20ºC for two weeks and checked for the presence of CFUs. As a control, leaf plugs infected with *P. ramorum*, but not-heat treated, were incubated.

Figure 1: Steam sterilization of a research plot at NORS-DUC using a SIOUX Steam Flo SF-11 unit.

Results and Discussion

Soil contained in a research bed used for studies on *P. ramorum* in a mock nursery was treated with aerated steam using a commercial steaming unit (Figure 1). The rising soil temperature was measured from sensors strategically positioned at different distances from the edges of the research bed and the hose as well as different soil depths. A typical temperature profile is shown in Figure 2. As to be expected from a top-down heating approach, temperature profiles show different dynamics depending on soil depths. Starting from an environmental temperature of approximately 20°C, the target temperature of 50°C at 5 cm soil depth is reached after approximately 1 hour, at 15 cm after 2 hours and at 28 cm after three to four hours. Very compact soils and high soil moisture can delay heat transfer even longer (data not shown). After turning off the heat source, temperatures closer to the surface drop faster than those at deeper soil layers, which sometimes still rise for several °C due to continued heat transfer from the upper layers, before declining steadily. Maximum temperatures are difficult to control with this approach; close to the soil surface temperatures above 90°C can be achieved. Soil temperatures remain well above environmental temperatures for up to 24 hours post-steaming or more. *P. ramorum* on infected leaf discs did not survive the heat treatment; no CFUs were detected after incubation of the leaf discs which were inserted in the soil. In contrast, *P. ramorum* cultures grew from each control leaf disc. Our results corroborate results from similar studies by Swain *et al.* (2006) on the effects of composting and Linderman & Davis (2008) on steaming of potting media to eradicate *P. ramorum*.

Additional studies to eliminate *P. ramorum* from infested soils are under way at NORS-DUC using a newly developed formulation of the bio-control agent *Trichoderma asperellum* and solarization. The final goal of the studies is to develop guidelines for the nursery industry offering several sanitization options optimized for different situations and needs.

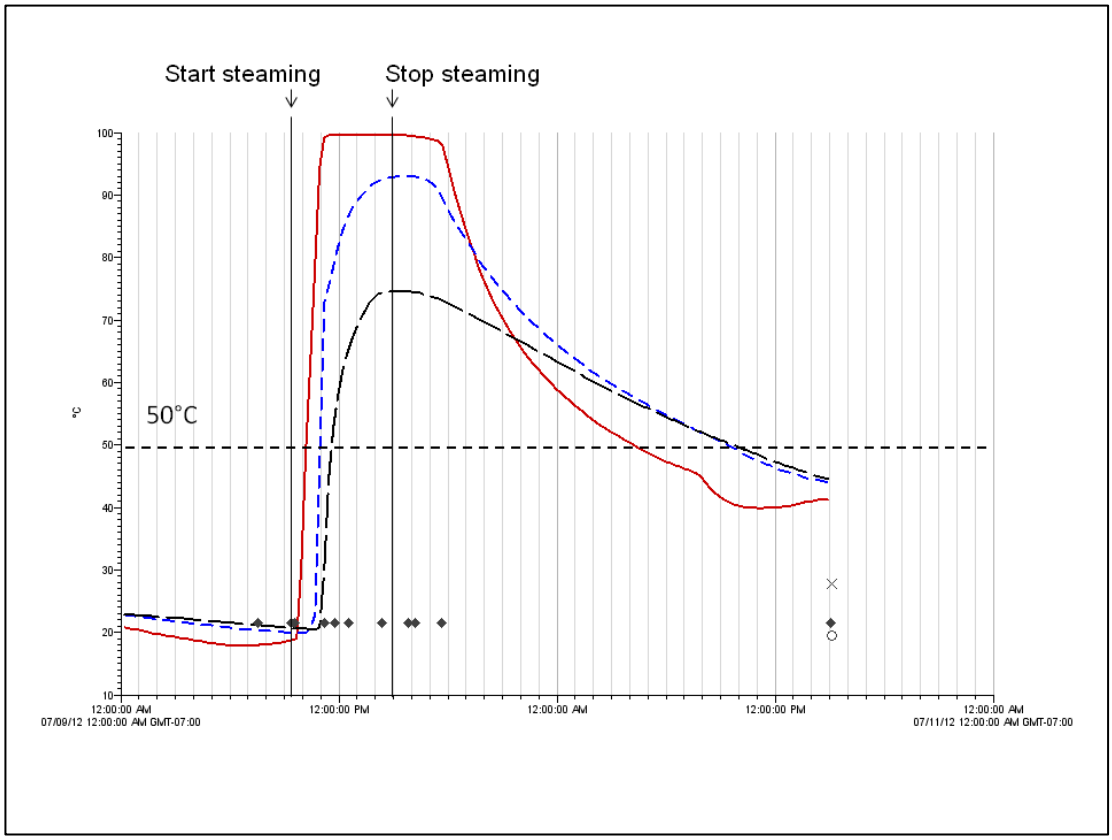


Figure 2: Temperature profile at different soil depths in response to top-down steaming (red line: 5, blue line: 15, black line: 28 cm below surface). The black vertical lines show the time for turning on and off, respectively, of the steaming unit. The dashed horizontal line shows the target temperature of 50°C.

Acknowledgements

The National Ornamentals Research Site at the Dominican University of California is funded by a grant from the 2008 Farm Bill, Section 10201, and administrated trough the United States Department of Agriculture (USDA) Animal and plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) Centre for Plant Health Science and Technology (CPHST).

Literature Cited

Anonymous, 2007. *Phytophthora ramorum*; Quarantine and Regulations. http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/01-064-3.pdf

Baker K.F., Roistacher C.N., 1957. Heat treatment of soil. In: **Baker KF,** (Ed.). The U.C. system for producing healthy container-grown plants. California Agricultural Experimental Station, Extension Service, Manual 23: 123-137.

Linderman R.G., Davis E.A., 2008. Eradication of *Phytophthora ramorum* and other pathogens from potting medium or soil by treatment with aerated steam or fumigation with metam sodium. *HortTechnology* **18:** 106-110.

Swain S., Harnik T., Mejia-Chang M., Hayden K., Creque J., Garbelotto M., 2006. Composting is an effective treatment option for sanitization of *Phytophthora ramorum*-infected plant material. *Journal of Applied Microbiology* **101:** 815-827.

Heat-treated Japanese larch (*Larix keampferi*) wood chips can counter persistence of *Phytophthora ramorum*

M. Kalantarzadeh[1,2], D. Mulholland[1], M. Langat[1], T. Hutchings[2], F. de Leij[3], J. Webber[2]

[1]*Department of Chemistry, University of Surrey, Guildford, Surrey, GU2 7XH, UK.*
[2]*Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK.*
[3]*CCure Solutions, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK.*

Corresponding author: mina.kalantarzadeh@surrey.ac.uk

Abstract

Phytophthora ramorum is the causal agent of 'sudden oak death' in North America. In the British Isles it infects primarily rhododendron but since 2009 has spread to Japanese larch infecting and killing thousands of trees [1]. Novel methods are being sought to combat the disease including the potential of wood chip mulches to reduce the survival and spread of the pathogen. Previous studies have shown that heating wood shavings obtained from a variety of tree species causes the shavings to become highly anti-microbial, with activity against both bacteria and fungi [2].The aim of this study was to investigate the antimicrobial activity of heat treated wood materials against *P. ramorum* focussing on the most frequently infected hosts, rhododendron and larch.
Samples of Japanese larch and rhododendron wood were chipped and heat-treated at 140^{o} C for three days. GC-MS analysis of methanol crude extract of pre- and post-heated chips identified anti-microbial chemicals, particularly in the larch, that could have activity against *P. ramorum*. To test this, colonised leaf disks of rhododendron were treated with the methanol crude extract derived from the heated woodchips and it prevented any growth of *P. ramorum*, including chlamydospore germination. Subsequently, treated and untreated wood chips were tested in a micro-cosm system, by challenging with zoospores of *P. ramorum*. It was found that the effectiveness of the anti-microbial action of the wood chips altered with wood type and pathogen genotype. However, the approach shows promise for reducing spread/persistence of *P. ramorum* on affected sites with the treated chips used as a pathogen-suppressive mulch in gardens and areas with limited infection.

Literature Cited

Webber J.F., Mullett M., Brasier C.M., 2010. Dieback and mortality of plantation Japanese larch (*Larixkaempferi*) associated with infection by *Phytophthora ramorum. New Disease Reports* **22:** 19, [http://dx.doi.org/10.5197/j.2044-0588.2010.022.019].
De Leij F., Kalantarzadeh M. "Antimicrobial material." US patent 0034289 A1. 09 Feb 2009.

Development of molecular markers and probes for detection of *P. ramorum*, *P. nicotianae*, *P. citricola*, *P. fragariae* and *P. cactorum*

M. Kopina, T. Surina

All-Russian Plant Quarantine Center, Moscow region, Russia.

Corresponding author: marija508a@mail.ru; T.A.Surina@yandex.ru

Abstract

Phytophthora diseases are some of the most dangerous diseases of trees and shrubs including raspberry and strawberry. Root rot of raspberry and strawberry, caused by *P. fragariae*, and sudden oak death, caused by *P. ramorum* should be given particular attention. These pathogens are regulated as quarantine pests in many countries, including Russia.

Laboratory diagnosis of these oomycetes is complicated due to similarity of symptoms caused by the pathogens as well as due to variability of their morphological characteristics. The importance and practical relevance of this study lies in the necessity for developing and further improving molecular methods for diagnosing *Phytophthora* diseases.

In developing real-time PCR, we selected primers and probes using Ypt gene. Nucleotide sequences of *Phytophthora* spp. Ypt gene were picked out from the GenBank and sequencing of pure cultures from the All-Russian Plant Quarantine Center Reference Collection.

For diagnosing sudden oak death, we selected *P. ramorum* - specific PramF and PramR primers, and PramP probe marked with MGB fluorescent dye.

In developing multiplex real-time PCR for differentiating among major causal agents of raspberry and strawberry root rot, we selected universal primers for *Phytophthora* spp., and probes marked with TAMRA fluorescent dye specific for *P. cactorum, P. fragariae, P.nicotianae, P. citricola.*

Primers and probes marked with various fluorescent dyes allowed real-time PCR for detection and identification of the four oomycetes species in a single tube. Selected primers (PramF, PramR) and the probe PramP did not cross react with other closely-related *Phypophthora* spp. causing tree diseases.

Literature Cited

Schena L., Duncan J.M., Cooke D.E.L., 2007. Development and application of a PCR-based `molecular tool box' for the identification of *Phytophthora* species damaging forests and natural ecosystems. *Plant Pathology* **57:** 64-75.

Early *Phytophthora* spp. detection by qPCR

N. Luchi[1], D. Migliorini[1,2], P. Capretti[2], A. Santini[1]

[1]*Institute for Plant Protection – C.N.R. Via Madonna del Piano, 10 50019 Sesto f.no, Italy.*
[2]*DiBA - Plant Protection, University of Florence.*

Corresponding author: n.luchi@ipp.cnr.it

Abstract

One of the main European nursery poles for ornamental plants is located in Pistoia, Tuscany, Italy, where nurseries are spread over more than 5300 hectares. The production is exclusively devoted to the production of plants in pots. In average, 80% of plant obtained by Pistoia's nurseries is exported to foreign countries. For this reason the production of healthy material is fundamental, in order to avoid the spread of nasty pathogens all over the world.
Among the diseases affecting plants in nursery, the root rots are one of the most important, particularly those caused by *Phytophthora* species. Since this class of pathogens is hard to isolate, and remains alive in the soil for long time periods before causing symptoms, the availability of an early detection tool is of primary concern to prevent the risk of spread of pathogens.
Aim of this work was to develop a real time PCR assay to detect and quantify *Phytophthora* spp. from plant and soil samples collected in nurseries in Tuscany. The survey was carried out by collecting plants in pots and soil samples. Isolation on selective media according to Moralejo et al. (2009) and DNA extraction were performed on leaves, roots and soil of both symptomatic and asymptomatic samples.
The sensitivity and specificity of real time PCR approach make possible to detect the presence of small amount of *Phytophthora* DNA in soil and plant samples even before symptoms occur.
The use of this molecular tool will prevent the spread of hitch-hiker pathogens by asymptomatic plants in anthropic and natural ecosystems.

Literature Cited

Moralejo E., Pérez-Sierra A., Álvarez L.A. Belbahri L., Lefort F., Descals E., 2009. Multiple alien *Phytophthora* taxa discovered on diseased ornamental plants in Spain. *Plant Pathology* **58:** 100-110.

Diversity of *Phytophthora* species in forest ecosystems of Bulgaria

A. Lyubenova[1], K. Kostov[1], I. Tsvetkov[2], T. Oszako[3], M. Borys[3], K. Sikora[3], T. Jung[4,5], S. Slavov[1]

[1]*AgroBioInstitute, 8 Dragan Tzankov blvd., 1164 Sofia, Bulgaria.*
[2]*Forestry Research Institute, 132 St. Kliment Ohridski blvd.,1756 Sofia, Bulgaria.*
[3]*Forest Research Institute – IBL, ul. Braci Lesnej 3, Sekocin Stary, 0509 – Raszyn, Poland.*
[4]*Phytophthora Research and Consultancy, Thomastrasse 75, Brannenburg, Germany.*
[5]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*

Corresponding author: kkostov@abi.bg

Abstract

The fungus-like organisms from the genus *Phytophthora* currently include more than 100 species, many of which are destructive plant pathogens. The most aggressive *Phytophthora* species are now acknowledged as causal agents in several devastating declines of forest trees in Europe, Australia and the USA. Therefore, *Phytophthora* surveys are carried out by scientists and forest services in most of the European countries. Despite the fact that Bulgaria is rich of natural ecosystems comprehensive information about the occurrence and diversity of *Phytophthora* species in forests and forest nurseries is still missing. The purpose of this study is to fill in this gap by detecting and identifying *Phytophthora* pathogens potentially dangerous for the Bulgarian forests ecosystems. Samples were primarily collected from regions that are close to the country borders and areas along major transportation roads, as *Phytophthora* pathogens are known to be distributed via trading, transportation and other human activities. The focus of our study was on known hosts of *Phytophthora* such as oak, sweet chestnut, alder, spruce etc. growing in forest ecosystems and nurseries. More than 100 samples of rhizosphere soil and plant tissue from trees with typical disease symptoms have been collected and examined for the presence of *Phytophthora*. Several *Phytophthora* isolates were obtained and their species identity was determined on the basis of their morphological, cultural and molecular characteristics. Up to now *P. cryptogea*, *P. cambivora*, *P. plurivora* and *P. rosacearum* were detected.

Determination of the minimum threshold of *Phytophthora cinnamomi* inoculum for infection of *Quercus* spp.

L. Martín, M.S. Serrano, M.E. Sánchez

Agronomy Department, University of Córdoba.Ctra. Madrid-Cádiz Km. 396. 14014 Córdoba, Spain.

Corresponding author: ag1sahem@uvo.es

Abstract

The root rot due to *Phytophthora cinnamomi* is the main cause of the massive death of Holm and cork oaks affecting rangeland ecosystems in the south of the Iberian Peninsula. The knowledge of the minimum level of inoculum of the pathogen in the soil for infection of tree roots is a crucial point for risk assessment. To determine this minimum level of inoculum for infection of *Quercus ilex* spp. ballota and *Q. suber* roots, 18 months-old seedings were planted in a substrate previously infested with aqueous suspensions of chlamydospores of two different isolates of *P.cinnamomi* (PE90 and PA25 isolated from roots of Holm and cork oaks, respectively) in increasing concentrations (0, 0.6, 6, 60, 600 and 6000 chlamydospores per gram of dry soil). Ten plants (repetitions) were planted per inoculum concentration and species of *Quercus*. All the plants were incubated in air-conditioned greenhouse at 25-10º C day/night, maintaining soil flooding for 2 days per week. Weekly, severity of foliar symptoms is evaluated on a 0-4 scale (0 = 0-10% of symptomatic tissue, 4 = dead tissue) and at the end of the experiment, the severity of radical symptoms will be assessed following the same scale. The results obtained for both species of *Quercus* will be discussed at the meeting.

Could climate change influence the survival of *Quercus ilex* seedlings germinating in *Phytophthora* infested soils?

J. Martín-García[1], T. Corcobado[1], A. Solla[1], S. Woodward[2]

[1]*Ingeniería Forestal y del Medio Natural. Universidad de Extremadura. Av. Virgen del Puerto 2, 10600 Plasencia, Spain.*
[2]*Institute of Biological and Environmental Sciences, Department of Plant and Soil Sciences. University of Aberdeen. Cruickshank Building, St. Machar Drive, AB24 3UU, Aberdeen, Scotland, UK.*

Corresponding author: jorgemg@unex.es

Abstract

Holm oak "dehesas" are amongst the most representative forest ecosytems in the Iberian Peninsula. Over the last three decades a serious forest decline associated with several species of *Phytophthora* has been observed. Although previous reports describe the pathogenicity of different species of *Phytophthora* to *Quercus* spp. in this ecosystem, empirical evidence concerning the possible responses of *Phytophthora* species to climate change are lacking. An increase in temperature between 2 - 4.5º C (with a most likely value of 3º C) is expected in the 21st century, which will lead to changing interactions between vegetation and pathogens and to a variety of forest health problems. The aim of this work was to determine whether an increase in temperatures would influence the germination and survival of *Quercus ilex* seedlings growing in *Phytophthora* infested soils. Acorns of *Quercus ilex* (provenance: Malpartida de Plasencia, Spain) were stored at 4º C before sowing. Inocula of *P. cinnamomi*, *P. gonapodyides, P. quercina* and *P. psychrophila* were raised on vermiculite - oat - V8 juice medium at 20º C for 6 weeks. Four hundred acorns were sown in infested soils with each of the four *Phytophthora* species separately, plus a control treatment of non-infested soil. Two temperature treatments were used: 17 or 20º C with the same photoperiod (n = 40 replicates/treatment). Germination, mortality rates, plant growth (above-ground part and root system) and photosynthetic capacity data will be presented.

Literature Cited

Sánchez M.E., Andicoberry S., Trapero A., 2005. Pathogenicity of three *Phytophthora* spp. causing late seedling rot of *Quercus ilex* ssp. *ballota*. *Forest Pathology* **35:** 115–125.
IPCC, 2007. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

COULD CLIMATE CHANGE INFLUENCE THE SURVIVAL OF *QUERCUS ILEX* SEEDLINGS GERMINATING IN *PHYTOPHTHORA* INFESTED SOILS?

MARTÍN-GARCÍA, Jorge[1,2,3]; SIASOU, Eleni[1]; CORCOBADO, Tamara[3]; SOLLA, Alejandro[3]; WOODWARD, Stephen[1].

[1] Institute of Biological and Environmental Sciences, Department of Plant and Soil Sciences. University of Aberdeen. St. Machar Drive, AB24 3UU, Aberdeen, Scotland, UK.
[2] Sustainable Forest Management Research Institute, University of Valladolid – INIA (Palencia, Spain). Avenida Madrid, 57. 34004 Palencia, Spain. E-mail: jorgemg@pvs.uva.es
[3] Ingeniería Forestal y del Medio Natural, Universidad de Extremadura. Avenida Virgen del Puerto 2, 10600 Plasencia, Spain.

INTRODUCTION

Holm oak "dehesas" are amongst the most representative forest ecosytems in the Iberian Peninsula. Over the last three decades a serious forest decline associated with several species of *Phytophthora* has been observed (Fig 1). Although previous reports describe the pathogenicity of different species of *Phytophthora* to *Quercus* spp. in this ecosystem (Sánchez et al., 2005), empirical evidence concerning the possible responses of *Phytophthora* species to climate change are lacking. An increase in temperature of 2 - 4.5 ºC (with a most likely value of 3 ºC) is expected in the 21st century (ICPP, 2007), which will lead to changing interactions between vegetation and pathogens and to a variety of forest health problems (La Porta et al., 2008; Pautausso et al., 2011, Sturrock et al., 2011). The aim of this work was to determine whether an increase in temperatures would influence the germination and survival of *Quercus ilex* seedlings growing in *Phytophthora* infested soils.

Fig 1.- Appearance of healthy (top) and declining holm oak (bottom).

MATERIAL AND METHODS

Acorns of *Quercus ilex* (provenance: Malpartida de Plasencia, Spain) were stored at 4 ºC before sowing. Inocula of *P. cinnamomi*, *P. gonapodyides*, *P. quercina* and *P. psychrophila* were raised on vermiculite - oat - V8 juice medium at 20ºC for 6 weeks (Jung et al., 1996). Four hundred and fifty acorns were sown in infested soils with each of the four *Phytophthora* species separately, plus a control treatment of non-infested soil. Three temperature treatments were used: 17, 20 and 23 ºC with the same photoperiod (n = 30 replicates/treatment). Each tray was flooded once every 2 weeks for 48 h to favour the sporulation of *Phytophthora* species. Germination, mortality rates and plant growth (aerial parts and root systems) were assessed.

In parallel, the effect of temperature on growth of the different species of *Phytophthora* in Petri Dishes (V8 agar) was studied using a range of temperatures (15 – 30 ºC, at 2.5 ºC intervals.

RESULTS

Inoculation tests demonstrated the high virulence of the four *Phytophthora* species to holm oak (Fig. 2), with mortalities on germinating acorns up to 100 % in most of cases. Furthermore, a clear effect of temperature was observed, while *P. cinnamomi*, *P. gonapodyides* and *P. quercina* showed similar mortality percentages in the range of temperatures 17-23 ºC, *P. psychrophila* only killed germinating seed at17-20 ºC (Fig. 3).

A similar pattern was in lengths of the emerging radicles, demonstrating differences amongst the four *Phytophthora* species according to the temperature (Fig. 4). Moreover, radicle damage correlated with optimum growth temperatures of each *Phytophthora* species (Fig. 5). Thus, relationships between radicle lengths and mycelial growth in Petri dishes of each *Phytophthora* species at increasing temperatures showed a good fit (Fig. 6).

Fig 2.- Appearance of acorns infected by *P. cinnamomi* (at top left), *P. gonapodyides* (at top right), *P. quercina* (at bottom left) and *P. psychrophila* (at bottom right).

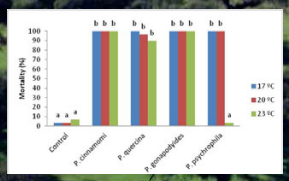


Fig 3. Mortality of germinating acorns caused by the different *Phytophthora* species at three temperatures.

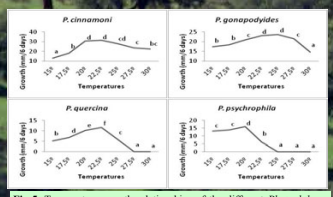


Fig 5. Temperature-growth relationships of the different *Phytophthora* species on V8A

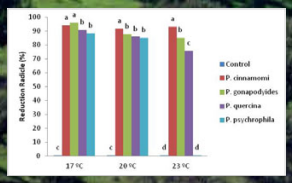


Fig 4. Reduction in radicle lengths of germinating acorns inoculated with the different *Phytophthora* species at three temperatures.

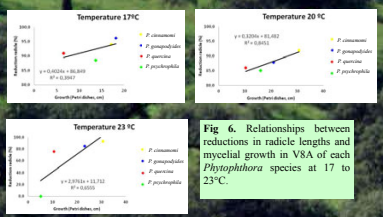


Fig 6. Relationships between reductions in radicle lengths and mycelial growth in V8A of each *Phytophthora* species at 17 to 23°C.

DISCUSSION

The sustainability of "dehesas" is questioned due to a lack of regeneration, which has been linked with grazing management (Plieninger et al., 2003) and the presence of soil-borne pathogens (Gómez-Aparicio et al., 2012). Our results confirm that *Phytophthora* species could be key drivers in regeneration processes in this ecosystem and should be taken into account by forest managers.

Is a common belief that forest health problems will increase as a result of climate change, although recent studies suggest it is not possible to generalize (La Porta et al., 2008; Pautasso et al., 2011; Sturrock et al., 2011) and empirical evidence is therefore necessary to reach firm conclusions.

Our findings indicate that temperature influences the virulence of *Phytophthora* species, and that the effect varies between species. While an increase in temperatures would aggravate damage caused by thermophilic species, damage due to low-temperature species such as *P. psychrophila* (Jung et al., 2002) would not.

In conclusion, (i) climate change could influence the survival of holm oak seedlings germinating in *Phytophthora* infested soils and (ii) results obtained under laboratory conditions could be used for predicting the potential effects of climate change on different pathogens.

REFERENCES:

Gómez-Aparicio, L.; Ibáñez, B.; Serrano, M.; DeVita, P.; Ávila, J.M.; Pérez-Ramos, I.; García, L.V.; Sánchez, E. & Marañón, T. 2012. Spatial patterns of soil pathogens in declining Mediterranean forests: implications for trees species regeneration. New Phytologist DOI 10.1111/j.1469-8137.2012.04108.x

IPCC 2007. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Jung, T.; Hansen, E.M.; Winton, L.; Oßwald, W. & Delatour, C. 2002. Three new species of Phytophthora from European oak forests. Mycological Research 106 (4): 397 – 411.

La Porta, N.; Capretti, P.; Thomsen, I.M.; Kasanen, R.; Hietala, A.M. & Von Weissenberg, K. 2008. Forest pathogens with higher damage potential due to climate change in Europe. Canadian Journal of Plant Pathology 30: 177 – 195.

Pautasso, M.; Döring, T.F.; Garbelotto, M.; Pellis, L. & Jeger, M. J. 2011. Impacts of climate change on plant diseases – opinions and trends. European Journal of Plant Pathology DOI 10.1007/s10658-012-9936-1.

Plieningeri, T.; Pulido, F. & Konold, W. 2003. Effects of land-use history on size structure of holm oak stands in Spanish dehesas: implications for conservation and restoration. Environmental Conservation 30 (1): 61 – 70

Sánchez, L.; Andicoberry, S & Trapero, A. 2005. Pathogenicity of three *Phytophthora* spp. causing late seedling rot of *Quercus ilex* spp. *ballota*. Forest Pathology 35: 115 – 125.

Sturrock, R.N.; Frankelb, S.J.; Brown, A.V.; Hennond, P.E.; Kliejunasb, J.T.; Lewise, K.J.; Worrall, J.J. & Wood, A.J. 2011. Review: Climate change and forest diseases. Plant Pathology 60: 133 – 149.

Acknowledgemens:

We thank Thomas Jung and Ana Pérez Sierra for supplying some of the isolates. Funded by the University of Extremadura (Action IV), ISEFOR project (KBBE FP7), Junta de Extremadura (IV-PRI regional projects), the Ministerio de Ciencia e Innovación (AGL2011-30438-C02-02), and performed within the frame of the COST Action FP0801.

Cryptogein and capsicein elicit defence responses in cork oak

C. Medeira[1], V. Quartin[1,2], I. Maia[1], I. Diniz[1,3], M.C. Matos[1], J. Nobre Semedo[1], P. Scotti-Campos[1], J. Ramalho[2], I. Pais[1], P. Ramos[1], E. Melo[1], A, Leitão[2], Alfredo Cravador[3]

[1]*Instituto Nacional de Recursos Biológicos / Instituto Nacional de Investigação Agrária, Quinta do Marquês, 2784-505 Oeiras, Portugal.*
[2]*Centro de Ecofisiologia, Bioquímica e Biotecnologia Vegetal / Instituto de Investigação Científica Tropical, Quinta do Marquês, 2784-505-Oeiras.*
[3]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*

Corresponding author: acravad@ualg.pt

Abstract

Cork oak (*Quercus suber*) decline in Iberian Peninsula stands is associated with infection by *Phytophthora cinnamomi*. Most *Phytophthora* species secrete elicitins, which can enhance defence reactions against some pathogens.
This work recently published on-line, is the first to report a potentially protective role of *Phytophthora*- derived elicitins against *P.* infection in a *Fagaceae* (*Q. suber*). It highlights the effect of cryptogein and capsicein (secreted by *P. cryptogae* and *P. capsici*, respectively) on the infection of *Q. suber* roots by *P. cinnamomi.*
Cytological and physiological effects of the two elicitins on cork oak root infection by *P. cinnamomi* were evaluated. The progression of the pathogen in root tissue and its effects on total fatty acid (TFA) of roots and leaves were analysed in seedlings. Net photosynthesis (Pn), stomatal conductance (gs), chlorophyll a fluorescence (quantum yield of linear electron transport ϕe, photochemical quenching qP, non-photochemical quenching NPQ) and carotenoid determinations were carried out in 4-month-old plants. In elicitin-treated roots, 2 days after inoculation, the pathogen was mainly restricted to the intercellular spaces of the cortical parenchyma, and did not reach the vascular cylinder of roots. Electron dense materials accumulated in the intercellular spaces of the cortex next to disorganized hyphae, suggesting to be related with defence reactions. Cryptogein (or its interaction with *P. cinnamomi*) induced enhanced lipid synthesis in leaves. *P.cinnamomi* decreased Pn, gs, ϕe, and qP, whereas elicitin treated plants displayed values similar to controls. Results indicated a resistance response of cork oak against this oomycete, induced by the elicitins.

Literature Cited

Medeira C., Quartin V., Maia I., Diniz I., Matos M.C., Semedo J., Campos P., Ramalho J., Pais I., Ramos,P., Melo E., Leitão A., Cravador A., 2012. Cryptogein and capsicein promote defence responses and photosynthesis protection in *Quercus suber* against *Phytophthora cinnamomi* infection. *European Journal of Plant Pathology* **134:** 145-159.

Susceptibility of *Quercus ilex* to mixed infections by multiple *Phytophthora* species

J. Miranda[1], T. Corcobado[1], T. Jung[1,2,3], J. Martín-García[1], A. Pérez-Sierra[4], P. Abad-Campos[4], A. Solla[1]

[1]*Ingeniería Forestal y del Medio Natural. Universidad de Extremadura. Avenida Virgen del Puerto 2, 10600 Plasencia, Spain.*
[2]*Phytophthora Research and Consultancy, Thomastrasse 75, 83098 Brannenburg, Germany.*
[3]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*
[4]*Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de la Vera s/n, 46022 Valencia, Spain.*

Corresponding author: asolla@unex.es

Abstract

Oak decline in Iberia is a complex phenomenon that requires the combination of several factors to occur. Although declining trees are often damaged by several pathogenic oomycetes [1, 2] the interaction among these organisms has still not been investigated. The present study aims to determine under greenhouse conditions whether possible interactions among different species of *Phytophthora* might have significant synergistic effects on *Quercus ilex* decline. The material comprised 1-year-old *Q. ilex* seedlings grown from acorns of a single tree (Malpartida de Plasencia, Spain), and single strains of *P. cinnamomi* (C), *P. gonapodyides* (G) and *P. quercina* (Q) isolated from rhizosphere soil of declining *Q. ilex* trees. Inocula were prepared by growing the *Phytophthora* strains in vermiculite - oat seeds - V8 juice medium at 20ºC for 6 weeks. Repeated inoculations were performed in December 2011 and in January 2012, so that nine combinations (CC, GG, QQ, CG, CQ, GC, GQ, QC, QG) plus a control treatment of non-inoculated seedlings were tested (n=22 seedlings). All plants were submitted to a regime of two days waterlogging per week. Twenty-one weeks after the first inoculation, mortality rates significantly varied among the treatments. Mortality rates were 100, 40, 9, 100, 100, 64, 50, 100, 9, and 0% for CC, GG, QQ, CG, CQ, GC, GQ, QC, QG and control treatments, respectively. At the conditions tested here (25ºC), *P. quercina* + *P. quercina* was not able to cause mortality of *Q. ilex* plants or to reduce fine root weight. However, if combined with *P. cinnamomi* (i.e. *P. quercina* + *P. cinnamomi*) mortality of plants was extremely rapid. *P. gonapodyides* + *P. quercina* and *P. gonapodyides* + *P. gonapodyides* caused a delayed mortality rate of about 40% in accordance to a previous study [2]. The highest mortality of seedlings occurred when C was present, irrespectively of the treatment or inoculation date. Inoculation of C, G or Q did not enhance plant resistance to a second inoculation, and no synergism or antagonism effects among *Phytophthora* species in *Q. ilex* seems to occur.

Literature Cited

Corcobado T., Cubera E., Pérez-Sierra A., Jung T., Solla A., 2010. First report of *Phytophthora gonapodyides* involved in the decline of *Quercus ilex* in xeric conditions in Spain. *New Disease Reports* **22:** 33-33.

Pérez-Sierra A., López-García C., León M., García-Jiménez J., Abad-Campos P., Jung, T., 2013. Previously unrecorded low temperature *Phytophthora* species associated with *Quercus* decline in a Mediterranean forest in Eastern Spain. *Forest Pathology* **43:** 331-339.

Susceptibility of *Quercus ilex* to mixed infections by multiple *Phytophthora* species

Javier Miranda[1], Tamara Corcobado[1], Thomas Jung[1,2], Jorge Martín-García[1], Ana Pérez-Sierra[3], Paloma Abad-Campos[3] and Alejandro Solla[1]*

[1]Universidad Forestal y del Medio Natural. Universidad de Extremadura. Avenida Virgen del Puerto 2, 10600 Plasencia, Spain.
[2]Phytophthora Research and Consultancy, Thomastrasse 75, 83098 Brannenburg, Germany.
[3]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de la Vera s/n, 46022 Valencia, Spain.

*asolla@unex.es

INTRODUCTION

Oak decline in Iberia is a complex phenomenon that requires the combination of several factors to occur. Although declining trees are often damaged by several pathogenic oomycetes (Corcobado *et al*. 2010; Pérez-Sierra *et al*. 2011) the interaction among these organisms has still not been investigated. The present study aims to determine under controlled conditions whether possible interactions among different species of *Phytophthora* might have significant synergistic effects on *Quercus ilex* decline.

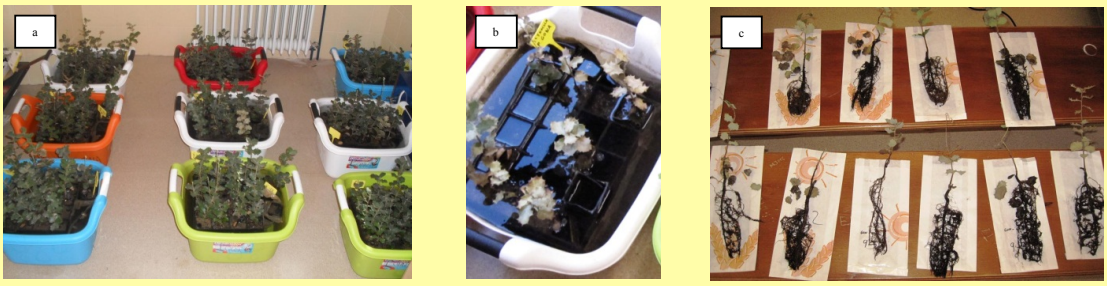


Fig. 1. One-year-old *Quercus ilex* plants divided into 10 independent groups (trays) in which different treatments were performed (a); waterlogging plants of the *P. cinnamomi* + *P. gonapodyides* treatment 20 weeks after first inoculation (b); root balls after treatments, ready for root assesment (c).

MATERIALS AND METHODS

The material (Fig. 1a) included 1-year-old *Q. ilex* seedlings grown from acorns of a single tree (Malpartida de Plasencia, Spain), and single strains of *P. cinnamomi* (C), *P. gonapodyides* (G) and *P. quercina* (Q) isolated from rhizosphere soil of declining *Q. ilex* trees. Inocula were prepared by growing the *Phytophthora* strains in vermiculite - oat seeds - V8 juice medium at 20ºC for 6 weeks. Repeated inoculations were performed in December 2011 and 6 weeks later, so that nine combinations (CC, GG, QQ, CG, CQ, GC, GQ, QC, QG) plus a control treatment of non-inoculated seedlings were tested (n=24 seedlings). All plants were subjected (25±2ºC) to a regime of two days waterlogging per two weeks (Fig. 1b), and their time to death and fine root weight was assessed (Fig. 1c).

RESULTS AND DISCUSSION

Twenty-one weeks after the first inoculation, mortality rates significantly varied among the treatments (Fig. 2). At the conditions tested here (25ºC), *P. quercina* + *P. quercina* was not able to cause mortality of *Q. ilex* plants (Fig. 3a) or to reduce fine root weight. However, if combined with *P. cinnamomi* (i.e. *P. quercina* + *P. cinnamomi*) mortality of plants was extremely rapid (Fig. 3b). *P. gonapodyides* + *P. quercina* and *P. gonapodyides* + *P. gonapodyides* caused a delayed mortality rate of about 40% in accordance to a previous study (Corcobado *et al*. 2010). The highest mortality of seedlings occurred when C was present, irrespectively of the treatment or inoculation date. Inoculation of C, G or Q did not enhance plant resistance to a second inoculation, and no synergism or antagonism effects among *Phytophthora* species in *Q. ilex* seems to occur.

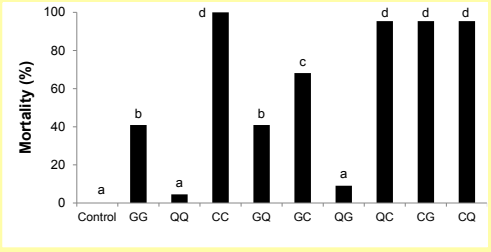


Figure 2. Mortality rates of *Q. ilex* plants after combined inoculations with sterilized inoculum (Control), *P. gonapodyides* + *P. gonapodyides* (GG), *P. quercina* + *P. quercina* (QQ), *P. cinnamomi* + *P. cinnamomi* (CC), *P. gonapodyides* + *P. quercina* (GQ), *P. gonapodyides* + *P. cinnamomi* (GC), *P. quercina* + *P. gonapodyides* (QG), *P. quercina* + *P. cinnamomi* (QC), *P. cinnamomi* + *P. gonapodyides* (CG) and *P. cinnamomi* + *P. quercina* (CQ) (a). Different letters indicate significant differences at p<0.05 (n=24). Wilting of CC plants 10 weeks after inoculation (b).

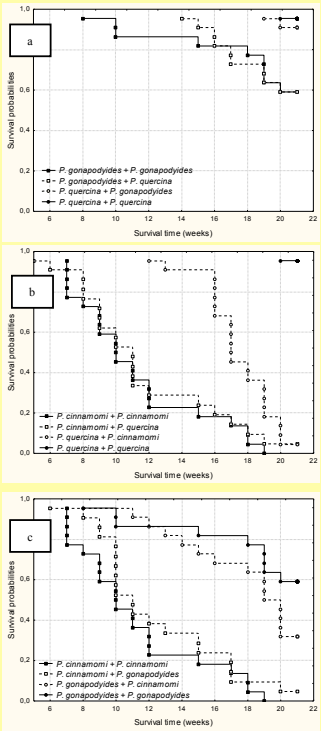


Figure 3. Plot of survival probabilities using the Kaplan–Meier estimate ($P < 0.01$) of the survival function for *Q. ilex* inoculated with *Phytophthora* spp. at weeks 0 and 6.

References

Corcobado, T., Cubera, E., Pérez-Sierra, A., Jung, T. and Solla, A. 2010. First report of *Phytophthora gonapodyides* involved in the decline of *Quercus ilex* in xeric conditions in Spain. New Disease Reports, **22** 33-33.

Pérez-Sierra, A., López-García, C., León, M., García-Jiménez, J., Abad-Campos, P. and Jung T. 2011. Detection of *Phytophthora* spp. on *Quercus* spp. in the Natural Park Carrascar de la Font Roja (preliminary). Proceedings of the COST Action FP0801 "Established and Emerging *Phytophthora*: Increasing threats to Woodland and Forest Ecosystems in Europe".

Acknowledgements. Thanks to O. Locos for encouragement and to Andrea Pérez and Marta Company for their technical help. Funded by Junta de Extremadura (IV-PRI regional project), and Ministerio de Ciencia e Innovación (AGL2011-30438-C02-02). Performed within the frame of the COST action FP0801.

Effect of *Phytophthora quercina*, *P. gonapodyides* and *P. cinnamomi* on germination of *Quercus ilex* acorns and seedling establishment in infested soils

A. Solla[1], J. Miranda[1], T. Corcobado[1], T. Jung[1,2,3], J. Martín-García[1], A. Pérez[1], E. Cubera[1]

[1]*Ingeniería Forestal y del Medio Natural. Universidad de Extremadura. Avenida Virgen del Puerto 2, 10600 Plasencia, Spain.*
[2]*Phytophthora Research and Consultancy, Thomastrasse 75, 83098 Brannenburg, Germany.*
[3]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*

Corresponding author: asolla@unex.es

Abstract

In Iberia, several species of *Phytophthora* are involved in the decline of *Quercus ilex*. The pathogen *P. cinnamomi* is the most frequent species isolated, whereas *P. quercina* and *P. gonapodyides* are less detected (Corcobado *et al.* 2010). Natural regeneration of declining holm oaks is a main goal for the forest administration, but no information is available about the viability of germinating acorns and subsequent establishment of seedlings in infested soils. Previous research reported 100% mortality of *Q. ilex* seedlings when pre-germinated seeds were placed into soils infested with *P. cinnamomi* (Rodríguez-Molina *et al.* 2002). Our objectives were to quantify the establishment of *Q. ilex* through acorns directly sown into infested soils, and to detect possible differences between different tree provenances. In December 2011, acorns were collected and stored at 4ºC for two months, and then were sown 1 cm deep into a mixture of sand and peat (1:1) containing inoculum of the three pathogens, separately. Non-infested soil mixture was used as control. Acorn germination (about 55%) was not reduced significantly by the presence of any of the *Phytophthora* species tested. Post-emergence damages caused by the three pathogens included tap root rot, leaf wilting and fine root reduction. Two months after sawing, plant mortality and main root necrosis differed among treatments, being very severe if plants were sawn into the *P. cinnamomi* infested soil (about 80% of seedling mortality). Plant mortality and main root necrosis did not differ among the provenances, but the number of fine roots per plant did. An interesting *Phytophthora* spp. x plant origin interaction involved the fine root production, which was not reduced by *P. gonapodyides* in the plants originating from humid sites (i.e. with annual rainfall above 800 mm). It is concluded that the presence of *Phytophthora* spp. is a clear limiting factor of the natural regeneration processes.

Literature Cited

Corcobado T., Cubera E., Pérez-Sierra A., Jung T., Solla A., 2010. First report of *Phytophthora gonapodyides* involved in the decline of *Quercus ilex* in xeric conditions in Spain. *New Disease Reports* **22:** 33-33.

Rodríguez-Molina M.C., Torres-Vila L.M., Blanco-Santos A., Palo-Núñez E.J., Torres-Álvarez E., 2002. Viability of holm and cork oak seedlings from acorns sown in soils naturally infected with *Phytophthora cinnamomi*. *Forest Pathology* **32:** 365–372.

Effect of *Phytophthora quercina*, *P. gonapodyides* and *P. cinnamomi* on germination of *Quercus ilex* acorns and seedling establishment in infested soils

Alejandro Solla[1]*, Javier Miranda[1], Tamara Corcobado[1], Jorge Martín-García[1], Elena Cubera[1], Andrea Pérez[1] and Thomas Jung[1,2]

[1]Universidad Forestal y del Medio Natural. Universidad de Extremadura. Av. Virgen del Puerto 2, 10600 Plasencia, Spain.
[2]Phytophthora Research and Consultancy, Thomastrasse 75, 83098 Brannenburg, Germany.

*asolla@unex.es

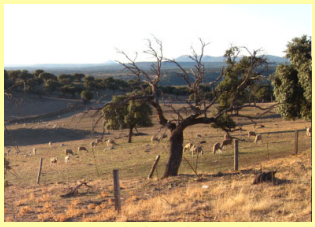


Fig. 1. Lack of natural regeneration.

INTRODUCTION

In the Iberian Penisula, several species of *Phytophthora* are involved in the decline of *Quercus ilex*. The pathogen *P. cinnamomi* is the most frequent species isolated, whereas *P. quercina* and *P. gonapodyides* are less detected (Corcobado et al. 2010). Natural regeneration of declining holm oaks is a main goal for the forest administration (Fig. 1), but no information is available about the viability of germinating acorns and subsequent establishment of seedlings in infested soils. Previous research reported 100% mortality of *Q. ilex* seedlings when pre-germinated seeds were placed into soils infested with *P. cinnamomi* (Rodríguez Molina et al. 2002), but no studies were conducted concerning germination, or using *P. quercina* and *P. gonapodyides*. Our objectives were to quantify the establishment of *Q. ilex* through acorns directly sown into infested soils, and to detect possible differences between different tree provenances.

MATERIALS AND METHODS

In December 2011, acorns were collected from 16 sites (Fig. 2a) and stored at 4ºC for two months. Acorns were sown inside trays 1 cm deep into a mixture of sand and peat (1:1) containing inoculum of the three pathogens, separately (Fig. 2b, c). Non-infested soil mixture was used as control.

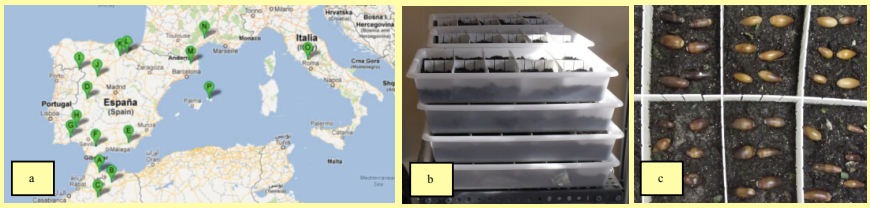


Fig. 2. Origin of the plant material (a) A: Talassentane (Oeste del Atlas), B: El Gouzate (Centro del Atlas), C: Azrou (Este del Atlas), D: Malpartida de Plasencia (Cáceres), E: Las Tres Villas (Almería), F: Algodonales (Cádiz), G: Alcoutim (Sur de Portugal), H: Barrancos (Bajo Alentejo), I: Cavaleiros (Norte de Portugal), J: Castronuño (Zamora), K: Barrio de Bureva (Burgos), L: Nanclares de Oca (Álava), M: Olot (Gerona),N: Puéchabon (Sur de Francia), O:Bagnania (Italia), y P: Menorca (Islas Baleares); trays including infested substrate and acorns (b); and detail (c).

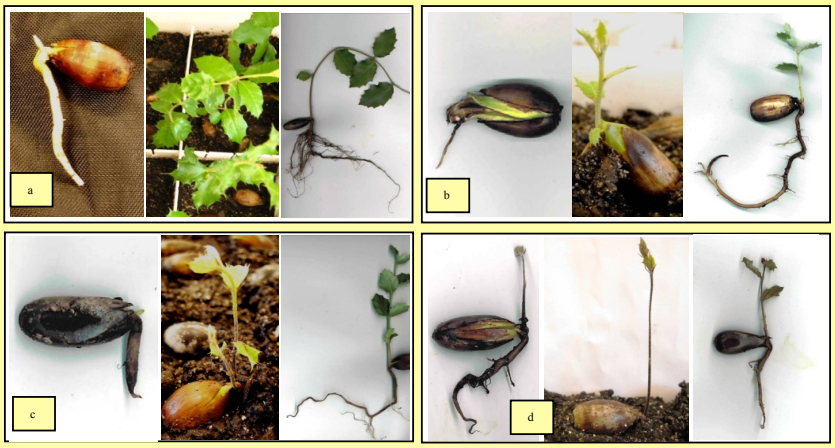


Figure 3. Appearance of *Q. ilex* during germination in a non-inoculated soil (a), and in *P. quercina* (b), *P. gonapodyides* (c) and *P. cinnamomi* (d) infested soils.

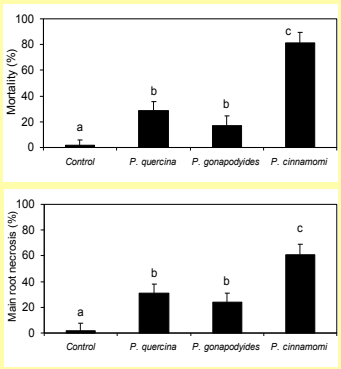


Figure 4. Plant mortality and necrosis of the tap root two months after sowing *Q. ilex* acorns into a non-inoculated soil (control), or into a *Phytophthora spp.* infested soil. Different letters indicate significances at $p<0.05$.

RESULTS AND DISCUSSION

Acorn germination (about 55%) was not reduced significantly by the presence of any of the *Phytophthora* species tested. Post-emergence damages caused by the three pathogens included tap root rot, leaf wilting and fine root reduction (Fig. 3). Two months after sawing, plant mortality and main root necrosis differed among treatments (Fig. 4), being very severe if plants were sawn into the *P. cinnamomi* infested soil. Plant mortality and main root necrosis did not differ among the provenances, but the number of fine roots per plant differed significantly among the provenances (Fig. 5). An interesting *Phytophthora* spp. x plant origin interaction involved the fine root production, which was not reduced by *P. gonapodyides* in the plants originating from humid sites (i.e. with annual rainfall above 800 mm). It is concluded that the presence of *Phytophthora* spp. is a clear limiting factor of the natural regeneration processes.

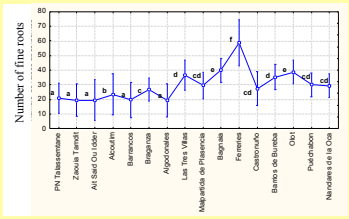


Figure 5. Number of fine roots per plant according to the provenances. Different letters indicate significances at $p<0.05$.

References

Corcobado, T., Cubera, E., Pérez-Sierra, A., Jung T. and Solla A. 2010. First report of *Phytophthora gonapodyides* involved in the decline of *Quercus ilex* in xeric conditions in Spain. *New Disease Reports*, **22** 33-33.

Rodríguez-Molina, M. C., Torres-Vila, L. M., Blanco-Santos, A., Palo-Núñez, E. J. and Torres-Álvarez, E. 2002.Viability of holm and cork oak seedlings from acorns sown in soils naturally infected with *Phytophthora cinnamomi*. *For. Pathol*., **32** 365–372.

Acknowledgements. Thanks a lot to Eneko Pérez, Marilia Horta, Anna Maria Vettraino, Alessia Tomassini, Andrea Vannini, Bor Krajnc, Oriol Perona, Luís Nuñez, Rafael Zas, Gloria Bustingorri and Stephanos Diamandis for their help in acorn collection. Thanks to O. Locos for encouragement and to Marta Company for technical help. Funded by Junta de Extremadura (IV-PRI regional project), and Ministerio de Ciencia e Innovación (AGL2011-30438-C02-02). Performed within the frame of the COST action FP0801.

In vitro inhibition of mycelial growth of *Phytophthora cinnamomi* by pellets of brassicas

C. Morales-Rodríguez[1], J. Picón-Toro[1], C. Palo[1], E. Palo[1], A. García[1], M. del Carmen Rodríguez-Molina[1]

[1]*Centro de Investigación Agraria "Finca La Orden-Valdesequera", Gobierno Extremadura, Finca La Orden, 06187 Guadajira (Badajoz), Spain.*

Corresponding author: carmen.mrodriguez@juntaextremadura.net

Abstract

Phytophthora cinnamomi is considered one of the main agents involved in oak decline, a phytosanitary problem currently affecting Spanish forests and dehesas (Mediterranean open woodlands). Biofumigation is an environmentally friendly method of controlling soil pathogens, so it may be a valid alternative to chemical use in agroforestry and dehesas and also economically viable. The hydrolysis products of glucosinolates in brassica tissues are potentially useful to control fungal pathogens. In this *in vitro* study the effect of the pellets of brassicas (BioFence ®) on the inhibition of mycelial growth of *P. cinnamomi* was evaluated.

Eight biofumigant concentrations were tested (5, 7.5, 10, 12.5, 15, 20, 40 and 60 mg; 40% humidity) on ten isolates of *P. cinnamomi* to calculate by Probit analysis the EC_{50} and EC_{90}. Plugs of PDA with actively growing mycelium were cut and transferred to the centre of petri dishes containing PARP-V8 juice agar. All plates were incubated at 25°C for 24h before being exposed to the biofumigant material. Biofumigant material was placed on the cover of the petri dishes and the plates were incubated inverted in the dark at 25°C for 72h. After that time, radial growth was measured taking two perpendicular diameter measurements for each colony. All isolates assayed showed to be highly susceptible to the effect of biofumigant although differed in their susceptibility, with EC_{50} values ranging 5.59 and 14.65 mg/plate and EC_{90} values ranging 14.97 and 28.10 mg/plate.

The results of our experiments indicated that isolates of *P. cinnamomi* differ in susceptibility to growth-inhibiting compounds released by the *Brassica* pellet. Differences between isolates in susceptibility to biofumigants have also been reported in other phytopathogenic fungi. Variation in sensitivity had been reported not only between species but also between isolates of the same species. Moreover, different forms of inoculum of the same organism or different states within its life cycle may have different sensitivity to biofumigant compounds. These results have implications for future investigations on the use of *Brassica* pellets for suppression of *P. cinnamomi* diseases in forests and dehesas. Further studies are required to confirm under field conditions the efficacy of biofumigation to control the diseases, since under field conditions several other factors as efficiency of incorporation of biofumigant material, forms of inoculum of the pathogen, activity of the hydrolysing myrosinase or microbial degradation, are involved.

In vitro inhibition of mycelial growth of *Phytophthora cinnamomi* by pellets of *Brassicas*

Morales-Rodríguez, M.C., Picón-Toro, J., Palo, C., Palo, E.J., García-Gallego, A., and Rodríguez-Molina, M.C.

Centro de Investigación Agraria "Finca La Orden-Valdesequera", Gobierno de Extremadura. 06187 Guadajira (Badajoz).
Email: carmen.mrodriguez@juntaextremadura.net

INTRODUCTION

Phytophthora cinnamomi is considered one of the main agent involved in oak decline, a phytosanitary problem currently affecting Spanish forests and *dehesas* (Mediterranean open woodlands). Biofumigation is an environmentally friendly method of controlling soil pathogens, so it may be a valid alternative to chemical use in agroforestry and *dehesas* and also economically viable. The hydrolysis products of glucosinolates in the brassicas tissues are potentially useful to control fungal pathogens. In this *in vitro* study there was evaluated the effect of the pellets of brassicas (BioFence®) on the inhibition of mycelial growth of *P. cinnamomi*.

MATERIAL AND METHODS

Trials were conducted by using pellets of *Brassica* (BioFence®, Triumph Italia SPA, Cerealetoscana Group). Eight biofumigant concentrations were tested: 5, 7.5, 10, 12.5, 15, 20, 40 and 60 mg (40% humidity) on ten isolates of *P. cinnamomi.*

Mycelial plugs (4-mm diameter) were cut from the margin of actively growing colonies. One plug was placed in the center of a 90 mm Petri plate with 10 mL of PARP-V8 agar (French-Monar et al., 2007), all plates were incubated for 24 h at 25 °C to exclude the initial growth lag phase. After this time biofumigant material was placed on the cover of petri plate and the plates were immediately sealed with parafilm® and incubated inverted in the dark at 25°C for 72h.

To calculate the percentage of inhibition, radial growth in the presence of biofumigant material was expressed as the mean percentage of the growth in the control plates. The experiment was conducted with four replicate plates per isolate/dose in consecutive weeks and using different sample of pellets in each repetition. Dose-inhibition regressions, EC_{50}'s, EC_{90}'s and their fiducial limits were estimated by probit analysis (Finney, 1971) using POLO software (LeOra-Software, 1987; Russell et al., 1977).

RESULTS AND DISCUSION

The biofumigant tested inhibited the mycelial growth of all *P. cinnamomi* isolates. Concentrations of 40 and 60 mg resulted in a 100% inhibition of mycelial growth in all isolates studied. The results of mycelial growth inhibition with different concentrations of biofumigant material are presented in Table 1. In all case, the *t*-ratio of the slope was significant ($P < 0.05$) so that a significant dose-response line was obtained. All isolates assayed showed to be highly susceptible to the effect of biofumigant although differed in their susceptibility, with EC_{50} values ranging from 5.59 to 14.65 mg/plate and EC_{90} values ranging from 14.97 to 28.10 mg/plate.

Table 1. Dose-inhibition regressions, effective concentration (EC_{50} and EC_{90}) values and their fiducial limits on brassica pellets of isolates of *P. cinnamomi*. In all isolates probit dose-response lines were significant (*t*-ratio test, $P < 0.05$)

Isolate	Probit regression		EC_{50} (mg)	95 % fiducial limits: lower - upper	EC_{90} (mg)	95 % fiducial limits: lower - upper
	Slope ± S.E.	Intercept				
CECT 20186	5.27 ± 0.4	-1.14	14.69	13.58 – 15.94	25.73	22.75 – 30.61
TCMC	6.40 ± 0.5	-2.99	17.72	16.38 – 19.42	28.10	24.56 – 34.82
TVIA	4.04 ± 0.2	0.52	12.84	11.86 – 13.93	26.63	23.32 – 31.81
EC-1a	3.35 ± 0.33	1.76	9.29	7.71 – 10.65	22.42	18.83 – 29.50
P. cn-5	3.57 ± 0.28	1.54	9.30	8.07 – 10.61	21.25	17.49 – 28.83
EC-3	2.99 ± 0.29	2.76	5.59	4.54 – 6.47	14.97	13.05 – 18.14
EC-5	3.36 ± 0.31	2.22	6.72	6.01 – 7.37	16.17	14.36 – 18.94
P. cn-9	5.78 ± 0.10	-1.23	11.93	10.62 – 13.84	19.86	16.33 – 29.86
P. cn- 10	4.54 ± 0.31	-0.29	14.65	13.16 – 16.45	28.06	23.78 – 35.66
P. cn-11	4.82 ± 0.39	-0.37	12.98	12.22 – 13.78	23.93	21.73 – 27.15

The results of our experiments indicated that isolates of differ in susceptibility to growth-inhibiting compounds released by the *Brassica* pellet. Differences between isolates in susceptibility to biofumigants have been also reported in other phytopathogenic fungi (Kirkegaard et al., 1996). Variation in sensitivity had been reported not only between species but also between isolates of the same species (Smith and Kirkegaard, 2002). Moreover, different forms of inoculum of the same organism, or different states within its life cycle, may have different sensitivity to biofumigant compounds (Yulianti et al., 2006).

This results have implications for future investigations on the use of *Brassica* pellets for suppression of *P. cinnamomi* diseases in forests and *dehesas*. Further studies are required to confirm under field conditions the efficacy of biofumigation to control the diseases, since under field conditions several other factors as efficiency of incorporation of biofumigant material, forms of inoculum of the pathogen, activity of the hydrolysing myrosinase or microbial degradation, are implicated.

REFERENCES

Finney, D. F. 1971. Probit analysis: Cambridge University Press. **Kirkegaard J. A, Wong P. T. W and Desmarchelier J. M.**,1996. *In vitro* suppression of fungal root pathogens of cereals by *Brassica* tissues. *Plant Pathol.* 45:593-603. **LeOra-Software.** 1987. POLO-PC: A User's guide to Probit or Logit Analysis. Berkeley: LeOra Software. **French-Monar, R. D., Jones, J. B., Ozores-Hampton, M., and Roberts, P. D.**, 2007. Survival of inoculum of *Phytophthora capsici* in soil through time under different soil treatments. *Plant Dis*. 91:593-598. **Russell, R. N., Robertson, J. L and Savin, N. E.,** 1977. Polo: a new computer program for probit analysis. *Bull. Entomol. Soc. Am.* 23:209-215. **Smith B. J and Kirkegaard J. A.,** 2002. *In vitro* inhibition of soil microorganisms by 2-phenylethyl isothiocyanate. *Plant Pathol.* 51:585-593. **Yulianti, T., Sivasithamparam, K. and Turner, D. W.,** 2006. Response of different forms of propagules of *Rhizoctonia solani* AG2-1 (ZG5) exposed to the volatiles produced in soil amended with green manures. *Ann. Appl. Biol.* 148:105-111 (2006).

RTA 2008-00058-C03-02 Proyect , Group GR10130 Government of Extremadura, European Regional Development Funds and European Social Funds.

PCR detection of *Phytophthora ramorum* from woody tissue

B. Moreland, J. Pollock, R. Swan, A. McCracken, M. Wilson, C. Fleming

Applied Plant Science and Biometrics Division, Agri-Food and Biosciences Institute, Newforge Lane, Belfast BT9 5PX, UK.

Corresponding author: alistair.mccracken@afbini.gov.uk

Abstract

Rapid and accurate detection of *Phytophthora ramorum* from advisory and quarantine samples is a key step in managing the spread of this pathogen. Along with standard culturing methods, PCR and real time PCR are effective tools for confirming infection, but in the case of woody hosts such as larch (*Larix* sp) these methods can have a low success rate, giving rise to false negatives and delays in managing disease outbreaks.

Comparison of different target genes were made including the use of the Elicitin gene and the ITS region. Significant differences were found in the CT values gained from the two assays compared.

A range of alternative extraction protocols and commercial kits were tested with varying degrees of PCR inhibitor removal. One kit consistently out performed the others. Further optimisation of this kit was carried out by modification of protocols and proportions of reagents. Additional components were also tested for their effectiveness in conjunction with the preferred extraction kit. Significant improvements were achieved by the adoption of these changes, resulting in a protocol with improved detection rates through increased efficiency of extraction from difficult tissues.

Comprehensive testing of available PCR reagents also resulted in improved detection of target. An increase in sensitivity was achieved and a significant reduction in false negatives being reported.

Phytophthora plurivora and *Phytophthora multivora* in the Czech Republic – comparison of distribution and pathogenicity to forest trees

M. Mrázková[1], K. Černý[1,2], V. Strnadová[1], V. Holub[1], B. Gregorová[1], M. Tomšovský[3], M. Pánek[1,3], L. Havrdová[1,2], S. Gabrielová[1], M. Hejná[1]

[1]*Silva Tarouca Research Institute for Landscape and Ornamental Gardening, publ. res. inst., Květnové nám. 391, 252 03, Průhonice, Czech Republic.*
[2]*Czech University of Life Sciences, FFWS, Kamýcká 129, 165 21, Praha, Czech Republic.*
[3]*Mendel University in Brno, FFWT, Zemědělská 3, 613 00, Brno, Czech Republic.*

Corresponding author: mrazkova@vukoz.cz

Abstract

The two new pathogens – *Phytophthora plurivora* and *P. multivora* – were described from the *P. citricola* complex. *P. plurivora* is widespread in forest, semi-natural ecosystems and nurseries across Europe, whereas *P. multivora* is responsible for damages in natural ecosystems in Western Australia, but it was found in Europe and also in the Czech Republic.
It was found out, that *P. plurivora* predominated over *P. multivora* (4/5 of isolates came under *P. plurivora*) in the investigated area and it was distributed in broad spectrum of altitude (162 – 611 m a.s.l.). The other species was rarely found and only in the lowest (up to 216 m a.s.l.) and warmest locations. Both species were found in nurseries, parks, forest and riparian stands and were isolated from oak, alder, willow and rhododendron. Moreover, *P. plurivora* was recovered from 13 other hosts. The morphological analysis revealed that Czech isolates of *P. multivora* differed from the Australian ones by thinner oospore wall and lower oospore wall index. Likely, it can be an adaptation to the more humid climate in Central Europe in comparison to Western Australia.
The pathogenicity test with 6 native forest trees (beech, oak, etc.) revealed that *P. plurivora* is more aggressive than *P. multivora*, but *P. multivora* also posed an important risk to European forests. Substrate specificity was detected in *P. plurivora* – the isolates from forests trees were more aggressive to them than the isolates from ericaceous plants. Likely, the two subpopulations (in anthropogenous and natural stands) were partially isolated and specialised to the accessible hosts.

Literature Cited

Jung T., Burgess T.I., 2009. Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* **22:** 95–110.
Scott P.M., Burgess T.I., Barber P.A., Shearer B.L., Stukely M.J.C., Hardy G.E.StJ., Jung T., 2009. *Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia. *Persoonia* **22:** 1–13.

Oomycete survey at two cork oak stands at Alentejo. Correlation with the declining condition

D. Neves[1], C. Maia[1], S. Durães[1], A. Cravador[2]

[1]Universidade do Algarve, Faculdade de Ciências e Tecnologia, Campus de Gambelas, 8005-139 Faro, Portugal.
[2]Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Corresponding author: neves.dina@gmail.com

Abstract

The aim of this work is to make a survey for the presence of phytopathogens in cork oak roots and associated soil in typical productive Montado Ecosystems. It is part of a wider integrative study aiming to assess the risk and the ecological sustainability of these ecosystems (RESCOE - PTDC/BIA-BEC/102834/2008). Two typical montados in Alto Alentejo, Herdade do Freixo do Meio (FM) and Gouveia de Baixo (GB) were selected and in each one two sub-areas defined according to contrasting tree aerial aspect: sub-area A with healthy, and sub-area B with declining cork oak trees and/or associated tree mortality. In each sub-area 10 healthy and 10 declining trees, were selected. The trees were labelled, photographed and GPS located in order to allow their easy identification throughout the project. Their symptoms were evaluated and the trees ranked according to their degree of defoliation: Class 0 = no symptoms (1-10% of defoliation); Class 1 = 11-25%; Class 2 = 26-60 %; Class 3 = 61-90%; Class 4 = 91-100% and trees that have died suddenly with or without leaf loss. This work has been carried out since October 2010, and samples are collected every autumn and Spring. Soil samples from the rhizosphere of each selected tree were collected. The isolation of the oomycetes was achieved by using biological baits, such as young leaves of *Q. suber* and *Q. ilex*. Briefly, 1000 ml of soil from each sample were baited with cork oak leaves. Necrosed leaves were cut, plated in selective medium (NARPH). Then each colony was observed in a light microscope and the ones that were morphologically distinct were transferred to NARPH or directly to V8 agar (V8A) to be identified. Several oomycetes were identified by ITS sequencing. *P. cinnamomi* was isolated from 73.3% and 6,7%, GBB and FMB samples respectively. Six *Pythium* species were also isolated, of which only *Pythium spiculum* is known to be pathogenic for cork oak, to a less extent then *P. cinnamomi*. *Py. spiculum* was isolated from GBA and GBB sub-areas. These results show that *P. cinnamomi* is the only aggressive soil born pathogen present in declining areas. The healthy state of the trees appears to correlate with its presence. The average degree of defoliation has increased in all sub-areas over time. *P. cinnamomi*, is an exotic highly aggressive pathogen that has not co-evolved with cork oaks, and therefore the trees have limited defenses against it. Thus, we would expect trees from sub-areas B to continue to decline.

Looking for a new quick and effective method of *Phytophthora* identification in environmental samples

J. Nowakowska[1], K. Sikora[1], T. Malewski[2], P. Bonants[3], C. Schoen[3], T. Oszako[1]

[1]*Forest Research Institute, ul. Braci Leśnej 3, 05-090 Sękocin Stary, Poland.*
[2]*Museum and Institute of Zoology (PAN) ul. Wilcza 64, 00-679 Warszawa, Poland.*
[3]*Plant Research International, Droevendaalsesteeg 1 6708PB Wageningen, The Netherlands.*

Corresponding author: j.nowakowska@ibles.waw.pl

Abstract

Contemporary forest protection needs a rapid and reliable method of identification of new invasive species both in nurseries and forest stands. Analysis of soil and water samples supplied a significant information about occurrence of fine root pathogens in Poland. Traditional baiting and morphological observations complement with physiological test and DNA analysis. Designing of real time probes specific to pathogen has advantages in nursery trials or pathological tests worth considering. So far specific probes designed for *P. plurivora*, *P. cactorum, P. alni* , *P. cambivora*, *P. quercina* and *P. pseudosyringae* were successfully tested. Two more probes prepared for *P. citrophthora* and *P. cryptogea* need to be improved as they are not specific and cross with other *Phytophthora* species. Some new techniques like Padlock probe amplification followed by micro-array analysis, Fluidigm amplification followed by 454 sequencing and Luminex multiplex analysis are tested or are under study.

The occurrence of *Phytophthora* species in European Ecological Network NATURA 2000 in Poland

I. Olejarski[1], K. Kubiak[1], J. Nowakowska[1], T. Jung[2,3], T. Oszako[1]

[1]Forest Research Institute, ul. Braci Leśnej 3, 05-090 Sękocin Stary, Poland.
[2]Phytophthora Research and Consultancy, Thomastrasse 75, Brannenburg, Germany.
[3]Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Corresponding author: I.Olejarski@ibles.waw.pl

Abstract

The research was done in Western (along Oder river) and West-Central (Krotoszyn Plateau) parts of Poland. DNA was extracted directly from soil samples (pre-incubation method) and from water (which was first filtered). In oak stands *Phytophthora quercina* and *P. plurivora* were the most common species causing damaged to fine roots. *P. cactorum* and *P. pseudosyringae* were detected, too. This put the new light on oak decline phenomenon which has been occurring in these regions since the 80's.

Ecophysiological reactions of alder (*Alnus* spp.) after the infection with *Phytophthora alni* ssp. *alni* in the field and during controlled green-house conditions

H. Pfanz, J.Mombour

Institut für Angewandte Botanik, Universität Duisburg-Essen, Campus Essen, 45117 Essen, Germany.

Corresponding author: hardy.pfanz@uni-due.de

Abstract

The new hybrid *Phytophthora alni* sp. nov. is spreading in Germany. Along rivers and creeks, surrounding bogs and lakes *Alnus glutinosa* is showing the classic symptoms of leaf desiccation and crown defoliation as well as stem cankers and bleeding. Many of the infected trees die within the following years. To study the ecophysiological effects of *P. alni* on *A. glutinosa* experiments with intact twigs, intact detached leaves and seedlings were performed in the field as well as in the laboratory. It was shown that infected trees have less and smaller leaves with a clearly reduced chlorophyll content. Photosynthetic capacity, measured either as electron transport capacity (ETR) via Chl-fluorescence or by CO_2 gas exchange was clearly reduced in infected trees. Also corticular photosynthesis was affected around artificial inoculations of stems. Following anatomical changes around the inoculation, also the chlorophyll content of the inner bark was drastically reduced. ETR measurements underlined this fact. Field observations showed a dramatic spreading of the stem infection within months. Absorptivity measurements showed clear changes in light absorption of infected and uninfected tissues of the stems. When seedlings or detached twigs were fed with extracts from infected trees, leaf wilting was observed.

A new *Phytophthora* species in ITS Clade 2 killing *Ceanothus* grown for rehabilitation of disturbed forest sites

P.W. Reeser[1], W. Sutton[1], E.M. Hansen[1], E.M. Goheen[2]

[1]*Oregon State University, Department of Botany and Plant Pathology, Corvallis OR 97331, USA.*
[2]*USDA Forest Service, Forest Health Protection, Medford, OR USA.*

Corresponding author: reeserp@science.oregonstate.edu

Abstract

Three species of *Phytophthora*, comprising *P. cactorum*, *P. pini*, and an undescribed species related to *P. himalsilva* and *P. citrophthora*, were isolated from stems and roots of dying *Ceanothus* plants (*C. sanguineus, C. integerrimus and C. velutinus*) being grown at a native plant nursery for transplanting to a mine rehabilitation site in the Siskiyou Mountains of Oregon, USA. *Ceanothus* is a genus comprising around 60 species of small trees and shrubs which are important components of many wildland ecosystems in North America. Of 20 symptomatic *Ceanothus* plants sampled, one plant yielded *P. pini*, 2 plants yielded *P. cactorum*, and 12 plants yielded the undescribed species. All three *Phytophthora* species were confirmed pathogenic on *C. sanguineus* and *C. velutinus*. It was evident from a subsequent nursery visit that damage was severe and cultural practices favored *Phytophthora* infection. To prevent similar occurrences in the future, native plant nurseries (making unregulated in-State sales) must conform to the same Best Management Practices currently used by many larger nurseries (certified for interstate shipping) to assure production of disease-free planting stock. Botanists charged with forest restoration must demand healthy stock from nurseries in the same manner as regeneration foresters now demand quality from forest tree nurseries.

Acknowledgments

We thank the Oregon Department of Forestry and the USDA Forest Service, Pacific Southwest Experiment Station and Southwest Oregon Forest Insect and Disease Center for participation in, and support of, this work.

Evaluation of biofumigant plants for control of *Quercus* root rot caused by *Phytophthora cinnamomi* in rangeland ecosystems

P. Ríos[1], S. Obregón[2], A. de Haro[2], P. Fernández[3], M.E. Sánchez[1]

[1]*Agronomy Department, University of Córdoba, Spain.*
[2]*Institute of Sustainable Agriculture-CSIC, Córdoba, Spain.*
[3]*Forestry Department, University of Córdoba, Spain.*

Corresponding author: ag1sahem@uco.es

Abstract

Root rot caused by *Phytophthora cinnamomi* is the most serious disease affecting *Quercus ilex* and *Q. suber* in oak rangeland ecosystems in southern Spain. A suitable strategy for disease control in these seminatural ecosystems is the use of biofumigant crops able to inhibit *P. cinnamomi* infections. Effects of two different genotypes of three potential biofumigant species (*Brassica carinata, B. juncea, B. napus*) on pathogen mycelial growth have been studied. Fresh plant material was collected at different phenological stages (stem extension and flowering) and macerate for direct testing, or collected and lyophilized before testing. Cultures of the pathogen plated on Carrot-Agar media were exposed to plant material (fresh or lyophilized and rehydrated) at different doses: 0 g (control), 5 g, 10 g and 20 g per plate and incubated in growth chamber at 25º C in the dark. Four different replicates were prepared for biofumigant plant material and dose. Two colony radiuses were daily measured. Data obtained were analyzed (ANOVA) and average values compared among them and with controls by Tukey's test. At the same time, glucosinolate content is being analyzed for each species, genotype and phenology tested by EU reference method (HPLC of desulphoglucosinolates).

All biofumigant treatments reduced mycelial growth of *P. cinnamomi*, but complete suppression was reached by all doses of both genotypes of *B. carinata* and *B. juncea*. A higher number of potentially biofumigant plants should be tested in order to choose the most effective ones for testing their ability to decrease the inoculum potential for root infections in artificially and naturally infested soils.

Variation in pathogenicity of *Phytophthora lateralis* and interactions with its host *Chamaecyparis lawsoniana*

C. Robin[1], W. Sutton[2], P.W. Reeser[2], E.M. Hansen[2]

[1] *INRA, UMR 1202, Forest Disease Ecology team, 69 Route d'Arcachon, 33612 Cestas Cedex, France.*
[2]*Oregon State University, Department of Botany and Plant Pathology, Corvallis OR 97331, USA.*

Corresponding author: robin@bordeaux.inra.fr

Extended Abstract

Phytophthora lateralis is an invasive pathogen which has had a severe impact in Oregon and California and which is nowadays reported in Europe (first in France and the Netherlands (Robin et al. 2011) then in Scotland). Additionally, in 2008 (Brasier et al. 2010) and again in 2010 (Webber et al. 2011), *P. lateralis* was detected in soil and in the foliage of young trees in natural old growth forests of *C. obtusa* in Taiwan, in agreement with a possible Asiatic origin for this species.
The principal host of *P. lateralis*, *Chamaecyparsis lawsoniana* (POC), is widely planted in Europe and grown in nurseries, where the root disease can spread very easily as observed in Oregon in the 50s. *P. lateralis* is thus considered as a high risk species which should be considered with caution and for which we need to develop management methods.
Several POC trees that have survived natural epidemics or in artificially infested raised beds have been tested for their resistance to *P. lateralis* (Hansen et al. 1989, Oh et al. 2006) using different inoculation procedures (Hansen et al. 2012). A program was initiated by the USDA Forest Service in cooperation with Oregon State University to derive benefits of this genetic resistance which appeared to occur in *C. lawsoniana* and to use resistant trees in forest regeneration and restoration plantings (Sniezko et al. 2012)
Brasier and colleagues (2012) gathered isolates of *P. lateralis* from all sources and showed that they formed four phylogenetically distinct lineages. Isolates from Taiwan (TWK and TWJ lineages) were morphologically and genetically distinct from isolates from North America and Europe (PNW lineage) and from a fourth group of isolates from Scotland (UK lineage). In the present work, we tested the aggressiveness and virulence of *P. lateralis* isolates from each of the newly described lineages, challenging POC trees previously demonstrated to be susceptible or resistant to Oregon isolates of the pathogen.
In repeated tests using three different inoculation methods, no evidence was found of increased aggressiveness or changed virulence among new isolates from Europe (PNW or UK lineages) or Taiwan (TWK and TWJ lineages) compared to the standard Oregon isolates (PNW lineage) that have been used in the resistance screening program. Differences in aggressiveness between *P. lateralis* isolates were expressed with a stem-wound inoculation technique and a zoospore root dip test. Specific genotype x genotype interactions were analysed with an inoculation test of five progenies of resistant or susceptible parents. In fact, the TWK isolates were significantly less aggressive than PNW isolates (Figure 1). Similarly, trees that were resistant to standard PNW isolates were resistant to new PNW lineage isolates from Europe and to isolates from Taiwan (Figure 2).

The combined results are reassuring for the resistance breeding program in the United States; the identified resistance remains durable against isolates from Taiwan that may represent the ancestral population of *P. lateralis*. The results also support the recently recognized

phylogenetic lineages within the species. The lineages differ in pathogenicity in addition to the described differences in growth and DNA sequence.

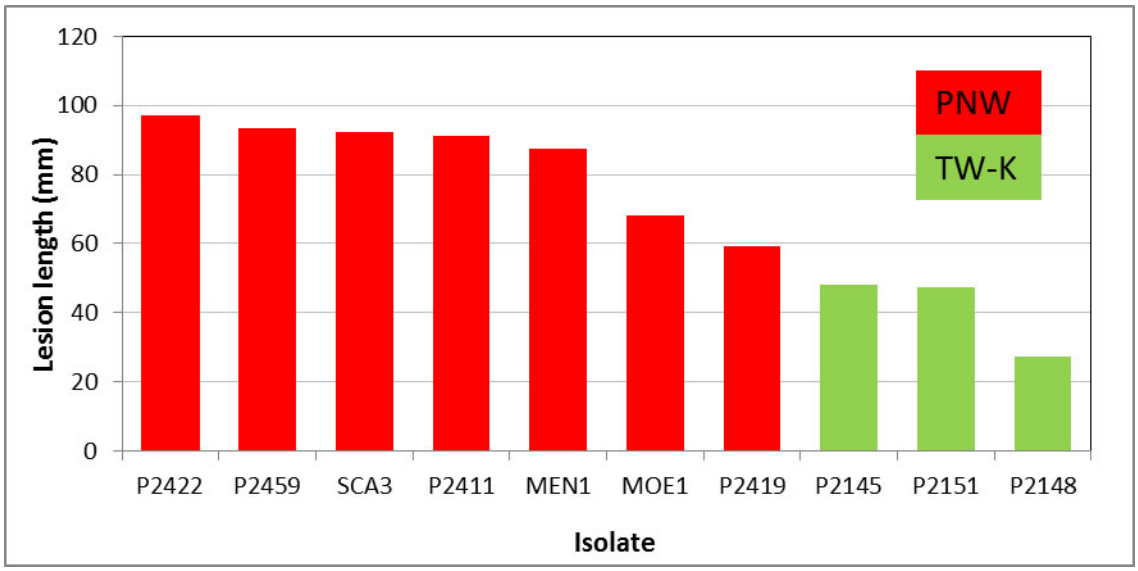


Figure 1. Lesion lengths resulting from wound inoculation of susceptible *Chamaecyparis lawsoniana* seedlings with isolates of *Phytophthora lateralis* representing two phylogenetic lineages of the pathogen.

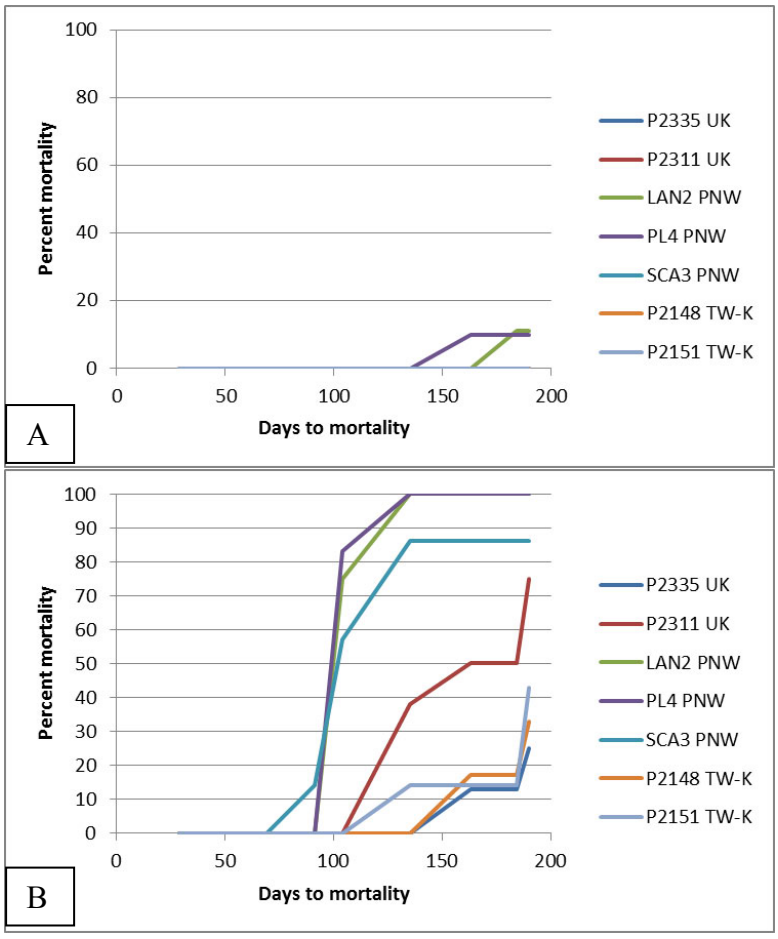


Figure 2. Mortality of *Chamaecyparis lawsoniana* seedlings representing resistant (A-117490) and susceptible (B-CON1) cedar families, with isolates of *Phytophthora lateralis* representing three phylogenetic lineages of the pathogen.

Acknowledgments

We thank Richard Sniezko and Sunny Lucas, USDA Forest Service, Umpqua National Forest, and the European COST "Forest Phytophthora" initiative for support of this work.

Literature Cited

Brasier C.M., Franceschini S., Vettraino A.M., Hansen E.M., Green S., Robin C., Webber J.F., Vannini A., 2012. Four phenotypically and phylogenetically distinct lineages in *Phytophthora lateralis.* *Fungal Biology* **116:** 1232–1249.

Brasier C.M., Vettraino A.M., Chang T.T., Vannini A., 2010. *Phytophthora lateralis* discovered in an old growth *Chamaecyparis* forest in Taiwan. *Plant Pathology* **59:** 595-603.

Hansen E.M., Hamm P.B., Roth L.F., 1989. Testing Port-Orford-Cedar for resistance to *Phytophthora*. *Plant Disease* **73:** 791-794.

Hansen E., Reeser P., Sutton W., Sniezko R., 2012. Screening Port-Orford cedar for resistance to *Phytophthora lateralis*. Pages 181-188, In: *Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry July 30 – August 5, 2011*. Palmieri, KM, tech. coord. USDA Forest Service PSW Research Station,

Oh E., Hansen E.M., Sniezko R.A., 2006. Port-Orford-cedar resistant to *Phytophthora lateralis*. *Forest Pathology* **36:** 385-394.

Robin C., Piou D., Feau N., Douzon G., Schenk N., Hansen E.M., 2011. Root and aerial infections of *Chamaecyparis lawsoniana* by *Phytophthora lateralis* : a new threat for European countries. *Forest Pathology* **41:** 417–424.

Sniezko R.A., Hamlin J., Hansen E.M., 2012. Operational program to develop *Phytophthora lateralis*-resistant populations of Port-Orford-cedar (*Chamaecyparis lawsoniana*). Pages 65-79, In: *Proceedings of the 4th International Workshop on Genetics of Host-Parasite Interactions in Forestry, Eugene Oregon.* Palmieri, K.M., tech. coord. Gen. Tech. Rep. PSW-GTR-240. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station.

Webber J.F., Vettraino A.M., Chang T.T., Bellgard S.E., Brasier C.M., Vannini A., 2012. Isolation of *Phytophthora lateralis* from *Chamaecyparis* foliage in Taiwan. *Forest Pathology* **42:** 136-143.

Evaluation of fungal secondary metabolites against *Phytophthora* spp.

B. Scanu[1], B. Linaldeddu[1], A. Franceschini[1], A. Evidente[2], L. Maddau[1]

[1]Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy.
[2]Dipartimento di Scienza del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università di Napoli Federico II, Via Università 100, 80055 Portici, Italy.

Corresponding author: lmaddau@uniss.it

Abstract

Phytophthora is the major genus within the Oomycota and it is considered one of the most serious threats for forest and natural ecosystems on a global scale. Within the disease management strategies, there is a need for new effective compounds owing to the development of fungicide resistance by pathogens and adverse effects on environmental ecosystems. Bioactive secondary metabolites of microbial origin, which have historically been of great importance in medicine and agriculture, could be expected to overcome these critical points. In this study, seven bioactive metabolites produced by phytopathogenic fungi and/or biocontrol agents were examined against several pathogenic species of *Phytophthora* isolated in natural ecosystems in Sardinia (Italy). The effects of these metabolites were assessed *in vitro* on mycelial growth, sporulation, oospores formation and zoospores motility. The fungicide metalaxyl-M (48% active ingredient) was used as positive control. All metabolites were assayed at increasing concentrations and EC_{50} values were evaluated. Interestingly, mycelial growth inhibition was observed with sphaeropsidin A, which showed to be the most active metabolites. The EC_{50} for sphaeropsidin A was ranging 1-10 µg/mL depending on the *Phytophthora* species, and it was tenfold higher than that of metalaxyl-M in some species. The EC_{50} for the other metabolites tested exceeded 100 µg/mL. Although the chemical control of these pathogens is impractical in natural ecosystems, our results may provide a basis for the development of new compounds that may have useful applications in agriculture and nurseries.

Evidence of lipophilic phytotoxic metabolites produced by *Phytophthora* spp. involved in chestnut ink disease

B. Scanu, B. Linaldeddu, A. Franceschini, L. Maddau

Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università di Sassari, Via E. De Nicola 1, 07100 Sassari, Italy.

Corresponding author: lmaddau@uniss.it

Abstract

Several species of *Phytophthora* have been proven to be involved in the chestnut ink disease. The nature of symptoms caused by these pathogens suggests that phytotoxic metabolites might be involved in the host–pathogen interaction. Furthermore, *Phytophthora* species are known to secrete proteins which play a key role in the host-pathogen interaction. Three selected isolates of *P. cambivora*, *P. gonapodyides* and *P. pseudosyringae* were examined for their ability to produce *in vitro* phytotoxic secondary metabolites. Cultures were grown in Roux bottles containing a defined liquid medium, and incubated in steady conditions at 21°C at the dark. After 30 days cultures were filtered using MF-Millipore™ membrane filters. In order to obtain the active lypophilic compounds, culture filtrates were extracted exhaustively with ethyl acetate. Culture filtrates and organic extracts along with the exhausted aqueous phase were tested on 20 days-old tomato cuttings. Culture filtrates induced symptoms of wilting after 3 days from the treatment up to 10% dilution. All organic extracts resulted active in the bioassay trials, suggesting that lipophilic metabolites could be involved in host-pathogens interaction in addition to hydrophilic metabolites. The isolation and purification of virulence factors of these *Phytophthora* species are in progress. To our knowledge, this is the first report regarding the production of lipophilic compounds by *Phytophthora* species.

Phytophthora species occurring in declining oak ecosystems in Sardinia (Italy)

B. Scanu[1], B. Linaldeddu[1], T. Jung[1,2,3], L. Maddau[1], A. Franceschini[1]

[1]Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy.
[2]Phytophthora Research and Consultancy, Thomastrasse 75, D-83098 Brannenburg, Germany.
[3]Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Corresponding author: bscanu@uniss.it

Abstract

Quercus ilex and *Quercus suber* are the main forest species in Sardinia. Since 2009 a survey on the occurrence of *Phytophthora* species has been conducted in ten oak stands. Rhizosphere soil samples were collected from symptomatic oak trees and baited using oak leaflets. In addition, bark samples were taken from lesions and cankers present on stems. Isolations were made using SMA selective medium for *Phytophthora*. Isolates were identified based on morphological characters, growth rates, cardinal temperatures for growth and ITS sequence analysis. Six *Phytophthora* species were identified, including *P. cinnamomi, P. citrophthora, P. cryptogea, P. gonapodyides, P. psychrophila* and *P. quercina*. Two unusual *Phytophthora* species are still in phase of identification since their morphological and molecular properties did not match any formally described species or informally designated taxon. *Phytophthora cinnamomi* was the most frequently isolated species. It was particularly found associated with severe decline of *Q. suber* trees. The oak-specific *P. quercina* was detected only at one site where it was causing extensive dieback of *Q. ilex* trees. The isolations of *P. citrophthora*, *P. cryptogea* and *P. psychrophila* from rhizosphere soil represent the first records of these species in *Q. ilex* and *Q. suber* stands in Italy. Pathogenicity tests are in progress in order to assess the susceptibility of both oak species to all eight *Phytophthora* species/taxa. The occurrence of some unidentified *Phytophthora* spp. suggests that many aspects related to diversity of *Phytophthora* in Sardinia and their role in the decline of oaks remain unexplored and further research is urgently required.

Development, comparison and validation of a Real-Time PCR tool for the detection of *Phytophthora lateralis*

N. Schenck[1], C. Fourrier[1], C. Robin[2], R. Ioos[1]

[1]*ANSES, Laboratoire de la Santé des Végétaux, Unité de mycologie, IFR 110, Domaine de Pixérécourt, BP 90059, 54220 Malzéville, France.*
[2]*UMR 1202 BIOGECO, INRA 69 route d'Arcachon, 33612 Cestas Cedex France.*

Corresponding author: nathalie.schenck@anses.fr

Abstract

Outbreaks of *Phytophthora lateralis* were recently identified in north-western France (Brittany) on Port-Orford Cedar trees (*Chamaecyparis lawsoniana*) planted as hedgerows in the 1970s. This soil and airborne aggressive oomycete represents a new and serious threat to European countries.
Therefore, rapid, specific and sensitive detection of the pathogen is essential. A new *in planta* detection protocol based on real-time polymerase chain reaction was developed. A *P. lateralis*-specific combination of primers and hydrolysis probe has been designed in the RAS-Ypt gene, in regions showing interspecific polymorphisms. This new test proved to be sensitive and highly specific since it does not cross react with the closely related species *P. ramorum*. The relative accuracy, specificity and sensitivity of this new tool will be evaluated in comparison with a previously published conventional PCR test and with isolation followed by morphological identification.

Literature Cited

Robin C., Piou D., Feau N., Douzon G., Schenck N., Hansen, E., 2010. Root and aerial infections of *Chamaecyparis lawsonia* by *Phytophthora lateralis*: a new threat for European countries. *Forest Pathology* **41:** 417-424.
Ioos R., Andrieux A., Marçais B., Frey P., 2006. Genetic characterization of the hybrid *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Genetics and Biology* **43:** 511-529.

Development, comparison and validation of a Real-Time PCR tool for the detection of *Phytophthora lateralis*

Nathalie Schenck[a], Céline Fourrier[a], Cecile Robin[b] and Renaud Ioos[a]
[a] ANSES, Laboratoire de la Santé des Végétaux, Unité de mycologie, Domaine de Pixérécourt, 54220 Malzéville, France
[b] UMR 1202 BIOGECO, INRA, 69 route d'Arcachon, 33612 Cestas Cedex, France

Objectives

Phytophthora lateralis is an oomycete that has been killing Port-Orford Cedars (*Chamaecyparis lawsoniana* - POC) since the 1920s in western North America. It is recommended for quarantine regulation by EPPO (A2 list) and has been recently detected in Europe, especially on POC from hedgerows in north-western France (Brittany). Lesions were observed on root and collar, but also on aerial parts of the trees (Robin *et al*, 2011). In order to implement an efficient control of the disease, a specific and sensitive detection test is needed. Conventional PCR techniques exist (Winton and Hansen, 2001, Schena *et al*, 2008) but lack specificity and/or are not fully validated.

Our objective was to develop, optimize and validate a new *P. lateralis*-specific qPCR assay for the sensitive detection of the pathogen in naturally infected samples.

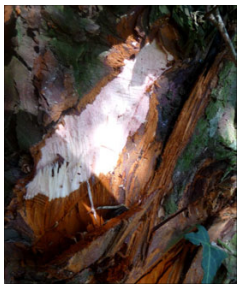


Figure 1: Trunk lesion caused by *Phytophthora lateralis*

Figure 2: *Chamaecyparis* hedgerow with aerial infection by *P. lateralis*

Design of primers and probe and qPCR conditions

Primers / Probe design

RAS-Ypt1 sequences from phylogenetically close *Phytophthora* species (e.g. *Phytophthora ramorum) were* aligned with *P. lateralis* sequences from distinct geographical origins (USA, F, UK, NL). A series of primers/hydrolysis probes combinations was screened *in silico* : length, secondary structures, intermolecular interactions and Tm were first assessed and a specific and inclusive *P. lateralis* primers / hydrolysis probe (qPlat-F/-R/-P) combination was successfully designed (Table 1).

Real-Time PCR conditions

The reactions were carried out using a Rotorgene 6000, Corbett Lifescience. Primers and probe were first tested with standard real-time PCR conditions, using the commercial Eurogentec core kit :
- Final primers and probe concentrations of 0,3 µM each.
- Final MgCl2 concentration of 5 mM.
- qPCR step parameters: 95°C for 10 min and then 40 cycles as follows: 95°C for 10 sec, 60°C for 45 sec.

As the first results obtained with these standard parameters for sensitivity and specificity tests were satisfying, they were retained for further development and validation of the assay.

Primer / probe	target	Sequence (5'-3')	5' fluorescent Label	3' non fluorescent quencher
qPlat-F	*P. lateralis*	ACGGGATCGTGTTCTAGCAG	n/a	n/a
qPlat-R	*P. lateralis*	TAGCTGCACGTCGTTGCTAC	n/a	n/a
qPlat-P	*P. lateralis*	TTTTCCCGCTTTCCTTGGGG	FAM (green)	BHQ1

Table 1: Characteristics of the primers and hydrolysis probe developed in this study

qPCR analytical sensitivity and specificity

DNA extracts were prepared from 55 *P. lateralis* isolates from different countries (USA, France, Scotland and the Netherlands), and normalised to 0.5 ng/µL. All the extracts yielded positive results with our qPCR, showing the new test was fully inclusive. In addition, the assay did not cross react neither with DNA from any of the fungi isolated from *Chamaecyparis* (22 isolates, 14 genera), nor with DNA from any other *Phytophthora or Pythium* species (24 isolates, 17 species, including 8 *P. ramorum* isolates), thus confirming the anticipated *in silico* analytical specificity.

The sensitivity of the qPCR assay and the existing conventional PCR were first tested with genomic DNAs from 3 distinct isolates. The qPCR test was able to yield consistent positive results with down to 10^{-3} or 10^{-4} ng of gDNA /PCR tube, depending on the isolate used, which was 1 log better than the conventional PCR of Schena *et al* (2006) .

The target region of the qPCR test was inserted in a PCR4 TOPO plasmid and multiplied by bacterial cloning, then purified. Standard dilutions of plasmids containing the DNA target were used to produce a standard curve.
The qPCR test successfully yielded 100% repeatable positive results with down to 47.2 copies of target DNA/PCR tube (figure 3).

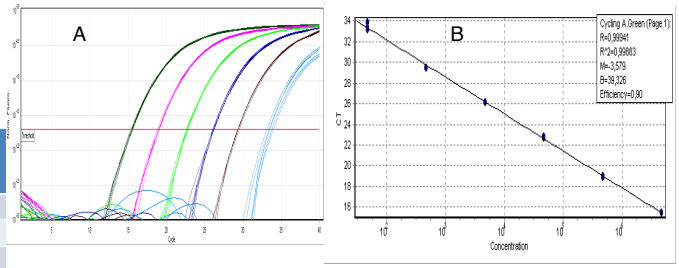


Figure 3: Raw fluorescence curves (A), standard curves (B), and correlation coefficients assessed with dilutions of plasmidic DNA (containing the *P. lateralis* target locus) to yield final concentrations ranging from 4.7 10^6copies / PCR tube to 4.7 copies / PCR tube.

What comes next?

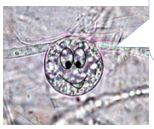
DNA extraction step to be optimized: test of different extraction kits and grinding methods.
Test of duplex qPCR to detect *P. lateralis* and 18S rDNA (assessment of the DNA extract quality).
Comparison of different detection methods (isolation, conventional PCR, new RT-PCR protocol) using naturally infected/non infected samples.
Validation procedure by ringtesting the new method (INRA Cestas).

References

Robin C, Piou D, Feau N, Douzon G, Schenck N, Hansen EM, 2011. Root and aerial infections of *Chamaecyparis lawsoniana* by *Phytophthora lateralis*: a new threat for European countries. Forest Pathology **41**, 417-424.

Schena L, Duncan JM, Cooke DEL (2008) Development and application of a PCR-based 'molecular tool box' for the identification of *Phytophthora* species damaging forests and natural ecosystems. *Plant Pathology* **57**, 64-75.

Winton LM, Hansen EM (2001) Molecular diagnosis of *Phytophthora lateralis* in trees, water and foliage baits using multiplex polymerase chain reaction. *Forest Pathology* **31**, 275-283.



...and warm thanks to Everett Hansen, Cécile Robin, Karin Rosendahl-Peters and Alexandra Schlenzig for providing their precious P. lateralis *isolates!*

anses.fr

Differential susceptibility of the commonest Andalusian morphotypes of Holm oak to *Phytophthora cinnamomi*

M.S. Serrano[1], P. De Vita[1], P. Fernández[2], M.E. Sánchez[1]

[1]*Agronomy Department, University of Córdoba. Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain.*
[2]*Forestry Department, University of Córdoba. Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain.*

Corresponding author: ag1sahem@uco.es

Abstract

The high inter and intrapopulation variability in *Quercus ilex* subsp. *ballota* led to the description of different morphotypes for this oak supspecies. Seedlings of the four main morphotypes of Holm oak present in Andalusia (*macrocarpa*, *expansa*, *microcarpa* and *rotundifolia*) were checked for their susceptibility to *P. cinnamomi* root infection. Seedlings were produced from selected acorns previously characterized as belonging to the four morphotypes and also acorns from a natural hybrid *Q. ilex ballota*-*Q. faginea* were included in artificial inoculation experiments. Plants were infected with water suspensions of *P. cinnamomi* chlamydospores added to the substrate. At the end of the experiments, the infected seedlings showed the aerial symptoms of the root disease: yellowing, wilting and in some cases crown defoliation. Root symptoms consisted in necrosis or absence of feeder roots. The four morphotypes of Holm oak could be separated in three groups according with foliar symptoms developed: very susceptible (*microcarpa*), susceptible (*expansa*) and moderately susceptible (*rotundifolia* and *macrocarpa*), but there were no great differences in root symptoms, always showing a high degree of necrosis. However, infected hybrids exhibited a low degree of foliar and root symptoms and always significantly lower than infected Holm oak morphotypes. We concluded than *Quercus* species able to hybridize with Holm oak and more tolerant to *P. cinnamomi* root infection (such as *Q. faginea*) should be considered as genitors in future breeding programs against the root disease.

Literature Cited

Serrano M.S., De Vita P., Carbonero M.D., Fernández F., Fernández P., Sánchez M.E., 2012. Susceptibility to *Phytophthora cinnamomi* of the commonest morphotypes of Holm oak in southern Spain. *Forest Pathology* **42:** 345-347.

Effectiveness of calcium and potassium fertilizers for control of *Quercus ilex* root rot caused by *Phytophthora cinnamomi*

M.S. Serrano[1], P. De Vita[1], P. Fernández[2], M.E. Sánchez[1]

[1]*Agronomy Department, University of Córdoba. Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain.*
[2]*Forestry Department, University of Córdoba. Ctra. Madrid-Cádiz Km 396, 14014 Córdoba, Spain.*

Corresponding author: ag1sahem@uco.es

Abstract

Based on the observation that the root rot caused by *P. cinnamomi* on *Q. ilex* has a low incidence and severity in soils with medium-high Ca^{2+} content, the effectiveness of different Ca^{2+} and K^{+} products (CaO, $CaCO_3$, $CaCl_2$, $Ca(NO_3)_2$, $CaSO_4$, KOH, KNO_3, KCl, KIO_3 and K_2SO_4) on mycelial growth, sporangial and chlamydospore production and sporangial germination (production of zoospores) of *P. cinnamomi* has been tested *in vitro* [1]. Although none of the products inhibited mycelial growth at pH ~ 6, CaO, $CaCO_3$, $CaSO_4$, KOH and KIO_3 effectively inhibited the sporangial production of the pathogen and therefore, zoospore production, although none of them were as effective in inhibiting the germination of already formed sporangia. CaO, $CaCO_3$, K_2SO_4 and $CaCl_2$ also inhibited the production of chlamydospores. Experiments performed in artificially infested soils treated with the most effective compounds in the *in vitro* experiments (CaO, $CaCO_3$, $CaSO_4$, KOH and KIO_3) showed that, in general, Ca^{2+} and K^{+} products induced a decrease in chlamydospore viability greater than registered in non-amended soils. Additionally, greenhouse experiments using the same infested soils showed a significant reduction in the severity of symptoms of Holm oak seedlings planted in amended soils in comparison with seedlings growing in infested but untreated soils. These results suggested that the application of Ca^{2+} amendments (mainly CaO and $CaCO_3$, but also $CaSO_4$), and even KOH to the soil in rangelands affected by the pathogen could be an effective tool against the root rot, decreasing the incidence of this serious disease. Now, these products are being tested in field conditions.

Literature Cited

Serrano M.S., De Vita P., Fernández-Rebollo P., Sánchez M.E. 2012. Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*. *European Journal of Plant Pathol*ogy **132:** 271-279.

Proteomics analysis of responses to *Phytophthora cinnamomi* in Holm oak

B. Sghaier–Hammami, J. Valero–Galvàn, M.C. Romero–Rodríguez, R.M. Navarro–Cerrillo, J. Jorrín–Novo

University of Cordoba, Department of Biochemistry and Molecular Biology and Department of Forestry, Cordoba, Spain.

Corresponding author: bf1jonoj@uco.es

Abstract

By using classical ecophysiology and biochemical techniques as well as the more recent –omics ones (proteomics, transcriptomics, and metabolomics) we do pretend to characterize natural variability as well as responses to biotic (*P. cinnamomi*) and abiotic stresses in Holm oak. Our current research and recent publications can be found at the web pages: http://www.uco.es/investiga/grupos/probiveag/; http://www.uco.es/restauracionforestal/. Data presented at the meeting will be focused on the Holm oak-*P. cinnamomi* interaction.

An overview of research activities on Sudden Oak Death (*Phytophthora ramorum*) at the Canadian Forest Service: results and progress

S.F. Shamoun[1], D. Rioux[2]

[1]Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, 506 West Burnside Road, Victoria, BC V8Z 1M5 Canada.
[2]Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., Québec, QC G1V 4C7 Canada.

Corresponding author: Simon.Shamoun@NRCan-RNCan.gc.ca

Abstract

Phytophthora ramorum (*Pr*) is an invasive alien pathogen causing a devastating disease known as sudden oak death (SOD) (= ramorum bleeding canker) of oak and tanoak trees in native forests of California and southwestern Oregon (USA). It also causes ramorum leaf blight or ramorum shoot dieback of woody ornamentals, such as rhododendron and camellia, in forests, nurseries, and garden environments. This pathogen can infect more than 120 hosts, several of which being present in Canadian forested and urban areas.The Canadian Food Inspection Agency has detected *Pr* in plants from a few retail garden centers in the Vancouver and Victoria areas of BC. Strict eradication protocols were put into effect to prevent *Pr* from spreading into the surrounding environment. Research activities at the Canadian Forest Service have been mainly carried out to better understand the biology, population genetics, and mitigation measures to help assess the risk associated with *Pr* in Canada. Our presentation will summarize results on: 1) development of DNA markers to identify the *Pr* lineages; 2) efficacy of commercial biocontrol products & fungicides against *Pr* lineages; 3) assessment of the aggressiveness and phenotypic differences among lineages of *Pr;* 4) evaluation of susceptibility of selected tree species common to eastern Canada to infection by *Pr*; 5) assessment of bioherbicidal efficacy of *Chondrostereum purpureum* registered product “Chontrol®” for control of tanoak and bay laurel resprouts in Oregon and California forests, respectively; and 6) research of putative resistance mechanisms in trees to *Pr*.

Literature Cited

Elliott M., Sumampong G., Varga A., Shamoun S.F., James D., Masri S., Grunwald N.J., 2011. Phenotypic differences among three clonal lineages of *Phytophthora ramorum*. *Forest Pathology* **41:** 7-14.

Jinek A., Simard M., Brière S.C, Watson A.K., Tweddell R.J., Rioux D., 2011. Foliage susceptibility of six eastern Canadian forest tree species to *Phytophthora ramorum*. *Canadian Journal of Plant Pathology* **33:** 26-37.

Foliar infection of *Rhododendron* by zoospores and cysts of *Phytophthora pini* Leonian

S. Shay[1], J. Eberhart[2], J. Parke[1]

[1]Dept. of Botany and Plant Pathology and [2]Dept. of Forest Ecosytems and Society, Oregon State University, Corvallis, Oregon, USA.

Corresponding author: Joyce.Eberhart@oregonstate.edu

Abstract

Phytophthora pini Leonian, recently re-established from *P. citricola* I, is a pathogen with a wide range of forest and nursery hosts. It causes foliar infections in horticultural nurseries in Oregon, where recirculating irrigation systems are common.
Detached leaf assays were conducted to determine the impact of inoculum dose and zoospore agitation on development of foliar infection of *Rhododendron.* Wounded and nonwounded leaves were dipped into suspensions of zoospores that were either untreated, mechanically agitated by vortexing, or pumped through an irrigation sprayer system. Disease severity (lesion area) and incidence (number of lesions per leaf area) were measured over seven days.
At inoculum levels of 10,000 propagules/mL, motile zoospores infected both wounded and nonwounded leaves. Vortexing or pumping resulted in zoospore encystment, and inoculation with these treatments caused disease almost exclusively on wounded leaves. Flow cytometry was used to distinguish propagule type present in motile, vortexed, and pumped inocula. SEM of leaves inoculated with encysted propagules showed germinated cysts with hyphae growing over and around stomata without entering leaf tissue until reaching a wound site.
These findings indicate the importance of zoospore motility in reaching suitable infection sites, and demonstrate the impact of zoospore encystment on disease development. This has implications for disease management in nurseries where pruning wounds are common and the pumping of infested irrigation water may influence zoospore motility.

Literature Cited

Hong C., Gallegly M.E., Richardson P.A., Kong P., 2011. *Phytophthora pini* Leonian resurrected to distinct species status. *Mycologia* **103:** 351-360.

Infection of oak (*Quercus robur*) and beech (*Fagus sylvatica*) seedlings growing in elevated CO_2 conditions with pathogenic *Phytophthora* species

K. Sikora, M. Siebyła, J. Nowakowska, T. Oszako

Forest Research Institute, ul. Braci Leśnej 3, 05-090 Sękocin Stary, Poland.

Corresponding author: t.oszako@ibles.waw.pl

Abstract

Up to 1 year-old potted oak and beech seedlings growing minimum 6 months in greenhouse chambers under 400 ppm and 800 ppm of CO_2 were infected via soil with *P. quercina* or *P. plurivora and P. cactorum,* respectively. Elevated CO_2 concentration facilitated *Phytophthora* infections probably because of stimulating fine roots growth. Contemporary climatic changes facilitate growth and infection by Oomycetes which may cause more damage to the future forest ecosystems.

An ecological role for *Phytophthora* taxon Oaksoil in western Oregon riparian alder ecosystems[2]

L. Sims[1], E.M. Hansen[1]

[1] *Oregon State University, Department of Botany and Plant Pathology, Corvallis OR 97331, USA.*

Corresponding author: simsla@science.oregonstate.edu

Abstract

Phytophthora taxon Oaksoil, an informally described ITS clade 6 *Phytophthora* species, was collected from 58 of 88 transects in riparian alder ecosystems in western Oregon, USA. Over the course of the two-and-a-half year alder health survey, ecosystem sampling was done. During 2010 stream water, soil and unwashed root samples were collected and in 2011-2012 stream water, surface sterilized roots, bark and alder leaf debris samples were collected. From a few sites attached symptomatic alder leaves from trees overhanging streams were collected and processed.

More than 500 *P.* taxon Oaksoil isolates were recovered from ecosystem samples between June and October 2010, approximately 42% of the total *Phytophthora* isolates from ecosystem samples during this time. Again during ecosystem sampling in 2011-2012, a large percentage of isolate recovery was *P.* taxon Oaksoil. Recovery of *P.* taxon Oaksoil from stream samples was possible year round. However, more isolates were recovered per liter during the summer and fall when alder leaves were accumulating in streams. In a field comparison, the proportion of *P.* taxon Oaksoil from stream water at two locations was similar to the proportion of *P.* taxon Oaksoil from alder leaf debris at these same locations, 0.57 and 0.52 respectively. In a lab study, it was found that *P.* taxon Oaksoil can sporulate and grow on dried and fresh green alder leaves and petioles floated in filtered stream water. Additionally, *P.* taxon Oaksoil was able to colonize detached alder leaves. In the field *P.* taxon Oaksoil was also easily, repeatedly and frequently isolated from fallen alder leaves but only rarely from necrotic fine roots ; < 3% of the *Phytophthora* sp. recovered from sterilized roots were *P.* taxon Oaksoil isolates. *P.* taxon Oaksoil was not recovered from attached alder leaf material > 1 m above the surface of the water or from bark samples. The combined evidence suggests that in western Oregon riparian ecosystems, *P.* taxon Oaksoil is growing and sporulating from alder leaf debris driving up propagule numbers in water. This does not mean that pathogenicity to alders is not possible.

Little is known about the roles of *Phytophthora* species in ecosystems beyond the aggressive pathogens, but data suggests *P.* taxon Oaksoil can use alder leaf debris as a carbon source and as a substrate for asexual reproduction. The main ecological role of *P.* taxon Oaksoil in western Oregon riparian ecosystems appears to be as an important saprotroph of riparian alder stream debris, a seasonally important stream inhabitant and only a minor opportunistic pathogen of alder.

Acknowledgments

Thank you to the Oregon Department of Forestry and the USDA Forest Service SW Oregon Forest Health Service Center for planning, assistance and field support. Also, thank you to the Forest Health Monitoring Program Pacific Northwest Region USDA Forest Service.

Biological pollution: *Phytophthora* species threatening forest nurseries and natural ecosystems in Portugal

M. Horta Jung[1], A. Pèrez-Sierra[2], A. Solla[3], A. Cravador[1], T. Jung[1,4]

[1]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*
[2] *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain.*
[3] *Ingeniería Técnica Forestal, Universidad de Extremadura, Avenida Virgen del Puerto 2, 10600 Plasencia, Spain.*
[4]*Phytophthora Research and Consultancy, Thomastraße 75, 83098 Brannenburg, Germany.*

Corresponding author: mhorta@ualg.pt

Abstract

Phytophthora is a major genus of plant pathogens, responsible for some of the most devastating diseases of tree species and natural ecosystems. On a global scale, more than 66 % of all fine root diseases and more than 90 % of all collar rot diseases of woody plant species are caused by *Phytophthora* species. Planting of infested nursery stock as one of the major pathways of *Phytophthora* species into agricultural and horticultural systems and into forests and other natural ecosystems is a serious case of biological pollution. In Portugal, monitoring of *P. cinnamomi* in mature holm and cork oak stands has been done with some regularity, but the true dimension of the risk posed by other *Phytophthora* spp. in natural ecosystems is unknown. Recent localized surveys in Portugal demonstrated the occurrence of *P. quercina* and *P. uliginosa* in two declining cork oak stands in Alentejo, *P. citrophthora*, *P. hydropathica* aff. and *P. parsiana* aff. in two water courses in Algarve and *P. alni* causing *Alnus* dieback along a river in Trás-os-Montes. In five Portuguese forest nurseries *P. cinnamomi*, *P. multivora*, *P. cambivora*, *P. cactorum*, *P. cryptogea* and *P. quercetorum* were also detected. In two young cork oak plantings with high levels of mortality (one pure stand and one mixed stand with *Pinus pinaster*) *P. cinnamomi* and *P. cryptogea* were detected. In Europe all these *Phytophthora* species are considered as alien invasive pathogens. It appears timely for a national *Phytophthora* survey to evaluate the Portuguese situation in order to elaborate a national management strategy.

BIOLOGICAL POLLUTION: *Phytophthora* SPECIES THREATENING FOREST NURSERIES AND NATURAL ECOSYSTEMS IN PORTUGAL

Marília Horta[1], Alejandro Solla[2], Ana Pèrez-Sierra[3], Alfredo Cravador[1] & Thomas Jung[2,4]

[1] *Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, (IBB/CGB), Plant and Animal Genomic Group, University of Algarve, Campus de Gambelas, 8005-139 FARO (Portugal)*
[2] *Ingeniería Técnica Forestal, Universidad de Extremadura, Avenida Virgen del Puerto 2, 10600 PLASENCIA (España)*
[3] *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 VALENCIA, (España)*
[4] *Phytophthora Research and Consultancy, Thomastrasse 75, 83098 BRANNENBURG (Germany)*

e-mail: ***mhorta@ualg.pt; dr.t.jung@t-online.de***

INTRODUCTION and METHODOLOGY

Phytophthora **is a major genus of plant pathogens, responsible for some of the most devastating diseases of tree species and natural ecosystems across the world. Planting of infested nursery stock as one of the major pathways of *Phytophthora* species into agricultural and horticultural systems and into forests and other natural ecosystems is a serious case of biological pollution. Preliminary surveys were made in three declining mature cork oak stands in Alentejo and Algarve, in five forest nurseries, in two young *Quercus suber* and *Pinus pinea* plantings with high levels of mortality ,in two rivers in Algarve and in a declining *Alnus glutinosa* stand along a river in Trás-os-Montes.**

Isolations from soil samples were carried out using an oak leaf baiting method and selective PARPNH agar [5,6]. Isolations from bark samples were made according to the method of Jung and Blaschke [4]. Waterways were surveyed using non-wounded young citrus leaves as *in-situ* baits. Isolates were identified by comparing colony growth patterns and morphological features with known isolates and with species descriptions in literature [2, 5, 7, 9, 10]. Morphological identification of key isolates was confirmed by molecular identification [3].

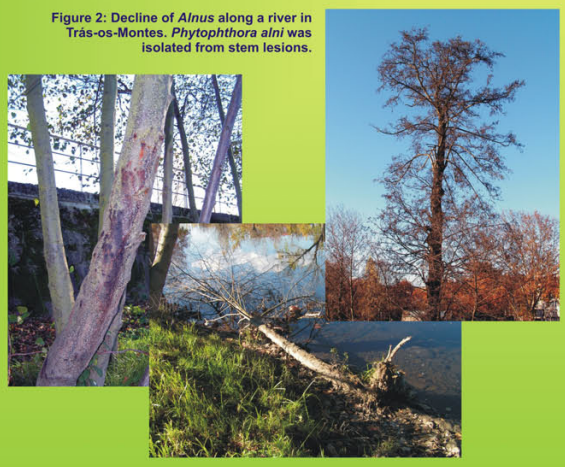
RESULTS

Phytophthora quercina **and *P. uliginosa*, respectively, were detected in two mature declining cork oak stands in Alentejo; in the declining cork oak stand in Algarve *P. cinnamomi* was identified.**

Figure 1: Slow decline of cork oak in Alentejo in the stand where *P. uliginosa* was isolated.

The results of the nursery survey are summarized in Table 1. In the two cork oak and pine plantings *P. cinnamomi* and *P. cryptogea* were recovered. *Phytophthora alni* was isolated from bleeding bark cankers of riparian *A. glutinosa* trees in Trás-os-Montes, while from the two rivers in Algarve *P. hydropathica* aff, *P. citrophthora* and *P. parsiana* aff. were baited.

Figure 2: Decline of *Alnus* along a river in Trás-os-Montes. *Phytophthora alni* was isolated from stem lesions.



Phytophthora quercina **and *P. uliginosa*, both aggressive pathogens associated with oak decline across Europe [5-7] are found for the first time in Portugal and on *Q. suber*.**

Phytophtora quercetorum**, an aggressive oak pathogen from the USA [8], is reported for the first time in Europe. *Phytophthora multivora* is also found for the first time in Portugal: it is native to Australia where it causes dieback on multiple tree species [9].**

Phytophthora cinnamomi**, *P. cambivora* and *P. cryptogea* are globally distributed aggressive pathogens which are associated with severe declines of oaks and many other tree and ornamental plant species [1, 5-7, 10].**

Phytophthora alni **ssp. *alni* is an aggressive hybrid pathogen of *Alnus* species [2, 4] which is responsible for the extensive mortality of riparian stands across Europe resulting in loss of riverbank protection.**

Figure 3: Dead holm oak plants in nurserie 2.

The role of the *Phytophthora* species found in Algarve rivers needs further research on the potential affected tree hosts present in the catchments of these watercourses.

Table 1: *Phytophthora* spp. found in five Portuguese forest nurseries

Nursery	Plant host	*Phytophthora* sp.
1	*Pinus pinea*	*P. cinnamomi* var. *parvispora* *P. cryptogea*
	Pinus pinaster	*P. cinnamomi*
	Quercus faginea	*P. cryptogea* *P. multivora*
	Quercus ilex	*P. cinnamomi*
	Quercus suber	*P. cinnamomi*
2	*Pinus pinea*	*P. cryptogea* *P. cactorum*
	Quercus ilex	*P. cactorum* *P. cambivora*
	Quercus suber	*P. cinnamomi* *P. multivora*
3	*Pinus pinea*	*P. cactorum*
	Quercus faginea	
	Quercus suber	
4	*Quercus ilex*	*P. cinnamomi* *P. cryptogea*
5	*Quercus pyrenaica*	*P. quercetorum*
	Quercus faginea	
	Quercus rubra	
	Quercus robur	

DISCUSSION

These new aggressive *Phytophthora* species most probably act synergistically with *P. cinnamomi* and cause progressive fine root destructions, predisposing the trees to droughts and accelerating their death. Moreover, in Portugal many other forest and horticultural tree species are also threatened by these *Phytophthora* spp., in particular other *Quercus* spp., *Pinus* spp., *Alnus* spp., *Castanea sativa*, *Citrus* spp., *Prunus dulcis* and *Ficus carica*.

Our findings reinforce the hypothesis that planting of infested nursery stock is one of the major pathways of *Phytophthora* species into forests and other natural ecosystems and that the international nursery trade is a primary pathway for alien *Phytophthora* species into and within Europe.

Synergistic interactions between root losses and bark infections caused by introduced soilborne *Phytophthora* species and the increasing frequency of climatic extremes are a major cause for the severe declines of oak and beech forests. Current models of climate change are predicting a further intensification of the underlying climatic trends, and a proliferation of *Phytophthora* damages may be expected, increasing the instability and vulnerability of forest ecosystems dominated by tree species susceptible to *Phytophthora*.

A national *Phytophthora* survey is urgently needed to evaluate the Portuguese situation in order to apply appropriate management and control measures.

Figure 4: Mixed planting of cork oak and *Pinus pinea*. *Phytophthoramcryptogea* was isolated from roots of dead seedlings.

References

[1] Brasier CM *et al.* (1993). Plant Pathology 42: 140-145.
[2] Brasier CM *et al.* (2004). Mycological Research 108: 1172-1184.
[3] Cooke DEL *et al.* (2000). Fungal Genetics and Biology 30: 17-32.
[4] Jung T, Blaschke M. (2004). Plant Pathology 53: 197-208.
[5] Jung T *et al.* (1999). Mycological Research 103: 785-798.
[6] Jung T *et al.* (2000). Plant Pathology 49: 706718.
[7] Jung T *et al.* (2002). Mycological Research 106: 397411.
[8] Balci Y *et al.* (2008). Forest Pathology 38: 394-409.
[9] Scott PM *et al.* (2009). Persoonia 22: 113.
[10] Erwin DC, Ribeiro OK. 1996. Phytophthora Diseases Worldwide. APS Press, 562 pp.

Acknowledgements:

FCT Fundação para a Ciência e a Tecnologia
Projecto PTDC/AGR-CFL/100217/2008

A simple, rapid and inexpensive chemical method for the detection phosphite in plant tissue

P. Stasikowski[1], D. Clark[1], J. McComb[1], B. Shearer[2], P. O'Brien[1], G.E.St.J. Hardy[1]

[1]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.
[2]Science Division, Department of Environment and Conservation, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.

Corresponding author: g.hardy@murdoch.edu.au

Abstract

Phosphite (phosphonate) is widely applied to plant communities to control the spread and impact of *Phytophthora* species in natural and peri-urban woodland and forest ecosystems. Determining (1) if phosphite applications have been successfully taken up *in planta*, (2) how phosphite is distributed around plants across seasons, and (3) when plants need to be retreated to maintain effective pathogen control is problematic due to the time and costs associated with current methods. This paper describes a direct chemical method of rapidly and effectively estimating the concentration of phosphite in plant material using a silver nitrate reagent. Glass fiber filter papers (Whatman GF/B) are saturated with acidified silver nitrate (1 M) and dried for 2 hours at 60^0C. 20 uL of a PVPP treated aqueous plant extract is then adsorbed on to the filter paper and incubated in the dark at room temperature for 1 hour. The presence of phosphite in the extract reduces the silver ions to elemental silver resulting in a grey-black precipitate that is clearly visible. The method was successfully tested on the roots and leaves of a range of exotic and Australian native plants species from different families and genera which had been treated with 0.3% phosphite. The method is rapid, sensitive and inexpensive, and can detect phosphite at concentrations of 1 mM in 20 ul of aqueous extract from 100 mg of fresh plant material, equivalent to 82 ug g^{-1} fresh weight, or 20 nmol phosphite per sample. The concentrations detected by the silver nitrate method equated well with the more expensive and less rapid HPLC method that we used to confirm the accuracy of the assay.

Calcium supplementation of soil augments the control of *Phytophthora cinnamomi* by phosphite

P. Stasikowski[1], J. McComb[1], B. Shearer[2], P. O'Brien[1], G.E.St.J. Hardy[1]

[1]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA, Australia.
[2]Science Division, Department of Environment and Conservation, Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.

Corresponding author: g.hardy@murdoch.edu.au

Abstract

Foliar application of phosphite, a systemic fungicide, to *Phytophthora cinnamomi* infected plants results in the control of disease symptoms and a reduction in the spread and impact of the pathogenic in native plant communities. Calcium ions have also been shown to affect the interaction between *Phytophthora* species and their plant hosts and to reduce the impact and spread of disease caused by soil-borne *Phytophthora* species. Calcium may enhance plant defence mechanisms or interfere with sporangial production, zoospore release and encystment on plant roots. Phosphite has been shown to have similar effects. The addition of calcium salts to soil inhibits the infection of plants by *P. cinnamomi*, and there is a correlation between the incidence in dieback disease caused by *P. cinnamomi* in natural ecosystems and the distribution of calcareous soil. This study used a susceptible Australian native plant species *Banksia leptophilia,* to investigate whether the disease control of *P. cinnamomi* by phosphite could be augmented by soil supplementation with calcium sulphate. The results showed that the effects of applying both calcium and phosphite were synergistic, and that the addition of calcium sulphate to the soil augmented and significantly prolonged the effect of foliar phosphite application. A mechanism involving the disruption of intracellular calcium signatures caused by phosphite induced accumulation of pyrophosphate in the cytosol of *P. cinnamomi* is discussed.

Phytophthora-baiting in Norwegian waterways

V. Talgø, M.L. Herrero, M.B. Brurberg, A. Stensvand

Bioforsk - Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway.

Corresponding author: venche.talgo@bioforsk.no

Abstract

In 2011, a *Phytophthora*-survey was carried out in some selected lakes, rivers and streams in southern Norway. We used rhododendron leaves from the cv. 'Cunningham's White' as baits. Prior to baiting, all leaves were surface sterilized with 70% ethanol and placed in perforated bags (2-3 leaves per bag), each with a styrofoam floater to keep the bait near the surface. The bags were anchored to the shore and left in the water for 6-8 days. All locations were recorded with a field mapping GPS-device. At many locations the leaves had dark and/or water soaked spots when removed from the water. Small sections from the leading edges of the spots were dissected and plated on *Phytophthora*-selective media (PARP or PARPH). We detected six *Phytophthora* spp.; *P. gonapodyides*, *P. lacustris*, *P. plurivora*, *P. pseudosyringae*, *P. ramorum*, and *P. syringae*. *P. plurivora* is known to damage beech (*Fagus sylvatica*) and Norway maple (*Acer platanoides*) on the west coast of Norway, *P. ramorum* has mainly been found outdoors on rhododendron, but is also confirmed on *Pieris japonica, Viburnum* spp., American oak (*Quercus* sp.), and bilberries (*Vaccinium myrtillus*) in Norway. Pathogenicity of all six *Phytophthora* spp. will be tested on beech in 2012, because *Phytophthora*-symptoms have been found on beech in the areas where we baited.

Phytophthora on trees in Norway

V. Talgø, M.L. Herrero, M.B. Brurberg, A. Stensvand

Bioforsk - Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway.

Corresponding author: venche.talgo@bioforsk.no

Abstract

During the last decade, a number of *Phytophthora* spp. have been detected from diseased conifer and deciduous trees in landscape plantings, urban forests, and Christmas tree and bough plantations in Norway. We have found *P. megasperma* on subalpine fir (*Abies lasiocarpa*) and linden (*Tilia* sp.), a *P. inundata*-like species on nordmann fir (*A. nordmanniana*), *P. plurivora* on Norway maple (*Acer platanoides*) and beech (*Fagus sylvatica*), *P. ramorum* on oak (*Quercus* sp.), *P. gonapodyides* on grey alder (*Alnus incana*), and *P. cambivora* on noble fir (*A. procera*), subalpine fir, and beech. For some of these findings we have clear indications that this is a result of contaminated, imported plants, a worldwide trend. We find it alarming that some of these pathogens have been detected in urban forests, a first step towards our natural ecosystems.

Subspecies identification of *Phytophthora alni* in riparian alder stands in the Czech Republic

M. Tomšovský[1], P. Štěpánková[2], K. Černý[3]

[1]*Mendel University in Brno, Faculty of Forestry and Wood Technology Brno, Czech Republic.*
[2]*Mendel University in Brno, Faculty of Agronomy, Brno, Czech Republic.*
[3]*Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Průhonice, Czech Republic.*

Corresponding author: tomsovsk@gmail.com

Abstract

In the Czech Republic, *Phytophthora alni* was firstly confirmed in 2001. Since the time, the pathogen has been spreading quickly and occupies almost all area of the country. The pathogen attacks *Alnus glutinosa* or *A. incana* to a lesser extent and causes considerable losses of alder trees along hundreds of kilometres of riverbanks. Aim of our work was to perform the identification of the *P. alni* isolates at subspecies level using PCR. The allele-specific PCR primers focused on allele diversity of orthologs of *ASF*-like, *TRP*1, *RAS-Ypt*, and *GPA*1 genes were selected for the identification. The 88 % of 59 analysed isolates belong to *P. alni* ssp. *alni* while 12 % of thats are *P. alni* ssp. *uniformis.*

P. alni ssp. *multiformis* has not been recorded in the country till know. The results follow expectations of more effective spreading of *P. alni* ssp. *alni* based on its higher aggressiveness and ecological advantage compared to remaining two taxa. In comparison, the scattered distribution of *P. alni* ssp. *uniformis* may represent the remains of its former occurrence. Therefore, *P. alni* ssp. *uniformis* may be an indigenous subspecies as hypothesed in literature suppressed by the more aggressive related taxon.

Literature Cited

Černý K., Strnadová,V., 2010. *Phytophthora* alder decline: disease symptoms, causal agent and its distribution in the Czech Republic. *Plant Protection Science* **46:** 12–18.
Ioos R., Andrieux A., Marçais B., Frey P., 2006. Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Genetics and Biology* **43:** 511–529.

The presence of *Phytophthora ramorum* in Greece: the risk of spread into forest ecosystems

P. Tsopelas[1], N. Soulioti[1], S.E. Tjamos[2], E.J. Paplomatas[2], E.K. Kulakiotu[3]

[1]Hellenic Agricultural Organization "Demeter"-Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28 Athens, Greece.
[2] Laboratory of Plant Pathology, Agricultural University of Athens, 75 Iera Odos, 118 55 Athens, Greece.
[3] Directorate of Rural Economy and Veterinary of Thessaloniki. Department of Quality & Phytosanitary Control, 63 Papanastasiou, 544 53 Thessaloniki, Greece.

Corresponding author: tsop@fria.gr

Extended Abstract

Introduction

Phytophthora ramorum is an important quarantine pathogen with a broad host range. Although it is mainly a pathogen of forest trees it has been spread all over Europe and North America through ornamental plant trade. EU legislation requires member states to survey for *P. ramorum* and take emergency measures for its eradication and/or containment. In Greece, the pathogen was recorded for first time in 2010 on rhododendron nursery plants imported from Belgium (Tsopelas *et al.* 2011). In the following years *P. ramorum* was also found in a few more areas of the country on nurseries only. The aim of this work is to present the current situation on the presence of *P. ramorum* in Greece and to examine the risk of disease spread into forest ecosystems of the country.

Materials and Methods

Surveys for the detection of *P. ramorum* in Greece that started in 2009 were focused on nurseries and garden centres that produce or sell ornamental plants host of *P. ramorum*. Special attention was given to nurseries that import plants from other countries. Parks and forest sites were also surveyed for this pathogen to a limited degree though. Surveys were carried out during winter, early spring and autumn. Late spring and summer periods are hot and dry in Greece and not favourable for sample collection and isolation of *P. ramorum*. Nurseries were visually inspected by trained personnel of the local Forestry and Agricultural Departments. Foliage with symptoms of *Phytophthora* leaf blight was collected from plant species, known hosts of *P. ramorum* and transferred to the laboratory.

Isolations from symptomatic leaves and shoots were performed by plating pieces of infected leaf tissue on selective PARBhy clarified (5%) V8 agar selective medium (Pimaricin 5 µg/ml, Ampicillin 250 µg/ml, Rifamycin 10 µg/ml, Benomyl 10 µg/ml, Hymexazol 50 µg/ml) (Fichtner and others 2007). In another method, pieces of leaves and shoots were plated on sterile distilled water, incubated for 24-48 hr and then examined for the presence of sporangia. In this case isolations were performed by transferring sporangia with a sterile fine needle onto selective medium. A trapping technique was also used in certain cases to examine the presence of *P. ramorum* in soil (Fichtner and others 2007), using leaves of *Rhododendron* and *Arbutus* as baits. Then isolations were performed from leaves showing symptoms of infection on the same selective medium. Cultures with *Phytophthora* phenotypic characters were subcultured on V8 agar (V8A) and potato dextrose agar (PDA) without antimicrobial amendments. Besides *P. ramorum* other *Phytophthora* species were isolated in this study. Cultures were stored in water-hemp-seed medium at 20 ^{o}C.

Identification of isolates was initially based on colony patterns, growth rates, cardinal temperatures and morphological features of hyphae, sporangia and chlamydospores (Werres and others 2001). For sporangial production plugs from cultures on V8A were transferred to Petri dishes containing soil extract, and incubated for 1-2 days at 20 ^{o}C in light conditions.

DNA extraction was performed from potato sucrose liquid still cultures. Ribosomal DNA internal transcribed spacer (ITS) sequences of approximately 875 bp long were amplified using the ITS4/5 primer pair. Comparison of amplicon sequences was carried out using MEGABLAST search for highly similar sequences already published on the Genbank database. DNA samples from a few isolates of *P. ramorum* were genotyped by the Institute for Agricultural and Fisheries Research, Belgium (Heungens and others 2011).

Results

Phytophthora ramorum was initially recorded in Greece on imported nursery plants. It was detected for first time in the spring of 2010 in a nursery in Fthiotis prefecture (central Greece), on rhododendron container-grown plants imported from Belgium. The genotype of this isolate was EU1MG4, which is mainly confined into Belgium. In 2011, *P. ramorum* was found in a nursery in northern Greece (Drama prefecture) also on rhododendron plants imported from Belgium, and this time the genotype was EU1MG1, which is the most common genotype in Europe (Table 1).

Table 1. Infestation of nurseries in Greece by *Phytophthora ramorum*

Locality	No of nurseries	Hosts	Host origin	Genotype	Year
Attica	1	*Viburnum tinus*	Greece	EU1MG1	2011
Drama	1	*Rhododendron* sp.	Belgium	EU1MG1	2011
Fthiotis	1	*Rhododendron* sp.	Belgium	EU1MG4	2010
Pelion	3	*Camellia japonica* *Viburnum tinus*	Greece		2011, 2012

Phytophthora ramorum was also found on nursery plants produced in Greece. In 2011, it was detected in a nursery in Athens (southern Greece) and two nurseries in the Pelion region of Magnesia prefecture (central Greece). The pathogen was isolated from plants of Viburnum tinus in both Athens and one of the nurseries in the Pelion region, while in the second nursery of Pelion *Camelia japonica* was the host. In 2012, *P. ramorum* was also found in the Pelion region in a third nursery on *C. japonica* (Table 1).

Discussion

The presence of *P. ramorum* in Greek nurseries seems to be sporadic; it has been detected in isolated cases only and into a very limited scale. In most cases it doesn't cause extensive damage to ornamental plants and symptoms are not very conspicuous to non-experts. Although *P. ramorum* does not pose a primary threat to plants in nurseries, infected nursery plants may provide a pathway for introducing the pathogen into natural ecosystems of Greece. Furthermore, the presence of the pathogen imposes significant economic costs to the nursery industry, due to the application of phytosanitary measures.

It is of particular concern the presence of the pathogen in nurseries of different areas of the country, located in southern, central and northern Greece. Weather during late spring and summer is hot and dry in most of the areas where nurseries are located in Greece, thus it is not favourable for the spread of *P. ramorum*; this also facilitates control measures during this period. The pathogen though can survive high summer temperatures in the form of chlamydospores (Fichtner and others 2007) and can cause new infections during autumn and spring. One area of high risk is the Pelion region in central Greece; *P. ramorum* has been found there in three different nurseries mostly on camellia plants. Climatic conditions in this region, with extended periods of moist weather and mild temperatures, are favourable for the spread of *P. ramorum*. There are many nurseries in the Pelion region producing camellia plants that are sold all over the country.

Greece has a range of climatic variations and there are many suitable habitats for the spread and establishment of *P. ramorum*. Natural forests of deciduous oaks cover a very large part of the country, constituting about one third of the high forests. Many of the oak stands grow in dry environments, which are not favourable for the establishment of *P. ramorum*, but there are many oak forests in higher elevations in moist sites which are at potential risk. Furthermore, several foliar hosts of *P. ramorum*, such as *Arbutus unedo*, *Ceratonia siliqua*, *Pistacia lentiscus*, *Rhamnus alaternus* etc. (Moralejo and others 2006), can be found in the understory of these forests. Beech is also quite common in natural forests in the highlands of northern and central Greece, in pure stands and in mixture with fir and other coniferous and broadleaved species. There is a natural forest of beech (*Fagus sylvatica*) in the high elevations of Pelion region, while *P. ramorum* has been detected in the nurseries in lower elevations of the same region.

A large part of the highlands of Greece, from south to north, is covered by natural stands of fir forests. Infections of *P. ramorum* on *Abies* spp. have been reported in Ireland, UK and United States (Anonymous 2012). It is very difficult though to predict if fir forests in Greece are at potential danger to *P. ramorum*. The recent outbreak of the disease in larch plantations of UK (Brasier and Webber 2010) shows that the behaviour of *P. ramorum* in new environments is unpredictable and this should make us particularly cautious.

Literature Cited

Anonymous, 2012. Fera list of natural hosts for *Phytophthora ramorum* with symptom and location. http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/phytophthora/documents/pRamorumHost.pdf

Brasier C., Webber J., 2010. Plant Pathology: Sudden larch death. *Nature* **466:** 824–825.

Fichtner E.J., Lynch S.C., Rizzo D.M., 2007. Detection, distribution, survival, and sporulation of *Phytophthora ramorum* in a California redwood-tanoak forest soil. *Phytopathology* **97:** 1366-1375.

Heungens K., Vercauteren A., Van Poucke K., Maes M., Werres S., 2011. Genotyping of European *Phytophthora ramorum* isolates. COST-Action FP0801 Meeting, 21–22 November 2011, Budapest.

Moralejo E., Puig M., Garcia J.A., Descals E., 2006. Stromata, sporangiomata and chlamydosori of *Phytophthora ramorum* on inoculated Mediterranean woody plants. *Mycological Research* **110:** 1323-1332.

Tsopelas P., Paplomatas E.J., Tjamos S.E., Soulioti N., Elena K., 2011. First Report of *Phytophthora ramorum* on *Rhododendron* in Greece. *Plant Disease* **95:** 223.

Werres S., Marwitz R., Man In't Veld W.A., De Cock A.W.A.M., Bonants P., de Weerdt M., Themann K., Ilieva E., Baayen R.P., 2001. *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. *Mycological Research* **105:** 1155-1165.

The presence of *Phytophthora ramorum* in Greece: the risk of spread into forest ecosystems

Tsopelas P.[1], Soulioti N.[1], Tjamos S.E.[2] Paplomatas E.J.[2], and Kulakiotu E.K.[3]

[1]*Hellenic Agricultural Organization "Demeter"-Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 115 28 Athens, Greece.*
[2]*Laboratory of Plant Pathology, Agricultural University of Athens, 75 Iera Odos, 118 55 Athens, Greece.*
[3]*Directorate of Rural Economy and Veterinary of Thessaloniki. Department of Quality & Phytosanitary Control, 63 Papanastasiou, 544 53 Thessaloniki, Greece.*

INTRODUCTION

Phytophthora ramorum was recorded for first time in Greece in 2010 on rhododendron nursery plants imported from Belgium [4]. In the following years *P. ramorum* was also found in a few more areas of the country on nurseries only.

The aim of this work is to present the current situation on the presence of *P. ramorum* in Greece and to examine the risk of disease spread into nurseries and forest ecosystems of the country.

MATERIALS & METHODS

Surveys

Surveys for the detection of *P. ramorum* started in Greece in 2009 and were focused on nurseries and garden centres that produce or sell ornamental plants host of this pathogen. Special attention was given to nurseries that import plants from other countries. Parks and forest sites were also surveyed for this pathogen to a limited degree though.

Surveys were carried out during winter, early spring and autumn. Late spring and summer periods are hot and dry in Greece and not favourable for sample collection and isolation of *P. Ramorum.*

***A** Nursery producing Camellia plants in the Pelion region, **B** Camellia plant with symptoms of infection.*

Isolation

Isolations from symptomatic leaves and shoots were performed by plating pieces of infected leaf tissue on selective PARBhy clarified (5%) V8 agar selective medium (Pimaricin 5 μg/ml, Ampicillin 250 μg/ml, Rifamycin 10 μg/ml, Benomyl 10 μg/ml, Hymexazol 50 μg/ml) [2].

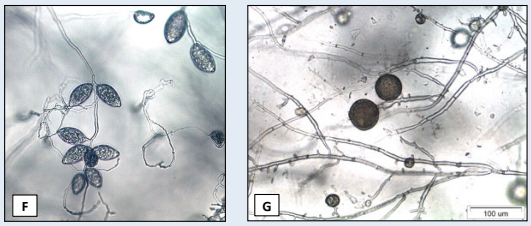
A trapping technique was also used in certain cases to examine the presence of *P. ramorum* in soil, using leaves of *Rhododendron* and *Arbutus* as baits [2].

*Isolations were performed from symptomatic leaves; **C** Rhododendron, **D** Camellia, on **E** PARBhy V8A.*

Identification

Identification of isolates was initially based on colony patterns, growth rates, cardinal temperatures and morphological features of hyphae, sporangia and chlamydospores. For sporangial production plugs from cultures on V8A were transferred to Petri dishes containing soil extract, and incubated for 1-2 days at 20° C in light conditions.

Identification was verified in many cases by amplification of DNA internal transcribed spacer (ITS) sequences (ITS4/5 primer pair) and comparison with published sequences. DNA samples from a few isolates of *P. ramorum* were genotyped by the Institute for Agricultural and Fisheries Research, Belgium [3].



***F** Sporangia, **G** Mycelia with characteristic chlamydospores of Phytophthora ramorum.*

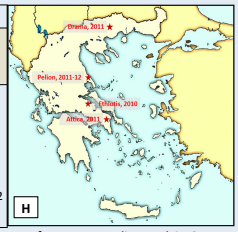
RESULTS

Phytophthora ramorum was initially recorded in Greece on imported nursery plants. It was detected for first time in of 2010 in a nursery in central Greece, on rhododendron plants imported from Belgium. The genotype of this isolate was EU1MG4, which is mainly confined into Belgium. In 2011, *P. ramorum* was found in a nursery in northern Greece also on rhododendron plants imported from Belgium, and this time the genotype was EU1MG1, which is the most common genotype in Europe (Table 1).

Phytophthora ramorum was also recorded on nursery plants produced in Greece. In 2011 and 2012, it was detected in a nursery in Athens and three nurseries in the Pelion region (central Greece), on plants of *Viburnum tinus* and *Camelia japonica* (Table 1).

Table 1. Presence of Phytophthora ramorum in Greece

Locality	No of nurseries	Hosts	Host origin	Genotype	Year
Attica	1	*Viburnum tinus*	Greece	EU1MG1	2011
Drama	1	*Rhododendron* sp.	Belgium	EU1MG1	2011
Fthiotis	1	*Rhododendron* sp.	Belgium	EU1MG4	2010
Pelion	3	*Camellia japonica* *Viburnum tinus*	Greece		2011,2012



***H** Map of P. ramorum dispersal in Greece*

DISCUSSION & CONCLUSIONS

Risk in nurseries

P. ramorum in Greek nurseries seems to be sporadic; it has been detected in isolated cases only and into a very limited scale. In most cases it doesn't cause extensive damage to ornamental plants and symptoms are not very conspicuous to non experts. Although *P. ramorum* does not pose a primary threat to plants in nurseries, infected nursery plants may provide a pathway for introducing the pathogen into natural ecosystems of Greece. Furthermore, the presence of the pathogen imposes significant economic costs to the nursery industry, due to the application of phytosanitary measures. It is of particular concern the presence of the pathogen in nurseries of different areas of the country, located in southern, central and northern Greece.

Weather during late spring and summer is hot and dry in most of the areas where nurseries are located in Greece, thus it is not favourable for the spread of *P. ramorum*; this also facilitates control measures during this period. The pathogen though can survive high summer temperatures in the form of chlamydospores [2] and can cause new infections during autumn and spring.

One area of high risk is the mountainous region of Pelion in central Greece; *P. ramorum* has been found there in three different nurseries mostly on camellia plants. Climatic conditions in this region, with extended periods of moist weather and mild temperatures, are favourable for the spread of *P. ramorum*. There are many nurseries in the Pelion region producing camellia plants that are sold all over the country.

***I, J** Mountainous region of Pelion is favourable for spread and development of P. ramorum*

Potential threat to forest ecosystems

Greece has a range of climatic variations and there are many suitable habitats for the spread and establishment of *P. ramorum*. Natural forests of **deciduous oaks** cover a very large part of the country, constituting about one third of the high forests. Many of the oak stands grow in dry environments, which are not favourable for the establishment of *P. ramorum*, but there are many oak forests in higher elevations in moist sites which are at potential risk. Furthermore, several foliar hosts of *P. ramorum*, such as *Arbutus unedo, Ceratonia siliqua, Pistacia lentiscus, Rhamnus alaternus* etc., can be found in the understory of these forests. **Beech** is also quite common in natural forests in the highlands of northern and central Greece, in pure stands and in mixture with fir and other coniferous and broadleaved species. There is a natural forest of beech (*Fagus sylvatica*) in the high elevations of Pelion region (J), while *P. ramorum* has been detected in the nurseries in lower elevations of the same region.

A large part of the highlands of Greece, from south to north, is covered by natural stands of **fir forests**. Infections of *P. ramorum* on *Abies* spp. have been reported in Ireland, UK and United States [1]. It is very difficult though to predict if fir forests in Greece are at potential danger to *P. ramorum*. However, the recent outbreak of the disease in larch plantations of UK and Ireland shows that the behaviour of *P. ramorum* in new environments is unpredictable and this should make us particularly cautious.

REFERENCES

[1] Anonymous, 2012. Fera list of natural hosts for *Phytophthora ramorum* with symptom and location. http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/phytophthora/documents/pRamorumHost.pdf
[2] Fichtner, E. J., Lynch, S. C., and Rizzo, D. M., 2007. Detection, distribution, survival, and sporulation of *Phytophthora ramorum* in a California redwood-tanoak forest soil. Phytopathology 97:1366-1375.
[3] Heungens, K., Vercauteren, A., Van Poucke, K., Maes, M., and Werres, S., 2011. Genotyping of European *Phytophthora ramorum* isolates. COST-Action FP0801 Meeting, 21–22 November 2011, Budapest.
[4] Tsopelas, P., Paplomatas, E. J., Tjamos, S. E., Soulioti, N., and Elena, K., 2011. First Report of *Phytophthora ramorum* on *Rhododendron* in Greece. Plant Disease 95:223.

Phytophthora species from native forests of Patagonia

M.L. Vélez, P.V. Silva, A.G. Greslebin

Protección Forestal, Centro de Investigación y Extensión Forestal Andino Patagónico (CIEFAP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de la Patagonia SJB (UNPSJB), Esquel, Argentina.

Corresponding author: agreslebin@ciefap.org.ar

Abstract

The diversity and ecology of *Phytophthora* species in natural ecosystems are little known. In Argentina, particularly in Patagonia, this subject has been poorly studied. The aim of this study was to contribute to the knowledge of *Phytophthora* diversity in Patagonian native forests. Sixteen streams located in mixed forests dominated by *Nothofagus* spp. and *Austrocedrus chilensis* were baited for *Phytophthora* using pears and foliage of native trees and shrubs. One hundred and fifty isolates were obtained. About 10 morphospecies were preliminarily discriminated. Species were identified by studying morphological and growth characters as well as ITS region sequences of rDNA. The most frequently isolated species was *P. syringae* (frequency 54%, detected in 12 streams), followed by *P. gonapodyides* (25%, 13 streams) and *P.* taxon pgchlamydo (10%, 6 streams). *P.* taxon syrchlamydo (frequency 1,3%, 2 streams), an unidentified species, clearly differentiated from *P. syringae* by morphology but not by the ITS sequence which differed from the *P. syringae* reference sequence in only 2 bases. Most of other species are in clade 6 and differ morphologically from *P. gonapodyides* and *P.* taxon pgchlamydo but the sequences of ITS regions were insufficient to solve the identity of these isolates which are still under study. Differences in the frequency of isolation of each species from each kind of bait were observed. A review of the *Phytophthora* species recorded in Patagonia together with short descriptions and a discussion about their phylogeny is presented.

Pyrosequencing as a tool for detection of Phytophthoras: error rate and risk of false MOTU's

A.M. Vettraino[1], P. Bonants[2], A. Tomassini[1], N. Bruni[1], A. Vannini[1]

[1]Laboratory of Mycology and Forest Pathology, University of Tuscia, Via S. C. De Lellis, snc, 01100, Viterbo, Italy.
[2]Research Institute for Plant Protection (IPO-DLO), PO Box 9060, 6700 GW Wageningen, The Netherlands

Corresponding author: vettrain@unitus.it

Abstract

The most widely used technique to identify Phytophthoras in environmental samples relies upon culture-based morphological approaches (selective media and baiting techniques). Beside the high specificity, the low sensitivity coupled to long time required to achieve the results are the main bottlenecks limiting its efficacy. Although several molecular–based approaches can circumvent many of these drawbacks, available molecular detection assays for *Phytophthora* species detect only one or few species. Next-generation sequencing (NGS) technologies offer an opportunity to overcome most of the above-described limitations. In this study pyrosequencing of partial ITS amplicons was used to describe the structure of a DNA mix consisting in 8 *Phytophthora* spp. and *Pythium vexans*. Pyrosequencing resulted in 16 965 reads, specific for specimens present in the artificial sample. A cut-off of 98% is suggested to analyse samples naturally affected by *Phytiaceae*, to absorb sequencing errors and limit the risk of false MOTUs. The pyrosequencing analysis in silico of the ITS region showed PA is a useful molecular tool for the rapid detection of *Phytophthora* spp. However, it cannot provide alone all the information need to understand the ecology of Phytophthoras but it can offer an opportunity to increase our understanding of this group of pathogens and their impact on natural and managed vegetation systems.

Biodiversity of *Phytophthora* community in a costal oak ecosystem in Italy

A.M. Vettraino, G. Natili, A. Vannini

Laboratory of Mycology and Forest Pathology, University of Tuscia, Via S. C. De Lellis, snc, 01100, Viterbo, Italy.

Corresponding author: vannini@unitus.it

Abstract

Oak decline occurs widely in Italy from north to south of the peninsula in a wide range of environmental conditions. In 2002, Vettraino et al reported the possible association between declined oak stands and the presence of *Phytophthora* species. However so far, no particular attention has been paid to the characterization of Phytophthoras community in costal oak ecosystems characterized by seasonal flooding and semi permanent pools of temporary water formed by outcrops of groundwater. In 2012 a survey was conducted in the forest of Palo Laziale (latitude 41° 55' to 41° 56' North and longitude 12° 5' to 12° 6' West, 7 m asl) in central Italy. The site represents one of the last residual of Tyrrhenian plain forest, which originally covered the coastal areas of Latium, dominated by *Quercus ilex*, *Q. cerris*, *Q. pubescens* and having *Ulmus minor*, *Fraxinus angustifolia*, *F. ornus*, *Sorbus domestica*, *Pinus pinea* as accessory species

A total of 12 *Phytophthora* spp. have been recovered. Based on phylogenetic analysis and morphological and physiological analysis, ten species and one informally designated taxon have been described. Among them some commonly recorded species in oak forests as like as *P. cryptogea*, *P. cinnamomi*, *P. gonapodyides*, *P. megasperma*, *P. plurivora* and some rare and unespected species (*P.* taxon oaksoil, *P. psychrophila*, *P. multivora P. rosacearum*). The identification of two additional *Phytophthora* species is ongoing.

Literature Cited

Vettraino A.M., Barzanti G.P., Bianco M.C., Ragazzi A., Capretti P., Paoletti E., Luisi N., Anselmi N., A. Vannini, 2002. Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. *Forest Pathology* **32:** 19–28.

Phytophthora obscura and the widespread decline of *Aesculus hippocastanum* in Europe

N.J. Grünwald[1], S. Werres[2], E.M. Goss[1,3], C.R. Taylor[4], V.J. Fieland[4], S. Wagner[2]

[1]*Horticultural Crops Research Laboratory (HCRL), USDA ARS, 3420 NW Orchard Ave., Corvallis, OR 97330, USA.*
[2]*Julius Kühn Institute - Federal Research Centre for Cultivated Plants (JKI), Institute for Plant Protection in Horticulture and Forests, Messeweg 11/12, 38104 Braunschweig, Germany.*
[3] *Current address: Department of Plant Pathology and Emerging Pathogens Institute, University of Florida, PO Box 110680, Gainesville, FL, 32611, USA.*
[4]*Department of Botany and Plant Pathology, Oregon State University, 2082 Cordley Hall, 14 Corvallis, OR 97331, USA.*

Corresponding author: stefan.wagner@jki.bund.de

Abstract

In the early nineties of the last century an unusual decline of *Aesculus hippocastanum* could be observed in the southern part of Germany. Old horse chestnut trees in public greens showed symptoms including small and pale green leaves and severe “bleeding cankers” with a cambium necrosis mainly on the stem. The *Phytophthora* isolates baited from soil samples around diseased horse chestnuts were originally thought to be *P. syringae* but shown to belong to the new species *P. obscura* Grünwald & Werres after recent reexamination. They are identical to isolates obtained from *Kalmia latifolia* leaves and from soil underneath *Pieris japonica* in the USA. Phylogenetic analysis revealed that *P. obscura* is genetically closely related to *P. syringae* and *P. austrocedrae*. Together these three taxa define a new subclade 8d of *Phytophthora*. Koch’s postulates could be fullfilled with *Kalmia latifolia*. Controlled infection trials showed that *P. obscura* is pathogenic on *Pieris, Rhododendron* and *Aesculus hippocastanum*.

Literature Cited

Werres S., Richter J., Veser I,. 1995. Studies on diseased and dead horse chestnuts (*Aesculus hippocastanum* L.) in public green spaces. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **47 (4):** 81-85.
Grünwald N.J., Werres S., Goss E.M., Taylor C.R., Fieland V.J., 2012. *Phytophthora obscura* sp. nov., a new species of the novel *Phytophthora* subclade 8d. *Plant Pathology* **61:** 610-622.

Phytophthora obscura and the widespread decline of *Aesculus hippocastanum* in Europe

S. Werres, N. Grünwaldt, E.M. Goss, C.R. Taylor, V.J. Fieland, S. Wagner
Contact: sabine.werres@jki.bund.de

Julius Kühn-Institut
Federal Research Centre for Cultivated Plants

Introduction

In Germany three periods with horse chestnut decline occured

- Circa 1990: In street allees and public parks in southern Germany
- Since circa 2000: A new decline mainly in street allees and public green
- Since 2011: First detection in nurseries (Germany)

In the following a short overview on the disease symptoms and the organisms isolated will be presented and discussed.

Horse chestnut decline in Germany (ca. 1990)[1]

Disease symptoms

- The whole crown is affected:
  - pale green leaves
  - smaller leaf size
- Bleeding mainly at the stem base
- Cambium necrosis

Fig. 1. *Phytophthora* symptoms on *Aesculus hippocastanum*
Upper row: crown symptoms
Lower row: bleeding and cambium necrosis

Occurence

Mainly in street allees and public parks

Phytophthora species isolated

- From tissue: *P. cactorum*
- From soil: *P. citricola, P. syringae*

Fulfilment of Koch's Postulate

- Possible with *P. cactorum* and *P. citricola*
- Failed with *P. syringae*
  (Incubation period: 8 weeks)

1 - Werres, S., Richter, J., Veser, J. (1995): Untersuchungen von kranken und abgestorbenen Roßkastanien (*Aesculus hippocastanum* L.) im öffentlichen Grün. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 47(4), 81-85

Re-determination of the *P. syringae* isolate from 1993[2]

In 2010/2011 the old *P. syringae* isolate from horse chestnut was studied once more together with similar isolates from *Kalmia*, *Pieris*, and *Rhododendron* isolated in the USA.

Results

Phylogenetic and mophological studies

- Genetically closly related to *P. syringae* and *P. austrocedrae*
- Morphologically closly related to *P. syringae*
- *P. obscura* sp. nov, *P. austrocedrae* and *P. syringae* define a new *Phytophthora* subclade 8d (Fig. 2)

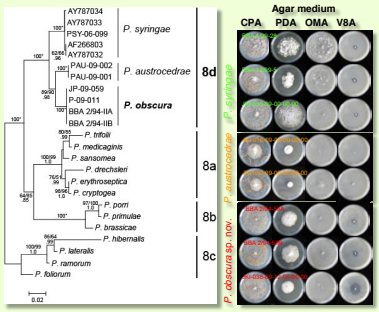


Fig. 2. Maximum likelihood phylogeny for *Phytophthora* clade 8 based on ITS sequence

Fig. 3. Colony pattern on different media

Infection studies

P. obscura caused disease symptoms on horse chestnut seedlings, but seems to need low temperatures and a long incubation period

2- Grunwald, N., S. Werres, E. Goss, C. Taylor, and V. Fieland (2012): *Phytophthora obscura* sp. nov., a new species that is a member of the novel *Phytophthora* subclade 8d. *Plant Pathology*. 61(3), June 2012, 610–622

Horse chestnut decline since ca. 2001

Disease symptoms (in Germany)

- Single dying twigs, whole crown dies
- Bleeding mainly as small spots
- Cracks in the bark
- Cambium (phloem) necrosis
- Yellow slime (rare)

But: **Symptoms vary**!

Fig. 4. Disease symptoms on *Aesculus hippocastanum* since circa 2001

Occurence

Mainly in street allees and public parks

Organisms isolated

- *Pseudomonas syringae* pv. *aesculi* (Fig.4 A,C,G,H,I)
- *Phytophthora* (very rarely) (Fig.4 F)
- Never *P. obscura*
- Very often no results (Fig.4 B,D,E)

Information on *Ps. syringae* pv. *aesculi*:
Green, S., Laue, B., Fossdal, C. G., A'Hara, S. W., and Cottrell, J. E. 2009. Infection of horse chestnut (*Aesculus hippocastanum*) by *Pseudomonas syringae* pv. *aesculi* and its detection by quantitative real-time PCR. Plant Pathology 58 (4):731-744.
Steele, H., Laue, B. E., MacAskill, G. A., Hendry, S. J., and Green, S. 2010. Analysis of the natural infection of European horse chestnut (*Aesculus hippocastanum*) by *Pseudomonas syringae* pv. *aesculi*. Plant Pathology 59 (6):1005-1013.

Discussion

- Horse chestnut shows varying disease symptoms. Some can be connected to infection with *Phytophthora* or with *Ps. syringae* pv. *aesculi*. But very often symptoms are unspecific and vary and the disease cause is uncertain.
- The role of *P. obscura* playing in horse chestnut decline under natural conditions is unclear.
- Isolation of *P. obscura* from bleeding cankers of naturally infected horse chestnuts have failed. It might be that in the past isolates were mis-determined as *P. syringae* or that isolation failed because *P. obscura* is a slow growing species
- It cannot be excluded that infection with *Ps. syringae* pv. *aesculi* and *Phytophthora* spec., perhaps also with *Verticillium* spec., the same time is possible.

Julius Kühn-Institut (JKI) – Bundesforschungsinstitut für Kulturpflanzen, Institut für Pflanzenschutz in Gartenbau und Forst (GF)
Messeweg 11/12, D – 38104 Braunschweig, Germany
www.jki.bund.de

Specificity of a Lab-on-a-Chip system for *Phytophthora* diagnosis

S. König[1], S. Wagner[1], S. Werres[1], L. Schwenkbier[2], K. Weber[2], J. Weber[3]

[1]*Julius Kühn Institut – Federal Research Center for Cultivated Plants (JKI), Braunschweig, Germany.*
[2]*Institute of Photonic Technology (IPHT), Jena, Germany.*
[3]*Analytik Jena AG, Jena, Germany.*

Corresponding author: sabine.werres@jki.bund.de

Abstract

Phytophthora monitoring in forests requires highly specific and sensitive diagnostic tools. The diagnostic techniques should give results within short time and should be not too expensive. At present samples must be brought to a diagnostic lab with specific equipment for molecular diagnosis (conventional and real-time PCR) and for direct isolation. Furthermore with the available molecular methods only limited numbers of *Phytophthora* species can be detected per run. Therefore techniques that can be used directly in the field and that can detect different *Phytophthora* species within one run would be much better diagnostic tools.

A current project aims the point-of-care-diagnosis of plant diseases caused by six EPPO listed pathogens. Therefore all public available sequences of *Phytophthora* sp. and related oomycete genera for two of the most frequently used genetic markers (ITS, COI) and one alternative marker (YPT1 *ras related protein*) were evaluated for their suitability to generate species specific molecular probes. The most suitable marker was YPT1, which showed the significantly highest genetic distances between the different species in full length and probe sequence. In a practical approach we tested specificity of YPT1 derived capture probes using cultured *Phytophthora* sp. isolates, and artificially infected plant leaf material. Virtually specific probes showed specific signals in all of the screened samples for both techniques: Lab-on-a-Chip diagnostics, and in parallel examined Real-Time PCR TaqMan assays.

Literature Cited

Julich S., Riedel M., Kielpinski M., Urban M., Kretschmer R., Wagner S., Fritzsche W., Henkel T., Möller R., Werres S., 2011. Development of a lab-on-a-chip device for diagnosis of plant pathogens. *Biosensors and Bioelectronics* **26:** 4070-4075.

Specificity of a Lab-on-a-Chip system for *Phytophthora* Diagnosis

S. König, S. Wagner, S. Werres – JKI Braunschweig, Germany
L. Schwenkbier, K. Weber, K. Kirsch – IPHT Jena, Germany
M. Hentschel, J. Weber – Analytik Jena AG, Germany

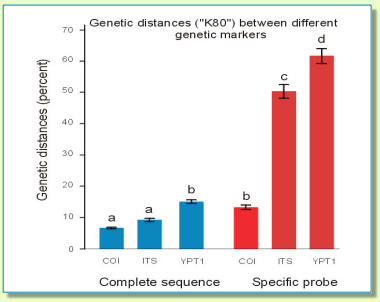
Introduction

In a three year project a Lab-on-a-chip laboratory sample based on DNA-Micro-Array technique was developed (Julich *et al.* 2011) as a primary step for field detection of *Phytophthora* species. Afterwards an additional project has been established with the aims of (i) genetic target identification, (ii) generation of *Phytophthora*-species specific hybridization Probes, and (iii) application of the technique for diagnostically issues on infected plant material. Specific probes were designed for *P. ramorum*, *P. lateralis*, *P. kernoviae*, *P. fragariae*, *P. rubi*, and *P. pinifolia*. In addition a *Phytophthora* genus specific probe, and a general plant probe serve as PCR controls on the chip. Here, the methodological development is examined on *P. ramorum*, the causal agent of the *Sudden Oak Death*, and *Larix* decline (UK).

1 - Identification of the genetic target region for probe generation

Materials and Methods

- Screening of public available sequences for genetic target regions COI[2], ITS, and YPT1[3]
- Probe target position was identified and adjusted for specificity using Sequencher 5.0 (Gene Codes Corporation, Ann Arbor, Michigan)
- COI, ITS, and YPT1 sequences of 54 *P.* species were selected according to Blair *et al.* (2008), and compared for their genetic distances to *P. ramorum* BBA 9/95 in (i) full length sequences, and (ii) the homologues positions of the best performing *P. ramorum* probes



Results

- COI and ITS showed significantly lower genetic distances than YPT1 (**Fig. 1**, blue bars)
- YPT1 probe showed the largest distances (**Fig. 1** red bars)

Fig. 1: Percent genetic distance of complete sequences (blue bars) and specific probes (red bars) of the individual genetic markers COI, ITS, and YPT1. ANOVA revealed F = 288.7, $p < 0.001$. Means with standard error. Bars with different letters vary significantly (Tukey's HSD post hoc test, $\alpha < 0.05$).

2 - Probe specificity – studies with *Phytophthora* spp. *in vitro* cultures

Materials and Methods

- *P. ramorum* YPT1-probe was screened against DNA extracts of 114 *Phytophthora*-isolates belonging to 55 species
- YPT1 PCR amplification with primers YPh1F and YPh2R[3]
- Probe specificity analyzed by hybridization at the Lab-on-a-Chip system (**Fig. 2**)

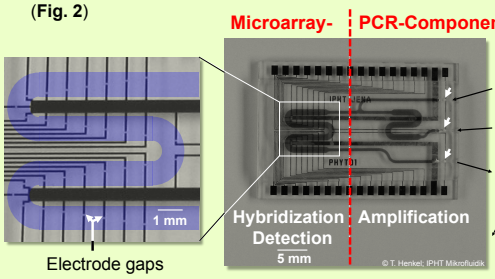
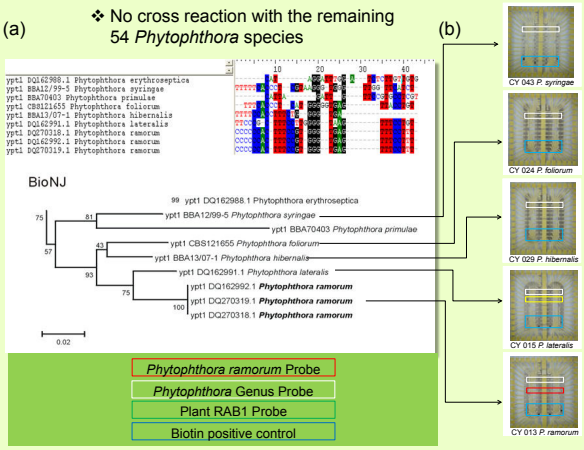


Fig. 2: Micro fluidic Lab-on-chip device used for the studies (Julich *et al.* 2011)

Fig. 3: YPT1 aligned probe of *P. ramorum* and partial complete sequence phylogram **(a),** YPT1 PCR-amplified DNA of *P. ramorum* and most related species **(b)**

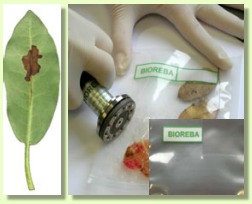
Results

- Specific detection of all 15 *P. ramorum* isolates
- No cross reaction with the remaining 54 *Phytophthora* species



3 - Probe specificity – studies with inoculated leaves

Materials and Methods



- Mesh extraction bag and hand homogenizer (Bioreba AG, Reinach, Ch) were used to ground cuts (1 cm in diameter) of artificially *P. ramorum* BBA 9/95 infected leaves (**Fig. 4**)
- DNA was extracted using magnetic beads DNA binding kits (AJ InnuScreen, Jena, Germany)

Fig. 4: Leaf tissue disruption by Mesh Bag homogenization

First Results

Positive signals showed:

- *P. ramorum* YPT1-probe
- *Phytophthora* spp. genus specific probe
- general plant RAB1 positive control

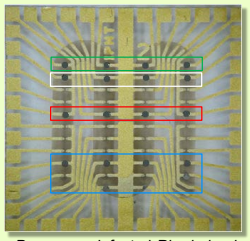


Fig. 5: Lab-on-a-chip diagnostic feature of a *P. ramorum* infected *Rhododendron* spp. leaf. For color-scale of marked frames see description under **Fig. 3b**.

Conclusion:

- YPT1 target region owns sufficient genetic distance for specific MicroArray detection of *P. ramorum* in comparison to COI and ITS.
- The YPT1 *P. ramorum* probe was species specific in the *in vitro* cultures and in first tests with artificially infected leaves.

(1) JULICH, S., M. RIEDEL, M. KIELPINSKI, M. URBAN, R. KRETSCHMER, S. WAGNER, S. WERRES, 2011: Development of a lab-on-a-chip device for diagnosis of plant pathogens. Biosens. Bioelectron. **26 (10), 4070-4075.**
(2) ROBIDEAU, G.P., A. DE COCK, M.D. COFFEY, H. VOGLMAYR, H. BROUWER, K. BALA, C.A. LEVESQUE *et al.*, 2011: DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Mol. Ecol. Resour. **11 (6), 1002-1011.**
(3) SCHENA, L. und D.E.L. COOKE, 2006: Assessing the potential of regions of the nuclear and mitochondrial genome to develop a "molecular tool box" for the detection and characterization of Phytophthora species. J. Microbiol. Methods **67 (1), 70-85.**
(4) BLAIR, J.E., M.D. COFFEY, S.Y. PARK, D.M. GEISER und S. KANG, 2008: A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. Fungal Genet. Biol. **45 (3), 266-277.**

Julius Kühn-Institute (JKI) – Federal Research Center for Cultivated Plants – Institute for Plant Protection in Horticulture and Forests
Messeweg 11/12, D – 38104 Braunschweig, Germany – eMail: sabine.werres@jki.bund.de; stephan.koenig@jki.bund.de; stefan.wagner@jki.bund.de

Federal Office for Agriculture and Food
FKZ 28-1-54.081-10
Supported by: Federal Ministry of Food, Agriculture and Consumer Protection
based on a decision of the Parliament of the Federal Republic of Germany
www.jki.bund.de

Variability in response to infection of *Phytophthora cinnamomi* in different families of *Quercus ilex*

I.L. Sánchez[1], J.J.G. Martínez[1], M.F. Martínez[1], J. Vázquez-Piqué[1], A. Cravador[2], R. Tapias Martín[1]

[1]*University of Huelva, Spain.*
[2]*Center for Mediterranean Bioresources and Food (MeditBio), Laboratory of Molecular Biotechnology and Phytopathology, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.*

Corresponding author: isabel.leon@dcaf.uhu.es

Abstract

Oak decline, a disease caused by oomycetes of the genus *Phytophthora*, causes substantial yield losses throughout the world, particularly in the southwest of the Iberian Peninsula with the very aggressive species *Phytophthora cinnamomi*. In order to reduce the impact of that pathogen in *Quercus ilex* seedlings, priority is given to genetic control through more resistant progeny. Ten plants of each family were tested. Measure of grown in new roots parameters were used as indicators of *P. cinnamomi* resistance. Resistance levels varied continuously across families from high to low values in all experiments, but family rankings were consistent among experiments. The narrow-sense heritability of the resistance character was high at both families. The resistance of holm oak to *P. cinnamomi* is under moderate genetic control. Selections of lines with high levels of resistance are feasible, and such lines can be used in rehabilitation plantings of holm oak forest sites.

Characterising the distribution of *Phytophthora* taxon Agathis (PTA) in the bark, cambium, and wood of diseased New Zealand kauri (*Agathis australis)*

M.P. Wheat[1], S.E. Bellgard[2]

[1]*Kauri Dieback Joint Agency: Private Bag 92300, Auckland 1142, New Zealand.*
[2]*Landcare Research: Private Bag 92170, Auckland Mail Centre, Auckland, 1142, New Zealand.*

Corresponding author: monique.wheat@aucklandcouncil.govt.nz

Abstract

Increased numbers of standing dead kauri *Agathis australis* are resulting from the Kauri Dieback disease caused by the root- and collar rot pathogen *Phytophthora* taxon Agathis (PTA). It is not known whether PTA is likely to be found systemically in kauri, and thus the timber may not be utilised without some knowledge of the risk of spread of the disease to healthy kauri. This knowledge gap has resulted in numerous enquiries to ascertain if dead and dying kauri can be harvested for the trees highly valued timber, for both cultural and commercial use. In order to ascertain the risks associated with the timber being a pathway for PTA spread, a direct plating method has been devised to detect the presence of PTA in the bark, cork cambium, sapwood and heartwood. To augment the direct plating approach, commercial Elisa test kits (e.g. PocketDiagnostic®) and a PTA-specific, Real Time PCR assay will be used to map the presence of PTA in late-stage (chronic-phase) diseased trees. From this empirical data, a set of phytosanitary protocols will be developed to govern the utilisation or secure disposal of diseased kauri trees.

Acknowledgments

Dr Nicholas Waipara, Stacey Hill, Jeremy Warden (Auckland Council). Dr Peter Buchanan, Elsa, Paderes, Chris Winks and Daniel Than (Landcare Research).

Literature Cited

Beever R.E., Waipara,N.W., Ramsfield,T.D., Dick,M.A., Horner,I.J., 2009. Kauri (*Agathis australis*) under threat from *Phytophthora?* Proceedings 4th IUFRO Working Party 7-02-09 "*Phytophthora* in Forests and Natural Ecosystems", 26-31st August 2007, Monterey, California, USA: 74-85.
Beever R.E., Bellgard S.E., Dick M.A., Horner I.J., Ramsfield T.D., 2010. Detection of *Phytophthora* taxon Agathis (PTA): Final Report. Landcare Research, Auckland.

Phytophthora cinnamomi infection results in changed patterns of fine root production in Scots pine

E. Siasou[1], H. Braganca[1,2], S. Woodward[1]

[1]University of Aberdeen, Institute of Biological and Environmental Sciences, Cruickshank Building, AB24 3UU, Aberdeen, UK.
[2]National Research Institute for Agriculture and Fisheries, Av. Brasilia 1449-006, Lisbon, Portugal.

Corresponding author: eleni.siasou@abdn.ac.uk

Abstract

Climate change scenarios for the near future include a higher frequency of extreme events, including floods and storms, drought, and abrupt changes between warm and cold weather conditions, coupled with a gradual increase in mean temperatures and changes in rainfall patterns. These factors will seriously impact host-parasite interactions at the individual tree, forest and landscape levels. *Phytophthora* spp. are virulent pathogens on a wide range of plant species and invasions in previously uncolonized regions have caused major problems. The UK has experienced numerous introductions of invasive alien *Phytophthora* spp. in recent years. Species in the *P. citricola* complex are present, for example. Moreover, *P. cinnamomi* was reported in Scots pine (*Pinus sylvestris*) forests in northern Scotland [1], although its impact in these ecosystems is unclear.
The impacts of *Phytophthora citricola sensu lato* and *P. cinnamomi,* inoculated independently or co-inoculated, on Scots pine under flooding conditions, simulating increased precipitation due to climate change, were examined. Inoculation with *P. citricola* and *P. cinnamomi*, coupled with periodic inundation, led changes in several parameters measured. Root morphology, biomass, and disease severity on the roots changed with time following treatment during interactions with *Phytophthora* spp., although very few plants were killed by these treatments.

Literature Cited

Chavarriaga D., Bodles W.J.A., Leifert C., Belbahri L., Woodward S., 2007. *Phytophthora cinnamomi* and other fine root pathogens: a threat to north temperate pine forests? *FEMS Microbiological Letters* **276**: 67-74.

Phytophthora plurivora and other Oomycota in an Aberdeenshire watershed

D. Belo[1], D. Nianiaka[1], P. van West[2], L. Belbahri[3], I. Baccelli[1,3], E. Siasou[1], S. Woodward[1]

[1]*University of Aberdeen, Institute of Biological and Environmental Sciences, Cruickshank Building, Aberdeen AB24 3UU, UK.*
[2]*University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK.*
[3]*Laboratory of Soil Biology, Institute of Biology, University of Neuchâtel, CH-2000 Neuchâtel, Switzerland .*

Corresponding author: s.woodward@abdn.ac.uk

Abstract

Oomycota diversity present in the Waters of Feugh, Aberdeenshire was assessed using baiting with leaves, rice grains and green pepper. Oomycota were recovered from all 12 sites sampled, from the upper, open moorland, down through forested and agricultural zones, to a point near the confluence with the River Dee. Isolates obtained were identified using morphological and molecular methods. *Phytophthora plurivora* was present in a section of the river surrounded by pasture and crop land, with riparian woodland. Numerous *Pythium* species were recovered, mainly *Py. undulatum* and, *Py. diclinum.* An unknown *Pythium* species was partially identified. The diversity of Saprolegniales included *Saprolegnia diclina*, *S. hypogyna* and *Achlya colorata.* A possible novel species of *Pythiopsis* and an unrecognized Oomycete were also detected. No direct influence of the surrounding land use on the distribution of these species was determined. Current work is focused on determining changes in Oomycota populations with time in Aberdeenshire watersheds and on the identities of the unknown species.

Quantification of infection and colonization of holm oak (*Quercus ilex*) roots by *Phytophthora cinnamomi* using histological methods

F.J. Ruiz-Gómez[1*], R. Sánchez-Cuesta[1], R.M. Navarro-Cerrillo[1], A. Pérez-de-Luque[2]

[1]Evaluación y Restauración de Sistemas Agrícolas y Forestales (RNM360). Departamento de Ingeniería Forestal. E.T.S. Ingeniería Agronómica y de Montes, Universidad de Córdoba. Córdoba, Spain.
[2]Instituto de Investigación y Formación Agraria y Pesquera de Andalucía-IFAPA, Centro "Alameda del Obispo", Área de Mejora y Biotecnología, Avda. MenédezPidal s/n, PO Box 3092, Córdoba, 14004 Spain.

Corresponding author: g72rugof@uco.es

Abstract

Phytophthora cinnamomi Rands. is an important pathogen widely distributed in the Northern Hemisphere, with a broad host range. Among others diseases, it is known for its important contribution, together with *Pythium spiculum,* to the rot root, this being considered a main factor involved in the decline of holm oak and cork oak, the most important tree species in the "dehesa" ecosystem of South-Western Spain. The life cycle of these pathogens greatly hampers the study of the interaction within their natural ecosystem, being necessary the development of experiments under controlled conditions in order to unveil the host-pathogen interaction mechanisms. An experiment inoculating *P. cinnamomi* (Pe-90 strain) in *Quercus ilex* L. roots growing on inert substrate was carried out. Semithin root sections of 0.2 µm thickness were obtained and stained with Toluidine Blue-O (TBO). Images were captured and digitally treated to identify the areas corresponding to the different pathogen structures and plant tissues of the sections. Several areal indexes for extracellular, intracellular and survival pathogen structures were obtained and their timeline evolution and spatial development were correlated with the visual observation of the infection and colonization process. *P. cinnamomi* explore external root tissues through the apoplast, reaching parenchymatous and vascular tissues of central cylinder, and using these last tissues for nutrient supply and to extend into new root areas. The studied indexes would be a useful tool for studies focusing on differences between treatments or resistance levels, and the specific responses of the host against the pathogen affecting *P. cinnamomi* development.

New hypothesis on the ploidy of the hybrid species *Phytophthora alni* subsp. *alni*

C. Husson, J. Aguayo, P. Frey, B. Marçais

[1]*INRA, UMR1136 INRA Université de Lorraine "Interactions Arbres/Micro-organismes", IFR110 EFABA, Centre INRA de Nancy, 54280 Champenoux, France.*

Corresponding author: claude.husson@nancy.inra.fr

Abstract

Alder decline caused by the *Phytophthora alni* complex is one of the most important diseases in forest and riparian ecosystems in Europe in the last 20 years. The emergence of *Phytophthora alni* subsp. *alni* (Paa), the pathogen responsible for the epidemics, is linked to an interspecific hybridization event between two parental species: *Phytophthora alni* subsp. *multiformis* (*Pam*) and *Phytophthora alni* subsp. *uniformis* (Pau) (Brasier et al. 2004, Ioos et al. 2006). One of the parental species, *Pau* that has been isolated in several European countries and in North America (Alaska and Oregon), is exotic to Europe and is a diploid species (Aguayo et al. 2013). *Pam* possesses a polyploid genome and should normally be tetraploid (Ioos et al. 2006). In this study, our aim was to determine the ploidy of the hybrid species Paa using flow cytometry and Real-Time PCR. Firstly, flow cytometry analysis on zoospore suspension allows us to compare the genome size of the three species. The results showed that DNA content of Paa is equal to half of the sum of DNA content of Pau and Pam. Secondly, we designed allele-specific primers and probes for three single copy nuclear genes. Using real-time PCR, we demonstrated that the number of copies of each allele is approximately 2 fold higher in the parental species than in the hybrid species. Both results are consistent and indicate that Paa contains half of the genome of each parental species and is therefore a triploid species, suggesting a chromosome loss following the hybridization process. This is consistent with a low level of oospore viability and the fact that oospore germination has never been observed (Delcan and Brasier 2001).

Literature Cited

Aguayo J, Adams GC, Halkett F, Catal M, Husson C, Nagy ZA, Hansen EM, Marçais B, Frey P, 2013. Strong genetic differentiation between North American and European populations of *Phytophthora alni* subsp. *uniformis*. Phytopathology **103:** 190-199.

Brasier CM, Kirk SA, Delcan J, Cooke DEL, Jung T, Man in´t Veld WA, 2004. *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogen spreading on *Alnus* trees. Mycological Research **108:** 1172–1184

Delcan J, Brasier CM, 2001. Oospore viability and variation in zoospore and hyphal tip derivatives of the hybrid alder Phytophthoras. Forest Pathology **31:** 65-83

Ioos R, Andrieux A, Marçais B, Frey P, 2006. Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. Fungal Genetics and Biology **43:** 511-529.

New hypothesis on the ploidy of the hybrid species *Phytophthora alni* subsp. *alni*

[1]Husson C. , [1]Aguayo J. , [2]Révellin C. , [1]Marçais B. and [1]Frey P.

[1]INRA, UMR1136 INRA Université de Lorraine "Interactions Arbres/Micro-organismes", IFR110 EFABA, Centre INRA de Nancy, 54280 Champenoux, France
[2]INRA, UMR1147 INRA Université de Bourgogne "Agroécologie" , Centre INRA de Dijon, 21000 Dijon, France
claude.husson@nancy.inra.fr

INTRODUCTION

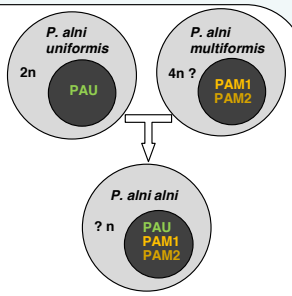
Foliage symptoms on alder

Flame-shaped bark lesion

Alnus glutinosa (common alder) is an important species of riparian ecosystems. At the beginning of the 1990s, a new lethal disease was described in the United Kingdom in riparian populations and is now a serious concern for the management of the riverbanks throughout Europe.

The causal pathogen, named *Phytophthora alni* subsp. *alni* (*Paa*), is a hybrid species between *Phytophthora alni* subsp. *multiformis* (*Pam*) and *Phytophthora alni* subsp. *uniformis* (*Pau*). One of the parental species, *Pau,* which is present in Europe and North America but probably exotic to Europe, is supposed to be a diploid species. *Pam* possesses a polyploid genome and is thought to be tetraploid (Brasier et al. 2004, Ioos et al. 2006, Aguayo et al. in press).

In this study, our aim was to determine the relative **genome size** and the **ploidy level** of the hybrid species *Paa* using **flow cytometry** (D'Hondt et al. 2011) and **Real-Time PCR**.



Hybridization event: based on 4 nuclear genes, *Paa* possesses at least 1 copy of alleles PAU, PAM1 and PAM2 from its parental species (Ioos et al. 2006)

MATERIALS and METHODS

1- Preparation of zoospore suspensions in Tris-EDTA buffer
- Production of sporangia in river water from 3 days old colony of *P. alni*. Addition of cold ultra-pure water in place of river water to release zoospores. Vortexing of zoospore suspensions, storage for 1 h at 4°C (encystment)
- Filtration of suspensions using 10 µm pore membrane filters to trap zoospores. Incorporation of filters in a new tube containing Tris-EDTA buffer and vortexing to release zoospores
- Zoospore concentrations measured by using a haemocytometer

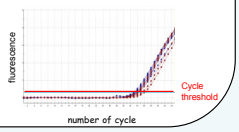
sporangia

Release of zoospores

Fluorescent staining of nuclear DNA in zoospores

2- Flow cytometry analysis
- Addition of Rnase into zoospore suspensions and staining nuclear DNA content with Propidium Iodide
- Measurement of fluorescence emission using a flow cytometer (Cyflow blue, Partec)
- Relative genome sizes calculated from the ratios between the peak position of each *Phytophthora* species

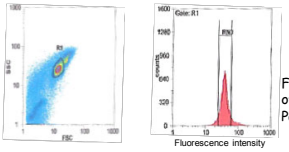
3- Allele-specific real-time PCR
- Design of 9 allele-specific primer pairs and probes for three single copy nuclear genes: *ASF*-like, *GPA1*, *RAS-Ypt*
- DNA extraction of calibrated zoospore suspensions for 6 isolates per species
- Quantitation of the number of copies of alleles for each gene using real-time PCR (Taqman®)



RESULTS

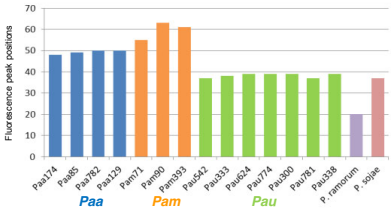
Comparison of genome size using flow cytometry

Genome size estimation is based on the comparison of the amount of fluorescence emitted by DNA stained with an intercalating fluorochrome (PI)



Gate R1 represents the zoospore population

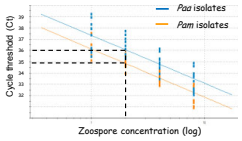
Fluorescence peak of the isolate Pau300

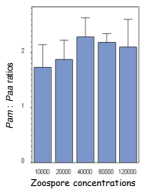


⇨ Total nuclear DNA content: ***Paa*** = (***Pam*** + ***Pau***) x 0.5

Mean (95% CI) = 0.49 (0.45-0.53)

Determination of ploidy level using real-time PCR





Pam : *Paa* ratios for the allele PAM1 of *GPA1* gene

- Quantitative PCR on the three alleles (PAU, PAM1, PAM2) per gene
- Comparison between *Pau* or *Pam* and *Paa* of the amount of amplified DNA
  ⇒ Calculation of *Pam* : *Paa* and *Pau* : *Paa* ratios
- A difference of 1 Ct represents a 2 fold higher DNA quantity

	Pau or *Pam* : *Paa* ratios		
genes	Allele PAU	Allele PAM1	Allele PAM2
ASF-like	1.9	2.0	1.9
GPA1	2.6	2.0	2.2
RAS-Ypt	2.5	2.1	1.9
Mean (95%CI)	*2.3 (1.9-2.7)*	*1.9 (1.7-2.2)*	

⇩

The number of copies of each allele is approximately **2 fold higher** in the parental species than in the hybrid species

CONCLUSION

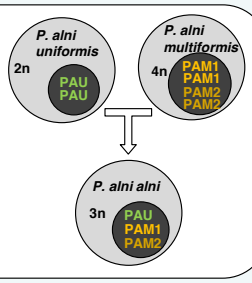
Results of both methods are consistent and indicate that *Paa* contains **half of the genome** of each parental species.

Thus, hybridization led to a reduction in chromosome number, as in the case of homoploid hybridization. As a result, the hybrid *Paa* is most probably a **triploid species**.

This ploidy level may explain that oospores viability of *Paa* is low and that no germination was observed (Delcan and Brasier 2001).

Such a ploidy level in hybrid species has already been described in plants species (Palop-Esteban et al. 2007).

Determination of the ploidy level is of fundamental importance to characterize the population genetic structure which is mostly based on allele frequencies.



Aguayo, J., Adams, G. C, Halkett, F., Catal, M., Husson, C., Nagy, Z. A., Marçais, B and Frey, P. Strong genetic differentiation between North American and European populations of *Phytophthora alni* subsp. *uniformis*. Phytopathology (in press)
Brasier C.M., Kirk S.A., Delcan J., Cooke D.E.L., Jung T. and Man in't Veld W.A. *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogen spreading on *Alnus* trees. Mycological Research, 108: 1172–1184 (2004)
D'Hondt L., Höfte M., Van Bockstaele E. and Leus L. Applications of flow cytometry in plant pathology for genome size determination, detection and physiological status. Molecular Plant Pathology, 12: 815–828 (2011)
Delcan J. and Brasier C. M. Oospore viability and variation in zoospore and hyphal tip derivatives of the hybrid alder Phytophthoras. Forest Pathology, 31: 65-83 (2001)
Ioos, R., Andrieux, A., Marçais, B. and Frey, P. Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. Fungal Genetics and Biology, 43, 511-529 (2006).

ANNOUNCEMENTS

Forest Phytophthoras, a new international journal and website

J. Parke[1], J. Eberhart[2]

[1]Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, USA.
[2]Dept. of Forest Ecosytems and Society, Oregon State University, Corvallis, Oregon, USA.

Corresponding author: Jennifer.Parke@oregonstate.edu

Oomycete plant pathogens in the genus *Phytophthora* threaten the biodiversity and sustainability of forest ecosystems worldwide. The new online journal, Forest Phytophthoras, was created to provide a permanent site for publication of peer-reviewed website articles with digital object identifier (DOI) numbers for archiving and retrieval. The journal provides immediate open access on the principle that making research freely available to the public supports a greater global exchange of knowledge. The journal is available at http://journals.oregondigital.org/ForestPhytophthora/issue/view/261.
The website, Forest Phytophthoras of the World [www.ForestPhytophthoras.org], is an international resource where scientists, students, forest managers, regulators, policy makers and the public can share the latest information on species of *Phytophthora* that affect the world's forests. Management and educational materials for each species are included, often in multiple languages. Other website features include a disease finder, an illustrated glossary, a photo gallery, and a section on *Phytophthora* basics. A searchable reference system with links to scientific publications is available.
The "What's New" column highlights recent publications and news releases pertaining to forest Phytophthoras, and a calendar section announces conferences and other events. Links are provided to a list of *Phytophthora* experts worldwide, archived conference proceedings for the IUFRO Working Party on Phytophthoras in Forest and Natural Ecosystems, proceedings of the Sudden Oak Death Science Symposia, and other *Phytophthora* web resources.
Funding for both projects is provided by the USDA Forest Service PSW Research Station. The journal is hosted by a joint project of Oregon State and University of Oregon Libraries (OJS at OregonDigital.org).

Morphological-Molecular ID Tools of Phytophthora: Lucid & Tabular Keys and Sequencing Analysis

Z.G. Abad[1], Y. Balci[2], M.D. Coffey[3], S. Kang[4]

[1]USDA-APHIS-PPQ-Center for Plant Health Science and Technology (CPHST) Beltsville,MD,USA.
[2]Department of Plant Science and Landscape Architecture, University of Maryland, MD, USA.
[3]World Oomycetes/Phytophthora Collection, Department of Plant Pathology and Microbiology, University of California, Riverside, CA, USA.
[4]Phytophthora Database, Pennsylvania State University, PA, USA.

Corresponding author: gloria.abad@aphis.usda.gov

The genus Phytophthora currently contains 121 species with 67 of them having been described during the last 15 years. It is expected that the number will continue to increase, potentially reaching up to 200-600 spp. Many species are responsible for severe damage to crops and natural ecosystems. Although considerable progress on the taxonomy of the genus has been achieved in the last ten years, accurate identification of species is still challenging in part because of unresolved species complexes. Accurate identification of species is fundamental not only for disease management but also for the implementation of regulatory measures to prevent pathogen spread. Given the rapidly increasing international trade, rapid responses based on accurate pathogen identification are critical for protecting agriculture and natural ecosystems from devastating disease. We have used morphological and molecular data from types and well-authenticated species to build a robust identification resource for all 121 reported species (expandable to cover new species). This resource includes interactive Lucid and Tabular identification keys, images, diagrams, fact sheets, and DNA sequence-based identification platform that is linked with Phytophthora Database (http://www.phytophthoradb.org). The Identification Technology Program at the USDA-APHIS-CPHST is consulting with our team to complete this resource. Taxonomic support of Phytophthora will be enhanced with the ID tools that we are developing. The ID Tools will be available online and will be updated frequently to cover well-authenticated newly discovered species. We expect the tools will be used by national and international scientists particularly those working in regulatory programs.

Literature Cited

Brasier C.M., 2009. Phytophthora Biodiversity: How many Phytophthora species are there?. Proceedings of the Fourth Meeting of IUFRO Working Party S07.02.09: 101-115.
Martin F.N., Abad Z.G., Balci Y., Ivors K., 2012. Identification and Detection of Phytophthora: Reviewing our Progress, Identifying our Needs. *Plant Disease* **96:** 1080-1103.

LIST OF PARTICIPANTS

Z GLORIA ABAD
USDA-APHIS-PPQ-CPHST Beltsville, USA
gloria.abad@aphis.usda.gov

PALOMA ABAD CAMPOS
Universitat Politècnica de València, SPAIN
pabadcam@eaf.upv.es

ANGEL ACEDO
Instituto del Corcho, la Madera y el Carbón Vegetal, SPAIN
angel@iprocor.org

SONIA AGHIGHI
Murdoch University, AUSTRALIA
S.Aghighi@murdoch.edu.au

OGUZHAN ANGAY
Technische Universität München, GERMANY
angay@wzw.tum.de

KAMYAR ARAM
University of California at Davis, USA
kamaram@ucdavis.edu

FRANS ARENTZ
Private Consultant, AUSTRALIA
arentz@activ8.net.au

YILMAZ BALCI
University of Maryland, USA
ybalci@umd.edu

STANLEY BELLGARD
Landcare Research Limited, NEW ZEALAND
bellgards@landcareresearch.co.nz

JOHANNA BOBERG
Swedish University of Agricultural Sciences, SWEDEN
Johanna.Boberg@slu.se

CLIVE BRASIER
Forest Research, UNITED KINGDOM
clive.brasier@forestry.gsi.gov.uk

TREENA BURGESS
Murdoch University, AUSTRALIA
t.burgess@murdoch.edu.au

SANTA OLGA CACCIOLA
Dipartimento dei Sistemi Agroalimentari e Ambientali- University of Catania, ITALY
olgacacciola@unict.it

DAVID CAHILL
Deakin University, AUSTRALIA
david.cahill@deakin.edu.au

PAOLO CAPRETTI
Dipartimento Biotecnologie agrarie, Università di Firenze, ITALY
paolo.capretti@unifi.it

MARIA DOLORES CARBONERO MUÑOZ
University of Cordoba, SPAIN
ir2camum@uco.es

ENRIQUE CARDILLO
Instituto del Corcho, la Madera y el Carbón (IPROCOR), SPAIN
enrique@iprocor.org

SANTIAGO CATALA GARCIA
Universitat Politècnica de València, SPAIN
sangarca@epsg.upv.es

THOMAS CECH
Federal Research Centre for Forests, Natural Hazards and Landscape, AUSTRIA
thomas.cech@aon.at

ANNE CHANDELIER
Walloon Agricultural Research Centre, BELGIUM
chandelier@cra.wallonie.be

RICHARD COBB
University of California Davis, USA
rccobb@ucdavis.edu

ANA CRISTINA COELHO
Universidade do Algarve, PORTUGAL
acoelho@ualg.pt

IAN COLQUHOUN
Alcoa, AUSTRALIA
ian.colquhoun@alcoa.com.au

DAVID COOKE
The James Hutton Institute, UNITED KINGDOM
david.cooke@hutton.ac.uk

TAMARA CORCOBADO SÁNCHEZ
Universidad de Extremadura, SPAIN
tamicorsa@hotmail.com

ALFREDO CRAVADOR
Centre of Genomics and Biotechnology, PORTUGAL
acravad@ualg.pt

RONALDO DALIO
Technical University of Munich, GERMANY
ronaldobio@hotmail.com

LOURDES DE LA MATA SAEZ
Agri-Food and Biosciences Institute (AFBI), Belfast, UNITED KINGDOM
lourdes262@hotmail.com

PAOLO DE VITA
University of Cordoba, SPAIN
er2vivip@uco.es

GEOFFREY DENTON
The Royal Horticultural Society, UNITED KINGDOM
geoffdenton@rhs.org.uk

MARGARET DICK
Scion, New Zealand Forest Research Institute, NEW ZEALAND
margaret.dick@scionresearch.com

JULIO DÍEZ CASERO
University of Valladolid, SPAIN
jdcasero@pvs.uva.es

HATICE TUGBA DOGMUS-LEHTIJARVI
Suleyman Demirel University, TURKEY
tugbadogmus@sdu.edu.tr

PILAR FERNÁNDEZ REBOLLO
Universidad de Córdoba, SPAIN
ir1ferep@uco.es

SYLVIA FERNÁNDEZ-PAVÍA
UMSNH, MEXICO
fernandezpavia@hotmail.com

FRANK FLEISCHMANN
Technical University of Munich, Section Patholoy of Woody Plants, GERMANY
fleischmann@wzw.tum.de

SUSAN FRANKEL
USDA-Forest Service, Pacific Southwest Research Station, USA
sfrankel@fs.fed.us

MATTEO GARBELOTTO
University of California, Berkeley, USA
matteog@berkeley.edu

LUIS VENTURA GARCÍA FERNÁNDEZ
Instituto de Recursos Naturales y Agrobiología de Sevilla, SPAIN
ventura@cica.es

ALMA MARÍA GARCÍA
Universidad de Córdoba, SPAIN
almariagarcia@gmail.com

SIBDAS GHOSH
Dominican University of California, USA
sibdas.ghosh@dominican.edu

GEORGE GILL
New Zealand Ministry for Primary Industry, NEW ZEALAND
George.Gill@mpi.govt.nz

BEATRICE GINETTI
University of Florence, ITALY
beatrice.ginetti@unifi.it

DONALD GOHEEN
USDA Forest Service (Retired), USA
edgoheen@jeffnet.org

ELLEN GOHEEN
USDA Forest Service, USA
egoheen@fs.fed.us

LORENA GOMEZ APARICIO
IRNAS-CSIC, SPAIN
lorenag@irnase.csic.es

NIKLAUS GRUNWALD
USDA ARS, USA
grunwaln@science.oregonstate.edu

EVERETT HANSEN
Oregon State University, USA
hansene@science.oregonstate.edu

MASUM HAQUE
University of Valladolid, USA
mhaque@pvs.uva.es

PABLO HARDOIM
University of Algarve, PORTUGAL
prhardoim@ualg.pt

GILES HARDY
Murdoch University, AUSTRALIA
g.hardy@murdoch.edu.au

ANNA HARRIS
Forestry Research, UNITED KINGDOM
anna.harris@forestry.gsi.gov.uk

MARKÉTA HEJNÁ
The Silva Tarouca Research Institute for Landscape and Ornamental Gardening, CZECH REPUBLIC
hejna@vukoz.cz

BEATRICE HENRICOT
The Royal Horticultural Society, UNITED KINGDOM
beatricehenricot@rhs.org.uk

MARIA LUZ HERRERO
Bioforsk - Plant Health and Plant Protection Division, NORWAY
maria.herrero@bioforsk.no

IAN HOOD
Scion (New Zealand Forest Research Institute), NEW ZEALAND
ian.hood@scionresearch.com

MARÍLIA HORTA JUNG
University of Algarve, PORTUGAL
mhorta@ualg.pt

CLAUDE HUSSON
INRA, FRANCE
claude.husson@nancy.inra.fr

JUAN JOSE JIMENEZ
Gestión Integrada Agroforestal, SPAIN
juanjose@giafor.com

THOMAS JUNG
University of Algarve, PORTUGAL
trjung@ualg.pt
Phytophthora Research and Consultancy, GERMANY
dr.t.jung@t-online.de

MINA KALANTARZADEH
Forest Research, Alice Holt, UNITED KINGDOM
mina.kalantarzadeh@surrey.ac.uk

ALAN KANASKIE
Oregon Department or Forestry, USA
akanaskie@odf.state.or.us

MARIJA KOPINA
All-Russian Plant Quarantine Center, RUSSIA
marija508a@mail.ru

JUDIT KOVÁCS
University of West-Hungary, HUNGARY
kovacsj@emk.nyme.hu

BARBARA LACHENBRUCH
Oregon State University, USA
barb.lachenbruch@oregonstate.edu

JOSE RAMON LEAL MURILLO
University of Córdoba, SPAIN
jramonleal@hotmail.com

ISABEL LEÓN SÁNCHEZ
Universidad de Huelva, SPAIN
isabel.leon@dcaf.uhu.es

MAELA LEON SANTANA
Instituto Agroforestal Mediterráneo - Universidad Politécnica de Valencia, SPAIN
maela.leon@uv.es

CARLOS ALFONSO LOPEZ ORONA
Universidad Autónoma de Sinaloa, MEXICO
orona_25@hotmail.com

ADRIÁN LÓPEZ VILLAMOR
SPAIN
adrian.lopez.villamor@xunta.es

NICOLA LUCHI
CNR-Istituto per la Protezione delle Piante, ITALY
n.luchi@ipp.cnr.it

BENOIT MARCAIS
INRA, FRANCE
marcais@nancy.inra.fr

LAURA MARTIN GÓMEZ
UCO, SPAIN
lauraldeanovita@hotmail.com

ALISTAIR MCCRACKEN
Agri-Food & Biosciences Institute, UNITED KINGDOM
alistair.mccracken@afbini.gov.uk

JOHAN MEFFERT
Plant Protection Service, NETHERLANDS
j.p.meffert@minlnv.nl

IVAN MILENKOVIC
University of Belgrade, SERBIA
ivan.milenkovic@sfb.bg.ac.rs

JOSE JAVIER MIRANDA TORRES
Universidad de Extremadura, SPAIN
javier_mt_1989@hotmail.com

CARMEN MORALES-RODRÍGUEZ
Centro de Investigación Agraria Finca La Orden, SPAIN
carmen.mrodriguez@juntaextremadura.net

MARCELA MRÁZKOVÁ
The Silva Tarouca Research Institute for Landscape and Ornamental Gardening, CZECH REPUBLIC
mrazkova@vukoz.cz

DINA NEVES
Universidade do Algarve, PORTUGAL
neves.dina@gmail.com

JUSTYNA NOWAKOWSKA
Forest Research Institute, POLAND
j.nowakowska@ibles.waw.pl
IRENEUSZ OLEJARSKI
Forest Research Institute, POLAND
I.Olejarski@ibles.waw.pl

TOMASZ OSZAKO
Forest Research Institute, POLAND
t.oszako@ibles.waw.pl

MARIA ANTONELLA PANE
University of Catania, ITALY
apane@unict.it

MATEJ PÁNEK
Mendel University in Brno, CZECH REPUBLIC
matej.panek@seznam.cz

JENNIFER PARKE
Oregon State University, USA
Jennifer.Parke@oregonstate.edu

ANA PÉREZ SIERRA
Universitat Politècnica de València, SPAIN
aperesi@eaf.upv.es

SIMONE PROSPERO
WSL, SWITZERLAND
simone.prospero@wsl.ch

MELODIE PUTNAM
Oregon State University, USA
putnamm@science.oregonstate.edu

PAUL REESER
Oregon State University, USA
reeserp@science.oregonstate.edu

PEDRO RIOS CASTAÑO
University of Córdoba, SPAIN
pedro.rios@gmail.com

DAVID RIZZO
University of California, Davis, USA
dmrizzo@ucdavis.edu

SARAH RIZZO
USA
rizfamily@hotmail.com

CÉCILE ROBIN
INRA, FRANCE
robin@bordeaux.inra.fr

Mª DEL CARMEN RODRÍGUEZ-MOLINA
Centro de Investigación Agraria Finca La Orden-Valdesequera, SPAIN
carmen.rodriguez@juntaextremadura.net

MARÍA CRISTINA ROMERO
Universidad de Córdoba, SPAIN
z92rorom@uco.es

FRANCISCO JOSÉ RUIZ GÓMEZ
Universidad de Córdoba, SPAIN,
g72rugof@uco.es

MARÍA ESPERANZA SÁNCHEZ HERNÁNDEZ
Universidad de Córdoba, SPAIN
ag1sahem@uco.es

BRUNO SCANU
Dipartimento di Agraria - Università degli Studi di Sassari, ITALY
bscanu@uniss.it

NATHALIE SCHENCK
ANSES - Laboratoire de la Santé des Végétaux - Unité de Mycologie, France
nathalie.schenck@anses.fr

CORINE SCHÖBEL
WSL, SWITZERLAND
corine.schoebel@wsl.ch

MARÍA SOCORRO SERRANO MORAL
Universidad de Córdoba, SPAIN
a12semom@uco.es

LAURA SIMS
Oregon State University, USA
simsla@science.oregonstate.edu

ALEJANDRO SOLLA HACH
Universidad de Extremadura, SPAIN
asolla@unex.es

TATIANA SURINA
All-Russian Plant Quarantine Center, RUSSIA
T.A.Surina@yandex.ru

WENDY SUTTON
Oregon State University, USA
suttonw@science.oregonstate.edu

GRAZYNA SZKUTA
Main Inspectorate of Plant Health and Seed Inspection, POLAND
g.szkuta@piorin.gov.pl

VENCHE TALGØ
Bioforsk, NORWAY
venche.talgo@bioforsk.no

GILLIAN THORP
The Food and Environment Research Agency, UNITED KINGDOM
gilli.thorp@fera.gsi.co.uk

STEVEN TJOSVOLD
University of California Cooperative Extension, USA
satjosvold@ucdavis.edu

PANAGIOTIS TSOPELAS
Hellenic Agricultural Organization "Demeter", GREECE
tsop@fria.gr

ANDREA VANNINI
University of Tuscia, ITALY
vannini@unitus.it

MARÍA LAURA VÉLEZ
CIEFAP (Centro de Investigación y Extensión Forestal Andino Patagónico), ARGENTINA
mvelez@ciefap.org.ar

ANNA MARIA VETTRAINO
University of Tuscia, ITALY
vettrain@unitus.it

STEFAN WAGNER
Julius Kuehn Institute (JKI), GERMANY
stefan.wagner@jki.bund.de

JOAN WEBBER
Forest Research, UNITED KINGDOM
joan.webber@forestry.gsi.gov.uk

ERIKA WEDGWOOD
ADAS UK Ltd, UNITED KINGDOM
101447@supanet.com

SABINE WERRES
Julius Kuehn Institute (JKI-GF), GERMANY
sabine.werres@jki.bund.de

MONIQUE WHEAT
Kauri Dieback Joint Agency, NEW ZEALAND
monique.wheat@aucklandcouncil.govt.nz

NARI WILLIAMS
Scion, NEW ZEALAND
nari.williams@scionresearch.com

STEPHEN WOODWARD
University of Aberdeen, UNITED KINGDOM
s.woodward@abdn.ac.uk