



Proceedings of the 9th Meeting of the  
International Union of Forest Research Organizations  
IUFRO Working Party 7.02.09

## *Phytophthora* in Forests and Natural Ecosystems

17-26 October 2019

La Maddalena - Sardinia, Italy



# Proceedings of the 9th Meeting of the International Union of Forest Research Organizations IUFRO Working Party 7.02.09

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## International & National Partners

International Union of Forest Research Organizations



Phytophthora Research Centre of Mendel University



Dipartimento per la Innovazione nei sistemi Biologici,  
Agroalimentari e Forestali - Università degli Studi della Toscana



Agenzia forestale regionale per lo sviluppo  
del territorio e dell'ambiente della Sardegna



**Front cover:** dieback and mortality of *Quercus ilex* and *Q. suber* trees caused by *P. cinnamomi* in a 10-year-old afforestation, photographed in October 2011 in Sardinia, Italy.

# Proceedings of the 9th Meeting of the International Union of Forest Research Organizations IUFRO Working Party 7.02.09

## Organizing Committee

*University of Sassari (Italy):*

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Thomas Jung - *Phytophthora Research Centre (Czech Republic)*

Andrea Vannini - *University of Tuscia (Italy)*



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## MEETING OVERVIEW

The 9th Meeting of the IUFRO Working Party 7.02.09 *Phytophthora diseases on forest trees* was held on 17-25 October 2019 in La Maddalena, Sardinia, Italy and counted 150 participants from 30 countries. For more than 30% of the delegates this was their first Working Party conference, which underlines the high interest in the WP activities. It was hosted by the Department of Agricultural Sciences – University of Sassari and the National Park of La Maddalena Archipelago.

Besides updating the scientific community on recent research developments on *Phytophthora* in forests and natural ecosystems, the objective of the meeting in Sardinia was to view the impact of *Phytophthora* species in Mediterranean type ecosystems, with particular regard to the severe outbreak currently affecting oak trees and the maquis vegetation in the National Park of La Maddalena Archipelago. More than 100 oral presentations were given during the meeting, including 6 keynote lectures and 32 poster presentations.

The conference was focused on forests and natural ecosystems and aimed to advance the discussion of concepts and ideas from pathology and biology to biosecurity. There is new evidence for the introduction of exotic *Phytophthora* species in Mediterranean ecosystems through restoration projects. Metagenomics is a powerful tool for *Phytophthora* detection, however there are some critical points to be considered, including sampling, lab contamination, selection of primers, sequencing technology and reference database. Studies in previously unexplored ecosystems improve our understanding of the biogeography and global diversity of *Phytophthora*. Phosphite represents the most effective treatment *Phytophthora* infection, however, more studies are needed to understand possible effects on the soil microbiomes as well as development of tolerant isolates through adaptive evolution.

During a 3-day pre-congress field trip across Sardinia, delegates visited the typical Mediterranean holm oak forests, featuring their historical uses and current management systems, including an old growth holm oak forest showing symptoms of chronic decline caused by endemic *Phytophthora* species (Nuoro province). Additionally, the delegation visited one the world's first FSC-certified cork oak forest as well as another site with severe dieback and mortality of cork oak trees caused by *Phytophthora cinnamomi*. During the main field trip, participants visited Caprera Island to see the impact of *Phytophthora* species on holm oak trees and Mediterranean maquis vegetation.

The tenth meeting is planned for 2022 in California and Oregon, to view the impact of Sudden Oak Death caused by *P. ramorum* across the two states as well as the dieback and mortality of trees and shrubs in restoration sites due to several exotic *Phytophthora* species.

*Report by Bruno Scanu, Meeting Organizer; IUFRO Working Party 7.02.09*





Delegates in the pre-congress field trip visiting an old growth *Quercus ilex* forest in Sardinia



Delegates during the conference in the Marina Militare of La Maddalena, Sardinia



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# PRE-CONGRESS FIELD TRIP PROGRAMME

## THURSDAY 17 OCT

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Arrival at Elmas airport (Cagliari) and accommodation in hotels

## FRIDAY 18 OCT

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- 9.00 Departure from Cagliari (Piazza Matteotti) to Ogliastra  
10.30 Visit to the archaeological site “Su Nuraxi” (Barumini)  
12.00 Lunch  
15.00 Visit to a Mediterranean holm oak forest – historical uses and current management systems of holm oak forests  
18.30 Arrival to the Rural Hotel ‘Abba e’ Murta’ (Tortolì)  
20.00 Dinner

## SATURDAY 19 OCT

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- 8.00 Departure from the Hotel to Supramonte (Orgosolo)  
10.00 Field trip to ‘Montes’ forest, with chronic decline by cryptic *Phytophthora* species  
13.00 Lunch in the forest  
16.00 Return to the Rural Hotel ‘Abba e’ Murta’ (Tortolì)  
20.00 Dinner  
21.30 Folkloristic exhibition

## SUNDAY 20 OCT

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- 7.00 Departure from the Hotel to Gallura  
9.00 Visit to a declining cork oak forest (San Teodoro)  
12.00 Visit to a FSC certified cork oak forest (Tempio Pausania)  
13.00 Lunch  
14.00 Visit to the Museum of the cork (Calangianus)  
18.00 Arrival to La Maddalena Island  
20.30 Welcome refreshment at the “Resort Ma&Ma”

# CONGRESS PROGRAMME

## MONDAY 21 OCT

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- 8.30 Registrations and posters set up  
10.00 Welcoming address: Organising Committee, Scientific Committee and Local Authorities

### OPENING SESSION

Chair: Bruno Scanu

- 10.30 C1. ***Phytophthora* in Italy: the history of a still undiscovered and ever-changing world.** G. Magnano di San Lio, S.O. Cacciola, A. Franceschini, S. Moricca, B. Scanu, L. Schena, A. Testa, A. Vannini, A.M. Vettraino
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### 11.00 Coffee break

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- 11.30 C2. **History of *Phytophthora cinnamomi* management in Western Australia.** G. Hardy  
12.00 C3. **Biology and control of *Phytophthora ramorum* in California.** M. Garbelotto  
12.30 C4. **The importance of the nursery pathway for the spread of *Phytophthora* species to natural ecosystems in Europe.** T. Jung
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### 13.00 Light Lunch

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#### SESSION 1: PHYTOPHTHORA IN NURSERIES AND PLANTING AREAS

Chair: Susan J. Frankel

- 14.30 C5. **Exotic *Phytophthora* species are being systematically introduced in California wildlands during restoration projects.** L. Sims, M. Garbelotto  
14.45 C6. **Assessing the incidence and diversity of *Phytophthora* species occurring in planned restoration areas of the Angeles National Forest.** S.N. Fajardo, T.B. Bourret, C. Endelenbos, E. Lozano, K. VinZant, D.M. Rizzo, S.J. Frankel  
15.00 C7. **Metabarcoding of *Phytophthora* communities at restoration sites in San Francisco Peninsula open spaces.** E. Peterson, J. Eberhart, N. Redekar, A. Mills, J. Parke  
15.15 C8. **Preventing *Phytophthora* introductions into California restoration sites.** S.J. Frankel, J. Hillman, D. Benner, J. Alexander, A. Shor  
15.30 C9. ***Phytophthora* communities in UK plant nurseries and links to management practice: opportunities for mitigation through accreditation.** S. Green, D. Cooke, D. Frederickson-Matika, B. Clark, E. Randall, M. Clark, T. Pettit, M. Dunn, A. Schlenzig, L. Pritchard, P. Thorpe, P. Cock, J. Barbrook  
15.45 C10. **Temporal patterns of airborne *Phytophthora* spp. in a woody plant nursery area detected using real-time PCR.** D. Migliorini, L. Ghelardini, N. Luchi, M. Onorati, A. Santini
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### 16.00 Coffee break

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- 16.30 C11. **Epidemic spread of *Phytophthora nicotianae* in a Mediterranean park in Athens causing decline and mortality of shrub and tree species.** A. Vannini, C. Morales-Rodriguez

- 16.45 C12. ***Phytophthora oleae* a widespread species in soil of olive (*Olea europaea*) orchards in Southern Italy.** M. Riolo, L. Schena, F. Aloï, E. Santilli, D. Ruano-Rosa, G.E. Agosteo, A. Pane, F. La Spada, S.O. Cacciola
- 17.00 C13. **Diversity of *Phytophthora* species in a public garden in Essex, UK.** E. Beal, F. Drizou, G. Clover
- 17.15 **SESSION 1 DISCUSSION**

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## TUESDAY 22 OCT

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### SESSION 2: GENETICS AND GENOMICS

Chair: David Cooke

- 9.00 C14. **Keynote Lecture: Novel insights into the evolution and emergence of *Phytophthora* pathogens using genomics approaches.** N.J. Grünwald, S.K. Shakya, B.J. Knaus, J.E. Weiland, V.J. Fieland, M. Horta Jung, C. Maia, A. Drenth, D.I. Guest, E.C.Y. Liew, C. Crane, B. Scanu, T. Jung, F. Albornoz, Z.S.L. Foster, M.M. Larsen, F.N. Martin
- 9.30 C15. **Highly contiguous genome assemblies for three *Phytophthora* species generated from PacBio sequencing.** E. Mollison, P. Sharp, C. Riddell, D. Cooke, L. Pritchard, P. Thorpe, A. Jeffries, S. Green
- 9.45 C16. **A microsatellite analysis identifies global pathways of movement of *Phytophthora cinnamomi* and the likely sources of wildland infestations in California and Mexico.** M. Socorro Serrano, T. Osmundson, A. Almaraz-Sánchez, P.J.P. Crouche, T. Swiecki, D. Alvarado-Rosales, M. Garbelotto

### SESSION 3: NEW DETECTION METHODS

Chair: Treena I. Burgess

- 10.00 C17. ***Phytophthora* detection via eDNA metabarcoding.** D.E.L. Cooke, E. Randall, B. Clark, P. Thorpe, P. Cock, L. Pritchard, D. Frederickson-Matika, S. Green
- 10.15 C18. **Comparison of soil baiting and metabarcoding methods for the detection of *Phytophthora* species in Scottish environments.** D.E. Frederickson Matika, S. Green, D.E.L. Cooke, L. Pritchard, E. Randall, B. Clark
- 10.30 C19. **Comparison of oomycete and *Phytophthora* specific primers for identification and metabarcoding.** T. Burgess, D. White, S. Sapsford
- 10.45 C20. **Comparison between direct isolation, baiting and molecular approaches to determine the oomycetes present in hardy woody plants in the plants for planting pathway.** A. Puertolas, P.J.M. Bonants, E. Boa, S. Woodward

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### 11.00 **Coffee break**

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- 11.30 C21. **Exploring *Phytophthora* community with HTS: a proposed pipeline for data analysis.** C. Morales-Rodriguez, W. Oßwald, A. Vannini
- 11.45 C22. **The structure and functionality of soil microbiota influenced health status of holm oak in dehesas affected by *Phytophthora* root rot.** F.J. Ruiz Gómez, R.M. Navarro-Cerrillo, A. Vannini, C. Morales-Rodriguez
- 12.00 C23. **Assessing the spread of *Phytophthoras* in Scottish forests by recreational and harvesting activities using comparative qPCR and metabarcoding techniques.** A. Armstrong, A. Penny, C. Vacca, K. Tsarna, C. Riddell, P. Hedley, S. Green



- 12.15 C24. **Detection of *Phytophthora* pathogens in forest nurseries using quantitative PCR techniques.** A. Bačová, M. Tomšovský, T. Májek
- 12.30 C25. **Metabarcoding identification of *Phytophthora* species from mock environmental samples via MinION HTS and the importance of a database of the Ex-types.** Z.G. Abad, S.K. Srivastava, L.M. Knight, K. Zeller, M. Nakhla
- 12.45 C26. **IDphy: Molecular and morphological identification of *Phytophthora* based on the types. Demonstration of the international online resource.** Z.G. Abad
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13.00 **Light Lunch**

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14.30 **PANEL DISCUSSION: NEW DETECTION METHODS** - Moderator: Treena I. Burgess

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16.00 **Coffee break**

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16.30 **POSTER SESSION** - Moderator: Nari Williams

- P1. **Are *Cistus* sp. shrubs contributing to the epidemic of Iberian Oak Decline? A pathogenicity test answer.** A. Maria Sanchez-Redondo, E. Cardillo, C. Perez
- P2. **Susceptibility of the herbaceous species used as pastures in dehesa/montado to *Phytophthora cinnamomi*.** M. Rodríguez-Romero, I.M. Calha, J.A. Passarinho, A.C. Moreira
- P3. **Characterization of constitutive and induced chemical defenses of *Quercus ilex* against infection by *Phytophthora cinnamomi*.** M. Rodríguez-Romero, B. Godoy, A.O. Conrad, F. Pulido, P. Bonello
- P4. **Characterization of the volatile compounds from three Brassicaceae species and their effects in the presence of *Phytophthora cinnamomi*.** M. Rodríguez-Romero, B. Godoy, J. Neno, I.M. Calha, J.A. Passarinho, A.C. Moreira
- P5. **Evaluation of difference of susceptibility of Andalusian *Quercus ilex* L. population through functional traits and physiology assessment.** A. Nizzoli, C. Morales Rodríguez, A. Vannini, R. Sánchez-Cuesta, F.J. Ruiz Gómez
- P6. **Habitats favorable for *Phytophthora* species in oak stands of Natura 2000.** I. Olejarski, J. Nowakowska, T. Oszako
- P7. **Toward a new soil detection method of the causal agents of chestnut ink disease.** M. Marchand, M. Massot, E. Chancerel, C. Robin
- P8. **Vigil'Ink: a citizen science project dedicated to chestnut ink disease.** C. Robin, M. Marchand, J. Gaudin, J.M. Armand
- P9. **Physiological and histopathological characterization of infections caused by A1 and A2 mating types of heterothallic *Phytophthora* spp. in *Fagaceae* woody hosts.** T. Corcobado, T. Jung, T. Kudláček, T. Májek, R. Plichta, I. Saiz, P. Kerchev, M. Matoušková, A. Bačová, H. Ďatková, L.B. Dálya, M. Trifković, D. Mureddu, I. Milenković
- P10. **A case study on the impact of *Phytophthora* on beech (*Fagus sylvatica*) decline in the Belgian Ardennes.** B. Henricot
- P11. **Analysis of heat shock protein genes expression in *Betula pendula* under defoliation and pathogen stress.** D. Berezowska, T. Oszako, T. Malewski
- P12. **Decline of alpine green alder (*Alnus viridis*) and relation to *Phytophthora* species, preliminary results.** T. Majek, K. Schwanda, T. Cech

- P13. **Responses of *Alnus glutinosa* populations to different inoculation methods of *Phytophthora x alni*.** I. Gomes Marques, J. Neno, R. Jansson, T. Corcobado, T. Cech, Y. Laurent, I. Bernez, S. Dufour, B. Mandák, H. Ennoui, A. Sahli, M. Ater, T.S. David, A. Solla, A.C. Moreira, P.M. Rodríguez-González
- P14. ***Phytophthora* species in the rhizosphere of *Alnus glutinosa* stands in western Turkey.** A. Gülden Aday Kaya, T. Doğmuş, A. Lehtijärvi, J. Nowakowska, T. Oszako, S. Woodward
- P15. **Diversity of *Phytophthora* species in forest streams and rivers in the southern part of Czech Republic and in northern Slovakia.** H. Ďatková, M. Tomšovský, I. Milenković, T. Májek, T. Corcobado, T. Jung
- P16. **Diversity and distribution of *Phytophthora* species from wild apple forest in Xinjiang Uighur Autonomous Region, China.** X. Xu, W. Huai, W. Zhao, Y. Cheng, Z. Zhou
- P17. **Pathogenicity of *Phytophthora* species on two commercially important non-native tree species from South Africa.** T. Bose, J. Roux, T.I. Burgess, C. Shaw, M.J. Wingfield
- P18. ***Phytophthora* infestations in Turkish forest nurseries.** A. Gülden Aday Kaya, T. Doğmuş, A. Lehtijärvi, S. Woodward, T. Jung
- P19. **Potential for the management of oomycota in the international trade in plant for planting.** C. Benavent, P. van West, S. Woodward
- P20. **Changes in *Ulmus minor* root fungal endobiome triggered by flood, drought, and drought after flood.** C. Martínez-Arias, J.A. Martín, J. Sobrino-Plata, D. Macaya-Sanz, C. Collada, L. Gil, J. Rodríguez-Calcerrada
- P21. **Phytopathogenic fungi and oomycetes detection trials by the means of e-nose and SPME-GCMS devices.** F. Lefort, J. Loulier, M. Stocki, M. Asztemborska, R. Szmigielski, K. Siwek, T. Grzywacz, T. Oszako
- P22. **Molecular Toolbox of *Phytophthora* species Ex-types with seven genes. Our over 1000 sequences at the NCBI.** Z.G. Abad, S.K. Srivastava, L.M. Knight, M. Nakhla
- P23. **Initiating pathogen reduction processes in post-agricultural soils with the addition of organic matter of forest origin.** I. Olejarski, J. Nowakowska, T. Oszako
- P24. ***Phytophthora* spp. on trees in Britain through the lens of the Tree Health Diagnostic and Advisory Service.** A. Pérez-Sierra, C. Gorton, A. Lewis, R. Chitty, S. van der Linde, A. Armstrong, S. Hendry, A. Harris, S. Green, C. Brasier, J. Webber
- P25. **Diversity of *Phytophthora* species detected in British soils using NGS analysis for ITS and COI.** A. Pérez-Sierra, M. Montes, B. Henricot, L. Shuttleworth, B. B. Landa
- P26. ***Phytophthora lateralis* isolated from Umbrella pine.** A. Schlenzig, R. Eden
- P27. **Investigating *Phytophthora* root rot in UK raspberry.** R. D'urban-Jackson, E. Wedgwood, T. Pettit
- P28. **Response of *Larix* bark to invasion by *Phytophthora ramorum*.** J. Webber, M. Kalantarzadeh, D. Mulholland
- P29. **Is *Phytophthora ramorum* associated with a new, severe disease of chaparral plants in Coastal California?** W. Schweigkofler, T. Pastalka, K. Suslow
- P30. **Comparative epidemiology of NA1 and EU1 *Phytophthora ramorum* populations in Curry County, OR.** E. Peterson, S. Navarro, J. Parke

- P31. **Using citizen science and outreach education to reduce the risk of *Phytophthora ramorum* spread in Oregon forests.** N. Kline, S. Navarro, J. LeBoldus
- P32. **Beech health decrease in Cologne Green Belts.** C. Sabatini, O. Menke, D. Migliorini, N. Luchi, A. Santini

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### WEDNESDAY 23 OCT

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#### SESSION 4: DIVERSITY AND DISTRIBUTION

Chair: Santa Olga Cacciola

- 9.00 C27. Keynote Lecture: **Insights into the biogeography and global diversity of *Phytophthora*.** T. Jung, I. Milenkovic, T. Corcobado, M. Tomšovský, J. Janousek, M. Panek, H. Ďatková, Y. Balci, B. Scanu, C.M. Brasier, J.F. Webber, A. Pérez-Sierra, J. Bakonyi, D. Seress, A. Durán, M. Tarigan, L. Oliveira, E. Sanfuentes von Stowasser, G. Magnano di San Lio, L. Schena, S. Mosca, P. Quang Thu, C. Nguyen Minh, C. Maia, A. Engelen, G. Carella, S. Moricca, S.O. Cacciola, A. Pane, F. La Spada, K. Kageyama, A. Hieno, H. Masuya, S. Uematsu, V. Talgø, M. Redondo, J. Oliva, A. Cravador, T.T. Chang, C.H. Fu, M. Horta Jung
- 9.40 C28. ***Phytophthora lateralis* discovered in Japan.** C.M. Brasier, J.F. Webber, M. Horta-Jung, S. Uematsu, A. Heino, K. Kagayama, H. Masuya, T. Jung
- 9.55 C29. **Diversity of *Phytophthora* species in natural ecosystems in Serbia, Bosnia and Herzegovina and Montenegro (Western Balkans).** I. Milenković, N. Keča, D. Karadžić, Z. Stanivuković, M. Tomšovský, S. Milanović, A. Vemić, Z. Radulović, T. Corcobado, M. Horta Jung, T. Májek, J.A. Nowakowska, T. Oszako, K. Sikora, T. Jung
- 10.10 C30. **Waterborne and soilborne *Phytophthora* diversity in declining cork oak stands in Sardinia (Italy).** S. Seddaiu, A. Brandano, G. Cadinu, C. Sechi, P.A. Ruiu, B. Scanu
- 10.25 C31. **Diversity of *Phytophthora* populations in Sicilian river ecosystems.** T. Jung, F. La Spada, F. Aloj, A. Pane, M. Horta Jung, M. Evoli, M. Riolo, J.B. Ristaino, L. Schena, S.O. Cacciola
- 10.40 C32. **A new *Phytophthora* species pathogenic on Chinese Hickory (*Carya cathayensis*) in South East China: a possible introduction from North America?** C. Morales-Rodriguez, Y. Wang, A. Vannini
- 
- 11.00 **Coffee break**
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- 11.30 C33. **Predictors of *Phytophthora* diversity and community composition in natural areas across diverse Australian ecoregions.** T. Burgess, K. McDougall, P. Scott, G. Hardy, J. Garnas
- 11.45 C34. **15 years of *Phytophthora* monitoring in Switzerland.** S. Prospero, C. Buser-Schöbel, D. Rigling
- 12.00 C35. **Diversity of Oomycetes associated with declining *Pinus* spp. and *Cupressus* spp. in Kerman province, Iran.** S. Aghighi, F. Sistani, Z. Lori, S. Sarikhan, T. Bose
- 12.15 C36. **First report of *Phytophthora parvispora* from a natural riparian ecosystem in Sicily.** F. La Spada, F. Aloj, M. Riolo, A. Pane, M. Evoli, R. Faedda, S.O. Cacciola
- 12.25 C37. **First report of *Phytophthora* spp. in declining broadleaf forests in western Ukraine.** I. Matsiakh, V. Kramarets, M. Cleary



12.35 SESSION 4 DISCUSSION

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13.00 Light Lunch

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14.30 FIELD TRIP TO CAPRERA ISLAND

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THURSDAY 24 OCT

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SESSION 5: ECOLOGY AND EPIDEMIOLOGY

Chair: Joan Webber

- 9.00 C38. ***Phytophthora* and damping-off within natural and rehabilitated plant communities.** C. Shaw, M. Dobrowolski, G.E.S.J. Hardy, T. Burgess
- 9.15 C39. **Trait-based approaches for predicting future global impacts in the genus *Phytophthora*.** L.J. Barwell, A. Perez-Sierra, B. Henricot, A. Harris, T.I. Burgess, G. Hardy, P. Scott, N. Williams, D. Cooke, P. Sharp, S. Green, D.S. Chapman, B.V. Purse
- 9.30 C40. **Functional traits associated with establishment and spread of invasive forest pathogens in Northern Europe.** M.A. Redondo, J. Boberg, J. Stenlid, J. Oliva
- 9.45 C41. **Genetic variation in host resistance as a prerequisite for natural selection by an invasive pathogen.** M.A. Redondo, J. Stenlid, J. Oliva
- 10.00 C42. **Modelling the key drivers of an aerial *Phytophthora* foliar disease epidemic, from the needles to the whole plant.** M. Gomez-Gallego, R. Gommers, M. Karl-Friedrich Bader, N. Williams
- 10.15 C43. **Pathogen loads of *Phytophthora pluvialis* and *Nothophaeocryptopus gaeumannii* populations co-existing within exotic plantations of New Zealand in contrast to Douglas-fir's endemic range in the US Pacific Northwest.** M. Gómez-Gallego, J. M. LeBoldus, M. Karl-Friedrich Bader, E. Hansen, L. Donaldson, N. Michelle Williams
- 10.30 C44. ***Phytophthora* species associated with roots and soils from native and non-native forestry environments in South Africa.** T. Bose, M.J. Wingfield, J. Roux, M. Vivas, T.I. Burgess
- 10.45 C45. **What's killing juniper? Identifying environmental risk factors for *Phytophthora austrocedri* infection.** F. Donald, S. Green, K. Searle, N. Cunniffe, B.V. Purse

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11.00 Coffee break

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- 11.30 C46. **Understanding sudden larch death – from epidemiology to host resistance.** H. Dun, J. Mackay, S. Green
- 11.45 C47. **Sudden Oak Death in Oregon forests: disease intensification and renewed engagement in disease management.** S. Navarro, E. Michaels Goheen, J. LeBoldus, E. Hansen, P. Reeser, W. Sutton, N. Grunwald, A. Kanaskie, R. Wiese, C. Nichlos
- 12.00 C48. **Epidemiology and management of an outbreak of the EU1 lineage of *Phytophthora ramorum* in Oregon forests.** J.M. LeBoldus, K.L. Søndreli, H. Daniels, S. Navarro
- 12.15 C49. **Sudden Oak Death: Comparing the EU1 and NA1 lineages of *Phytophthora ramorum* in Oregon forests.** K.L. Søndreli, J.M. LeBoldus

12.30 **SESSION 5 DISCUSSION**

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13.00 **Light Lunch**

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**SESSION 6: HOST-PATHOGEN INTERACTION**

Chair: Niklaus J. Grünwald

- 14.30 C50. **Keynote Lecture: Dissecting the in's and out's of *Phytophthora* biology and pathology.** F. Govers
- 15.00 C51. **Hormones and secondary metabolites in chestnut during susceptible and resistant interactions with *Phytophthora cinnamomi*.** Á. Camisón, M.Á. Martín, P. Sánchez-Bel, V. Flors, G. Pinto, F. Alcaide, E. Cubera, [A. Solla](#)
- 15.15 C52. **Disclosing pathogen target-proteins in cork oak plants infected with *P. cinnamomi*.** [A. Cristina Coelho](#), R. Pires, G. Schütz, C. Santa, B. Manadas, P. Pinto
- 15.30 C53. **Identifying effector proteins involved in infection by *Phytophthora kernoviae* and *P. plurivora*.** [S.C. Whisson](#), S. Wang, P.C. Boevink, P.R.J. Birch, L. Welsh, P. Thorpe, S.K. Kushwaha, R.R. Vetukuri
- 15.45 C54. **Identification and functional characterisation of CAZymes from the kauri dieback pathogen, *Phytophthora agathidicida*.** [E.L. Bradley](#), P. Panda, R. Bradshaw, C.H. Mesarich
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16.00 **Coffee break**

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**SESSION 7: PATHOGENICITY**

Chair: Bruno Scanu

- 16.30 C55. **Decline and mortality of evergreen oaks in a protected area in Central Italy driven by the pathogenic activity of the invasive *Phytophthora cinnamomi* and *Phytophthora multivora*.** [A. Vannini](#), L. Osimani, C. Morales-Rodríguez
- 16.45 C56. **Extending the host range of *Phytophthora multivora*, a pathogen of woody plants in horticulture, nurseries, urban environments and natural ecosystems.** [D. Migliorini](#), M.Y. Khdiar, C. Rodríguez Padrón, M. Vivas, P.A. Barber, G. Hardy, T. Burgess
- 17.00 C57. **Assessment of potential risk of Radiata pine (*Pinus radiata*) as host for Sudden Oak Death (*Phytophthora ramorum*).** [H.A. Daniels](#), L. Bulman, J.M. LeBoldus

**SESSION 8: PUBLIC ENGAGEMENT**

Chair: Nari Williams

- 17.15 C58. **Kauri Rescue™: a citizen science programme developing controls for kauri dieback in New Zealand.** [I. Horner](#), M. McEntee, M. Barton, L. Hill, W. Wood, N. Waipara, N. Kingsbury, P. Graham, L. Jesson
- 17.30 C59. **Inclusive, adaptive management of *Phytophthora agathidicida* in Waipoua Forest, Aotearoa-New Zealand: an Indigenous perspective.** [T. Patuawa](#), T. Donovan, T. Tane, I. Horner, P. Scott, N. Waipara, M. Calder, T. Beauchamp, N. Williams, S. Bellgard, A. Black, L. Hill, P. Barber, G. Hardy
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20.00 **SOCIAL DINNER**

## FRIDAY 25 OCT

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### SESSION 9: BIOLOGY AND EVOLUTION

Chair: Ana Pérez-Sierra

- 9.00 C60. **Changing distribution of *Phytophthora ramorum* lineages in Britain.** J. Webber, Anna Harris, James Snowden
- 9.15 C61. **Multiple phenotypes and genotypes of Clade 6 *Phytophthora gonapodyides* and *P. gonapodyides* x *P. chlamydospora* within a single aerial lesion on *Fagus sylvatica*.** A. Pérez-Sierra, B.B. Landa, K. Heungens, K. Van Poucke, B. Henricot, C.M. Brasier
- 9.30 C62. **Detection and description of viruses in *Halophytophthora* spp. from Portugal.** L. Botella, J. Janousek, M. Raco, T. Jung
- 9.45 C63. **Detecting novel viruses of *Phytophthora castaneae* using high-throughput sequencing of small RNAs.** M. Raco, T. Jung, E.J. Vainio, A. Eichmeier, E. Penazova, L. Botella
- 10.00 C64. **Describing two new species of *Nothophytophthora* (oomycota) from Ireland and Northern Ireland.** R. O’Hanlon, M. Destefanis, S. Bellgard, B. Weir, I. Milenković, T. Jung
- 10.15 **SESSION 9 DISCUSSION**
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11.00 **Coffee break**

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### SESSION 10: MANAGEMENT AND CONTROL

Chair: Giles Hardy

- 11.30 C65. ***Phytophthora ramorum* mitigation at the Bloedel Reserve in Western Washington – A four-year update.** G.A. Chastagner, M. Elliott, D. Strenge
- 11.45 C66. **Managing foliar *Phytophthora* pathogens across a whole forest: integrated management using all the tools in the toolbox.** N. Williams, S. Fraser, C. Rolando, G. Pearse, N. Graham, M. Gomez-Gallego, R. McDougal, R. O’Neil, P. Panda, M. Bader, P. Scott, R. Ganley, J. Klápště, A. Ismael, H. Dungey, R. Gommers, L. Bulman
- 12.00 C67. **A multi-pronged approach for establishing resistance to foliar *Phytophthora* infection in *Pinus radiata*.** N. Williams, N. Graham, J. Klápště, A. Ismael, H. Dungey
- 12.15 C68. **Efficacy of long-term phosphite applications to control *Phytophthora* dieback of mature *Fagus sylvatica*, *Quercus robur* and *Quercus petraea* trees under natural conditions.** T. Jung, I. Milenković, M. Horta Jung, A. Nannig, M. Blaschke, T. Kudláček, T. Corcobado
- 12.30 C69. **Phosphite trials for control of kauri dieback in New Zealand.** I. Horner, M. Arnet, E. Carroll, M. Horner
- 12.45 C70. **Phosphite inducing resistance on sweet chestnut against *Phytophthora* infection.** A. Brandano, B. Scanu, L. Maddau, S. Serra, N. Schianchi, G. Hardy
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13.00 **Light Lunch**

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- 14.30 C71. **Screening New Zealand Kauri (*Agathis australis*) for tolerance to *Phytophthora agathidicida*.** S.E. Bellgard, C.M. Probst, L.G. Raymond, S.J. Hill, P.M. Scott, N.M. Williams



- 14.45 C72. **Lignin nanoparticles containing essential oils: a new natural biocide delivery system for *Phytophthoras* control.** A.M. Vettraino, F. Zikeli, D. Tabet, G. Scarascia Mugnozza, M. Romagnoli
- 15.00 C73. **Evaluation of *Gluconobacter* spp. as biocontrol agent against *Phytophthora* spp.** D. Tabet, S. Woodward, A.M. Vettraino
- 15.15 C74. **Antagonist potential of indigenous *Trichoderma* against *Phytophthora palmivora* of Soe Mandarin in East Nusa Tenggara, Indonesia.** A.V. Simamora, M.V. Hahuly, L.F. Ishaq, J.B.D. Henuk, E. Yulia, E. Hosang
- 10.30 **SESSION 10 DISCUSSION**
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- 16.00 **Coffee break**
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- 16.30 **Short presentation about the proposed next meeting**
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**GENERAL DISCUSSION – MEETING SUMMARY**  
**European Chairs: Andrea Vannini and Thomas Jung**

**SATURDAY 26 OCT**

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9.00 **Departure**



## OPENING SESSION

***Phytophthora* in Italy: the history of a still undiscovered and ever-changing world.** Gaetano Magnano di San Lio<sup>1</sup>, Santa Olga Cacciola<sup>2</sup>, Antonio Franceschini<sup>3</sup>, Salvatore Moricca<sup>4</sup>, Bruno Scanu<sup>3</sup>, Leonardo Schena<sup>1</sup>, Antonino Testa<sup>5</sup>, Andrea Vannini<sup>6</sup>, Anna Maria Vettraino<sup>6</sup>

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This joint review summarizes the history of research on *Phytophthora* in Italy starting from the second half of the nineteenth century to the present day. The aim is to pay tribute to researchers who, with a pioneering spirit, have made fundamental contributes to the knowledge of these plant pathogens and also to update the state of the research on this subject in Italy. The potato blight epidemics caused by *Phytophthora infestans*, which had such devastating effects in Ireland, affected only marginally Italy. Two epidemics, which occurred in the second half of the nineteenth century, the *Phytophthora* trunk gummosis of citrus caused by *P. citrophthora* and the ink disease of chestnut caused by *P. × cambivora*, had a greater social impact and attracted more scientific interest. The first one was dealt with large-scale use of resistant rootstocks and since then it has become endemic; the latter was almost forgotten after the first world war because chestnut fruit lost most of its economic importance as staple food following the exodus from mountain territories. Presently, the Italian research on *Phytophthora* is recognized internationally also thanks to the collaboration with eminent scientists from other countries. Advanced research lines include the study of *Phytophthora* communities in different ecosystems and the development of new molecular techniques to improve the diagnosis of these pathogens and analyze the genetic variability of their populations. Several new species have been discovered and described in ornamental, agricultural and forest plants, in different environments, including agricultural and natural ecosystems, gardens and amenity parks.

## **History of *Phytophthora cinnamomi* management in Western Australia.**

Giles Hardy

*Centre of Phytophthora Science and Management, Murdoch University, Western Australia*

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*Phytophthora cinnamomi* is recognized as a key threatening process to biodiversity in Australia by the Commonwealth Government. It was first observed 1921 in southwestern Australia killing jarrah (*Eucalyptus marginata*) trees in the northern jarrah forest. It took over 40 years for the causal agent to be identified in 1964. Over the next 40 years, State Government Departments formulated policy and implemented management measures to deal with the problem. These measures have changed greatly over time as new knowledge about the host range and extent of the epidemic became available. Unfortunately, the pathogen had spread over large areas of estate prior to the identification of the causal agent and the development of a management response. The spread of *P. cinnamomi* into significant areas of the conservation estate, including biodiversity hotspots, highlighted the urgency of ensuring that *Phytophthora* dieback and its management was adequately resourced and is underpinned by appropriate research and communication programs. This talk describes the main historical events leading up to the formulation of the 2004 State *Phytophthora* Dieback Response Framework. These include: quarantining half a million hectares of State Forest in 1976/7 in order to map the extent of the disease and implement hygiene measures; developing policy and management practices for the conservation estate; the acceptance by the Hon. Minister for the Environment in 1996 of the 33 recommendations in the WA Dieback Review Panel Report; the establishment of community based Dieback Working Groups; the formation of the Centre for *Phytophthora* Science and Management at Murdoch University, and the preparation of the National Threat Abatement Plan in 2001, and in 2004 the development of National Best Practices Management Guidelines and a risk assessment methodology suitable for national adoption. Subsequent reviews of the National Threat Abatement Plan and reviews of State's hygiene management plans as well as the selection of 'priority protection areas' will be discussed together with other management tools such as phosphite applications and attempts to eradicate spot infestations. In spite of these actions, flora and fauna remain threatened by the continued expansion and impact of *Phytophthora* dieback. We have few tools available to reduce the extension, spread and impact of the pathogen and the diseases it causes. The community needs to be better informed of the direct and indirect impacts this disease has had on individual species and ecosystem function and health and encouraged to take greater ownership of an environmental problem that encompasses all types of land tenure.



**Biology and control of *Phytophthora ramorum* in California.**

Matteo Garbelotto

*University of California, Berkeley, CA*Corresponding email: [matteog@berkeley.edu](mailto:matteog@berkeley.edu)

The discovery of *Phytophthora ramorum* in California marks a shift in focus from the study of soilborne to the study of airborne Phytophthoras. Research on this novel pathogen highlighted many unique traits. Transmissive hosts are not the same as dead end hosts and are not necessarily killed by the pathogen. Production of sporangia is regulated by rainfall and temperature but not by coastal fog. Aerial populations from leaves and twigs are possibly the only epidemiologically relevant populations, they were proven to have been generated from infected nursery stock and to be slowly evolving even in the absence of sexual reproduction. Infection is modulated by inoculum density, host density, availability of free water, temperature, genetics and phenology of the host. Tanoak infection requires low inoculum dosage while oak infection requires high inoculum loads and occurs only after substantial rainfall and only in close proximity of sporulating hosts, nonetheless synchronicity between pathogen sporulation and oak phenology facilitates infection. Soil and water pathogen populations appear to be generated by aerial populations; however different selection processes lead to genotypic differentiation of populations in the three substrates. Control of the disease has focused on five areas: removal of infectious hosts, reduction of host density, use of disease tolerance, increase of disease tolerance by chemical treatment, and limiting landscape work to dry months. All of the available treatments are preventive and rely on the early detection of the disease mostly obtained by volunteers through the citizen science program SOD blitz.

**The importance of the nursery pathway for the spread of *Phytophthora* species to natural ecosystems in Europe.** Thomas Jung<sup>1,2</sup>, Leszek Orlikowski<sup>3</sup>, Beatrice Henricot<sup>4</sup>, Paloma Abad-Campos<sup>5</sup>, A. G. Aday<sup>6</sup>, Olga Aguín Casal<sup>7</sup>, Jozsef Bakonyi<sup>8</sup>, Santa Olga Cacciola<sup>9</sup>, Thomas Cech<sup>10</sup>, D. Chavarriaga<sup>11</sup>, Tamara Corcobado<sup>12</sup>, Alfredo Cravador<sup>1</sup>, T. Decourcelle<sup>13</sup>, Geoffrey Denton<sup>5</sup>, Stefanos Diamandis<sup>14</sup>, H. T. Doğmuş-Lehtijärvi<sup>7</sup>, A. Franceschini<sup>15</sup>, Beatrice Ginetti<sup>16</sup>, Milka Glavendekić<sup>17</sup>, Jarkko Hantula<sup>18</sup>, Günther Hartmann<sup>19</sup>, Maria Herrero<sup>20</sup>, D. Ivic<sup>21</sup>, Marilia Horta Jung<sup>1</sup>, Arijia Lilja<sup>18</sup>, Nenad Keca<sup>17</sup>, Vlodimir Kramarets<sup>22</sup>, A. Lyubenova<sup>23</sup>, Helena Machado<sup>24</sup>, Gaetano Magnano di San Lio<sup>9</sup>, P. J. Mansilla Vázquez<sup>7</sup>, Benoit Marçais<sup>25</sup>, Irina Matsiakh<sup>22</sup>, Ivan Milenkovic<sup>17</sup>, Salvatore Moricca<sup>16</sup>, Jan Nechwatal<sup>26</sup>, Christer Olsson<sup>27</sup>, Tomasz Oszako<sup>28</sup>, Antonella Pane<sup>9</sup>, E. J. Paplomatas<sup>29</sup>, Cristina Pintos Varela<sup>7</sup>, Simone Prospero<sup>30</sup>, C. Rial Martínez<sup>7</sup>, Daniel Rigling<sup>30</sup>, Cecile Robin<sup>13</sup>, A. Rytönen<sup>18</sup>, Maria E. Sánchez<sup>31</sup>, Bruno Scanu<sup>15</sup>, Alexandra Schlenzig<sup>32</sup>, Jörg Schumacher<sup>33</sup>, S. Slavov<sup>23</sup>, Alejandro Solla<sup>12</sup>, Eduardo Sousa<sup>24</sup>, Jan Stenlid<sup>27</sup>, Venche Talgø<sup>20</sup>, Z. Tomic<sup>21</sup>, Panos Tsopelas<sup>34</sup>, Andrea Vannini<sup>35</sup>, Anna M. Vettraino<sup>35</sup>, Marcel Wenneker<sup>36</sup>, Steve Woodward<sup>11</sup>, A.V. Sanz Ros<sup>37</sup>, Ana Pérez-Sierra<sup>38</sup>

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An analysis of incidence of *Phytophthora* spp. in 732 European nurseries producing forest transplants, larger specimen trees, landscape plants and ornamentals, plus 2525 areas in which trees and shrubs were planted, is presented based on work conducted by 38 research groups in 23 European countries between 1972 and 2013. Forty-nine *Phytophthora* taxa were recorded in 670 nurseries (91.5%); within these nurseries, 1614 of 1992 nursery stands (81.0%) were infested, although most affected plants appeared healthy. In forest and landscape plantings, 56 *Phytophthora* taxa were recovered from 1667 of 2525 tested sites (66.0%). Affected plants frequently showed symptoms such as crown thinning, chlorosis and dieback caused by extensive fine root losses and/or collar rot. Many well-known highly damaging host-*Phytophthora* combinations were frequently detected but 297 and 407 new *Phytophthora*-host associations were also observed in nurseries and plantings, respectively. On average, 1.3 *Phytophthora* species/taxa per infested nursery stand and planting site were isolated. At least 47 of the 68 *Phytophthora* species/taxa detected in nurseries and plantings were exotic species several of which are considered well established in both nurseries and plantings in Europe. Seven known *Phytophthora* species/taxa were found for the first time in Europe, while 10 taxa had not been previously recorded from nurseries or plantings; in addition, 5 taxa were first detections on woody plant species. Seven *Phytophthora* taxa were previously unknown to science. The reasons for these failures of plant biosecurity in Europe, implications for forest and semi-natural ecosystems and possible ways to improve biosecurity are discussed.

## SESSION 1

# PHYTOPHTHORA IN NURSERIES AND PLANTING AREAS

**Exotic *Phytophthora* species are being systematically introduced in California wildlands during restoration projects.** Laura Sims<sup>1</sup>, Matteo Garbelotto<sup>2</sup>

<sup>1</sup>Louisiana Tech University, Ruston LA

<sup>2</sup>University of California, Berkeley, CA

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This study describes the identification of several *Phytophthora* species previously found in San Francisco Bay Area nurseries [1]. from multiple sites at the urban wildland interface within the greater San Francisco Bay Area. Isolation success was highest from the three plant species *Diplacus aurantiacus* (sticky monkey-flower), *Ceanothus thyrsiflorus* (blueblossum), and *Frangula californica* (California coffeeberry), growing in restoration sites and in adjacent disturbed sites. Isolation success instead was zero in control undisturbed “natural” areas adjacent to infested sites. In order to confirm plant production facilities were the source of the *Phytophthora* strains isolated from restoration sites, genetic and phenotypic analyses were performed on *Phytophthora crassamura*, one of the most common species identified during the course of this and other studies. Results indicated that field isolates were genetically identical to those found in plant production nurseries. Furthermore, resistance to fungicides such as phosphites and mefenoxam was identified in a select number of isolates found both in plant production facilities and wildland sites, further corroboration of a “nursery” origin of wildland infestations [2]. Spatial landscape-level analysis identified patterns of *Phytophthora* spread consistent with an outward expansion from sites that were planted and with topography, although the presence of some species was not associated with water accumulation patterns. *Phytophthora* species assemblages were site-specific and possibly related to site type, nursery where plants were grown, and time since planting. To our knowledge, this is one of the first studies providing robust evidence that exotic *Phytophthoras* are being systematically introduced through the use of infected plants in restoration projects.

References:

- [1] Sims L., Tjosvold S., Chambers D., Garbelotto M., 2018. Control of *Phytophthora* species in plant stock for habitat restoration through best management practices. *Plant Pathology*, 68: 196–204.
- [2] Sims L., Chee C., Bourrett T., Hunter S., Garbelotto M., 2018b. Genetic and phenotypic variation of *Phytophthora crassamura* isolates from California nurseries and restoration sites. <https://doi.org/10.1016/j.funbio.2018.11.01>



**Assessing the incidence and diversity of *Phytophthora* species occurring in planned restoration areas of the Angeles National Forest.** Sebastian N. Fajardo<sup>1</sup>, Tyler B. Bourret<sup>1</sup>, Chris Endelenbos<sup>1</sup>, Evan Lozano<sup>1</sup>, Katie VinZant<sup>2</sup>, David M. Rizzo<sup>1</sup>, Susan J. Frankel<sup>3</sup>

<sup>1</sup>UC Davis Plant Pathology, Davis, CA

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<sup>3</sup>USDA Forest Service, Pacific Southwest Research Station, Albany, CA

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The Angeles National Forest (ANF), located in the greater Los Angeles metropolitan area encompasses approximately 700,000 acres (238,230 ha), comprising a vital biodiversity hot spot. From 2002 to 2008, three major fires affected approximately 40,000 acres (16,187 ha) of coastal sage scrub, montane chaparral, grassland, riparian corridor, as well as isolated big cone Douglas-fir (*Pseudotsuga macrocarpa*) stands. Droughts and floods in the following years, plus off-road recreational off-highway vehicle use aggravated erosion and altered chaparral vegetation regeneration. As a mitigation effort, restoration attempts in these areas were initiated to plant native nursery stock.

In 2016 - 2017, prompted by concerns that *Phytophthora* species may have been introduced on restoration plantings, *Phytophthora* surveys were conducted in several restoration locations associated with utility project mitigation on ANF lands. These preliminary surveys detected numerous *Phytophthora* species associated with outplanted native plants and at the source nurseries. The inadvertent outplanting of infested nursery stock is considered one of the main pathways of entry for exotic *Phytophthora* into natural areas.

To better understand the *Phytophthora* distribution on arid lands of the ANF, three *Phytophthora* surveys were performed between May 2018 to March 2019 in areas that had burned in the fires and were prioritized for restoration. In total, 508 soil samples were collected from a range of forest types, including areas which had been planted with container nursery stock. Thirteen *Phytophthora* spp. were detected (*P. borealis*, *P. cactorum*, *P. chlamydospore*, *P. crassamura*, *P. gonapodyides*, *P. inundata*, *P. lacustris*, *P. riparia*, *P. lacustris x riparia* hybrid, *P. multivora*, *Phytophthora* sp. *cadmea*, *Phytophthora* sp. *NJB-2015*, and *P. taxon agrifolia* 2). *Phytophthora* spp. detections were primarily associated with riparian areas. However, *Phytophthora*-positive detections were made on chaparral, grasslands, oak woodlands and on off-road vehicle tracks. *P. crassamura* was found to be the most common and present in all forest types.

Previous studies have associated *P. crassamura* with a Mediterranean climate and with restoration activities, thus indicating the potential danger that this species presents to the ANF area, but the pathogenicity of this species is not fully known. Sampling will be repeated seasonally to determine what additional factors are correlated with the incidence of *Phytophthora* pathogens.

**Metabarcoding of *Phytophthora* communities at restoration sites in San Francisco Peninsula open spaces.** Ebba Peterson<sup>1</sup>, Joyce Eberhart<sup>2</sup>, Neelam Redekar<sup>2</sup>, Amanda Mills<sup>3</sup>, and Jennifer Parke<sup>1,2</sup>

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The inadvertent spread of *Phytophthora* from nurseries into native ecosystems has increased interest in assessing their diversity in native plant communities. Of special concern is the introduction of new species to vulnerable wildlands during restoration outplantings. *Phytophthora* spp. are abundant within plant nurseries; their introduction can result in failed plantings, reduced natural regeneration, and spread to established vegetation. To assess the distribution of *Phytophthora* in restoration plantings and surrounding wildlands we surveyed restoration sites located on the San Francisco Peninsula within the Midpeninsula Regional Open Space District (MROSD) preserves.

In December of 2017 and 2018 we collected and baited a total of 579 soil samples from the base of native shrubs and seedlings at 30 planted restoration sites, 12 planned restoration sites, and 29 non-planted areas adjacent to restoration projects. We also extracted DNA from each sample and submitted ITS1 amplicons (250 base pairs in length) for sequencing on the Illumina MiSeq platform. Distinct sequences, or operational taxonomic units (OTUs), were assigned to a species when the amplicon was a  $\geq 99\%$  match to known sequences. In many cases, species could not be distinguished over the sequenced region, in which case the OTU was assigned a complex or cluster designation representing multiple potential species. To reduce the inclusion of false-positives we required an individual OTU comprise a minimum of 0.05% of the within-sample relative abundance. Some sites were sampled both years to test our ability to detect the same species (baiting) or OTU (Illumina MiSeq) between years.

*Phytophthora* was equally prevalent in planted and non-planted areas, however many species were only found in areas where nursery plants were introduced. *P. cactorum* and *P. cambivora* were the most commonly baited species; the *P. cactorum*-cluster was the second most abundant OTU detected, while the *P. cambivora*-complex was underrepresented in the MiSeq analysis. Clade 3 species were rarely baited from soils (recovered from 2 soil samples) but were widespread and abundant in the MiSeq analysis (detected in 63 of the 383 samples successfully sequenced to date), consistent with the hypothesis that some are native to the western United States.

Metabarcoding detected numerous species not recovered by baiting, including sequences matching at  $\geq 99\%$  similarity to the *P. quercina*-cluster (*P. quercina* and/or *P. versiformis*) and *P. tentaculata*; both *P. quercina* and *P. tentaculata* are species of ecological concern that have only recently described in California. To confirm the identify of *P. quercina*, we additionally sequenced these extracts with the MinION sequencer, which provides longer (1,000 bp) read lengths; this revealed this OTU was an approximate 90% match to the *P. quercina*-cluster and represents a taxon not present in our database. Similarly, numerous samples from three sites had detections of an OTU matching the *P. uliginosa*-cluster (*P. uliginosa* and/or *P. europea*, neither of which were baited); more than likely this OTU is *P. sp. 'cadmea'*, a species for which

we successfully recovered 3 isolates in 2 separate preserves and was only recently recovered by Bourret [1] in a neighbouring county.

We baited *Phytophthora* from 55 samples for which MiSeq data was obtained. Of these, we were unable to detect the baited species in 54% of the samples. When the baited species was detected, the OTU comprised only 0.01 to 0.499% of the within-sample relative abundance for the majority of samples. The detected species comprised greater than 1% of the OTU reads in only 4 samples.

Soil was sampled from the base of 108 plants in both years. Of these, 72% of the samples were negative for *Phytophthora* via baiting in both years. When a *Phytophthora* was detected, the majority (24 repeat-pairs) were positive in only year; we baited the same *Phytophthora* species both years from the soil collected from only 5 plants. Reproducibility between years was similarly inconsistent via MiSeq. Notably, we did detect the OTU matching *P. tentaculata* from the same location both years, albeit at a low relative abundance. While this species was not detected via baiting, it is unlikely this detection is a false positive and may represent a prior introduction. As this process detected DNA remnants in addition to viable pathogens, it is possible *P. tentaculata* was introduced to the site but never established.

*Phytophthora* spp. are widespread within MROSD preserves, although some preserves had noticeably greater species diversity and detection frequency. While the short read lengths and deficiencies in existing databases make positive identification of OTUs difficult, Illumina MiSeq sequencing is a sensitive tool able to detect prior introductions and describe *Phytophthora* diversity. The detection of *Phytophthora* was poorly indicative of plant health status, however the prevalence of plant-pathogenic species should encourage the use of best management practices to limit spread of *Phytophthora* to surrounding lands.

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## Preventing *Phytophthora* introductions into California restoration sites. Susan J. Frankel<sup>1</sup>, Janell Hillman<sup>2</sup>, Diana Benner<sup>3</sup>, Janice Alexander<sup>4</sup>, Alisa Shor<sup>5</sup>

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Over the past five years, *Phytophthora tentaculata*, *P. cactorum* and other new or new hybrid *Phytophthora* spp. have been unintentionally but extensively introduced into restoration areas in the greater San Francisco Bay Area [1]. The infections are on hosts in more than 20 different families [2], comprised of a variety of shrubs and herbaceous native plants. The susceptibility of these and other California native plants to plant pathogens, including Phytophthoras, is not well understood despite several of the plants being federal and state listed endangered species. To prevent further *Phytophthora* introductions and spread on California native plants, the Phytophthoras in Native Habitats Work Group ([www.calphytos.org](http://www.calphytos.org)) continues to develop and test treatments, detection methods and strategies to improve phytosanitation in restoration nurseries and outplantings [3]. Innovations include an accreditation program for nurseries that commit to improved phytosanitation (AIR), solar ovens, an irrigation leachate pear baiting detection technique, changes in nursery stock deployment and acquisition, as well as education and outreach emphasizing protection of both sensitive habitats and restoration site investments. Economic, biological and practical considerations continue to impede strategies to minimize infection and thus reduce the risk of irreversible environmental degradation from *Phytophthora* infections in sensitive habitats, other landscapes and remnant wildlands. However, several nurseries have demonstrated that they can produce plants for several years without *Phytophthora* detection. Increased interceptions of infected plant material prior to outplanting demonstrate that improved management can contribute to wildland plant health protection.

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***Phytophthora* communities in UK plant nurseries and links to management practice: opportunities for mitigation through accreditation.** [Sarah Green](#)<sup>1</sup>, David Cooke<sup>2</sup>, Debra Frederickson-Matika<sup>1</sup>, Beatrix Clark<sup>2</sup>, Eva Randall<sup>2</sup>, Mhairi Clark<sup>1</sup>, Tim Pettit<sup>3</sup>, Mike Dunn<sup>1</sup>, Alexandra Schlenzig<sup>4</sup>, Leighton Pritchard<sup>2</sup>, Peter Thorpe<sup>2,6</sup>, Peter Cock<sup>2</sup>, Jane Barbrook<sup>5</sup>

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The PHYTO-THREATS project is an interdisciplinary collaboration of seven institutions aimed at addressing risks to UK forest and related ecosystems from *Phytophthora*. One key objective of this three-year project, which started in April 2016, is to examine the diversity of *Phytophthora*s across a range of UK plant nurseries operating different management practices. Metabarcoding is being used to identify *Phytophthora* species in root and water samples collected from each nursery. Fifteen partner nurseries were sampled 3-4 times over the course of the project, generating over 4000 samples in total. A further 600 samples were collected from over 100 nurseries as part of a broader scale sampling by statutory Plant Health inspectors. Approximately 50% of all samples were positive for *Phytophthora* and/or other closely related oomycetes. *Phytophthora* findings, as determined by metabarcoding analyses of the DNA present in samples, will be presented in relation to host associations and management practice. Evidence generated from the nursery sampling is being used to underpin a 'management standard' being developed as part of a new UK wide 'Plant Healthy' accreditation scheme. This scheme, to be rolled out in 2019, targets producers and consumers across the plant supply chain.



**Temporal patterns of airborne *Phytophthora* spp. in a woody plant nursery area detected using real-time PCR.** D. Migliorini<sup>1</sup>, L. Ghelardini<sup>2</sup>, N. Luchi<sup>1</sup>, M. Onorati<sup>3</sup>, A. Santini<sup>1</sup>

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In this study, spore trap monitoring was applied to provide a proof of concept for the use of qPCR to detect *Phytophthora* in aerial samples and provide valuable information for epidemiological studies in nurseries. Two qPCR TaqMan assays were developed to detect pathogen DNA: the first used a generic probe to detect *Phytophthora* spp. [1], and the second was based on a specific probe for detecting *P. ramorum* and *P. lateralis*. All samples tested positive for the genus *Phytophthora*, although *P. ramorum* and *P. lateralis* were not detected. In late spring and in autumn, two main peaks of *Phytophthora* sporulation were observed. Peaks were preceded by rainfall, high relative humidity, and mild temperature. From mid-May to the end of August, *Phytophthora* DNA detected in the air increased with relative humidity, while it decreased with increasing mean temperature. There was also a positive correlation between *Phytophthora* DNA detected and rainfall in the same period. No significant correlations between *Phytophthora* DNA and temperature or rainfall were found from the end of August to December. Our results are in agreement with those obtained with classical diagnostic methods based on microscopy [2,3], but the approach used here enabled rapid detection and relative quantification of the target organisms, thus assisting in the implementation of disease management strategies.

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**Epidemic spread of *Phytophthora nicotianae* in a Mediterranean park in Athens causing decline and mortality of shrub and tree species. Andrea Vannini, Carmen Morales-Rodriguez**

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*Phytophthora nicotianae* Breda de Haan (= *Phytophthora parasitica* Dastur) is a destructive pathogen on a wide range of hosts including many woody ornamental species. In multiple surveys carried out in planting beds of a large park in Athens, soil was found heavily infested by *P. nicotianae*. Semi-quantitative baiting technique applied to soil in planting beds resulted in frequency of infestation up to 100%. The epidemic spread was favoured by the presence of highly susceptible species such as *Euphorbia* spp., *Rosmarinus* spp., *Salvia* spp., *Lavandula* spp. Surprisingly, *P. nicotianae* was constantly isolated from bleeding cankers on *Platanus orientalis* up to 2 meters on the stem. This is the first record of *P. nicotianae* causing collar and stem canker on *P. orientalis*. *Phytophthora nicotianae* is considered the most common species infesting Mediterranean nurseries [1], thus, its introduction in urban parks with plants for planting is usual and not surprising. Based on the review of the past management of the park, the outbreak was likely caused by unappropriated practices including lack of preventive and early eradication measures.

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***Phytophthora oleae* a widespread species in soil of olive (*Olea europaea*) orchards in Southern Italy.** M. Riolo<sup>1</sup>, L. Schena<sup>2</sup>, F. Aloï<sup>1,3</sup>, E. Santilli<sup>4</sup>, D. Ruano-Rosa<sup>5</sup>, G.E. Agosteo<sup>2</sup>, A. Pane<sup>1</sup>, F. La Spada<sup>1</sup>, S.O. Cacciola<sup>1</sup>

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In 2015 a new homothallic species of *Phytophthora* in clade 2, with an optimum growth temperature of around 20°C, was found to cause rot of mature olive fruit in Calabria, Southern Italy, and was formally described with the epithet *P. oleae* [1]. Assuming that *P. oleae* had only temporarily adapted to an aerial life style, we investigated its distribution in rhizosphere soil of olive orchards along the Tyrrhenian coast of Calabria using leaf baiting and a selective medium. Isolates were identified on the basis of morphological characteristics and by sequencing the ITS1-5.8S-ITS2 region and *cox1* gene. *P. oleae* was consistently recovered from rhizosphere soil and fine roots of olive trees of an olive-growing area of about 30,000 ha at an altitude ranging from 87 to 365 m a.s.l. Apparently, it was not associated with symptoms of tree decline. Interestingly, in a survey of protected natural areas in Sicily, whose results have been partly published [2], *P. oleae* has been found in rhizosphere soil of phytocenosis *Olea europaea-Quercetum virgiliana*. The presence of *P. oleae* in a very large rainy area in Calabria and in a relatively undisturbed ecosystem in Sicily without any visible symptom on the tree canopy would suggest this species is endemic or naturalized in Southern Italy. It may cause occasional outbreaks of fruit rot and leaf blight when environmental conditions are conducive. Recently, *P. oleae* has been reported as a root pathogen of wild olive in a very restricted protected natural area in Spain [3] where it has been considered an exotic invasive pathogen.

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**Diversity of *Phytophthora* species in a public garden in Essex, UK.** Elizabeth Beal, Fryni Drizou, Gerard Clover

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Hyde Hall garden, in Essex, is one of four RHS gardens open to the public. The garden welcomes more than 325,000 visitors a year. This amount of footfall is accompanied by the risk of disease spread into and within the garden, as well as spread out of the garden into the surrounding areas.

In common with many other public gardens, *Phytophthora* spp. infections have been confirmed across RHS Hyde Hall. For example, in 2017 several alder trees with bleeding cankers were discovered in the garden and molecular tests revealed the cause to be two different species of *Phytophthora*: *P. alni* subsp. *multiformis* and *P. siskiyouensis*. *Phytophthora alni* is highly specific to alder and was first recorded in the UK in 1993 [1]. It is now found throughout the UK. Interestingly this is only the second record of *P. siskiyouensis* in the UK. This pathogen appears to be native to south-west Oregon and was first recorded in the UK in 2013 [2].

In addition, the garden is host to the National *Viburnum* Collection, which has also been affected adversely by *Phytophthora* spp.

This work aims to develop a management plan to minimise the impact of *Phytophthora* in the garden, by identifying which *Phytophthora* species are present, and how they are distributed across the garden. During summer 2019, samples of infected plants will be collected from different areas across Hyde Hall. Additionally, samples will be collected from the water reservoir which provides all the irrigation for the garden and may have a role in pathogen spread.

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## SESSION 2

# GENETICS AND GENOMICS

**Novel insights into the evolution and emergence of *Phytophthora* pathogens using genomics approaches.** Niklaus J. Grünwald<sup>1</sup>, Shankar K. Shakya<sup>2</sup>, Brian J. Knaus<sup>2</sup>, Jerry E. Weiland<sup>1</sup>, Valerie J. Fieland<sup>2</sup>, Marilia Horta Jung<sup>3</sup>, Cristiana Maia<sup>3</sup>, André Drenth<sup>4</sup>, David I. Guest<sup>5</sup>, Edward C.Y. Liew<sup>6</sup>, Coline Crane<sup>7</sup>, Bruno Scanu<sup>8</sup>, Thomas Jung<sup>3</sup>, Felipe Albornoz<sup>2</sup>, Zachary S. L. Foster<sup>2</sup>, Meredith M. Larsen<sup>1</sup>, Frank N. Martin<sup>9</sup>

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*Phytophthora* species are challenging newly emerging and re-emerging plant pathogens. We have studied patterns and processes involved in emergence of several *Phytophthora* species using a range of approaches including genotyping-by-sequencing and whole genome sequencing. Here we report on novel findings on the emergence of *P. cinnamomi* using genotyping-by-sequencing conducted in collaboration with a global consortium of colleagues. The highest diversity was found in Asia, followed by Australia and South Africa. The rest of the world has predominantly 2 panglobal lineages of the A2 mating type. A stepping-stone model best describes emergence out of Asia via Australia/South Africa to the rest of the world. We are also currently sequencing genomes of *P. ramorum* using PacBio long-read sequencing and short-read Illumina sequencing on Asian, European and North American samples. This work is currently in progress. Finally, we are in the final stages of developing a new barcode for amplicon sequencing using MiSeq for the *rps10* mitochondrial locus. This barcode is more informative and PCR more reproducible than the ITS1 locus often used for oomycetes. A website (<http://oomycetedb.cgrb.oregonstate.edu/>) has been set up with protocols and a reference database for calling of known taxa. We encourage the oomycete community to contribute *rps10* sequences to this database.

**Highly contiguous genome assemblies for three *Phytophthora* species generated from PacBio sequencing.** Ewan Mollison<sup>1</sup>, Paul Sharp<sup>1</sup>, Carolyn Riddell<sup>2</sup>, David Cooke<sup>3</sup>, Leighton Pritchard<sup>3</sup>, Peter Thorpe<sup>4</sup>, Aaron Jeffries<sup>5</sup>, Sarah Green<sup>2</sup>

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We used a modified protocol to extract high quality *Phytophthora* genomic DNA suitable for long-read PacBio technology and successfully sequenced and assembled the genomes for three species of *Phytophthora* currently regarded as less damaging than their close relatives. *Phytophthora europeae* was first found associated with the rhizosphere of oak trees and is closely related to the highly damaging species *P. alni*; *P. foliorum* was first found on azalea and is closely related to *P. ramorum*; and *P. obscura* was first found in association with horse chestnut and kalmia, and is closely related to *P. austrocedri*. All three genomes were sequenced to approximately 100-fold coverage using PacBio long reads and following assembly, scaffolding and polishing produced highly contiguous assemblies for all three with N50 values of 6.40 Mbp (*P. obscura*), 7.50 Mbp (*P. foliorum*) and 10.97 Mbp (*P. europeae*). Completeness of coverage estimation using BUSCO indicated a very good coverage of the gene-space of the three organisms: of 234 BUSCOs associated with stramenopiles 98 – 99% were identified as being “complete”, with only around 1% of these classed as duplicates, suggesting that a good resolution of the haplotypes has been achieved during assembly. Repeat modelling and masking indicated repeat contents of 29 – 35% and Augustus gene prediction identified between 19,441 and 19,658 possible gene models for the three species. Availability of such highly contiguous and apparently complete genome assemblies should provide a valuable resource and tool for studying genes associated with pathogenicity in highly damaging *Phytophthora* species.

**A microsatellite analysis identifies global pathways of movement of *Phytophthora cinnamomi* and the likely sources of wildland infestations in California and Mexico.** María Socorro Serrano<sup>1,2</sup>, Todd Osmundson<sup>1,4</sup>, Alejandra Almaraz-Sánchez<sup>1,3</sup>, Peter JP Croucher<sup>1</sup>, Ted Swiecki<sup>5</sup>, Dionicio Alvarado-Rosales<sup>3</sup>, Matteo Garbelotto<sup>1</sup>

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The genetic structure of a sample of isolates of the oomycete plant pathogen *Phytophthora cinnamomi* from natural and agricultural outbreaks, and the long-distance movement of individual genotypes, were studied using four microsatellite markers on 159 isolates of Californian, Mexican, and worldwide origin. Allelic profiles identified 75 multilocus genotypes; STRUCTURE analysis placed them in three groups characterized by different geographic and host ranges, different genic and genotypic diversity, and different reproductive modes. When relationships among genotypes were visualized on a minimum spanning network (MSN), genotypes belonging to the same STRUCTURE group were contiguous with rare exceptions. A putatively ancestral Group 1 had high genic diversity, included all A1 mating type isolates and all Papuan isolates in the sample, was rarely isolated from natural settings in California and Mexico, and was positioned at the center of the MSN. Putatively younger Groups 2 and 3 had lower genic diversity, were both neighbors to Group 1 but formed two distinct peripheral sectors of the MSN, and were equally present in agricultural commodities and natural settings in Mexico and California. The presence of identical genotypes from the same hosts in different continents indicates that long-distance human-mediated movement of *P. cinnamomi* has occurred. The presence of identical genotypes especially from Groups 2 and 3 at high frequency in neighboring wildlands and agricultural settings and suggests that specific commodities may have been the source of recent wild infestations caused by novel invasive genotypes. We provide evidence that these overrepresented clonal genotypes are present in multiple sites and can be regarded as emergent: their emergence may be correlated with a relatively young age and with higher fitness. We provide evidence in support of both hypotheses and suggest these genotypes may represent a novel threat.

## SESSION 3

### NEW DETECTION METHODS



***Phytophthora* detection via eDNA metabarcoding.** David E.L. Cooke<sup>1</sup>, Eva Randall<sup>1</sup>, Beatrix Clark<sup>1</sup>, Peter Thorpe<sup>1</sup>, Peter Cock<sup>1</sup>, Leighton Pritchard<sup>1</sup>, Debbie Frederickson-Matika<sup>2</sup>, Sarah Green<sup>2</sup>

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The detection of invasive plant pathogens such as *Phytophthora* that destroy crops, horticulture, forestry and plants in natural ecosystems is an ongoing challenge. Pathogen isolation remains the ‘gold standard’ but is time consuming and, as with any detection system, prone to bias. The 170 or so formally described *Phytophthora* species are only a fraction of the expected diversity and a large potential reservoir of *Phytophthora* taxa thus occur in natural ecosystems. Knowledge of the spatial and temporal distribution of known and unknown *Phytophthora* species and their ecology and impact is crucial for the accurate interpretation of plant biosecurity protocols. We have combined a *Phytophthora*-specific PCR test based on the rDNA ITS1 region and high-throughput Illumina sequencing to survey *Phytophthora* diversity in samples of environmental DNA (eDNA). The eDNA samples comprise water from *in situ* water filtration within a stream catchment and soil close to the water sampling sites from several natural and semi-natural ecosystems across Scotland. The method is proving effective and yielding rich datasets of *Phytophthora* diversity, but technical challenges and questions remain. For example, the downstream computational biology pipeline to process the data must be validated and based on a robust database of reference sequences that copes with ‘fuzziness’ and overlap around species boundaries. Such validation is critical to objectively assess the benefits of the technology for plant health legislation and ecosystem surveillance.

**Comparison of soil baiting and metabarcoding methods for the detection of *Phytophthora* species in Scottish environments.** D.E. Frederickson Matika<sup>1</sup>, S. Green<sup>1</sup>, D.E.L. Cooke<sup>2</sup>, L. Pritchard<sup>2</sup>, E. Randall<sup>2</sup>, B. Clark<sup>2</sup>

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New invasive *Phytophthora* species pathogenic to native trees and shrubs, include *P. ramorum*, *P. kernoviae* and *P. austrocedri*. These pathogens, which were first detected in the UK from 2002 onwards, are still causing serious economic and ecological impacts in UK forests and ecosystems [1,2]. Effective surveillance to accurately inform plant health policy requires an accurate and reliable detection method for *Phytophthora* across a range of environments. In this project five sites in Scotland were chosen, comprising two commercial forestry plantations, one native woodland, one lowland agricultural site and an urban public park. Several of these sites have tree populations at epidemic or post-epidemic stages of an already identified *Phytophthora* disease outbreak. This study compared outputs from a traditional baiting and *Phytophthora* isolation method with those of a DNA-based metabarcoding method. Beginning in September 2016, and twice annually thereafter, replicate soil and water samples were collected beneath 3 trees sited close to a natural stream at each location. Water samples were filtered on-site and filters stored in buffer for DNA extraction and metabarcoding. Soil samples were mixed thoroughly then divided, providing one half for metabarcoding and one for baiting. The complexities of soil baiting and the *Phytophthora* species recovered will be discussed and compared with DNA metabarcoding as a detection method.

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**Comparison of oomycete and *Phytophthora* specific primers for identification and metabarcoding.** Treena Burgess, Diane White, Sarah Sapsford

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The use of metabarcoding to determine oomycete community composition is becoming increasingly common. There have been a range of primers developed purported to be oomycete or *Phytophthora* specific, but are all primer sets equivalent? This study aimed to determine the sensitivity and specificity of 14 different primer sets and combinations amplifying the ITS, *cox* and YPT gene regions using the Illumina platform. To do this a mock community of 68 *Phytophthora* species from 11 phylogenetic clades was generated where the DNA concentration of different species ranged from 0.3 to 30 ng/μl. Each primer set was used to amplify (in triplicate) (1) the mock community alone, (2) an environmental (eDNA) sample previously determined to contain *Phytophthora*, and (3) the same eDNA sample spiked with the mock community. For the mock community the different primer sets amplified between 50 to 100% of the species present in the mix and the relationship between read number and DNA concentration range from  $r^2 = 0.10$  to 0.75. Fewer of the species in the mock community were recovered from the spiked eDNA samples for almost all of the primer pairs. For the eDNA samples alone the average fraction of oomycete reads per sample ranged from 0.04-100%. Variability between the replicate samples was low for the mock community but varied considerably for eDNA samples highlighting the need to amplify and pool replicate samples before sequencing. The primers varied considerably in specificity and sensitivity and care must be taken when selecting primers depending on if the intention is to identify species or quantify the relative proportion of different oomycetes in a sample.

**Comparison between direct isolation, baiting and molecular approaches to determine the oomycetes present in hardy woody plants in the plants for planting pathway.** Alexandra Puertolas<sup>1,3</sup>, Peter J.M. Bonants<sup>2</sup>, Eric Boa<sup>1</sup>, Steve Woodward<sup>1</sup>

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Detection and identification of pathogens are crucial to reduce risks posed by Oomycota and to increase biosecurity in the plants for planting pathway. Hardy woody ornamental plants were obtained from nurseries, general retailers and internet sellers, and oomycete diversity present in roots and compost determined using a combination of classical isolation techniques and molecular analyses. Oomycete load was quantified using TaqMan PCR through analysis of environmental DNA from the nursery plants, the three loci ITS, trnM-trnP-trnM and atp9-nad9. Ninety sampled plants were contaminated with at least one oomycete species: 10 *Phytophthora* spp., 17 *Pythium* spp. and 5 *Phytopythium* spp. were isolated using baiting methods. Quantification with TaqMan PCR suggested a significantly higher oomycete load associated with each plant, including asymptomatic plants, with multiple oomycetes occurring in the composts in comparison to roots and filters from baiting water.

**Exploring *Phytophthora* community with HTS: a proposed pipeline for data analysis.** Carmen Morales-Rodriguez<sup>1</sup>, Wolfgang Oßwald<sup>2</sup>, Andrea Vannini<sup>1</sup>

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High Throughput Sequencing (HTS) techniques were often applied in the last years to assess the Oomycetes community in soil or other environmental samples. Pioneer works employed the 454 platform, while now the Illumina MiSeq technology is the most used in metabarcoding studies, using a priming approach most of the time based on rDNA markers. Beside the several advantages provided by HTS technologies, such as high sensitivity, some problem in data quality and risk of false positives need to be considered also to allow data comparison from different laboratories. With the present contribute, a pipeline of data analysis based on free-share software, run in Window and Linux systems, is proposed with some examples coming from environmental samplings carried out in Germany and Spain. The relevance of using the appropriate Mock community as positive control is also discussed. Finally, the use of HTS as a tool for detection of regulated pathogens is evaluated on the base of the state of the art.



**The structure and functionality of soil microbiota influenced health status of holm oak in dehesas affected by *Phytophthora* root rot.** Francisco J. Ruiz Gómez<sup>1</sup>, Rafael M. Navarro-Cerrillo<sup>1</sup>, Andrea Vannini<sup>2</sup>, Carmen Morales-Rodríguez<sup>2</sup>

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Forest decline is nowadays a major challenge for sustainability of Mediterranean evergreen oak ecosystems sustainability. In the case of the holm oak decline in Andalusia (Spain), the problem is exasperated by the impact of alien invasive pathogens such as *Phytophthora cinnamomi*. In the study of the interactions among plant, pathogen and environment, the qualitative and quantitative structure of the rhizosphere microbiota and its role in the disease expression level are of great interest even in a perspective of sustainable pest management strategies. In the present study, the rhizosphere microbiome of 25 plots distributed among the main Andalusian dehesas territory was analyzed for fungal and oomycete communities. Total DNA was extracted from soil samples and metabarcoded amplicon libraries were “de novo” sequenced through NGS. Sequences were processed to obtain Operational Taxonomic Units (OTUs) frequency matrix. OTU's were identified using the Basic Local Alignment Search Tool, and diversity indices were calculated. A possible association of structure and functionality of soil microbiota with tree defoliation indices and biogeographical zones, was investigated. Both the composition and the functionality of soil microbiome were found associated to tree health indices in specific cases: i) the presence of an OTU of *Trichoderma* spp. was significantly correlated with the scarcity/absence in soil of pathogenic *Phytophthoras*, and ii) the presence of the ectomycorrhizal species *Russula praetervisa* was inversely correlated with defoliation level of trees. Finally, defoliation rate was inversely correlated with diversity of oomycetes (evenness vector). The present study highlighted the potential of biotechnological application based on soil microbiome structure and functionality, to mitigate or prevent the impact of invasive *Phytophthoras* on Mediterranean evergreen oak ecosystem. The use of fungal taxa with specific function (e.g. antagonist, mycorrhizal) open to the development of strategies of biological control joining efficacy and environmental sustainability.

**Assessing the spread of Phytophthoras in Scottish forests by recreational and harvesting activities using comparative qPCR and metabarcoding techniques.** [April Armstrong](#)<sup>1</sup>, Alistair Penny<sup>1</sup>, Claudia Vacca<sup>1</sup>, Kalliopi Tsarna<sup>1</sup>, Carolyn Riddell<sup>1</sup>, Pete Hedley<sup>2</sup>, Sarah Green<sup>1</sup>

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*Phytophthora ramorum* has caused substantial losses of Scottish forests in recent years due to widespread mortality of Japanese larch trees (*Larix kaempferi*). Infected trees are subject to statutory felling notices in an effort to reduce sporulation potential. Nevertheless, there are concerns about the multiple pathways by which spores might be transferred to new sites. This project began in 2016 with its original aim to assess the impact of human mediated spread due recreational activities within the forest. The UKs Forestry Commission and Scottish Forestry's "Keep-it-Clean" campaign encourages recreational forest users to engage in biosecurity. Walking/running and cycling in the forest have been identified as the two main leisure activities with the potential to transport soil/plant material between forest and forest users are asked to clean their shoes and bicycle tyres between forest visits. Transects were walked/cycled in 2 forest locations in Scotland with either standing infected or recently felled infected *L. kaempferi* with a further 3<sup>rd</sup> site being assessed that had no prior record of infection. Material was collected from treads and tested for the presence of *P. ramorum*.

The project has now extended to the commercial sector looking at the ability of harvesting machinery to transport *P. ramorum* and other Phytophthoras. Samples have been collected from 6 larch forest locations in Scotland with varying degrees of infection both within and outwith the management zone. Samples will be assessed via both qPCR and metabarcoding allowing for a comparative study of techniques and presented alongside isolation data.

## **Detection of *Phytophthora* pathogens in forest nurseries using quantitative PCR techniques.** Aneta Bačová, Michal Tomšovský, Tomáš Májek

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The most common disease caused by *Phytophthora* infection in the Czech Republic is the fall of European beech (*Fagus sylvatica*) seedlings. The most frequent species involved are *P. plurivora* and *P. cactorum*. Epidemic disease incidences can cause relatively significant economic damage locally. In mature beech trees pathogens can invade the tree months to years before they cause crown symptoms, often after a significant reduction of the root system by more than 50%. Woody diseases caused by *Phytophthora* pathogens are generally very common. Although *Phytophthora* species are relatively difficult to detect, a large proportion of root and collar rot diseases can be attributed to them.

The aim of the study is to optimize the methodology for detection of *Phytophthora* species on asymptomatic plants in forest nurseries. Diseases can be spread to woodlands when infected but asymptomatic nursery seedlings are used for afforestations. Therefore, methods aimed at the early detection of pathogens in asymptomatic plants or from infested soil are particularly necessary for nursery operations. In this study, the pathogen will be detected by quantitative PCR using DNA and RNA extraction from soil and irrigation water. The method allows not only the detection but also the relative quantification of the inoculum in the sample. Optimizing this methodology will enable nurseries to detect infections within a few days, and it is possible to target directly to a particular species of pathogen if species-specific probes are used. The samples will be quantified using qPCR with species-specific probes for the three *Phytophthora* species most commonly found in the Czech Republic (*P. plurivora*, *P. xcambivora*, *P. cactorum*). For other species, a primer specific for oomycetes will be used. To verify the sensitivity of this method, the isolation of pathogens from the soil by baitings will be done.

**Metabarcoding identification of *Phytophthora* species from mock environmental samples via MinION HTS and the importance of a database of the Ex-types.** Z.G. Abad<sup>1</sup>, S.K. Srivastava<sup>1,2</sup>, L.M. Knight<sup>1,2</sup>, K. Zeller<sup>1</sup>, M. Nakhla<sup>1</sup>

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The genus *Phytophthora* contains numerous species that affect forest and natural ecosystems and that are of biosecurity concern. The MinION 3<sup>rd</sup> generation High-throughput Sequencing (HTS) portable device, is being used for HTS applications in medicine, veterinary, diagnostics and food safety, and this system is opening new opportunities for the evaluation of environmental DNA (e-DNA) in a cost-effective and real-time fashion in Plant Pathology. Here we describe testing the MinION sequencer for its utility in plant regulatory diagnostics. We have designed and tested ITS rDNA primers for environmental DNA metabarcoding for identification of *Phytophthora* species. In our research we have accurately identified ten *Phytophthora* species in mocked individual and mixed samples using the DNA barcoding ONT kit. By running the MinION after PCR amplification and library preparation we have generated reads that can identify the correct target *Phytophthora* and with consensus sequences from CAP3 assembled barcode(s) reads with BLAST accuracy of 99.51% to 100% of similarity to those target *Phytophthora* species. We verified the MinION 3<sup>rd</sup> generation HTS sequences by comparison to Sanger sequences and comparison to a highly curated database of specimens of the Ex-types of *Phytophthora* species that we have generated and submitted to GenBank. Correct identification of *Phytophthora* to species levels in e-DNA that contains individual and multiple species is possible with target region of the full ITS rDNA via the miniaturized MinION portable device.

**IDphy: molecular and morphological identification of *Phytophthora* based on the types. Demonstration of the international online resource.** Z.G. Abad.

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We recently completed “IDphy: Molecular and morphological identification of *Phytophthora* based on the types” Abad Z.G., Burgess T.I., Bienapfl J., Redford A., Knight L.K. and Coffey M.C. This was an international effort with the collaboration of scientists working at the USA and Australia.

The IDphy is an international online resource, free of charge <http://idtools.org/id/phytophthora/index.php> developed in order to provide robust and accurate identification for species in the genus *Phytophthora*. This important genus of plant pathology contains 186 species. Many of these are of economic importance or biosecurity concern. Morphological and molecular identification of *Phytophthora* to species levels can be very challenging due to overlapping or unclear morphological characters, and also due to misidentifications of sequences at the NCBI. We will demonstrate use of the IDphy resource for correct identification of species. Items that the online resource contains include: About section, fact sheets for culturable species, tabular and Lucid Keys, molecular Identification with five SOPs including sequencing identification using ITS and COI Barcoding genes and five additional genes. The resource has an innovative system for molecular identification via Blast and Phylogenetic draft analysis at the NCBI with work initiated at the IDphy. In addition, there is a morphological Identification page with two SOPs, glossary, gallery with more than 1000 figures and tools to identified particular species of *Phytophthora* for regulatory purposes. This is the first Online Resource with combination of Lucid Key with molecular identification via BLAST analysis implemented for plant pathogens.



## SESSION 4

# DIVERSITY AND DISTRIBUTION

**Insights into the biogeography and global diversity of *Phytophthora*.** Thomas Jung<sup>1,2</sup>, Ivan Milenkovic<sup>1</sup>, Tamara Corcobado<sup>1</sup>, Michal Tomšovský<sup>1</sup>, Josef Janousek<sup>1</sup>, Matej Panek<sup>1</sup>, Henrieta Ďatková<sup>1</sup>, Yilmaz Balci<sup>3</sup>, Bruno Scanu<sup>4</sup>, Clive M. Brasier<sup>5</sup>, Joan F. Webber<sup>5</sup>, Ana Pérez-Sierra<sup>5</sup>, József Bakonyi<sup>6</sup>, Diána Seress<sup>6</sup>, Alvaro Durán<sup>7</sup>, Marthin Tarigan<sup>7</sup>, Leonardo Oliveira<sup>7</sup>, Eugenio Sanfuentes von Stowasser<sup>8</sup>, Gaetano Magnano di San Lio<sup>9</sup>, Leonardo Schena<sup>9</sup>, Saveria Mosca<sup>9</sup>, Pham Quang Thu<sup>10</sup>, Chi Nguyen Minh<sup>10</sup>, Cristiana Maia<sup>11</sup>, Aschwin Engelen<sup>11</sup>, Giuseppe Carella<sup>12</sup>, Salvatore Moricca<sup>12</sup>, Santa Olga Cacciola<sup>13</sup>, Antonella Pane<sup>13</sup>, Federico La Spada<sup>13</sup>, Koji Kagayama<sup>14</sup>, Ayaka Hieno<sup>14</sup>, Hayato Masuya<sup>15</sup>, Seiji Uematsu<sup>16</sup>, Venche Talgø<sup>17</sup>, Miguel Redondo<sup>18</sup>, Jonas Oliva<sup>18</sup>, Alfredo Cravador<sup>19</sup>, Tun-Tschu Chang<sup>20</sup>, C.H. Fu<sup>20</sup>, Marília Horta Jung<sup>1,2</sup>

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Between 2013 and 2019, within the frame of several projects aiming at unravelling global diversity and biogeography of the genus *Phytophthora*, surveys were performed in natural ecosystems of Japan, Taiwan, Vietnam, Indonesia (Borneo, Java, Sulawesi and Sumatra), Chile, Nicaragua, Panama, Curacao, Egypt and eight countries in Europe. In total 320 forest sites, 410 forest streams, 9 mangrove forests, 6 lagoons and 5 other marine sites were sampled. Baiting assays and direct plating of necrotic plant tissues were used for isolating *Phytophthora* species from forest streams, forest soils and woody plants. Isolates were identified using both classical identification and sequence analysis of ITS, *cox1* and, if necessary, further gene regions. Overall, 13242 isolates were obtained which could be assigned to 65 known and 101 previously unknown species of *Phytophthora* belonging to 11 of the 12 known phylogenetic clades. In addition, an array of interspecific hybrids from *Phytophthora* Clades 6 and 8, 3 known and 24 novel *Halophytophthora* species and 9 species from the novel genus *Nothophytophthora* have been isolated. These surveys contributed to pin down the origin of several invasive aggressive *Phytophthora* pathogens, including *P. cinnamomi*, *P. ×cambivora*, *P. lateralis*, *P. ramorum* and the *P. citricola* complex. Together with records from previous *Phytophthora* surveys conducted by the authors and other researchers in natural ecosystems of Australia, Africa, Europe, the Americas and Asia, population genetic studies, and pathogenicity data this study provides insights into the global diversity and biogeography of the different clades and subclades of *Phytophthora* which will be discussed.

***Phytophthora lateralis* discovered in Japan.** C.M. Brasier<sup>1</sup>, J.F. Webber<sup>1</sup>, M. Horta-Jung<sup>2</sup>, S. Uematsu<sup>3</sup>, A. Heino<sup>4</sup>, K. Kagayama<sup>4</sup>, H. Masuya<sup>5</sup>, T. Jung<sup>2</sup>

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*Phytophthora lateralis*, one of the most destructive introduced tree pathogens in the Northern Hemisphere, has killed enormous numbers of native *Chamaecyparis lawsoniana* in its native range in the Pacific Northwest since the 1940s. It subsequently spread to Europe. Searches for the origin of the pathogen in Taiwan in 2008 and 2010 led to the discovery of four phenotypic lineages, two (TWK and TWJ) on healthy native *C. obtusa* v *formosana* in Taiwan, one (PNW lineage) in the Pacific Northwest and Europe and one (UK lineage) on diseased ornamental *C. lawsoniana* in Scotland [1]. It was hypothesised that the Taiwan TWJ lineage, and possibly the PNW lineage, may have originated in Japan. Soil baiting and direct isolation from lesions on fallen foliage was therefore carried out in multiple native, healthy *C. obtusa* and *C. pisifera* stands in Japan in 2016 and 2018. *Phytophthora lateralis* was readily obtained. Characterisation of the isolates shows at least two very distinct lineages of *P. lateralis* are present in Japan, one of which may be the PNW lineage.

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**Diversity of *Phytophthora* species in natural ecosystems in Serbia, Bosnia and Herzegovina and Montenegro (Western Balkans). I. Milenković<sup>1,2</sup>, N. Keča<sup>2</sup>, D. Karadžić<sup>2</sup>, Z. Stanivuković<sup>3</sup>, M. Tomšovský<sup>1</sup>, S. Milanović<sup>2</sup>, A. Vemić<sup>2</sup>, Z. Radulović<sup>4</sup>, T. Corcobado<sup>1</sup>, M. Horta Jung<sup>1</sup>, T. Májek<sup>1</sup>, J.A. Nowakowska<sup>5</sup>, T. Oszako<sup>6</sup>, K. Sikora<sup>6</sup>, T. Jung<sup>1,7</sup>**

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During the monitoring of health status of different forest ecosystems in Serbia (SER), symptoms indicative of *Phytophthora* infections were noticed on several broadleaved tree species, and particularly on pedunculate oak, sessile oak, European beech, narrow-leafed ash and maples. To determine the presence of *Phytophthora* pathogens in the affected natural ecosystems, over ten years, intensive studies were performed in different forest ecosystems in SER. Soil and tissue samples from numerous hosts were sampled using standardized methods [1,2]. Also, forest streams and rivers were sampled to determine the aquatic *Phytophthora* populations. As a comparison, a small-scale survey was performed in forests and streams of neighbouring Bosnia and Herzegovina (BiH) and Montenegro (MNE). From forest soils more than 600, 200 and 200 *Phytophthora* isolates were obtained in SER, BiH and MNE, respectively. In rivers and forest streams, more than 700, 250 and 350 isolates were obtained, respectively. The most frequently sampled and also most positive host was pedunculate oak, followed by European beech, sessile oak and other host species. The most frequently isolated species was by far *P. plurivora*, followed by *P. gonapodyides*, *P. lacustris*, *P. ×cambivora*, *P. quercina*, *P. cactorum*, *P. polonica*, *P. pini* and less frequently other *Phytophthora* species. Several new host-pathogen combinations were recorded in this study and the known area of distribution of several *Phytophthora* species were extended. Also, most of the recorded species in BiH and MNE were for the first time reported from these countries. Significant correlation between symptoms and *Phytophthora* presence was recorded in some areas, but also in many cases known pathogenic species from this genus were isolated from non-symptomatic stands, posing high risks for these ecosystems.

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**Waterborne and soilborne *Phytophthora* diversity in declining cork oak stands in Sardinia (Italy).** Salvatore Seddaiu<sup>1</sup>, Andrea Brandano<sup>2</sup>, Giovanni Cadinu<sup>2</sup>, Clizia Sechi<sup>1</sup>, Pino Angelo Ruiu<sup>1</sup>, Bruno Scanu<sup>2</sup>

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Since the beginning of the twentieth century, cork oak forests have been threatened by several factors including human activities, climate change and attacks of pathogens and pests. Several studies have demonstrated the primary role of the oomycete *Phytophthora cinnamomi* in the widespread decline of oaks in Portugal, Spain, southern France and Italy, although other *Phytophthora* species have been also associated with this phenomenon. Since almost ten years independent surveys have been undertaken to investigate the diversity of *Phytophthora* species associated with declining oak trees in Sardinia (Italy). Roots and soil samples were collected from symptomatic oak trees located in natural and managed forests, new plantations and reforestation areas, parks, gardens and forest nurseries. Different methods of isolation were used, including baiting with leaves and fruits as well as direct isolation from infected root and bark tissues on selective medium. In addition, a river baiting survey was recently undertaken across ten declining cork oak stands. All the species obtained were identified based on their morphological properties and multigene sequences analyses. A total of 16 *Phytophthora* species were detected, some of which represent first records for cork oak ecosystems, although their habitus seems to be restricted to the aquatic environment. Phenotypic and genetic variability was detected in some species, suggesting their possible native origin in the Mediterranean. This is the first long-term study looking at the diversity of *Phytophthora* species in cork oak ecosystems in Italy. The ecology and distribution of the different *Phytophthora* species detected will be discussed.

**Diversity of *Phytophthora* populations in Sicilian river ecosystems.** T. Jung<sup>1,2</sup>, F. La Spada<sup>3</sup>, F. Aloï<sup>3,4</sup>, A. Pane<sup>3</sup>, M. Horta Jung<sup>1</sup>, M. Evoli<sup>3</sup>, M. Riolo<sup>3</sup>, J.B. Ristaino<sup>5</sup>, L. Schena<sup>6</sup>, S.O. Cacciola<sup>3</sup>

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The aim of this study was to investigate the diversity, expressed as Shannon Diversity Index (SDI) Shannon Evenness Index (SEI) and Richness (R), of *Phytophthora* species from 14 river systems [1] distributed among typical Sicilian floristic districts [2]. The survey included waterways from Hyblaean District (Anapo, Ciane, Cavagrande, Irminio), Etnean District (Sciambro, Fiumefreddo), Nebrodensian District (Alcantara, Fiume di Troina, Flascio, Della Saracena, Martello, Cutò) and Peloritanean District (Fiumara d'Agrò, Fiumedinisi). Isolates of *Phytophthora* were collected by *in situ* baiting using floating mesh bag-styrofoam rafts. Isolates were identified on the basis of morphological features and the analysis of Internal Transcribed Spacer regions (ITS) of rDNA. The altitudinal range of the capture sites was between 4 and 1450 m a.s.l. In total, 181 isolates belonging to 12 *Phytophthora* species were obtained (i.e.: *P. ×cambivora*, *P. citrophthora*, *P. frigida*, *P. gonapodyides*, *P. hydropathica*, *P. sp. kelmania*, *P. lacustris*, *P. multivora*, *P. plurivora*, *P. polonica*, *P. pseudocryptogea* and *P. thermophila*). Among the floristic districts, the Etnean showed the highest values of SDI (1.557) and SEI (86.9%), while the highest R (i.e.: 7) was observed in the Hyblaean district. Considering single waterways, values of SEI were significant for only 57% of examined rivers. Among them, systems highly disturbed by human activities (e.g. Fiumefreddo river, a small watercourse in a small natural reserve recently established in a highly anthropized area), unlike the naturalized ones, showed high SDI, SEI and R values and a significant proportion of exotic *Phytophthora* species.

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**A new *Phytophthora* species pathogenic on Chinese Hickory (*Carya cathayensis*) in South East China: a possible introduction from North America?** Carmen Morales-Rodriguez<sup>1</sup>, Yongjun Wang<sup>2</sup>, Andrea Vannini<sup>1</sup>

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Chinese Hickory (*Carya cathayensis* Sarg.) a member of the Juglandaceae family native of China, is an economically important nut tree in China. Currently, more than 15,000 ha of Chinese hickory trees are cultivated in Zhejiang Province. In August 2016, crown decline associated to collar lesion and mortality were observed on Chinese Hickory trees in a plantation in the Zhejiang province. Isolation from active lesions and baiting from rhizosphere soil resulted in isolation of an unknown *Phytophthora* sp. and *P. cinnamomi*, respectively. The unknown species, here informally designated *Phytophthora* taxon *Carya* sp. nov (PtCA), is a homothallic taxon clustering in clade 4. Inoculation trials were carried out in greenhouse on 2 years old Chinese Hickory plants to complete the Koch postulates. Noticeable, a unique rDNA sequence with 100% identity with PtCA isolates was found in NCBI referred to an unknown *Phytophthora* isolated from *Carya illinoensis* (Pecan) in the USA. In the past century, Pecan has been introduced to China and many Pecan orchards are in Zhejiang Province, where Lin'an is the main city in which pecan processing is conducted.

**Predictors of *Phytophthora* diversity and community composition in natural areas across diverse Australian ecoregions.** Treena Burgess<sup>1</sup>, Keith McDougall<sup>2</sup>, Peter Scott<sup>3</sup>, Giles Hardy<sup>1</sup>, Jeff Garnas<sup>4</sup>

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Comprehensive understanding of the patterns and drivers of microbial diversity at a landscape scale is in its infancy, despite the recent ease by which soil communities can be characterized using massively parallel amplicon sequencing. Here we report on a comprehensive analysis of the drivers of diversity distribution and composition of the ecologically and economically important *Phytophthora* genus from 414 soil samples collected across Australia. We assessed 22 environmental and seven categorical variables as potential predictors of *Phytophthora* species richness,  $\alpha$  and  $\beta$  diversity, including both phylogenetically and non-phylogenetically explicit methods. In addition, we classified each species as putatively native or introduced and examined the distribution with respect to putative origin. The two most widespread species, *P. multivora* and *P. cinamomi*, are introduced, though five of the ten most widely distributed species are putatively native. Introduced taxa comprised over 54% of Australia's *Phytophthora* diversity and these species are known pathogens of annual and perennial crop habitats as well as urban landscapes and forestry. Patterns of composition were most strongly predicted by bioregion ( $R^2=0.29$ ) and ecoregion ( $R^2=0.26$ ) identity; mean precipitation of warmest quarter, mean temperature of the wettest quarter and latitude were also highly significant and described approximately 21%, 14% and 13% of variation in NMDS composition, respectively. We also found statistically significant evidence for phylogenetic over-dispersion with respect to key climate variables. This study provides a strong baseline for understanding biogeographical patterns in this important genus as well the impact of key plant pathogens and invasive *Phytophthora* species in natural ecosystems.

## **15 years of *Phytophthora* monitoring in Switzerland. Simone Prospero, Corine Buser-Schöbel, Daniel Rigling**

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In Switzerland, *Phytophthora* monitoring in forests, urban greens (parks, gardens) and nurseries has officially started in 2003, when *P. ramorum* was detected in a nursery. Since then, nurseries that are subjected to the European plant passporting system are controlled once a year by trained inspectors. During the inspections, symptomatic plant material is collected from potential host plants of *P. ramorum* and sent to the WSL institute for *Phytophthora* isolation and identification. Moreover, regular *Phytophthora* surveys are conducted by the WSL in natural forests and plantations in order to exclude the presence of *P. ramorum* in the forest environment. Finally, potential infections of plants by *Phytophthora* species are reported to the Swiss Forest Protection group at WSL by private citizens. Here, the results of these monitoring activities will be presented and discussed. During 15 years, more than 700 samples have been tested positive for the genus *Phytophthora*. Although about 120 samples belonged to *P. ramorum*, this quarantine pathogen was never detected in the forest environment. Using ITS sequencing, we identified 32 different *Phytophthora* species in Switzerland, which is more than twice the number previously reported for the country. Noteworthy, considerable differences were detected in the composition of the *Phytophthora* community between substrates (soil, water, plants) and environments (forests, urban green, nurseries).

**Diversity of Oomycetes associated with declining *Pinus* spp. and *Cupressus* spp. in Kerman province, Iran.** Sonia Aghighi<sup>1</sup>, Farnaz Sistani<sup>2</sup>, Zohreh Lori<sup>2</sup>, Sajjad Sarikhan<sup>3</sup>, Tanay Bose<sup>4</sup>

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Various oomycetes species have recorded to infect gymnosperms globally. However in Iran, the diversity of oomycetes species associated with gymnospermous trees is not well recorded. In this study, we catalogued the diversity of oomycetes associated with declining *Pinus* spp. and *Cupressus* spp. from Iran. Since 2017, several surveys were carried out in parks, urban areas, nurseries and the iconic Shahzadeh garden located in Kerman Province, Iran. During these surveys, declining *Pinus* spp. and *Cupressus* spp. trees were observed across all sites. In a recent survey we collected a total of twelve soil samples from the rhizosphere region of symptomatic *Pinus* spp. and *Cupressus* spp. trees. All the soil samples were baited using sour orange (*Citrus aurantium*) leaves. Environmental DNA was extracted for all the soil samples for cataloging the diversity of *Phytophthora* spp. using high-throughput sequencing platform. In total, we recovered fifty-three *Phytophthora* isolates using soil baiting. Majority of these isolates represented an ITS Clade 6 *Phytophthora* spp. Using ITS metagenome analysis of soil samples, we detected at least five *Phytophthora* spp.: *P. cactorum*, *P. citrophthora*, *P. gonapodyides*, *P. lacustris*, and *P. pseudocryptogea*. Among these taxa, *P. lacustris* and *P. gonapodyides* corresponded to highest number of OTUs. *P. lacustris* was detected from all twelve samples. Other oomycetes species detected in this study were: *Ovatisporangium* sp., *Phytopythium* spp. and *Pythium* spp. This is the first report of *P. gonapodyides* associated with soil from declining pine and cypress trees in Iran. Our study shows a significant number of OUTs linked to oomycetes which are associated with declining pine and cypress trees.

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**First report of *Phytophthora parvispora* from a natural riparian ecosystem in Sicily.** F. La Spada<sup>1</sup>, F. Aloï<sup>1,2</sup>, M. Riolo<sup>1</sup>, A. Pane<sup>1</sup>, M. Evoli<sup>1</sup>, R. Faedda<sup>1</sup>, S.O. Cacciola<sup>1</sup>

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Originally identified as a variety of *Phytophthora cinnamomi* (i.e.: *Phytophthora cinnamomi* var. *parvispora*), *Phytophthora parvispora* was recently described as a distinct species in the ITS clade 7a [1]. In Sicily, the species was previously reported only from nurseries in association with cultivated *Mandevilla* spp. [2]; furthermore, a previous large-scale survey conducted in protected natural areas excluded the occurrence of *P. parvispora* in natural ecosystems [3]. However, in a recent survey carried out in autumn 2017, *P. parvispora* was recovered in a protected natural area (i.e.: Complesso Speleologico Villasmundo - S. Alfio Regional Natural Reserve - Hyblaean Mountains - south-eastern Sicily) at an altitude of about 100 meters a.s.l. from a chronically declining riparian forest along the course of a river with a torrential regime. The species was isolated by leaf baiting from rhizosphere soil of a symptomatic willow (*Salix pedicellata*) tree and identified by combining morphological features with sequencing of internal transcribed spacer regions (ITS1 and ITS2) and 5.8S gene.

Thanks to its biological and ecological characteristics, *P. parvispora* can endure short periods of soil waterlogging and high relative humidity of the air alternating with long periods of drought, typical of the Mediterranean ecosystem where it has been found.

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## First report of *Phytophthora* spp. in declining broadleaf forests in western Ukraine. Iryna Matsiakh<sup>1</sup>, Volodymyr Kramarets<sup>1</sup>, Michelle Cleary<sup>2</sup>

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Elsewhere in Europe, forest *Phytophthoras* are known as primary agents responsible for declining health of trees [1]. In Ukraine, disease symptoms indicative of *Phytophthora* infections, have been observed during the last decade in forest stands and nurseries but have never been considerably studied [2]. Research started during 2017-2018 whereby field sampling was carried out in six regions in the western part of Ukraine. Soil samples were collected from 14 locations in western Ukraine representing diverse forest sites. The detection and identification of Oomycetes were performed using traditional techniques (soil baiting and isolation), as well as with DNA sequencing [3, 4]. In total, 401 isolates were recovered across all locations. We selected 114 isolates to analyze and of those, 48 were identified as species of *Phytophthora* including *P. lacustris*, *P. gonapodyides*, *P. plurivora*, *P. bilorbang*, *P. polonica*, *P. gallica*, *P. gregata*, *P. taxon Oaksoil*, and *P. cactorum*. The most diverse *Phytophthora* species spectrum was found in declining alder stands which included *P. lacustris*, *P. gonapodyides*, *P. bilorbang*, *P. taxon Oaksoil*, *P. plurivora* and *P. polonica*. Four species (*P. gonapodyides*, *P. plurivora*, *P. gallica* and *P. gregata*) were found in declining birch forests. This report gives a broad overview of the *Phytophthora* species distribution and diversity in declining forest tree species.

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## SESSION 5

### ECOLOGY AND EPIDEMIOLOGY

***Phytophthora* and damping-off within natural and rehabilitated plant communities.** Christopher Shaw<sup>1</sup>, Mark Dobrowolski<sup>2,3</sup>, Giles E.S.J. Hardy<sup>1</sup>, Treena Burgess<sup>1</sup>

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Several putatively native *Phytophthora* species have been detected from the natural kwongan heath and Banksia woodland plant communities of Western Australia [1]. The location of detections has generally favoured disturbed native vegetation, and disease expression is atypical of invasive *Phytophthora* species [2]. Native pathogens can contribute to the diversity of natural plant communities [3], and potentially the inefficiency of broadcast seeding methods in ecological restoration [4]. We designed four experiments to explore the role of putatively native *Phytophthora* species and oomycetes in natural and disturbed plant communities. Putatively native *Phytophthora* and *Pythium* species were found to be damping-off pathogens of native plant species in a glasshouse experiment; however, host range and virulence differed substantially between pathogen treatments. Natural and disturbed vegetation was surveyed using molecular tools and the richness and abundance of *Phytophthora* species in the region sampled were far lower than hypothesised. The emergence and survival of seedlings differed when plant species were sown into conspecific and heterospecific soils. The oomycete community associated with mature individuals and seedlings was assessed and related to levels of damping-off. In post-mining ecological restoration, the effects of fungicides applied to broadcast seed were evaluated. Fungicides provided a small to moderate increase in seedling emergence for several plant species indicating the presence of oomycete damping-off pathogens. These results have implications for the status of some *Phytophthora* species and the role of oomycetes in natural and disturbed plant communities.

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**Trait-based approaches for predicting future global impacts in the genus *Phytophthora*.** Louise J Barwell<sup>1</sup>, Ana Perez-Sierra<sup>2</sup>, Beatrice Henricot<sup>3</sup>, Anna Harris<sup>2</sup>, Treena I. Burgess<sup>4</sup>, Giles Hardy<sup>4</sup>, Peter Scott<sup>5,10</sup>, Nari Williams<sup>5</sup>, David Cooke<sup>6</sup>, Paul Sharp<sup>7</sup>, Sarah Green<sup>3</sup>, Daniel S. Chapman<sup>8,9</sup>, Bethan V. Purse<sup>1</sup>

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*Phytophthora* pathogens are introduced to new geographic regions ever more frequently as global connectivity increases. Predicting the risk they pose to plant health can be difficult without in-depth knowledge of behaviour, distribution and spread. Using a species-level trait database, we apply phylogenetically-informed trait-based models to evaluate the power of traits, phylogeny and years known to science to explain and predict the geographic extent and host range of 179 *Phytophthora* species. In the best-performing models, traits, phylogeny and years known to science explained up to 62 % and 69 % of variance in geographic extent and host range, respectively. Traits and phylogeny alone explained up to 18 % (geographic extent) and 29 % (host range) of variance. Root- and foliar-attacking *Phytophthoras* were more widespread and generalist in host range. Host generalists also had greater oospore wall indexes, higher optimum temperatures for growth and faster growth rates at their optima. Cold-tolerance may also be important for range expansion but more accurate inter-specific empirical data is needed to confirm this finding. We illustrate a framework for linking rapidly obtainable information about invasive pathogen traits and phylogenetic position to their global impacts. Our findings are of value for prioritising ecologically relevant traits for future measurement that may help to predict potential geographic and host range expansion for newly described species. We highlight data and methodological requirements to further develop early-warning trait-based risk assessments for plant pathogens.

**Functional traits associated with establishment and spread of invasive forest pathogens in Northern Europe.** M.A. Redondo<sup>1</sup>, J. Boberg<sup>1</sup>, J. Stenlid<sup>1</sup>, J. Oliva<sup>2</sup>

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By high-throughput sequencing and baiting, we studied the functional traits of *Phytophthora* communities along the introduction pathway, as well as across a climatic gradient in Sweden. Comparing community assembly pre- and post-establishment, we saw that resistant structures were a key functional trait to predict establishment. Once established, we saw that invasive *Phytophthora* species distributed in Sweden following a climatic gradient. Temperature was the main driver for aquatic *Phytophthora* species, while precipitation was the main driver for terrestrial species. Our work encourages the use of functional traits to predict establishment. It also highlights the importance of considering the physical environment (water or soil) where pathogens complete their life cycle when predicting their response to climate. During this work, we developed a novel high-throughput sequencing system based on PacBio to describe *Phytophthora* communities in water, soil or plant material, which can be used for future ecological studies.



**Genetic variation in host resistance as a prerequisite for natural selection by an invasive pathogen.** M.A. Redondo<sup>1</sup>, J. Stenlid<sup>1</sup>, J. Oliva<sup>2</sup><sup>1</sup>Dept. Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Sweden<sup>2</sup>Dept. Crop and Forest Sciences, University of Lleida, SpainCorresponding email: [jonas.oliva@pvcf.udl.cat](mailto:jonas.oliva@pvcf.udl.cat)

To predict the long-term impact of invasive pathogens on forests, we need to be able to predict the evolutionary responses of naïve host populations to novel pathogens. We searched for signs of natural selection in alder (*Alnus glutinosa*) stands growing on Swedish riverbanks invaded by either *Phytophthora x alni* or *Phytophthora uniformis* by comparing their survival and heritability with that of uninvaded stands growing inland. We found that naïve alder populations showed a higher level of heritability of resistance to *P. uniformis* than to *P. x alni*, and that only progenies of populations invaded by *P. uniformis* survived longer and had lower heritability than naïve populations. Simulated data supported low genetic variation in resistance as an impediment for natural selection and rejected the role of a trade-off between aggressiveness and dispersal for *P. x alni*. Whether invasive pathogens can exert selection pressure and trigger adaptive evolutionary responses in naïve hosts and how much that depends on the level of native resistance will be discussed.

**Modelling the key drivers of an aerial *Phytophthora* foliar disease epidemic, from the needles to the whole plant.** Mireia Gomez-Gallego<sup>1,2</sup>, Ralf Gommers<sup>2</sup>, Martin Karl-Friedrich Bader<sup>1</sup>, Nari Williams<sup>2</sup>

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Understanding the epidemiology of infectious diseases in a host population is a major challenge in forestry. Radiata pine plantations in New Zealand are impacted by a foliar disease, red needle cast (RNC), caused by *Phytophthora pluvialis*. This pathogen is dispersed by water splash with polycyclic infection affecting the lower canopy of the tree. In this study, we extended an SI (Susceptible-Infectious) model presented for RNC to analyse the key epidemiological drivers. We conducted a detached-needle assay and an *in vivo* inoculation experiment to empirically fit the model. With the detached-needle assay data we compared resistant and susceptible genotypes, and with the *in vivo* inoculation data we informed sustained infection of the whole plant. We also compared isolations and real-time quantitative PCR (qPCR) to assess *P. pluvialis* infection. While susceptible and resistant genotypes presented similar primary infection rates and incubation times, the pathogen death rate was 2.5 times higher for resistant genotypes. External proliferation of mycelium and sporangia was only observed on 28% of the resistant ramets compared to 90% of the susceptible ones. Detection methods were the single most important factor influencing model parameter estimates for the whole-plant infection, giving qualitatively different epidemic outputs. In the early stages of infection, qPCR proved more efficient than isolations but the reverse was true at later points in time. Our results have important implications to the management of RNC, by highlighting the main differences between susceptible and resistant genotypes, and comparing the most common assessment methods to detect RNC epidemics.

**Pathogen loads of *Phytophthora pluvialis* and *Nothophaeocryptopus gaeumannii* populations co-existing within exotic plantations of New Zealand in contrast to Douglas-fir's endemic range in the US Pacific Northwest.** Mireia Gómez-Gallego<sup>1,2</sup>, Jared M. LeBoldus<sup>3</sup>, Martin Karl-Friedrich Bader<sup>1</sup>, Everett Hansen<sup>3</sup>, Lloyd Donaldson<sup>2</sup>, Nari Michelle Williams<sup>2</sup>

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The emergence of *Phytophthora pluvialis* as a foliar pathogen of Douglas-fir in New Zealand (NZ) and the US Pacific Northwest (PNW) has raised questions about its interaction with the widespread Swiss needle cast (SNC) disease. During spring 2017, we repeatedly sampled 30 trees along an environmental gradient in each region, and 292 additional trees in a longitudinal transect, to assess the *P. pluvialis* epidemic and the association between *P. pluvialis* and *Nothophaeocryptopus gaeumannii*, causal agent of SNC. Both pathogens were consistently more abundant in the host's exotic environment of NZ, compared to the PNW. In both regions, the two pathogens coexist at different spatial scales ranging from region to needles. The relative abundance of both pathogens was negatively correlated in the PNW, where both pathogens have presumably coexisted for a longer period of time. Our findings confirm the co-existence of *P. pluvialis* and *N. gaeumannii* as foliar pathogens of Douglas-fir and suggest a within-site spatial variation in the PNW.

***Phytophthora* species associated with roots and soils from native and non-native forestry environments in South Africa.** Tanay Bose<sup>1</sup>, Michael J. Wingfield<sup>1</sup>, Jolanda Roux<sup>1</sup>, Maria Vivas<sup>1</sup>, Treena I. Burgess<sup>2</sup>

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*Phytophthora* species are well-known pathogens, the majority of which are soil-borne and cause root diseases on a wide range of plants. The advances in sequencing technologies have made it possible to better understand the microbiota in the soil and this includes *Phytophthora*. In this study, we used pyrosequencing to compare the community composition and species richness of *Phytophthora* species in: (i) roots of two non-native tree species (*Eucalyptus grandis* and *Acacia mearnsii*) and adjacent native forest trees, (ii) roots of two non-native tree species from an *in vivo* infection trial, (iii) roots collected from the field versus those from the infection trial, and (iv) roots and soil samples collected from the field. We found that the origin of the soil and the interaction between the roots and soil significantly influenced *Phytophthora* species richness. *Phytophthora* species richness and composition were significantly different between the field-collected root and soil samples with the latter group having higher species richness. Overall, the results revealed a substantial and previously unreported, *Phytophthora* species diversity from South Africa.

**What's killing juniper? Identifying environmental risk factors for *Phytophthora austrocedri* infection.** Flora Donald<sup>1,2,4</sup>, Sarah Green<sup>2</sup>, Kate Searle<sup>3</sup>, Nik Cunniffe<sup>4</sup>, Beth V. Purse<sup>1</sup>

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*Phytophthora austrocedri* is causing rapid decline of juniper (*Juniperus communis*) across the UK. Confirmed worldwide infection of wild tree populations is currently limited to Argentina and the UK [1, 2]. As a keystone species for biodiversity and one of only three UK native conifers, significant resource has been dedicated to juniper conservation in the form of repeated national surveys, scientific research, local management action and planting initiatives. With little existing knowledge about the specific host-pathogen-environment interaction and few resources dedicated to disease detection, where should juniper conservation effort be prioritized. As management for juniper is conducted at a local level, we undertook the first UK field investigation of abiotic and biotic predictors of *P. austrocedri* symptom distribution across three discrete populations with different infection histories. At each location, the proportion of juniper showing discoloured or dead foliage characteristic of infection was measured from fifty 10 x 10 m quadrats stratified by juniper density. Potential predictors included altitude, slope, soil moisture, watercourse proximity, browsing damage and associated vegetation. Bayesian Generalised Linear Mixed Models fit using Integrated Nested Laplace Approximations revealed that the area of symptomatic juniper increased with increasing soil moisture, consistent with findings at a local and landscape scale from Argentina [3]. Establishment of new, and management of existing, populations must, therefore, be prioritised on drier microsites. As remedial action for juniper situated in wetter microsites will be difficult, strong measures are also required to prevent introduction of *P. austrocedri* to new populations.

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## Understanding sudden larch death – from epidemiology to host resistance. Heather Dun<sup>1</sup>, John Mackay<sup>2</sup>, Sarah Green<sup>3</sup>

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*Phytophthora ramorum* Werres, De Cock & Man in't Veld is responsible for devastating disease outbreaks in a range of species, most prominently sudden oak death in the USA and sudden larch death in the UK [1]. Our study focuses on the interactions between *P. ramorum* and two important forestry species in the UK, European larch (*Larix decidua* Mill.) and Japanese larch (*Larix kaempferi* (Lamb)Carr.). Field surveys in south-west Scotland, which suffered an extensive epidemic in 2012 from a highly virulent new lineage (EU2), have allowed examination of how *P. ramorum* infects individual trees and spreads across a site. We identified a period of increased spread in spring 2018 and associated climate records allow us to consider how environmental conditions influence outbreaks. Field observations have also acted as a foundation to investigate a range of possible infection pathways.

Observations of surviving trees within stands suffering heavy mortality from the 2012 epidemic indicate different degrees of resistance might exist naturally within the larch population. This hypothesis is being tested by artificial inoculation of grafted scions from affected and putatively tolerant trees. Samples from these trees will be analysed along with other greenhouse trials to compare the susceptibility of the two larch species to the EU2 lineage of *P. ramorum*. Analysis of RNA expression will aim to identify the molecular interactions of key host defences and immune responses. Natural plant disease resistance strategies might provide a way forward for silvicultural methods to combat or reduce the effects of *P. ramorum* in commercial forestry.

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**Sudden Oak Death in Oregon Forests: Disease intensification and renewed engagement in disease management.** [Sarah Navarro](#)<sup>1</sup>, Ellen Michaels Goheen<sup>2</sup>, Jared LeBoldus<sup>3</sup>, Everett Hansen<sup>3</sup>, Paul Reeser<sup>3</sup>, Wendy Sutton<sup>3</sup>, Nicholas Grunwald<sup>4</sup>, Alan Kanaskie<sup>1</sup>, Randall Wiese<sup>1</sup>, Casara Nichlos<sup>1</sup>

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Sudden Oak Death (SOD), caused by *Phytophthora ramorum*, is lethal to tanoak (*Notholithocarpus densiflorus*) and threatens this species throughout its range in Oregon. In July 2001, the disease was first discovered in coastal southwest Oregon forests. Since 2001, an interagency team has attempted to eradicate and slow the spread of disease through a program of early detection, survey and monitoring, and destruction of infected and nearby host plants. Eradication treatments, totalling approximately 2550 ha, eliminated disease from most infested sites, but the disease has continued to spread slowly, mostly in a northward direction. Detection of the EU1 lineage in 2015 and 2016 caused a temporary strategy change emphasizing total eradication for EU1. Total EU1 eradication has not occur; EU1 infested trees have continued to be detected within a small geographic area resulting in 190 ha of eradication treatments from 2015 to 2018. The SOD Program follows a combined strategy of prioritizing treatments where EU1 is detected, while continuing treatment of the NA1 line along the SOD expansion edge. In 2017, a SOD Task Force convened local, state and federal government agencies, tribes, industry, and local residents and environmental groups. The mission of the Task Force is to develop a collaborative-based strategic action plan to contain *Phytophthora ramorum* in Curry County, Oregon using the best available science. Following the strategic action plan, the Oregon Department of Forestry commissioned an economic impact assessment of SOD, which concluded a 19:1 cost benefit to the state's economy by slowing the spread of the disease.

**Epidemiology and management of an outbreak of the EU1 lineage of *Phytophthora ramorum* in Oregon forests.** Jared M. LeBoldus<sup>1</sup>, Kelsey L. Søndreli<sup>1</sup>, Hazel Daniels<sup>1</sup>, Sarah Navarro<sup>2</sup>

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Sudden oak death is caused by *Phytophthora ramorum* and is an invasive oomycete pathogen in the coastal western US and Europe. Forest infestations in Oregon and California have until recently been the NA1 lineage. In 2015 the EU1 lineage of *P. ramorum* was found infecting a tanoak (*Notholithocarpus densiflorus*) tree in Oregon. The disease has since spread to multiple locations. Using population genetics this outbreak has been traced back to a nearby nursery infestation originally detected in 2012. Growth chamber experiments using log and seedling inoculations have indicated the EU1 lineage to be more aggressive on Oregon tree species in terms of lesion size and has the potential to produce a larger number of sporangia on tanoak than the NA1 lineage. Eradication of forest infestations of the EU1 lineage has been prioritized by the Oregon Department of Forestry and the US Forest Service in order to contain the outbreak. Monitoring of soil and vegetation from EU1 infested sites before and after treatment indicates that pathogen survival is similar to what has been reported for NA1 infested sites. Although the EU1 lineage has the potential to spread more quickly, act more aggressively, and sporulate more profusely current knowledge suggests that contemporary management efforts will likely be equally as effective at containing the EU1 lineage as they have been slowing the spread of the NA1 lineage.

## **Sudden Oak Death: Comparing the EU1 and NA1 lineages of *Phytophthora ramorum* in Oregon forests. Kelsey L. Søndreli, Jared M. LeBoldus**

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Sudden Oak Death is caused by *Phytophthora ramorum* and is an invasive pathogen in California and Oregon in the US and several countries in Europe. Forest infestations in Oregon and California have until recently been the NA1 lineage [1]. In 2015, the EU1 lineage of *P. ramorum* was found infecting a tanoak tree in Oregon [2]. Tanoak (*Notholithocarpus densiflorus*) is the most susceptible species and the main driver of this disease in Oregon. Infected trees develop lethal stem cankers and sporulate from infected leaves and branches. Two field experiments were conducted in order to compare the epidemiology of the EU1 and NA1 lineages in Oregon forests. The first field experiment was conducted in 2017, 2018, and 2019 in order to evaluate the relative susceptibility of Douglas-fir, Sitka spruce, western hemlock, and western larch. Overall, EU1 was shown to have a higher incidence of infection on Oregon trees than NA1. In general a larger proportion of trees, from all species were infected at the EU1 site compared to the NA1 site. In 2018 and 2019 a rain bucket experiment was conducted in order to compare the relative amount of spores produced at two EU1 and two NA1 infested sites. The accumulated evidence indicates that the EU1 lineage of *P. ramorum* is able to infect more species and at a higher rate than NA1, and potentially has higher sporulation under field conditions indicating that it may pose a greater risk to Oregon forests than the NA1 lineage.

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## SESSION 6

# HOST-PATHOGEN INTERACTION

## Dissecting the in's and out's of *Phytophthora* biology and pathology. Francine Govers

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*Phytophthora* species are widespread and cause devastating diseases in crops and natural ecosystems. Well-known is *Phytophthora infestans*, the late blight pathogen that was the culprit of the Irish potato famine in the mid-19<sup>th</sup> century. Despite its filamentous growth *Phytophthora* is not a fungus. It belongs to the oomycetes, a class in the stramenopile lineage that comprises plant and animal pathogens, mycoparasites and saprotrophs, and evolved independently of fungi. Yet, they occupy similar ecological niches and also their weaponry for plant infection is comparable including the exploitation of effectors for suppressing host defence. Nevertheless, there are remarkable differences. Here I will highlight features that illuminate the success of *Phytophthora* as pathogen. An illustrative example is the massive expansion of gene families encoding host specific RXLR effectors, the counterparts of resistance proteins and instrumental in *R* gene discovery [1,2]. Other peculiarities result from specific gene innovations leading to proteins with oomycete-specific domain combinations. We focus on the so-called GPCR-bigrams that have a N-terminal 7-transmembrane domain typical for G-protein coupled receptors (GPCRs) linked to an accessory domain with critical roles in signal transduction [3]. This domain structure suggests that GPCR-bigrams directly feed extracellular signals into downstream signalling pathways, a condition that could be explored for identifying novel targets for disease control. Other efforts aimed at unravelling the dynamics of the cytoskeleton by life cell imaging have revealed novel actin structures that seem to be unique for oomycetes [4]. The long-term goal is to uncover oomycete or *Phytophthora* specific features instrumental for drug design.

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**Hormones and secondary metabolites in chestnut during susceptible and resistant interactions with *Phytophthora cinnamomi*.** Álvaro Camisón<sup>1</sup>, M. Ángela Martín<sup>2</sup>, Paloma Sánchez-Bel<sup>3</sup>, Víctor Flors<sup>3</sup>, Gloria Pinto<sup>4</sup>, Francisco Alcaide<sup>1</sup>, Elena Cubera<sup>2</sup>, Alejandro Solla<sup>1</sup>

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Despite the advances reported at molecular and physiological level, key points to unveil the players behind plant defence responses to *Phytophthora cinnamomi* (*Pc*) are missing. To fulfil this knowledge gap, we quantified constitutive and *Pc*-induced specific stress signals (hormones and metabolites) complemented with changes in photosynthetic related parameters by exploring susceptible and resistant *Castanea* spp.–*Pc* interactions. In a greenhouse experiment, five days before and nine days after inoculation with *Pc*, leaves and fine roots from susceptible *C. sativa* and resistant *C. sativa* × *C. crenata* clonal 2-year-old plantlets were sampled. In the resistant clone,  $g_s$  and  $A$  decreased significantly and soluble sugars in leaves increased, while in the susceptible clone  $g_s$  and  $A$  remained unchanged and proline levels increased. In the resistant clone, higher constitutive content of SA in roots and higher constitutive ABA, JA and JA-Ile in leaves in comparison to the susceptible clone were observed. Total phenolics and condensed tannins were highest in roots of the susceptible clone, suggesting that root phenolics are poor predictors of resistance to *Pc*. In response to infection, a dynamic hormonal response in the resistant clone was observed, consisting of accumulation of JA, JA-Ile and ABA in roots and depletion of total phenolics in leaves. However, in the susceptible clone only JA diminished in leaves and increased in roots. From the hormonal profiles obtained in leaves and roots before and after infection, it is concluded that the lack of effective hormonal changes in *C. sativa* explains the lack of defense responses to *Pc* of this susceptible species. *Pc* adopts a necrotrophic lifestyle when visible symptoms appear in chestnut. Finally, JA-Ile is a good candidate as biomarker for screening chestnuts for *Pc* resistance.

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**Disclosing pathogen target-proteins in cork oak plants infected with *P. cinnamomi*.** Ana Cristina Coelho<sup>1,2</sup>, Rosa Pires<sup>1</sup>, Gabriela Schütz<sup>1,3</sup>, Cátia Santa<sup>4,6</sup>, Bruno Manadas<sup>4</sup>, Patrícia Pinto<sup>5</sup>

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The pathological interaction between oak trees and *Phytophthora cinnamomi* has implications in cork oak and holm oak decline observed over the last decades in the Iberian Peninsula [1]. During host colonization, the phytopathogen secretes effector molecules like elicitors to increase disease effectiveness [2]. The objective of this study was to unravel the proteome associated to cork oak systemic immune response triggered by *P. cinnamomi* infection, through SWATH-MS quantitative proteomics [3]. Using the *Arabidopsis* proteome database as reference, 424 proteins have been confidently quantified in cork oak leaves, of which 80 proteins showed a more than 2-fold change in abundance or a statistical p-value below 0.05 between control and infected samples. The infection of cork oak roots with *P. cinnamomi* induced in the leaves the upregulation of proteins associated with protein-DNA complex assembly, lipid oxidation and response to endoplasmic reticulum stress. In opposition, several proteins associated with cellular metabolic compound salvage had significantly decreased abundances. The most significant expression variations were observed for the Ribulose 1,5-Bisphosphate Carboxylase small subunit (RBCS1A) and Heat Shock protein 90 (Hsp90) revealing a relevant role for these proteins in the host-pathogen interaction mechanism. This work represents the first SWATH-MS analysis performed in cork oak plants infected with *P. cinnamomi* and highlights host proteins that may be targeted by the oomycete.

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**Identifying effector proteins involved in infection by *Phytophthora kernoviae* and *P. plurivora*.** Stephen C. Whisson<sup>1</sup>, Shumei Wang<sup>1,2</sup>, Petra C. Boevink<sup>1</sup>, Paul R.J. Birch<sup>2</sup>, Lydia Welsh<sup>1</sup>, Peter Thorpe<sup>3</sup>, Sandeep K. Kushwaha<sup>4</sup>, Ramesh R. Vetukuri<sup>4</sup>

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While much of the molecular pathology research into *Phytophthora* species has been directed at a few crop infecting species such as *Phytophthora infestans* and *P. sojae*, other species from the genus that cause damage to woody host plants are not as well investigated. We have been developing resources to investigate the molecular processes involved in infection by tree pathogenic *Phytophthora* species, for example: genome sequences, transcriptomes, GFP tagged strains, and infection assays in a model plant host. To date, we have focussed most of our research on *P. plurivora* and *P. kernoviae*, which both infect European beech. We have generated transcriptome data from infected plant tissue and are now identifying the pathogen effector proteins that may be used to promote infection development. Results suggest that *P. kernoviae* deploys a diversity of cell wall degrading enzymes and 'RxLR class' effector proteins during infection, whereas *P. plurivora* appears to deploy relatively few RxLR effectors during infection. RxLR effectors are known to be translocated into host cells to exert their action. We localized selected *P. kernoviae* effectors inside plant cells, showing them to be targeted to the cytoplasm, nucleus, nucleolus, and plasma membrane. Tested individually, these effectors were shown to strongly promote disease development by *P. kernoviae*. The resources and data that we have generated will form the basis for experiments to examine how these *Phytophthora* species manipulate plant defence responses to promote their spread through tree tissues.

**Identification and functional characterisation of CAZymes from the kauri dieback pathogen, *Phytophthora agathidicida*. Ellie L. Bradley<sup>1,2</sup>, Preeti Panda<sup>3</sup>, Rosie Bradshaw<sup>4,2</sup>, Carl H. Mesarich<sup>1,2</sup>**

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Kauri, *Agathis australis*, is an ancient conifer species endemic to New Zealand where it is both culturally and ecologically important. This is particularly true in Māori culture, where the largest tree, Tāne mahuta, is considered God of the Forest. As a consequence of human activity only 1% of the original kauri forest remains. Though much of the remaining kauri forest is now protected, kauri dieback disease caused by *Phytophthora agathidicida* has resulted in the addition of kauri to the list of threatened species for the first time. *P. agathidicida* colonises the fine roots of all life stages of kauri, although it is several years before disease symptoms are seen in mature trees. While a lot is still unknown about *P. agathidicida*, preliminary studies have shown that some kauri show levels of resistance toward the disease. This resistance forms the backbone of my PhD project which investigates the molecular basis of interaction between pathogen and host. Pathogen invasion patterns such as pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), and effectors are likely to play a key role in host colonisation. My research focuses on identification and functional characterisation of carbohydrate-active enzymes (CAZymes) that are PAMPs/effectors or induce DAMPs which are recognised by plant pattern recognition receptors to activate the plant immune system. CAZymes identified in this study will be used to screen for resistant kauri and help to identify immune receptors that could be used in a kauri breeding programme.

## SESSION 7

# PATHOGENICITY

**Decline and mortality of evergreen oaks in a protected area in Central Italy driven by the pathogenic activity of the invasive *Phytophthora cinnamomi* and *Phytophthora multivora*.** Andrea Vannini, Luigi Osimani, Carmen Morales-Rodríguez

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Intense phytosanitary monitoring of the evergreen oak forest of the Tenuta Presidenziale di Castelporziano, 6000 hectares of protected area at 25 km from the City of Rome, revealed intense decline processes and mortality specifically affecting *Quercus ilex* and *Quercus suber*. Isolation trials of rhizosphere soil of trees in declining areas resulted in the identification of several species of Peronosporales, including the genera *Pythium*, *Phytopythium*, *Elongiosporangium* and *Phytophthora*. Four species of *Phytophthora* were identified, *P. gonapodyides*, *P. plurivora*, *P. cinnamomi* and *P. multivora*. The latter two species were always associated with declining or dead trees. In the last few years *P. multivora* was recorded in several areas in Italy, including Sicily, Sardinia and Latium in domesticated and natural areas, associated to different hosts including evergreen Mediterranean oaks. Pathogenicity tests were carried out to compare the aggressivity between *P. multivora* and the well-known pathogen of evergreen Mediterranean oaks *P. cinnamomi*. Preliminary results suggest a comparable aggressivity of the two species.

**Extending the host range of *Phytophthora multivora*, a pathogen of woody plants in horticulture, nurseries, urban environments and natural ecosystems.** Duccio Migliorini<sup>1,2,3</sup>, Mohammed Y. Khdiar<sup>1,6</sup>, Cristina Rodríguez Padrón<sup>4</sup>, María Vivas<sup>2</sup>, Paul A. Barber<sup>1,5</sup>, Giles E. StJ. Hardy<sup>1</sup>, Treena I Burgess<sup>1,2</sup>

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*Phytophthora multivora* is a recently described species with a global distribution associated with disease of many woody plant species. However, very few pathogenicity studies have been conducted to determine the host range of this pathogen. A soil infestation pathogenicity experiment was conducted using two *P. multivora* isolates with *Phytophthora cinnamomi*, a known virulent pathogen, included for comparison purposes. Twenty-seven plant species were included, 19 native to Western Australia (WA) and eight exotic tree species often used as urban street trees. Plants were harvested 12 weeks after inoculation, damage of root systems were rated and root and shoot dry weight measured. Twenty-four out of twenty-seven tested host species were significantly susceptible to *P. multivora*. *P. cinnamomi* was often more pathogenic; - despite this, *P. multivora* represents an ecological risk for urban forests of Perth and for the whole of the South West Botanical Province of WA. Additionally, the susceptibility of other common woody plants found globally in cities suggests that *P. multivora* will, in time, become as 'well-known' and damaging as *P. cinnamomi*.



**Assessment of potential risk of Radiata pine (*Pinus radiata*) as host for Sudden Oak Death (*Phytophthora ramorum*).** Hazel A. Daniels<sup>1</sup>, Lindsay Bulman<sup>2</sup>, Jared M. LeBoldus<sup>1,3</sup>

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Radiata pine (*Pinus radiata*) is an important species in Australia, Chile, and New Zealand, where it comprises between 40-90% of plantations. There are over four million hectares of planted radiata pine globally. It is susceptible to several diseases, including root and foliar Phytophthoras [1]. Our objective was to test whether radiata pine bole logs are susceptible to *Phytophthora ramorum*, the causal pathogen of Sudden Oak Death. Radiata pine boles were collected from an endemic population in California, USA. Trees were felled, delimbed, and cut into ~60 cm lengths. Similarly-sized bolts of Douglas-fir (*Pseudotsuga menziesii*) and tanoak (*Notholithocarpus densiflorus*) were also collected from Oregon. Three bolts of each species were placed at four sites, two infested with the NA1 lineage of *P. ramorum* and two with the EU1 lineage. Bolts were left for four weeks before processing. Additionally, bolts of radiata pine, Douglas-fir, and tanoak were artificially inoculated [2] with known NA1 or EU1 isolates and incubated at 18C for four weeks. Bolts were debarked and measured. Lesion margins were excised and placed into a petri plate containing Phytophthora-selective media. Where *P. ramorum* was present, it was isolated and confirmed via morphology. *Phytophthora ramorum* was isolated from lesions of two field-inoculated tanoak logs. Successful reisolation of *P. ramorum* occurred in 64% of lab-inoculated radiata pine, 50% of tanoak, and 50% of Douglas-fir. Average radiata pine lesion size in lab inoculations was 0.8 cm, 88% smaller than average tanoak lesions (7.1 cm), and 54% smaller than average Douglas-fir lesions (1.8 cm).

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## SESSION 8

# PUBLIC ENGAGEMENT

**Kauri Rescue™: a citizen science programme developing controls for kauri dieback in New Zealand.** Ian Horner, Marie McEntee, Mels Barton, Lee Hill, Waitangi Wood, Nick Waipara, Ngaire Kingsbury, Pete Graham, Linley Jesson

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Kauri dieback, caused by *Phytophthora agathidicida*, is causing decline and death of kauri trees in northern New Zealand. Many of these trees are on private land, and landowners are desperate for treatments to save their trees. Kauri Rescue™ is a citizen science programme aimed at providing experimental control tools to landowners, whilst helping with the refinement of those tools. Participants with *P. agathidicida*-infected trees are provided with all the equipment required for the treatment of their trees, and asked to collect data on various tree health parameters (e.g. canopy health, lesion activity and growth) before treatment and at 6-monthly intervals thereafter. Clear instruction manuals and videos on treatment application and data collection methods are provided to participants, along with suggestions of treatment options and application rates. Participants are able to decide for themselves what application rates and doses are applied. The systems developed can be used for monitoring any kauri dieback treatment options, including mātauranga Māori (traditional knowledge) methods, although to date the vast majority of Kauri Rescue participants have selected various rates of phosphite as their main treatment. A community of volunteers is available to help participants with large properties or multiple trees to treat.

A summary of the scientific data collected will be provided, with discussion on the challenges of data collection, reliability, and biases among participants. We will also discuss some of the key findings about community participation and engagement, what works well, and the challenges involved.

**Inclusive, adaptive management of *Phytophthora agathidicida* in Waipoua Forest, Aotearoa-New Zealand: An Indigenous perspective.** Taoho Patuawa<sup>1</sup>, Tom Donovan<sup>1</sup>, Taoho Tane<sup>1</sup>, Ian Horner<sup>2</sup>, Peter Scott<sup>2</sup>, Nick Waipara<sup>2</sup>, Matthew Calder<sup>3</sup>, Tony Beauchamp<sup>3</sup>, Nari Williams<sup>4</sup>, Stanley Bellgard<sup>5</sup>, Amanda Black<sup>6</sup>, Lee Hill<sup>7</sup>, Paul Barber<sup>8</sup>, Giles Hardy<sup>8</sup>

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This paper presents the case study of Te Roroa in providing a management response to kauri dieback disease directly threatening the Waipoua Forest in Northland, New Zealand. *Phytophthora agathidicida* is the pathogen recognised to cause dieback in *Agathis australis*, the kauri tree of Aotearoa-New Zealand. First isolated and recognised as the likely causal agent of kauri dieback in 2006 [1], but later described in 2015 [2], researchers have since been building knowledge of the biology, ecology and dispersal mechanisms of *P. agathidicida* while also trying to address the ever-growing needs of forest managers in their efforts to make informed decisions to preserve kauri forests. An inclusive and adaptive approach initiated in 2018 by Te Roroa, the ancestral people of Waipoua Forest is showcasing kauri dieback forest management informed by scientific knowledge, contemporary technology and Mātauranga Maori (indigenous knowledge system). Waipoua Forest covers over 13,000 hectares of varying ecological assemblages within which the keystone species and main landscape attraction is the ancient kauri tree. Once widely distributed throughout northern New Zealand, kauri was extensively logged by European settlers to the point where an estimated 2% of the original land area remains [3]. Kauri are *taonga tuku iho* (enduring treasures) to Maori, however until recently, Maori have largely been removed from active participation in management. The role of Te Roroa *Rangatiratanga* (chiefly status) in leadership and embracing positive collaborations between the scientific community, legislative agencies and local communities will be discussed.

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- [3] Steward G.A., Beveridge A.E., 2010. A review of New Zealand kauri (*Agathis australis* (D. Don) Lindl.): its ecology, history, growth and potential for management for timber. *New Zealand Journal of Forestry Science* 40: 33-59

## SESSION 9

# BIOLOGY AND EVOLUTION

## Changing distribution of *Phytophthora ramorum* lineages in Britain.

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Up until 2010, the population structure of *Phytophthora ramorum* within its introduced range consisted of three, largely clonal evolutionary lineages. The EU1 lineage has been reported from most European countries, whereas in North America NA1 and NA2 lineages dominate although recently the EU1 was also found in Oregon. Then in 2011 a fourth lineage of *P. ramorum* was discovered in the UK [1] and so far has not been found elsewhere. The EU2 arrival may have come via the ornamental plant trade but in Britain it infects a wide range of hosts including Japanese larch (*Larix kaempferi*) and other conifer and broadleaf species. Laboratory evidence indicates that the EU2 is more aggressive when colonizing larch bark compared with the EU1, suggesting it could pose an increased threat to forestry than the widespread EU1 although this may be mitigated by other differences in behaviour. Each year since 2012, plant health surveys across England, Scotland and Wales have generated thousands of samples from woodlands and forests. These are initially tested for *P. ramorum*, and if positive also tested for lineage. The combined dataset collected over seven years reveals that although the EU2 was initially restricted to Northern Ireland and a small area of south west Scotland [2], the EU2 has slowly extended its range beyond south west Scotland and in some locations EU1 and EU2 now overlap. So far, however the EU2 is absent from England and Wales although other *Phytophthora* species, in addition to *P. ramorum*, can also infect larch.

### References:

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- [2] King K.M., Harris A.R., Webber J.F., 2015. *In planta* detection used to define the distribution of the European lineages of *Phytophthora ramorum* on larch (*Larix*) in the UK. *Plant Pathology* 64: 1168-1175



**Multiple phenotypes and genotypes of Clade 6 *Phytophthora gonapodyides* and *P. gonapodyides* x *P. chlamydospora* within a single aerial lesion on *Fagus sylvatica*. A. Pérez-Sierra<sup>1</sup>, B.B. Landa<sup>2</sup>, K. Heungens<sup>3</sup>, K. Van Poucke<sup>3</sup>, B. Henricot<sup>1</sup>, C.M. Brasier<sup>1</sup>**

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In routine surveys for *P. ramorum* in south west England small (<10cm) aerial stem lesions caused by the often riparian *P. gonapodyides* were found on European beech, *Fagus sylvatica*. In 2015 a survey revealed a mature beech with aerial bleeds overlying a large lesion of 50 x 20 cm. An isolation grid yielded ~30 slow growing *P. gonapodyides*-like colonies that grouped into five distinctive colony phenotypes. These in turn were found to exhibit significant differences in sporangial morphology. ITS,  $\beta$ -tubulin and cox sequences indicated some of the isolate types were close to *P. gonapodyides*. However other isolates showed a large number of heterozygous sites along the entire ITS, with Clade 6 *P. chlamydospora* being the closest match. The number of heterozygous sites suggested these isolates originated as hybrids with *P. gonapodyides* as the maternal parent. Analysis using Genotyping-by-Sequencing (GBS) confirmed three different *P. gonapodyides* genotypes and a *P. gonapodyides* x *P. chlamydospora* hybrid genotype. NGS amplicon sequencing was used on bark panels from the lesion and the soil around the roots. Pathogenicity tests on the three *P. gonapodyides* and *P. gonapodyides* x *P. chlamydospora* types were carried out on beech logs together with isolates of *P. gonapodyides* and *P. chlamydospora* for comparison. The areas of the resulting lesions were relatively homogeneous, but one isolate of *P. gonapodyides* genotype 2 and one of the hybrid isolates produced larger lesions. The possible ecological origins and the evolutionary and tree health significance of such a 'multi-genotype Clade 6' lesion will be discussed.

**Acknowledgement:**

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**Detection and description of viruses in *Halophytophthora* spp. from Portugal.** Leticia Botella<sup>1</sup>, Josef Janousek<sup>1</sup>, Milica Raco<sup>1</sup>, Thomas Jung<sup>1,2</sup>

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We investigated possible RNA virus infection in a collection of *Halophytophthora* spp. from estuarine ecosystems in Portugal. The first approach to detect the presence of viruses was based on the occurrence of double stranded RNA, typically pointed out as a viral molecule in plants and fungi. From 80 *Halophytophthora* isolates, different dsRNA-banding patterns were observed. Two isolates were chosen for performing stranded mRNA sequencing for the novo assembling of viruses. Total RNA was extracted separately for those two samples and then mixed for the library construction of short inserts. Inserts were sequenced from both ends generating paired-reads. A total of eight putative novel viruses were detected. Each isolate of *Halophytophthora* seems to host four viruses. All of them appear to be novel species with certain similarity to members from the *Bunyaviridae* family. This study confirms the presence of viruses in marine oomycetes.

**Detecting novel viruses of *Phytophthora castaneae* using high-throughput sequencing of small RNAs.** Milica Raco<sup>1</sup>, Thomas Jung<sup>1</sup>, Eeva J. Vainio<sup>2</sup>, Ales Eichmeier<sup>3</sup>, Eliska Penazova<sup>3</sup>, Leticia Botella<sup>1</sup>

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RNA silencing often called RNA interference (RNAi), represents a pathway used by many organisms including fungi, to regulate gene expression. This biological response to non-self double-stranded (ds) RNAs has an essential role in mediating defence mechanisms against RNA viral infection. When detected in a cell, dsRNA is cleaved into short interfering RNAs (siRNAs) by RNase III endoribonucleases, enzymes called Dicers. As RNA silencing is triggered by the occurrence of viral dsRNAs in the cell, virus-infected organisms can be enriched for small RNA fragments representing viral genomes. Small RNA sequencing has been used as an effective method for detecting viruses in plants and fungal pathogens.

To our knowledge, this study represents the first report of using high-throughput small RNA sequencing for detecting viruses in *Phytophthora* species. Fifty-six isolates of *Phytophthora castaneae* from a wide range of forest sites in Vietnam and Taiwan were screened for virus presence using the dsRNA method. From the twenty-three isolates showing putative virus presence, six isolates hosting dsRNAs of approximately 20kb, 10kb, 7kb, 5kb, 4kb, 3kb and 0.9kb have been chosen for small RNA sequencing. This research aimed to examine, whether *Phytophthora* species utilise RNA silencing and whether it can be used as a method to detect viruses. Phylogenetic analyses of the obtained virus sequences may help to understand the evolution and origin of their *Phytophthora* hosts. In addition, these viruses could be potentially useful in biological control if their hypovirulent activity is proven.

**Describing two new species of *Nothophytophthora* (oomycota) from Ireland and Northern Ireland.** Richard O’Hanlon<sup>1</sup>, Maria Destefanis<sup>2</sup>, Stanley Bellgard<sup>3</sup>, Bevan Weir<sup>3</sup>, Ivan Milenković<sup>4</sup>, Thomas Jung<sup>4</sup>

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*Nothophytophthora* was erected as a genus of oomycetes in 2017 [1]. It is closely related to the genus *Phytophthora*, sharing many morphological characteristics with *Phytophthora*. During surveys of plant material in and around river in 2014, 2015, 2017, 2018 oomycetes were isolated that were similar to *Phytophthora* spp. [2]. These isolates come from 3 locations in Ireland and 1 location in Northern Ireland. DNA sequencing of the ITS region indicated that these isolates were different from the other sequences on GenBank, and very similar to members of the genus *Nothophytophthora*. Morphological measurements of 15 isolates were carried out in the Phytophthora Research Centre in Czech Republic, and indicate that there is at least 2 different taxa isolated. Both taxa form chlamydospores on carrot and V8-juice agar. The two taxa differed morphologically from other described *Nothophytophthora* species and were differentiated at the  $\beta$ -tubulin locus. Limited mating type studies with known *Phytophthora ramorum* type cultures indicate some of the isolates are silent A1. The taxa do not appear to be very pathogenic to plants, although detailed pathogenicity studies have not yet been carried out. BLAST comparisons with GenBank entries indicate that isolates similar to those found here have been detected using environmental DNA in Scotland, Spain and Portugal. Stream baiting studies carried out in the Kauri forests of New Zealand have also isolated a genetically similar taxon [3]. Details on the two taxa will be discussed in terms of their ecology and biogeography.

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## SESSION 10

# MANAGEMENT AND CONTROL

***Phytophthora ramorum* mitigation at the Bloedel Reserve in Western Washington – A four year update.** Gary A Chastagner<sup>1</sup>, Marianne Elliott<sup>1</sup>, Darren Streng<sup>2</sup>

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In March 2015, *Phytophthora ramorum* was detected on a *Pieris* at the Bloedel Reserve. This 150 acre botanical garden in western Washington encompasses undeveloped forest, pastures, a marsh, woodland plantings, and intensely maintained gardens. Initial surveys detected infected plants in two initial sites, the Rhododendron Glen and the Camellia Trail areas in April and June of 2015. Subsequent delimitation surveys uncovered several additional positives in these managed landscape areas and monthly surveys from November of 2015 to December 2016 identified an additional 6 positive plants within the Glen and one additional plant located in the Camellia Trail area. Destruction of infected plants and adjacent hosts within a minimum 2-meter zone around positive plants was done as they were located. Steam mitigation of the soil in the infested areas was undertaken by WSU Puyallup in summer of 2015 and 2016. In addition, a series of best management practices were implemented to limit the potential spread of the pathogen. During early 2016, an IPM program was initiated that included additional removal of native host material, soil applications of *Trichoderma* and mulches, applications of protectant fungicides and stringent biosanitation procedures. No *P. ramorum* infected-plants have been detected during extensive surveys at this site since February 2016. A total of 3 positive water baits have been obtained from a small creek and pond in (2018 and 2019). To date, there has been no evidence of spread of the pathogen from infested water to nearby plants. Surveys and mitigation efforts are continuing at this site.



**Managing foliar *Phytophthora* pathogens across a whole forest: integrated management using all the tools in the toolbox.** Nari Williams<sup>1</sup>, Stuart Fraser<sup>1</sup>, Carol Rolando<sup>1</sup>, Grant Pearse<sup>1</sup>, Natalie Graham<sup>1</sup>, Mireia Gomez-Gallego<sup>1</sup>, Rebecca McDougal<sup>1</sup>, Renelle O’Neil<sup>1</sup>, Preeti Panda<sup>1</sup>, Martin Bader<sup>1,2</sup>, Peter Scott<sup>1,3</sup>, Rebecca Ganley<sup>1,3</sup>, Jaroslav Klápště<sup>1</sup>, Ahmed Ismael<sup>1</sup>, Heidi Dungey<sup>1</sup>, Ralf Gommers<sup>1</sup>, Lindsay Bulman<sup>1</sup>

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The emergence of foliar *Phytophthora* pathogens on *Pinus radiata* plantations has significant implications for the productivity of New Zealand’s most significant forestry species. Contrary to many *Phytophthora* pathogens, the non-fatal impacts, seasonal pathogen population fluctuations and environmental limitations of these pathogens within New Zealand forests are seeing needle disease management emerge as a leading example of large-scale integrated pest management of *Phytophthora* pathogens in a long-run economically significant crop. Our vision is to use epidemiological modelling at the landscape level, integrated with near-real-time remote sensing to inform genetic deployment, silvicultural management, chemical control and monitor crop productivity. We report on the integration of epidemiological models with remote sensing data acquisition to inform landscape needle disease risk models, genotype impacts, optimisation of spray application and their influence on disease control, and plans for informing genetic deployment, stocking and management to minimise disease impacts and maintain resilient and productive forests.

**A multi-pronged approach for establishing resistance to foliar *Phytophthora* infection in *Pinus radiata*.** Nari Williams<sup>1</sup>, Natalie Graham<sup>2</sup>, Jaroslav Klápště<sup>2</sup>, Ahmed Ismael<sup>2</sup>, Heidi Dungey<sup>2</sup>

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The emergence of *Phytophthora pluvialis* and *Phytophthora kernoviae* as a foliar pathogens of *Pinus radiata* plantations threatens the long-term health and productivity and wood quality of New Zealand's most significant forestry species. Breeding for resistance to these pathogens remains the most promising strategy for sustained disease management. For *Pinus radiata* production, resistance to diseases, such as *Dothistroma* needle blight and *Cyclaneusma* needle cast, have been incorporated as selection criteria into New Zealand's radiata pine breeding programmes for several decades, building on decades of research and selection for resistance. For emerging diseases, like red needle cast, development of laboratory-based artificial screening methods have offered an accelerated alternative for assessing resistance and establishing estimating breeding values (EBVs). The maturation of these populations in field trials now offers the first opportunities to test these values and validate earlier laboratory-based phenotypes for both *Phytophthora* pathogens and co-existing fungal needle pathogens. Field validation of disease resistance phenotypes along with genomic selection (GS) are proving particularly useful for identifying markers which circumvent the need for direct measurement, requirements for individuals to reach a certain age or delays waiting for forest conditions suitable for pathogen development. Genomic selection is enabling the preservation of desirable wood traits along with disease resistance. We report on the development of prediction models for RNC resistance, and generation of the first RNC resistance genomic estimated breeding values.

**Efficacy of long-term phosphite applications to control *Phytophthora* dieback of mature *Fagus sylvatica*, *Quercus robur* and *Quercus petraea* trees under natural conditions.** Thomas Jung<sup>1,2</sup>, Ivan Milenković<sup>1</sup>, Marilia Horta Jung<sup>1</sup>, Alexandra Nannig<sup>3</sup>, Markus Blaschke<sup>3</sup>, Tomáš Kudláček<sup>1</sup>, Tamara Corcobado<sup>1</sup>

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Phosphite applications have proven to be an effective treatment for controlling *Phytophthora* diseases in natural ecosystems in Australia [1,2]. However, in Europe the protective role of phosphite in long term trials has not been yet explored. Therefore, annual phosphite applications were performed over 11 years in five mature (>100 years old) stands of *Quercus robur*/*Quercus petraea* and *Fagus sylvatica* trees, naturally infested by several *Phytophthora* species including *P. xcambivora*, *P. cactorum*, *P. plurivora* and *P. quercina*. The trees were treated annually between 2006 and 2017 using two methods of phosphite applications: aerial low-volume mist application via helicopter (16 ha mature mixed oak and beech forest) and basal stem sprayment (96 trees). The efficacy of the treatments was tested by annual monitoring of the crown condition of treated trees and non-treated control trees. In addition, the fine root condition of each 30 treated and non-treated trees was examined in 2006 (before the start of the trial), in 2008 and in 2017. Root samples were collected, scanned and analysed using the WinRHIZO software. The results demonstrate that both aerial and stem applications of phosphite can be a feasible method to control *Phytophthora* dieback in mature oak and beech trees.

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**Phosphite trials for control of kauri dieback in New Zealand.** Ian Horner, Matthew Arnet, Ellena Carroll, Mary Horner

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Kauri dieback, caused by *Phytophthora agathidicida*, is a serious disease in northern New Zealand forests. Phosphite treatments have been tested in kauri forest trials since 2012. Five-year trials on young trees (up to 40 cm trunk diameter) demonstrated that 7% and 20% phosphite (20 mL injected every 20-cm trunk circumference) facilitated healing of *Phytophthora* lesions. However, phytotoxicity symptoms (leaf yellowing, canopy thinning, trunk bleeds) occurred in some phosphite-treated trees [1]. Trials established in 2016 are investigating lower phosphite concentrations (4% injected every 20, 40- or 80-cm trunk circumference). Trunk spray applications are also being tested. Large trees (up to 2.5-m trunk diameter) were included in trials with low-rate application, in an attempt to determine safe but effective doses for giant trees.

Early results from these trials suggest that 4% phosphite can give effective control, without obvious signs of phytotoxicity. Spreading injection points from 20 cm to one every 40 or 80 cm reduced the lesion healing in some instances, especially in the very large trees. Trunk spray applications of phosphite facilitated some lesion healing, but were not as effective as injection treatments.

Latest trial results will be reported, with discussion on possible deployment options.

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**Phosphite inducing resistance on sweet chestnut against *Phytophthora* infection.** Andrea Brandano<sup>1</sup>, Bruno Scanu<sup>1</sup>, Lucia Maddau<sup>1</sup>, Salvatorica Serra<sup>1</sup>, Nicola Schianchi<sup>1</sup>, Giles Hardy<sup>2</sup>

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Ink disease caused by *Phytophthora* spp. still represents a serious threat to sweet chestnut throughout its distribution area [1]. Amongst control strategies, new perspectives have been offered by the use of potassium phosphite, a fertilizer that indirectly control *Phytophthora* diseases by acting on host physiology and on host-pathogen interactions. In this study, we tested *in planta* the efficacy of trunk injection with potassium phosphite against seven different *Phytophthora* species previously associated with ink disease. For two of the seven species, *P. cinnamomi* and *P. × cambivora*, the treatments were repeated at two different environmental conditions (mean temperature 15°C vs 25°C) and tree phenology. The results obtained demonstrated that potassium phosphite was overall able to contain the development of *Phytophthora* infections in phloem tissues, however its efficacy varied based on the concentration applied and the *Phytophthora* species tested. The concentration of 280 g/l of potassium phosphite (40% of the commercial product Kalex) was the most effective and in some cases, callus formation around the necrotic lesion was detected. Overall, this study broadens the knowledge on endotherapeutic treatments as an effective measure for the management of chestnut ink disease. Interestingly, at higher temperature the ability of *P. cinnamomi* to colonize the phloem tissues increased significantly in non-treated controls, highlighting the potential for *P. cinnamomi*, under current climate change projections, to invade and cause huge damage to sweet chestnut ecosystems on a large scale.

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**Screening New Zealand Kauri (*Agathis australis*) for tolerance to *Phytophthora agathidicida*.** S.E. Bellgard<sup>1</sup>, C.M. Probst<sup>1</sup>, L.G. Raymond<sup>2</sup>, S.J. Hill<sup>2</sup>, P.M. Scott<sup>3</sup>, N.M. Williams<sup>2</sup>

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To address the emerging biosecurity risk of forest pathogens, the world consistently turns to breeding and selection for the long-term sustainability of pest and disease resistance. In tree species and especially native forest tree species, such breeding and selection are a long-term process. Native trees and those of cultural and environmental significance present further challenges with regards to the sourcing of germplasm for screening, as it is necessary to ensure that methodologies consider cultural and social values, within a framework of the phytosanitary imperatives of containment and quarantine. Kauri dieback caused by *Phytophthora agathidicida* is menacing the conservation of this threatened species. This paper describes screening programs applying *ex situ* assay systems to increase the screening throughput. We have progressed from leaf and shoot assays to sterile “root cassettes using juvenile seedlings less than 6 months old. Soil-based, pot-inoculation assays are now being routinely employed on selected “family” lines of kauri, grown from seeds collected from open-pollinated trees. Variation in disease tolerance using a uniform disease pressure under controlled glasshouse conditions was measured by seedling survivability from time of inoculation. Differential host tolerance has been identified within the native remnant kauri populations sampled, with biological samples being used for parallel, post-hoc analyses: recovery of the pathogen, genomic and biochemical analyses, and micro-nutrient analyses. Thus, phenotypic host tolerance responses can be related to expression patterns of genes associated to primary defence, and biochemical libraries of the important intermediate organic compounds used in biosynthetic pathways e.g. lignin.

**Lignin nanoparticles containing essential oils: a new natural biocide delivery system for *Phytophthora* control.** Anna Maria Vettraino, Florian Zikeli, Dania Tabet, Giuseppe Scarascia Mugnozza, Manuela Romagnoli

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The use of conventional synthetic fungicides for controlling diseases caused by *Phytophthora* spp. leads to development of resistant pathogens and negative impact on the environment and human health. Previous studies confirmed that essential oils (EOs) represent an interesting eco-friendly control system in *Phytophthora*s. Despite their promising properties, the application of EO-based fungicides encounters limitations related to their chemical composition (e.g., volatility, poor water solubility, environmental degradation). The encapsulation of EOs inside nanoparticles could improve the efficacy of treatments, due to their unique surface properties, small size, and anti-oxidative effects. Recent advances in engineering lead to the use of nanomaterials as carriers of fungicides in plants. However, most of the nanoparticle materials cannot be used in agriculture due to cytotoxic effects they produce in plants. The aim of this study was thus to assess the fungicidal activities of thyme (*Thymus serpyllum* L.) EOs against *P. cactorum*, using lignin nanoparticles (LNPs). Lignin isolated from beech sawdust was used for the preparation of LNPs loaded with essential oils (EO) from white thyme using a fast anti-solvent method. Release kinetics of EOs incorporated into the LNPs were assessed by reversed phase HPLC at different pH values imitating different soil conditions. Results showed that lignin nanoparticles combined with essential oils may contribute to the development of new antifungal agents to protect the crops from *Phytophthora* diseases.



**Evaluation of *Gluconobacter* spp. as biocontrol agent against *Phytophthora* spp.** Dania Tabet<sup>1</sup>, Stephen Woodward<sup>2</sup>, Anna Maria Vettraino<sup>1</sup>

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*Phytophthora* spp. are highly virulent plant pathogens that are common in many plant nurseries. Chemical treatments used in the control of these pathogens have negative impacts on the environment and present health risks to non-target organisms. Regular use of chemicals in control of soil-borne pathogens may also lead in development of resistance. There is a need to find and establish more effective long-term strategies for sustainable agriculture and improvement of crop growth and health. Implementation of environmentally friendly control methods is currently encouraged in an integrated management approach that minimizes the use of chemicals in nurseries. This review therefore considers in detail the application of biocontrol agents based on endophytic bacteria, as potential biological agents for the management of *Phytophthora* spp. *In vitro* evaluations of the capacities of *Gluconobacter* spp. to biosynthesize secondary metabolites that suppress plant diseases will be presented, along with examination of their capacity as plant growth promoting bacteria (PGPB).

**Antagonist potential of indigenous *Trichoderma* against *Phytophthora palmivora* of Soe Mandarin in East Nusa Tenggara, Indonesia.** Agnes V. Simamora<sup>1</sup>, Mayavira V. Hahuly<sup>1</sup>, Lily F. Ishaq<sup>1</sup>, Julinda B.D. Henuk<sup>1</sup>, Endah Yulia<sup>2</sup>, Evert Hosang<sup>3</sup>

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One of the deadly diseases of Soe mandarin in Nusa Tenggara Timur (NTT) province, Indonesia is root and basal stem rot caused by *Phytophthora palmivora* [1]. This disease is difficult to control and is generally controlled only with pesticides. As environmental awareness increases, it has become obvious that pesticides can have deleterious impacts on the environment and human health, so the use of biocontrol agents such as *Trichoderma* is demanded.

This study aimed to (a) isolate and identify indigenous *Trichoderma* from Soe mandarin plant rhizosphere, (b) examine the efficacy of indigenous *Trichoderma* in suppressing the growth of *P. palmivora* *in vitro* and *in vivo*.

Four species of *Trichoderma* (*T. asperellum*, *T. hamatum*, *T. harzianum*, *T. viride*) were obtained from the Soe mandarin plant rhizosphere by using dilute plate techniques on Potato Dextrose Agar (PDA). The antagonistic potential of the local isolates of *Trichoderma* against *P. palmivora* was investigated in dual culture method. The four species of *Trichoderma* significantly inhibited the growth of *P. palmivora* *in vitro*. The largest inhibition of *P. palmivora* growth was performed by *T. harzianum* (56.67%); followed by *T. hamatum* (55.65%), *T. asperellum* (41.11%) and *T. viride* (34.44%). However, after six months being stored, the spore density of *T. viride* is higher than the other *Trichoderma*. In the glasshouse and field trials, the application of *T. viride* alone and *T. viride* in combination with *Bacillus* significantly reduced the disease incidence of root and basal stem rot of Soe mandarin.

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## POSTER SESSION

**Are *Cistus* sp. shrubs contributing to the epidemic of Iberian Oak Decline? A pathogenicity test answer.** Alba Maria Sanchez-Redondo, Enrique Cardillo, Celestina Perez

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During the 1980s an epidemic of oak (*Quercus ilex* and *Quercus suber*) decline and mortality emerged in Spain and Portugal. *Phytophthora cinnamomi* (PC) has been considered as a main factor associated with the Iberian oak decline (IOD) epidemic [1]. When pathogen has a wide host range and where diversity include abundant reservoir hosts which protect and transmit the pathogen more efficiently than the focal host, an amplification of risk can occur [2]. In IOD affected areas, Moreira *et al.* [3] found that 56% of the surveyed species of shrub flora, including *Cistus* sp., were infected with *P. cinnamomi*. Whereas, in Sardinia, multiple *Phytophthora* spp. were involved in the severe dieback and mortality of *Quercus ilex* and Mediterranean maquis shrubs, including also *Cistus* sp. [4]. With the objective of clarifying the role of *Cistus* sp. in the IOD epidemic, 84 seedlings of six different species of this genera were inoculated with PC and cultivated in a growth chamber. Thereafter, plant survival and root infection were evaluated in a 90 days trial. Pathogenicity trial revealed all species were profusely (40%) infected by PC but four of them (*C. salvifolius*, *C. ladanifer*, *C. monspeliensis* and *C. populifolius*) showed a high survival rate (>80%) and many plants remained asymptomatic. On the contrary, *C. crispus* and *C. albidus* registered mortality rates comparable with *Quercus ilex* (60%). These results suggest a pathogen reservoir role of some *Cistus* sp. shrubs in the IOD epidemic. The contribution of shrubs to an increase of disease risk is of interest to oak woodlands managers so epidemiologic analysis are needed.

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**Susceptibility of the herbaceous species used as pastures in dehesa/montado to *Phytophthora cinnamomi*.** Manuela Rodríguez-Romero<sup>1,2</sup>, Isabel M. Calha<sup>3</sup>, José António Passarinho<sup>3</sup>, Ana Cristina Moreira<sup>3</sup>

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The most representative agrosilvopastoral systems in the Iberian Peninsula are suffering an important decline associated with *Phytophthora cinnamomi* presence. The behavior of *Quercus ilex* and *Q. suber* to *P. cinnamomi* has been widely studied but not so much the herbaceous species sown in these systems. In order to understand the role played by these species in the oak decline, the susceptibility to the pathogen was assessed in eleven species (Poaceae and Fabaceae). *Lupinus luteus*, a susceptible species [1], was used as a positive control.

The assay was conducted in the greenhouse. The soil in half of the experiment was infested with *P. cinnamomi* (isolate 5833 code) from the INIAV fungal collection. After three months, the root and shoot biomass dry weight were evaluated, as well as the isolation of the pathogen from roots and its abundance in the infested soil.

Almost all the plants in the infested soil, although asymptomatic, showed a root biomass reduction (11.6-78.2%). *P. cinnamomi* was not isolated from roots (except in *L. luteus*) [2]. Plants of *Lolium perenne*, instead of reduction, showed an increase in the root biomass comparatively to the control. The number of propagules of *P. cinnamomi* in the soil of the assays of *Lolium perenne* and *Festuca arundinacea* was very low.

We consider important to deepen the knowledge of relationship established between *P. cinnamomi* and the herbaceous pastures used in these agrosilvopastoral systems. The results show that these species could play a role in the activity and survival of the pathogen.

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**Characterization of constitutive and induced chemical defenses of *Quercus ilex* against infection by *Phytophthora cinnamomi*.** Manuela Rodríguez-Romero<sup>1,2</sup>, Belén Godoy<sup>1</sup>, Anna O. Conrad<sup>3</sup>, Fernando Pulido<sup>2</sup>, Pierluigi Bonello<sup>3</sup>

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The oomycete *Phytophthora cinnamomi* is the pathogen most frequently isolated from soils affected by *Quercus* decline in the Iberian Peninsula. The susceptibility of *Q. ilex* to *P. cinnamomi* has been widely studied, but not much is known about how its phytochemistry relates to disease tolerance/resistance. In *Quercus*, the main chemical defenses are phenolic compounds, which are produced constitutively and are also induced following infection. Phenolic compounds have been shown to be useful in determining whether an individual will be resistant in other ecologically significant *Quercus* pathosystems.

For this study, *Q. ilex* seedlings, selected from seven different Spanish provenances, were grown in the greenhouse. Half of the plants (504 seedlings) were then inoculated with *P. cinnamomi*, leaving the other half for analysis of constitutive phenolic compounds. A month after inoculation, seedlings were harvested, phenotyped, and leaves were flash frozen in liquid nitrogen for subsequent extraction and analysis of phenolics.

Differences in the phenolic profiles of symptomatic (susceptible) and asymptomatic (tolerant) individuals were identified and quantified by ultra performance liquid chromatography/mass spectrometry.

Principal component analysis (PCA) was performed to determine which compounds contributed to differences between symptomatic and asymptomatic individuals. Certain flavonoid-like compounds were among the drivers of the PCA differentiation, so these compounds could be useful as biomarkers of resistance to the pathogen. If this result is confirmed, these putative biomarkers could facilitate the selection of disease-tolerant plants to reforest those areas where natural regeneration is strongly compromised by high *P. cinnamomi* inoculum loads.

**Characterization of the volatile compounds from three Brassicaceae species and their effects in the presence of *Phytophthora cinnamomi*. Manuela Rodríguez-Romero<sup>1,2</sup>, Belén Godoy<sup>1</sup>, Joana Neno<sup>3</sup>, Isabel M. Calha<sup>3</sup>, José António Passarinho<sup>3</sup>, Ana Cristina Moreira<sup>3</sup>**

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Plants can change the composition of the surrounding biotic communities around their roots by releasing different compounds in the soil.

*In vitro* tests of anti-*Phytophthora* activity of *Raphanus raphanistrum*, *Eruca vesicaria* and *Diplotaxis tenuifolia* aqueous root extracts (AREs) obtained by maceration of roots, with heat enzymatic inactivation were done. *Diplotaxis tenuifolia* showed the highest rates of inhibition on *P.cinnamomi* mycelial growth (100%), as well as in the production of its asexual structures (sporangia, chlamydospore and zoospore).

Previous tests showed allelopathic effect of *D. tenuifolia* ARE on oak seedlings inoculated with zoospores of *P. cinnamomi* in non-sterile soil suspension. The pathogen reisolation from plant roots and the observation of tissues by histological cuts were carried out to confirm the presence or absence of the pathogen.

The role of the volatiles responsible for the allelopathic effect (mainly isothiocyanates) was analysed and quantified by gas chromatography-mass spectrometry (GC-MS).

The aim of this study was (i) to analyse and characterize the volatile composition of AREs from three Brassicaceae species in the presence or absence of *P. cinnamomi* and (ii) to assess the allelopathic effect of *D. tenuifolia* ARE on oak seedlings.

The allelopathic capacity of these species could be used to reduce the pathogen activity and consequently its infection capacity in the Iberian agrosylvopastoral systems.



**Evaluation of difference of susceptibility of Andalusian *Quercus ilex* L. population through functional traits and physiology assessment.** Alessia Nizzoli<sup>1</sup>, Carmen Morales Rodríguez<sup>1</sup>, Andrea Vannini<sup>1</sup>, Rafael Sánchez-Cuesta<sup>2</sup>, Francisco J. Ruiz Gómez<sup>2</sup>

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*Dehesas* farmland are important and widespread ecosystems in the Iberian Peninsula, covering over 2.9 mill. ha. The holm oak is the main species of the *dehesa* tree layer, occupying more than 1.4 mill ha. This species is threatened by the oak decline, a phenomenon lead by the oomycete root rot, mainly *Phytophthora cinnamomi*, which causes high rates of tree mortality, resulting in a high impact on the economic and ecological sustainability of *dehesas*. The search of the most tolerant progenies to this oomycete could be an alternative strategy to improve ecosystem resilience, being necessary to identify seeds collection stands which provide less susceptible individuals to oomycete infection. In this work, an experimental inoculation with *P. cinnamomi* was carried out with 1-year-old seedlings germinated from acorns collected in 10 different controlled stands of *Quercus ilex* L. subsp. *ballota*. Stands were selected along Andalusian territory for their acorn production, ecological conditions, drought susceptibility and geographical gradient. Survival, growth, biomass allocation, root traits and photosynthesis rates were measured during 3 months at intervals of 15 days. High differences were found between provenances in physiology, root traits and die-off rates. The susceptibility gradient did not coincide with the drought resistance, belonging the lower mortality rates to populations very different in environmental conditions and drought susceptibility, including the populations of Cádiz, Jaén and Sevilla. The characterization of genetic material proceeding of *Q. ilex*-controlled stands, for their use in degraded *dehesas*, could provide better integrative forest management and the control of the oak decline spread, increasing genetical variability and system resilience.

**Habitats favorable for *Phytophthora* species in oak stands of Natura 2000.**  
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Analysis of the chemical properties of degraded soils in Krotoszyn oaks NATURA 2000 showed progressive acidification (pH 2.99), the inhibition of decomposition of organic matter and shortages of some nutrients such as phosphorous, magnesium and calcium. This is probably a consequence of their habitat (oak monocultures self-acidifying) and long-term leaching by rainwater. For these reasons, soils revitalization treatments with the phosphorous, magnesium and liming can be applied. Analysis of historical data in conjunction with the meteorological parameters indicate a strong link between the dieback of oaks and the occurrence of extreme weather conditions. The DNA analysis allowed to detect and identified of pathogens in environmental samples (soil, water and plant tissues). For the first time, in forest sites of Natura 2000 the presence of alien invasive oomycetes: *Phytophthora hendraiandra*, *Pythium quercum* in the rhizosphere of oak, beech and ash trees was confirmed with molecular methods. Pathogenic oomycetes are most often found in hornbeam forests and alluvial forests, where favourable climatic conditions i.e. high rainfall and flooding can lead to damage, especially oaks, alders and ash trees. In addition, the occurrence of a dangerous pathogens of roots seedlings - *Pythium debaryanum* and numerous fungi like *Aphanomyces* and *Armillaria* spp. was denoted. Over the last decade, significant deterioration in the health of oak stands on Krotoszyn plateau was confirmed by the decline symptoms like dieback of shoots in the crowns, lesions on stems and roots of trees. *Phytophthora* species were found deep in the soil profile (ca 150 cm). Treatment with phosphites harmful for oomycetes helped to recover many of trees.

# Habitats favourable for *Phytophthora* species in oak stands of Natura 2000

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## BACKGROUND

- White oak (*Quercus robur* L.), European ash (*Fraxinus excelsior* L.) and black alder (*Alnus glutinosa* Gaertn.) stands belong to NATURA 2000 in western Poland (Krotoszyn Plateau)
- Many pathogens are responsible for oak deterioration in NATURA 2000 stands, but they are not yet identified exhaustively

## METHODS

- Analysis of environmental samples from rhizosphere (roots, water and soil profiles of 1 meter depth)
- DNA analysis based on ITS sequencing of Oomycetes present in soil

## KEY FINDINGS

We found in soil of oak stands:

- ✓ progressive acidification (pH 2.99),
- ✓ inhibition of decomposition of organic matter,
- ✓ shortage of some nutrients such as magnesium and calcium.

## TAKE-AWAY

- ✓ For the first time, the presence of alien invasive Oomycetes was confirmed with molecular methods in forest sites of Natura 2000 in Poland

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## Results

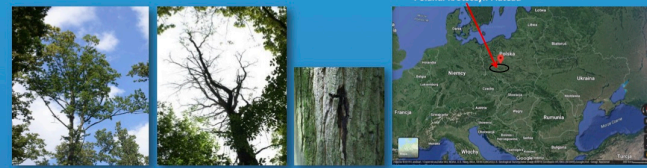


Figure 1. Oak trees from the 3rd resignation Roloff class present dying stems and leaking stain on the trunks (NATURA 2000 Krotoszyn Plateau)

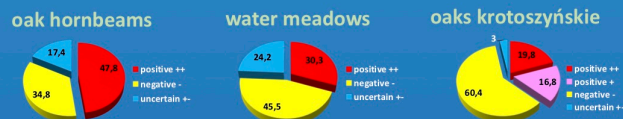


Figure 2. Presence of Oomycetes in soil samples is taken from different sites in Krotoszyn Plateau



Figure 3. The occurrence *Phytophthora* species in gley soils of oak stands in NATURA 2000 site, positive (+) and negative results (-)

## Discovery

- ✓ *P. hedraiaandra* and *P. hungarica* were identified in the rhizosphere of oak, beech and ash trees
- ✓ *Py. quercum* and numerous fungi like *Aphanomyces* and *Armillaria spp.* were denoted in roots seedlings

## Conclusions

- ✓ High rainfall and flooding are favourable climatic conditions for Oomycetes proliferation leading to the rhizosphere damage of oaks, alders and ash trees in Krotoszyn Plateau
- ✓ Special regards should be given to NATURA 2000 protection against Oomycetes sp. via e.g. treatment by phosphites ( $PO_4^{3-}$ )



9th Meeting of the IUFRO Working Party 7.02.09: Phytophthora in Forests and Natural Ecosystems  
La Maddalena, Sardinia, Italy; 18-25 October 2019



## **Toward a new soil detection method of the causal agents of chestnut ink disease.** Marylise Marchand<sup>1</sup>, Marie Massot<sup>2</sup>, Emilie Chancerel<sup>2</sup>, Cécile Robin<sup>1</sup>

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Reports of ink disease and dieback have been dramatically increasing in French chestnut stands for ten years. However, disentangling the role of *Phytophthora cinnamomi* and *P. cambivora* and of various abiotic stresses involved in these symptoms is difficult as long as reliable and fast diagnosis methods are not available. Perfect diagnoses of the disease rely on root and collar bleeding cankers. However, the latter are unfrequent in adult chestnut trees and the former are difficult to excavate. To assess the prevalence of the soilborne pathogens, isolation from soil could be the most convenient method. However, current detection methods display several limitations, *e.g.* the biological baiting is time consuming, unspecific and allow the detection of viable propagules only, positioning the time and place of sampling as parameters of the utmost importance. This underlines the crucial need for a detection method that should be sensitive, specific and applicable to soil samples. However, the soil is a complex matrix and the DNA extraction from such a frame can be stochastic. Therefore, we used DNA extraction kits to standardize the procedure. First, we compared results obtained with a multiplex PCR detection method [1] and a metabarcoding approach [2]. Then, we developed a droplet digital PCR (ddPCR) assay to detect *P. cinnamomi*/*P. cambivora* in soil samples. Indeed, the ddPCR is a promising technique to circumvent the current problems of PCR assays, *e.g.* greater detection sensitivity than real time PCR, lower sensitivity to PCR inhibitors present in samples and bypassing the need for replicates [3,4].

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**Vigil'Ink: a citizen science project dedicated to chestnut ink disease.**

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For twenty years, there has been a significant increase of chestnut ink disease mentions by the Department of Forest Health (DFH) in western and central France. These mentions report symptoms of collar bleeding cankers, in which *Phytophthora cinnamomi* (in most cases) or *P. cambivora* have been identified either by isolation or only by a Lateral Flow Device test. Such an increase of disease incidence might be explained by the climatic conditions prevailing in this part of France during this period. However, the same pattern should be observed in the eastern part of France where chestnut trees are well distributed but also where environmental conditions are favorable for *P. cinnamomi*. However, in this area the DFH reports an increase of chestnut dieback (reflected by tree defoliation and mortality). Such symptoms can be caused by *P. cinnamomi* alone, by abiotic stresses (e.g. drought or unadapt forest soils) or by a combination of both.

What is the actual distribution of the pathogens in France? Is the increase of chestnut ink disease and dieback reports linked to climatic changes, silvicultural practices or *Phytophthora* spp. emergence? In order to answer these questions and to recommend chestnut owners how to manage chestnut sites, we developed a citizen science program called Vigil'Ink. It is based on a nomad application that can be downloaded on smartphones, thus making possible participatory science actions and allowing citizens to recognize chestnut ink disease symptoms, to signal their presence and to sample both plant and soil material for further analyses.



**Physiological and histopathological characterization of infections caused by A1 and A2 mating types of heterothallic *Phytophthora* spp. in *Fagaceae* woody hosts.** Tamara Corcobado<sup>1</sup>, Thomas Jung<sup>1,2</sup>, Tomáš Kudláček<sup>1</sup>, Tomáš Májek<sup>1</sup>, Roman Plichta<sup>3</sup>, Iñigo Saiz<sup>3</sup>, Pavel Kerchev<sup>4</sup>, Marie Matoušková<sup>3</sup>, Aneta Bačová<sup>1</sup>, Henrieta Ďatková<sup>1</sup>, László Benedek Dálya<sup>5</sup>, Miloš Trifkovič<sup>5</sup>, Davide Mureddu<sup>6</sup>, Ivan Milenković<sup>1</sup>

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*Phytophthora* infections are characterized by histological alterations encompassed by physiological and chemical adjustments in the host. However, very few studies combine these diverse approaches [1]. In heterothallic *Phytophthora* spp., A1 and A2 mating types may affect the host differently and may have distinct modes of infection and colonization. The present study aims to subject plants from the *Fagaceae* family to infections of both mating types of heterothallic *Phytophthora* spp. It attempts to identify physiological and metabolomic changes accompanying by structural alterations in the host tissue and describe the invasion process of the A1 and A2 mating types. Artificial inoculations, consisted of immersion of the root tips into a zoospore suspension, were applied. After inoculation, 10-15 days measurements of gas exchange, chlorophyll fluorescence and spectral reflectance were carried out as well as the sampling for metabolomic analyses. Simultaneously, root fragments were collected for histological assessments. Through microscopic observations, structural changes of the root tissue caused by the infection and the colonization process of both *Phytophthora* spp. mating types were examined and recorded. Based on these assessments, preliminary results will be exposed.

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## **A case study on the impact of *Phytophthora* on beech (*Fagus sylvatica*) decline in the Belgian Ardennes. B. Henricot**

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Since 2000, beech decline in the Belgian Ardennes has been observed. At the time it was attributed to frost damage following mild temperatures in the autumn which led to cambium necrosis, beetle and wood decay damage. Between 2003-2009, surveys for *Phytophthora* in beech forests in Central Europe [1] and also in Belgium [2] have shown that at least 10 *Phytophthora* species were associated with the decline. Since 2008, the decline of the beech stands in Belgium has been more widespread [3]. The objective of the study is to determine whether the current decline can also be attributed to *Phytophthora*. For this, beech stands with different health histories and the natural regeneration will be surveyed for *Phytophthora* and other health problems such as beetle and wood decay damage. As part of the project, an area of around 1000 ha with beech stands having shown varying degrees of decline since 2000 have been selected. Stands have been categorized as healthy, showing decline in 2000 or having declined post-2008 and assessed using the DEPEFEU protocol [4]. These stands, the natural regeneration and water courses will be now tested for *Phytophthora* over a period of three years. Preliminary results have shown that trees of higher circumference are showing a higher degree of decline as shown by their higher DEPEFEU score. Five *Phytophthora* species were recovered in watercourses with a peak of recovery in late August and September. Among them, *P. plurivora* known to be pathogenic to beech was recovered. The other species belonged to clade 6 with *P. riparia* new to Europe and *P. gallica* new to Belgium.

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**Analysis of heat shock protein genes expression in *Betula pendula* under defoliation and pathogen stress.** Daria Berezowska<sup>1</sup>, Justyna A, Nowakowska<sup>2</sup>, Tadeusz Malewski<sup>3</sup>, Tomasz Oszako<sup>4</sup>

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In natural conditions, plants are exposed to a variety of environmental stresses, especially long-lived organisms as forest trees. Environmental stresses such as drought, heat, defoliation and pathogen are major limiting factors for plant productivity. Heat shock proteins are responsible for protein folding, assembly, translocation and degradation in many cellular processes. Therefore, they are essential for protecting plants against stress by reestablishing normal protein conformation and thus cellular homeostasis.

*Betula pendula* is a species of tree native to Poland. This is a pioneer species, and one of the first trees to appear on bare or fire-swept land. It is widely used as joinery timber and, firewood. Hence, *B. pendula* may be considered an important plant species to study the events responsible for the survival of woody plants under pathogen stress. However, no information about the gene expression responsible to stress pathogen in *B. pendula* is available.

In this study we compared different techniques of RNA extraction from birch leaves and chosen optimal for study of heat shock protein genes expression. Analysis of two heat shock genes *Hsp83* and *Hsp90* showed they are good indicators of stress condition of *B. pendula*.

# Analysis of heat shock protein genes expression in *Betula pendula* under defoliation and pathogen stress

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## BACKGROUND



- *Betula pendula* (Roth.) is one of the native species of Polish trees, which may be considered as an important plant to study the processes responsible for the survival of woody plants subject to the defoliation and plants infected by root pathogen.
- There is no information about the gene expression responsible for stress pathogen in *B. pendula*.

## METHODS

- Comparison of different RNA isolation techniques (1- Syngen Plant RNA Mini Kit, 2- A&A Biotechnology Total RNA Mini, 3- TRIzol RNA)
- Analysis of the leaves of *B. pendula* subject to 30% defoliation, to 60% defoliation, and infected by root pathogen – *Phytophthora plurivora*
- Chlorophyll fluorescence, as an additional indicator reflecting the influence of defoliation and pathogen on the physiological state of *B. pendula*
- Analysis of gene expression based on quantitative assay (qPCR)

## KEY FINDINGS

- ✓ Heat shock gene *Hsp90* is a good indicator of stress condition for *B. pendula*
- ✓ 30% defoliation is not a heavy stress factor for birch seedlings

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## Results

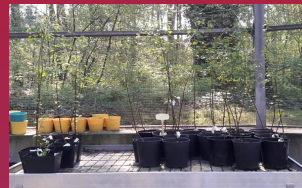


Figure 1. 3-year-old potted plants (control, 30% and 60% defoliation, soil inoculated by *P. plurivora*)

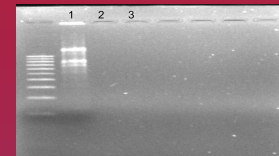


Figure 2. Optimization of RNA extraction from *B. pendula* leaves. Best yield with Syngen Plant RNA Mini Kit (1)



Figure 3. Design of *Hsp90* specific primers

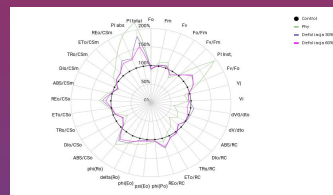


Figure 4. Chlorophyll fluorescence as physiological stress of plants

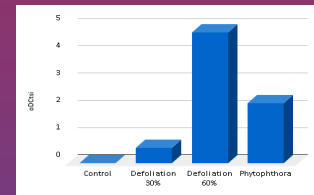


Figure 5. Effect of stress on *Hsp90* gene expression

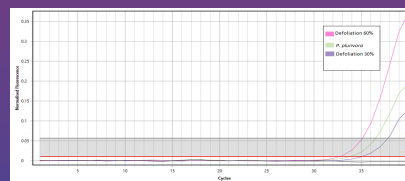


Figure 6. Expression of *Hsp90* gene in qPCR reaction

## Discovery

- ✓ Measurement of chlorophyll fluorescence showed that trees inoculated by *P. plurivora* have higher vitality
- ✓ *B. pendula* subject to 60% defoliation revealed the highest *Hsp90* gene expression

## Conclusions

- ✓ Defoliation and pathogen stress increase gene expression
- ✓ Heavy stress of great defoliation causes much higher gene expression than pathogen-induced stress



9th Meeting of the IUFRO Working Party 7.02.09: Phytophthora in Forests and Natural Ecosystems.  
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**Decline of alpine green alder (*Alnus viridis*) and relation to *Phytophthora* species, preliminary results.** T. Majek<sup>1</sup>, K. Schwanda<sup>2</sup>, T.L. Cech<sup>2</sup>

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Green alder (*Alnus viridis*) is a widespread tree species in the Northern hemisphere. In Europe it is mainly growing in mountainous areas producing shrubby stands on moist sites up to 2800m a.s.l. In the Alps, this species has been suffering from a decline related to imbalances in water supply as a likely consequence of climate change. Furthermore, Green alders often grow adjacent to riparian Grey alders (*Alnus incana*), which are commonly subject to root and butt rot caused by a number of *Phytophthora* species. Since pathogenicity of *P. alni* s.l. to *A. viridis* has been proved experimentally, an impact to Green alders is not unlikely. Therefore, we are assessing *Phytophthora* species present in and around declining green alder stands in the Austrian and Northern Italian Alps within the scope of the horizon 2020 project "Pest Organisms Threatening Europe" (POnTE). This is being performed by direct isolation from stem and root bark necroses onto PARNPH-medium, furthermore by soil baiting and direct isolation from symptomatic leaves of *A. viridis* and other plant species collected from nearby streams, onto PARNPH-medium. In addition neighbouring Grey alder sites are checked by the same method in order to compare the spectrum of *Phytophthoras* present. A number of species were identified, among them *P.pseudosyringae* as a species well adapted to cold climates and a mutual pathogen of *Vaccinium myrtillus*, a shrub widespread in the Alps. In addition also the presence of *P. plurivora* as a pathogen with a wide host range was confirmed.

**Responses of *Alnus glutinosa* populations to different inoculation methods of *Phytophthora xalni*.** I. Gomes Marques<sup>1</sup>, J. Neno<sup>2</sup>, R. Jansson<sup>3</sup>, T. Corcobado<sup>4,5</sup>, T. Cech<sup>4</sup>, Y. Laurent<sup>6</sup>, I. Bernez<sup>6</sup>, S. Dufour<sup>7</sup>, B. Mandák<sup>8,9</sup>, H. Ennoui<sup>10</sup>, A. Sahli<sup>10</sup>, M. Ater<sup>10</sup>, T.S. David<sup>1,2</sup>, A. Solla<sup>11</sup>, A.C. Moreira<sup>2</sup>, P.M. Rodríguez-González<sup>1</sup>

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Decline of *Alnus glutinosa* (L.) Gaertn caused by *Phytophthora xalni* is an emerging threat to European riverine ecosystems, from northern to southern Europe. Reported intraspecific genetic and physiological variation within this tree species suggests a variation in responses to the pathogen infection. This study aims to (i) investigate the response of *A. glutinosa* populations to *P. xalni* and (ii) quantify the infection level of two inoculation methods – flooding vs. stem wounding.

A total of 118 one-year old seedlings from 8 populations ranging the latitudinal extremes of its natural distribution (Sweden, Austria, Czech Republic, France, Jerte and Furelos from Spain, Portugal and Morocco) were grown in peat/sand at 2:1. Seedlings were submitted to the following four treatments: flooding inoculation, wound inoculation, control flooding inoculation and control wound inoculation. To inoculate by flooding seedlings were immersed in river water up to the basis of the stem and V8 plugs colonized with *P. xalni* were put into the water. Seedlings were removed from the river water ten days after flooding [1]. The wound inoculation method was performed through a vertical incision done close to the collar stem, reaching the stem cambium in which a V8 plug colonised by *P. xalni* was placed. Symptoms evaluation included weekly measurements of length and width of stem lesion, extent of girdling [2] and rate of chlorotic foliage [3]. Inoculated seedlings showed external symptoms 12 days after inoculation for both inoculated treatments. Death of seedlings was observed 12 and 15 days after wound and flooding inoculation treatments, respectively.

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***Phytophthora* species in the rhizosphere of *Alnus glutinosa* stands in Western Turkey.** Ayşe Gülден Aday Kaya<sup>1</sup>, Tuğba Doğmuş<sup>2</sup>, Asko Lehtijärvi<sup>3</sup>, Justyna Nowakowska<sup>4</sup>, Tomasz Oszako<sup>5</sup>, Steve Woodward<sup>6</sup>

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Rhizosphere soil samples (27) from alder-dominated flood plain forests in Karacabey and Iğneada, Turkey, were baited using young, fresh foliage of *Quercus suber*, *Rhododendron simsii* and *R. ponticum*. In total, 311 *Phytophthora* isolates were obtained and identified based on a combination of morphological characteristics and sequencing of the ITS rDNA and COX 1 regions. *Phytophthora plurivora* accounted for 83% of the isolates. In addition to *P. plurivora*, *P. gonapodyides* (9%), *P. chlamydospora* (3.8%), *P. lacustris* (2.5%) and *P. cactorum* (2%) were also isolated. The presence of dieback in the forests was indicative of *Phytophthora* infections, although no *P. x alni* was recovered from these sites. Pathogenicity tests showed that the isolates caused lesions on a range of host plants, including *Alnus glutinosa*.

**Diversity of *Phytophthora* species in forest streams and rivers in the southern part of Czech Republic and in northern Slovakia. Henrieta Ďatková<sup>1</sup>, Michal Tomšovský<sup>1</sup>, Ivan Milenković<sup>1,2</sup>, Tomáš Májek<sup>1</sup>, Tamara Corcobado<sup>1</sup>, Thomas Jung<sup>1,3</sup>**

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To determine the diversity of *Phytophthora* species in rivers and forest streams in southern part of Czech Republic (CR) and Slovakia (SK), ten rivers or streams were sampled in both the Moravia region in CR, and the Kysuce region in SK. The survey was performed in November 2018, and isolation tests were performed from naturally fallen leaves collected in the particular streams. Diversity of collected leaves was high, and numerous hosts like beech, oaks, ash, maples, hornbeam, hazel, willow, and several other hosts were sampled. Leaves were washed in the laboratory and dried carefully on kitchen towels. Isolations were performed by direct plating of sections from necrotic lesions onto selective V8-PARPNH agar medium [1]. The first emerging *Phytophthora* colonies were checked under the light microscope and immediately transferred onto fresh V8-agar media and stored at 20-22°C in the dark. After purification and final checking, the isolates were sorted into morphotypes. Representative isolates from each morphotype and from each stream were selected for molecular identification. Mycelial DNA extraction and amplification was performed according to [2]. Consensus sequences were subjected to an NCBI BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST/>) and to a blast search in a local database containing sequences of ex-type isolates or key isolates from published studies to identify the closest-related sequences.

In total, 458 isolates were obtained from rivers and streams in the Moravia region in CR, and *Phytophthora lacustris* was the most frequently isolated species. The second most common species was *P. gonapodyides*, followed by *P. gallica*, *P. bilorbang*, and *P. syringae*. In addition, *Halophytophthora fluviatilis*, *Phytopythium* spp. were recorded in several streams. From the rivers and streams in the Kysuce region in SK, 141 isolates were obtained and *Phytophthora gonapodyides* was most common. Also, *P. lacustris* and *P. bilorbang* were isolated frequently. In addition, *Pythium* spp., *Phytopythium* spp., and one undescribed *Nothophytophthora* species were isolated from the streams. Although some known pathogens were obtained in this study, no pathogenic dieback of forest trees has been observed along the sampled localities.

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**Diversity and distribution of *Phytophthora* species from wild apple forest in Xinjiang Uighur Autonomous Region, China.** Xiao-xue Xu, Wen-xia Huai, Wen-xia Zhao, Yuan Cheng, Zhong-fu Zhou

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*Phytophthora* species are well-known as destructive forest pathogens, especially in natural ecosystems. During the period from 2016 to 2018, a survey of *Phytophthora* diversity was performed at four plots (i.e. Xin Yuan (XY), Ba Lian(BL), Ku Erdeding(KE) Jin Qikesai(JQ)) of wild apple forest on the north slope of Tianshan Mountain in Xinjiang, China. *Phytophthora* species were isolated using baiting techniques from stream, canopy drip and soil samples and were identified based on ITS sequence data, phylogenetic analysis and Morphology. The 621 resulting *Phytophthora* isolates resided in 10 different *Phytophthora* species including 8 known species (*P. lacustris* was the most frequent species, followed by *P. gonapodyides*, *P. plurivora*, *P. gregata*, *P. chlamydospora*, *P. inundata*, *P. virginiana* and *P. cactorum*), two previously unrecognized species (*P. sp.* CYP74 and *P. sp.* forestsoil-like). The highest species richness of *Phytophthora* is at BL, secondly is XY. *P. lacustris* is the main species at BL, XY and JQ while *P. gonapodyides* is most popular at KE. The possible reason, implications and the diseases they are possible causing are discussed. This is the first comprehensive study from Xinjiang to examine the *Phytophthora* communities in wild apple forest, and advances our understanding of the role of these *Phytophthora* in natural and anthropized ecosystems.

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**Pathogenicity of *Phytophthora* species on two commercially important non-native tree species from South Africa.** Tanay Bose<sup>1</sup>, Jolanda Roux<sup>1</sup>, Treena I. Burgess<sup>2</sup>, Christopher Shaw<sup>2</sup>, Michael J. Wingfield<sup>1</sup>

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The commercial forestry industry in South Africa depends on non-native trees for plantation establishment. *Acacia mearnsii* and several *Eucalyptus* spp. or their hybrids of are amongst the most commonly planted trees. A number of *Phytophthora* spp. have been reported to infect these trees but it has been more than a decade since the pathogenicity of *Phytophthora* spp. was assessed on commercially important tree species from South Africa. In the present study, we used sand-infestation pot trials to evaluate the pathogenicity of five *Phytophthora* spp., commonly isolated from South African plantation environments. These included *P. alticola*, *P. cinnamomi*, *P. frigida*, *P. multivora* and *P. nicotianae* that were inoculated on *E. grandis* and *A. mearnsii*. Amongst the tested host tree species, *E. grandis* showed higher susceptibility towards *Phytophthora* spp. *Phytophthora cinnamomi* was the only pathogen that had a significant negative effect on both *E. grandis* and *A. mearnsii*, resulting in a reduction in root and shoot weight and in some cases death of *E. grandis* plants. *Phytophthora alticola* and *P. nicotianae* exclusively infected *E. grandis* and *A. mearnsii* respectively. The results augment the current base of knowledge regarding the relevance of *Phytophthora* spp. to commercial forestry in South Africa.

***Phytophthora* infestations in Turkish forest nurseries.** Ayşe Gülden Aday Kaya<sup>1</sup>, Tuğba Doğmuş<sup>2</sup>, Asko Lehtijärvi<sup>3</sup>, Steve Woodward<sup>4</sup>, Thomas Jung<sup>5</sup>

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Approximately 496 million seedlings of 400 woody plant species, including *Pinus nigra*, *Cedrus libani*, *Juniperus excelsa* and *Castanea sativa*, are produced annually by forest nurseries in Turkey. Most of these plants are sold as 1- or 2-year-old bare root seedlings and are used for reforestation, although some are also used in amenity plantings. Transfers of *Phytophthora* spp. from nurseries to the wider environment have frequently been reported; some resulting in severe dieback or death of the transplanted trees and shrubs, and irrevocable contamination of the planting sites [1]. Occurrence and pathogenicity of *Phytophthora* species causing root rot were investigated in sixteen forest tree nurseries in western Turkey. Scattered mortality in seedbeds, typical of *Phytophthora* infections, was very common in the nurseries. Soils were sampled from the seedbeds and baited using young leaves of *Quercus suber*, *Rhododendron simsii* and *R. ponticum*. One hundred and seventy eight Oomycota isolates were obtained. *Phytophthora* aff. *cactorum*, *P. citricola sensu lato*, *P. crassamura* and *P. syringae* were common amongst these isolates. Pathogenicity tests showed that the isolates caused damage to a range of host plants.

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**Potential for the management of Oomycota in the international trade in plant for planting.** Clara Benavent<sup>1</sup>, Pieter van West<sup>2</sup>, Steve Woodward<sup>1</sup>

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Oomycota are a major problem in the plants for planting pathway, and have established in horticulture and forest situations globally as a result of inadvertent dispersal in the international trade in plants. Work has recently begun in a Marie Skłodowska Curie ITN examining biological methods for managing these pathogens in live plants. We are testing bacterial (e.g. *Aneurinibacillus migulanus* Nagano; *Bacillus subtilis* MBI 600) and oomycete (*Pythium oligandrum*) biological control agents already known to inhibit growth of *Phytophthora* spp. for their abilities to reduce or eliminate *Phytophthora* infestations in plants and potting substrates (compost). The impacts of various edaphic factors on control is also being examined.

**Changes in *Ulmus minor* root fungal endobiome triggered by flood, drought, and drought after flood.** C. Martínez-Arias, J.A. Martín, J. Sobrino-Plata, D. Macaya-Sanz, C. Collada, L. Gil, J. Rodríguez-Calcerrada

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There is increasing evidence that the plant microbiome plays a key role on plant fitness. However, little is known about how the microbiome is affected by abiotic stresses, particularly in trees. In this work, we aimed to understand how tolerance of *Ulmus minor* to drought is altered by a preceding flood; and how flood- and drought-induced changes in plant physiology are related to concomitant changes in the root fungal endobiome. To this end, ramets of five *U. minor* genotypes resistant to Dutch elm disease were subjected to flood and subsequently drought. Changes in fungal taxa were determined by extracting total genomic DNA and sequencing the ITS1 region. Plants exhibited stress symptoms during flood and drought. Stomatal closure was produced by chemical signals during flood and hydraulic signals during drought. Flood did not enhance susceptibility to drought and barely affected drought-induced changes in both, plant physiology and fungal endobiome due to fast flood-stress recovery and moderate drought intensity applied. Flood and drought generally triggered different changes in the root fungal endobiome. Glomerellales increased in response to flood, Sordariales in response to drought, and Sporidiobolales also in response to drought, but only in non-previously-flooded plants. Some plant physiological changes related to abiotic stress were coupled with relative abundance of some fungal orders: Chaetothyriales, Pleosporales, Hypocreales, Sporidiobolales and Cystobasidiales were positively associated with chlorophyll, root non-structural carbohydrates, root soluble sugars, root starch and root proline, respectively. The way that these associations contributed to or cushioned plant stress deserves further research.

**Phytopathogenic fungi and oomycetes detection trials by the means of e-nose and SPME-GCMS devices.** François Lefort<sup>1</sup>, Jérémie Loulier<sup>1,5</sup>, Marcin Stocki<sup>2</sup>, Monika Asztemborska<sup>3</sup>, Rafał Szmigielski<sup>3</sup>, Krzysztof Siwek<sup>4</sup>, Tomasz Grzywacz<sup>4</sup>, Tomasz Oszako<sup>5</sup>

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The aim of this work was to apply an electronic nose developed by the Warsaw University of Technology to the field of phytopathology. Volatiles from several samples of different fungi and oomycetes strains were analyzed by the means of this e-nose and SPME-GCMS method. The assessed treatments included 8 fungal strains (*A. gallica*, *A. ostoyae*, *F. avenaceum*, *F. culmorum*, *F. oxysporum*, *F. poae*, *R. solani* and *T. asperellum*), 4 oomycetes species (*P. cactorum*, *P. cinnamomi*, *P. plurivora*, *P. ramorum*) and two control treatments (empty container and PDA medium alone).

The PDA control turned out to produce its own smell when submitted to the e-nose's analysis, whereas the empty flask control would bring about almost no reaction of the device. *F. poae*, *R. solani* and *T. asperellum* were inducing a strong and immediate response from the e-nose. The other *Fusarium* species along with *P. ramorum* were generating a less intense reaction from the sensors, which was however still stronger than for *Armillaria* and remaining *Phytophthora* strains whose smell appeared to be weak.

All the investigated fungi species (except *R. solani*) were producing sesquiterpenes, in contrary to the tested oomycetes strains and control treatments which seemed not to emit any. Moreover, other molecules such as aliphatic hydrocarbons, alcohols, aldehydes, esters and benzene derivatives were spotted in all samples. The major difference among respective VOCs emission ranges of the tested species may lie in sesquiterpene production, with fungi emitting some of these molecules while oomycetes releasing none or significantly smaller amounts of them.

**Molecular Toolbox of *Phytophthora* species Ex-types with seven genes. Our over 1000 sequences at the NCBI. Z.G. Abad<sup>1</sup>, S.K. Srivastava<sup>1,2</sup>, L.M. Knight<sup>1,2</sup>, M. Nakhla<sup>1</sup>**

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*Phytophthora* species identification at the present is challenging due to the presence of misidentified sequences in public databases including the NCBI. In order to make robust and correct molecular identification and diagnostic systems it is crucial to have information from well authenticated specimens including the Ex-type specimens for accurate comparisons. In order to help ensure the correct molecular identification and diagnostics of the species including those of regulatory concern we have submitted about 1000 sequences to NCBI from seven genes. These include: Internal Transcribed Spacer Ribosomal DNA (ITS rDNA), Cytochrome c Oxidase Subunit 1 (COI) gene, Ras-related Protein ypt1 (ypt) gene, Translation Elongation Factor 1-alpha (EF1A) gene, Ribosomal Protein L10 (RPL10) gene, Heat Shock Protein 90 (HSP90) gene, and Beta-tubulin gene. Authors of this project Abad and Srivastava have been invited to submit the sequences of the ITS rDNA for the project "Oomycetes ITS markers in RefSeq" under the initiative of NCBI Leader Conrad Schoch. This initiative will provide reference targeted loci to aid accurate assessment of species names in Oomycetes, focused on ITS barcode markers from type material. This will expand the existing framework for Fungi ITS RefSeq (PRJNA177353) published with the contribution of 92 international collaborators [1]. The database implemented in this tool box is currently a very important part of "IDphy" online resource and is used for correct identification of species via Sanger and for the implementation of the pipeline for metabarcoding 3<sup>rd</sup> generation high-throughput sequencing via MinION at the USDA-PPQ-CPHST Beltsville Laboratory.

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**Initiating pathogen reduction processes in post-agricultural soils with the addition of organic matter of forest origin.** Ireneusz Olejarski<sup>1</sup>, Justyna Nowakowska<sup>2</sup>, Tomasz Oszako<sup>3</sup>

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Soil residues, sawdust and compost were applied to the soil on post-agricultural lands. Physicochemical and biological changes of soil after addition of organic matter were observed in comparison with the control. More mycorrhizae associations (qualitatively and quantitatively) were observed after the addition of logging residue, as well as the presence of fungi of the genus *Trichoderma* and *Penicillium* antagonists to *Heterobasidion annosum* causing root and butt rot of many conifer species. In addition, the annual increment on the height, length and dry weight of 100 pairs of needles and their chemistry was investigated. Organic matter better stimulated growth of Scots pine seedlings *Pinus sylvestris* and biological activity in the soil, not only increasing its humidity (reducing evaporation) when mulching seedlings as well as porosity but also being a valuable medium colonized and deteriorated by microorganisms. Nowadays, after 10 years we plan to re-evaluate soil features and biological life with the help of molecular tools like Next Generation Sequencing. It will help us to look at the composition of oomycetes and fungi (a part of mycorrhizal once, saprotrophs and pathogens). We hope that accelerating of natural processes in the soil will be reflecting in better sustainability (durability) and biodiversity of growing pine stands on post agricultural soils.

# Initiating pathogen reduction processes in post-agricultural soils with the addition of organic matter of forest origin

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## BACKGROUND

- Sustainable management of Scots pine stands growing on post-agricultural land is crucial to enhance the natural biodiversity of soil microorganisms, being antagonistic to pathogens
- Mycorrhizae associations can be effectively restored by soil supply with forest organic matter

## METHODS

- In autumn 2001, mycorrhized 2-year-old pine seedlings were planted and mulched with forest logging residues (compost) and sawdust
- In 2002 and 2003, biometric measures were performed on seedlings and fungal species were examined in their rhizosphere

## KEY FINDINGS

- The most of mycorrhizae associations (qualitatively and quantitatively) were observed in seedlings grown with logging residues

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## Results

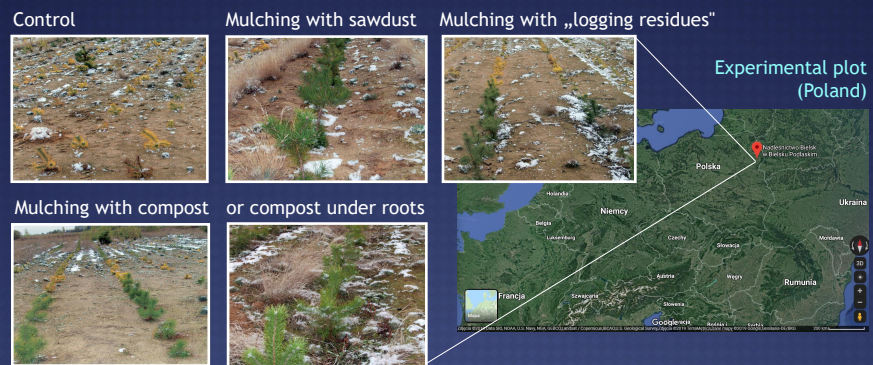


Figure 1. Experimental plot of 3-year-old Scots pine (*Pinus sylvestris* L.) seedlings grown in different conditions of soil fertilizers in Bielsk FD.

Table: Total biotic effect for soil fungi communities antagonistic to *H. annosum* in variant with logging residues

Soil fungi association (organic remnants)	Frequency	TBE H.a.1	TBE H.a.2	TBE H.a.2
<i>Penicillium dahliae</i>	17	3	51	3
<i>Sesquillium canelabrum</i>	15	-3	-45	-4
<i>Penicillium janczewski</i>	10	5	50	4
<i>Mortierella nana</i>	5	-5	-25	-5
<i>Trichoderma polysporum</i>	4	7	28	5
<i>Penicillium citreo-viride</i>	3	-7	-21	-6
<i>Cladosporium ochridis</i>	2	0	0	-1
<i>Trichoderma koningi</i>	2	8	16	8
<b>Total biotic effect:</b>			<b>54</b>	<b>22</b>

## Morphotypes encountered

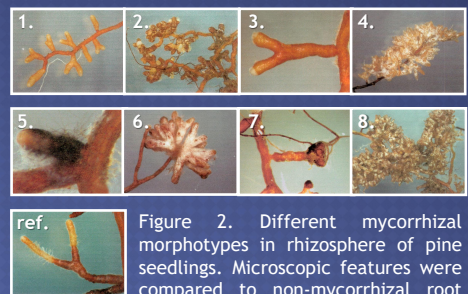


Figure 2. Different mycorrhizal morphotypes in rhizosphere of pine seedlings. Microscopic features were compared to non-mycorrhizal root (reference)

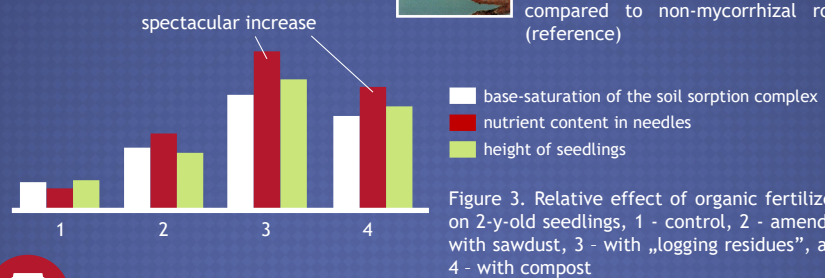


Figure 3. Relative effect of organic fertilizers on 2-y-old seedlings, 1 - control, 2 - amended with sawdust, 3 - with „logging residues”, and 4 - with compost

## TAKE-AWAY

- ✓ Application of the „logging residues” favoured the presence of fungi of the genus *Trichoderma* and *Penicillium*, both antagonistic to *Heterobasidion annosum* the cause of root and butt rot

## Conclusion

- ✓ Organic matter stimulated growth of Scots pine seedlings and promoted the biological activity in the soil



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***Phytophthora* spp. on trees in Britain through the lens of the Tree Health Diagnostic and Advisory Service.** A. Pérez-Sierra<sup>1</sup>, C. Gorton<sup>1</sup>, A. Lewis<sup>1</sup>, R. Chitty<sup>1</sup>, S. van der Linde<sup>1</sup>, A. Armstrong<sup>2</sup>, S. Hendry<sup>2</sup>, A. Harris<sup>1</sup>, S. Green<sup>2</sup>, C. Brasier<sup>1</sup>, J. Webber<sup>1</sup>

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The Tree Health Diagnostic and Advisory Service (THDAS) deals with pest and disease enquiries on trees from England, Scotland and Wales. Diseases caused by *Phytophthora* species have been in the top ten reported problems on trees for the last five years with 280 cases reported. In total 20 different *Phytophthora* species were identified on 26 different genera. Four of these species were first records for Britain, *P. foliorum* on *Rhododendron*, *P. gallica* on *Tilia* sp., *P. siskiyouensis* on *Alnus incana* and the hybrid *P. gonapodyides* x *P. chlamydospora* on *Fagus sylvatica*. The most common species identified were *P. plurivora* followed by *P. austrocedri* and *P. cinnamomi*. A high number of reports of *P. plurivora* were on *Tilia* spp. and *Acer* spp. although it was also recorded on seven other tree genera. *Phytophthora austrocedri* was detected on four different *Juniperus* spp., on two *Cupressus* spp. and on *Chamaecyparis lawsoniana*. *Phytophthora cinnamomi* was detected on nine tree genera and the highest number of cases were recorded on *Castanea sativa*. The fourth most commonly reported species was *P. pseudosyringae* which was detected on seven different tree genera. As the results from the last five years were very informative, a further review of all THDAS records dating back to the early 1970s when the first *Phytophthora* species was diagnosed at the THDAS was performed to compare how the *Phytophthora* scenario has evolved over time in trees in Britain and to determine whether the THDAS was a reliable system for early detection of *Phytophthora* species.

Acknowledgement:

The current staff at Forest Research would like to thank all the advisors that have contributed to THDAS records since the beginning of the service. 'Part of this work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635646, POnTE (Pest Organisms Threatening Europe)''

**Diversity of *Phytophthora* species detected in British soils using NGS analysis for ITS and COI.** A. Pérez-Sierra<sup>1</sup>, M. Montes<sup>2</sup>, B. Henricot<sup>1</sup>, L. Shuttleworth<sup>1</sup>, B. B. Landa <sup>2</sup>

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The objective of the study was to determine the *Phytophthora* species present in British soils in areas which have not been linked to *Phytophthora* outbreaks and compare species present on disturbed sites and undisturbed sites. DNA was extracted from soil samples collected from England, Scotland and Wales from 83 locations in nine 'disturbed' sites and from 51 locations sampled in five 'undisturbed' sites. NGS analysis of amplified ITS and COI regions using Illumina technology revealed the presence of *Phytophthora* spp. in all disturbed and undisturbed sites and in about 80% of all samples tested. NGS analyses of the ITS region revealed the presence of other oomycetes (7,9% of sequences) in the soil samples apart from *Phytophthora* spp., while those using the COI region revealed lower specificity as compared to ITS. Results from NGS analysis of ITS sequences revealed a total of 119 ZOTUs clustered within the genus *Phytophthora* that were assigned to 36 *Phytophthora* phylotypes or species, some of them matching uncultured *Phytophthora* or unidentified ones, with 34 species detected in disturbed soils and 30 in undisturbed ones. NGS results using the COI region detected 28 species/phylotypes, with 23 detected in disturbed soils and 21 in undisturbed soils. Although most *Phytophthora* spp. were identified by both ITS and COI, some of them were identified only with one of the DNA regions. Results indicate that British soils harbour a high diversity of both known and new *Phytophthora* species and other oomycetes that may pose a risk to forests, agriculture and horticulture.

**Acknowledgement:**

This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635646, POnTE (Pest Organisms Threatening Europe)

***Phytophthora lateralis* isolated from Umbrella pine. Alexandra Schlenzig,  
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In June 2018 *Phytophthora lateralis* was isolated from an established Umbrella pine (*Sciadopitys verticillata* (Thunb.) Siebold) in a botanic garden in the West of Scotland. *P. lateralis* had been confirmed in this garden since 2015 and the tree grew in the vicinity of an infected Sawara cypress (*Chamaecyparis pisifera*). Symptoms included olive-brown discoloration and dieback on single branches throughout the tree. The microscopic identification of the isolated culture was confirmed through sequencing of the ITS region of the ribosomal RNA gene. To complete Koch's postulate the culture was used to inoculate a healthy Umbrella pine in the laboratory and the pathogenicity was proved.



## Investigating *Phytophthora* root rot in UK raspberry. Ruth D'urban-Jackson<sup>1</sup>, Erika Wedgwood<sup>1</sup>, Tim Pettit<sup>2</sup>

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Raspberry (*Rubus idaeus*) root rot leading to cane wilting and death, is an economically important disease in the UK. It has been thought to be caused primarily by *Phytophthora rubi*, but with new molecular diagnostic methods [1], other species of *Phytophthora* are increasingly being detected [2]. The English raspberry industry is transitioning towards container production under protection, creating warmer growing conditions and an increasing number of wilting plants. This study set out to establish species of *Phytophthora* now present in these dying plants, and to further develop lateral flow assays with antibodies 3C4 and 3H7 for their use in pathogen detection. During the 2019 growing season, 167 samples of root and cane material were tested, including material from 23 different cultivars, from both soil-grown and containerised plants. Most (90%) samples contained *Phytophthora* spp., with stronger positives often found in extracts from canes, than from root. *Phytophthora* spp. were found in newly imported propagation material from Europe and the USA. *Phytophthora* infection was also found in asymptomatic plants, suggesting visual inspection insufficient for early stage infection. Detection by antibodies 3C4 and 3H7 of *Phytophthora* at very low levels indicates high sensitivity of the lateral flow assay. This work highlights the difficulty in obtaining *Phytophthora*-free raspberry propagation material [3]. These results suggest *Phytophthora* root rot is widespread in English plantations. Further work will investigate the species present in each sample, to explore any *Phytophthora* species dominance in particular parts of the plant, and in material sampled at different times of the year.

### References:

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- [2] Young V., Cooke D., Barker H., Dolan A., 2009. Does *Phytophthora idaei* pose a threat to the raspberry crop? [poster] SCRI Scotland
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## Investigating *Phytophthora* root rot in raspberry

Ruth D'urban-Jackson<sup>1</sup>, Erika Wedgwood<sup>1</sup>, Gary Keane<sup>2</sup> and Tim Pettitt<sup>2</sup>




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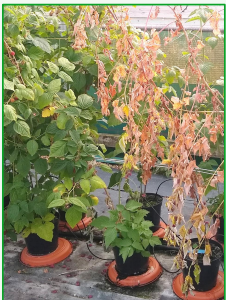


### Background

Raspberry (*Rubus idaeus*) root rot leading to cane wilting and death, is an economically important disease in the UK.

It has been thought to be caused primarily by *Phytophthora rubi*, but with new molecular diagnostic methods, other species of *Phytophthora* are increasingly being detected.

This study set out to establish species of *Phytophthora* present in dying plants, and to further develop lateral flow assays with antibodies 3C4UW387 (3C4) and 3H7UW375 (3H7) for their use in pathogen detection.




### Methods

During the 2019 growing season, wilting raspberry plants from across the UK were sampled, testing root, cane and leaf material. Plant extracts were used in prototype competitive immunoassay format LFD tests (**phase 1**) before being frozen for sequencing (**phase 2**).

**Phase 1:** Test samples via 2 LFD tests.  
 General *Phytophthora* spp. = 3H7  
 Pathogenic species= 3C4 (clades 1, 7 & 8)

Plant tissue tested using General LFD.  
 If negative → no further testing.  
 If positive → test with Pathogenic LFD.

**Phase 2:** Using nested PCR, sequencing and BLAST to determine the species present in cane and root tissue. (Work currently underway).




### Results

Most of the 166 samples were taken from symptomatic, with a small subset of 12 from asymptomatic raspberry plants. Material was from 73 plants, encompassing 23 different cultivars, from both soil-grown and containerised plantations.


Sites	Plants	Samples			<i>Phytophthora</i> species
		Root	Cane	Leaf	
46	73	87	71	8	To be confirmed

Prototype competitive format LFD tests using antibodies 3C4 and 3H7 found:


- Most samples (91%) contained *Phytophthora* spp. and the majority of these (95%) also tested positive for clades 1/7/8.
- Strong presence of *Phytophthora* in above-ground cane material, as well as in root and crown tissue.
- Phytophthora* spp. detected in some asymptomatic plants.
- Phytophthora* spp. present in stems of newly propagated mother stock plugs, but not in root of same plant.
- Phytophthora* infection in all ages of plant, from 2-week old plug plants to 6 year old mature plants.




**A**



**B**



**C**



**A) Infected cane base, B) infected cane, and C) LFD test kits**

### Conclusions

- Together, antibodies 3C4 and 3H7 can be used to determine if certain pathogenic *Phytophthora* species are present in raspberry plant material.
- Antibodies 3C4 and 3H7 may allow users to test more of their plant material, and with a higher sensitivity than many current diagnostic options.

Tests with these antibodies have potential use for detecting *Phytophthora* spp. throughout production systems at very low levels. This requires verification from sequencing work, which is currently underway.



**Acknowledgements:** This work was funded by the Agriculture and Horticulture Development Board, UK. 2019. Photo credit: Images B and C - T Pettitt and G Keane, SSE University of Worcester.



**Response of *Larix* bark to invasion by *Phytophthora ramorum*.** Joan Webber<sup>1</sup>,  
Mina Kalantarzadeh<sup>1,2</sup>, Dulcie Mulholland<sup>2</sup>

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Since the introduction of *Phytophthora ramorum* into Britain and the host jump from rhododendron to larch, at least 25,000 ha of commercially grown larch have been lost to 'sudden larch death'. Mostly Japanese larch (*Larix kaempferi*) has been affected although European (*L. decidua*) and hybrid larch (*L. x eurolepis*) are also susceptible. Trees of all ages can be quickly killed by girdling cankers on branches and stems. As *P. ramorum* invades larch bark, it not only incites copious resin flow from infected tissues, but the freshly invaded phloem tissue takes on a striking pink-red colour at the margins, only changing to a typical brown-coloured necrosis in older lesion regions [1]. Isolation or PCR detection of *P. ramorum* usually fails when targeted at the pink/red lesion margins but are more successful when older lesion areas are sampled. GCMS analysis of Japanese larch bark extracts detected an array of compounds, including resin acids dehydroabietic acid and methyl abietate, plus other compounds such as  $\alpha$ -pinene and 3-carene, at elevated levels in Japanese larch bark colonised by *P. ramorum* [2]. Chemical changes in the phloem were most varied and concentrated in the red-coloured areas and likely play a part in an induced resistance response of larch to *P. ramorum* attack. However, the response may be overwhelmed when multiple infections occur on individual trees, especially during the epidemic peak. Better understanding of this process during the early stages of pathogen attack could provide insights into what resistance mechanisms operate in larch against *P. ramorum*.

References:

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- [2] Kalantarzadeh M., Mulholland D.A., De Leij F.A.A.M., Webber J.F., 2019. Induced antimicrobial activity in heat-treated woodchips inhibits the activity of the invasive plant pathogen *Phytophthora ramorum*. Plant Pathology. <https://doi:10.1111/ppa.13010>

**Is *Phytophthora ramorum* associated with a new, severe disease of chaparral plants in Coastal California?** Wolfgang Schweigkofler, Tomas Pastalka, Karen Suslow

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*Phytophthora ramorum*, causal pathogen of Sudden Oak Death and ramorum blight, was discovered in California in the mid 1990s, and spread quickly along a narrow band close to the coast characterized by mild temperatures and abundant year-long moisture. The foliar host California bay laurel (*Umbellularia californica*) is an essential driver for the spread of the disease to 'dead-end hosts', such as coast live oak (*Quercus agrifolia*). Recently, *P. ramorum* was detected on several chaparral plants (manzanita, *Arctostaphylos* spp.; chaparral pea, *Pickeringia montana*) on a high, sun-exposed ridge in Marin County. During 2018, a severe outbreak of disease was observed on Mt. Tamalpais, with symptoms including wilting, branch dieback and occasionally plant death. Several plants showed a positive reaction for *Phytophthora* spp. using immuno-strips; and *P. ramorum* was detected using PCR from a manzanita stem. In addition, *Neofusicoccum australe* (Botryosphaeriaceae) was isolated from a symptomatic plant. Potted rhododendron plants were placed near symptomatic plants on Mt. Tamalpais to monitor the possible spread of airborne inoculum and the effect of environmental parameters such as rainfall on the timing and appearance of disease symptoms. While it is still unclear whether the observed symptoms are caused by a disease complex, and which role *P. ramorum* has in it, mounting evidence indicates that *P. ramorum* is expanding its host range and moving into new environments.

# Is *Phytophthora ramorum* associated with a new, severe disease of chaparral plants in Coastal California?



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Chaparral habitat on Mt. Tamalpais, Marin Co.



Symptomatic chinquapin



Symptomatic manzanita



'Bait plant' for transmission detection



Location of Mt. Tamalpais, Marin Co., CA

Since its introduction into California, *Phytophthora ramorum* was found predominately on a rather narrow band along the coast characterized by mild temperatures and abundant year-long moisture (the 'fog belt'). The presence of foliar hosts, especially California bay laurel (*Umbellularia californica*), common in this ecosystem, is an essential driver for the spread of the disease to 'dead-end hosts', such as coast live oak (*Quercus agrifolia*) and tanoak (*Notholithocarpus densiflorus*).

### Chaparral plant community

Shrublands in California, known as chaparral, are shaped by Mediterranean climate with mild, wet winters and hot dry summers and the regular occurrence of wildfires. Chaparral covers approximately 5% of California, especially along the coast and the foot hills, and contains many endemic species. More than 40 species of manzanitas (*Arctostaphylos* sp., Ericaceae) are found in the California chaparral, many of them with a very limited distribution and not found outside the state.

Recently, *P. ramorum* was detected on several plants typical for the chaparral plant community (*Arctostaphylos* spp.; and chaparral pea *Pickeringia montana*; Fabaceae) on a high, sun-exposed ridge in Marin County. In addition, *P. ramorum* was isolated from dying chinquapin (*Chrysolepis chrysophylla*; Fagaceae) from the same location (Rooney-Latham et al. 2017). Chinquapins are small to mid-size trees native to California and the Pacific Northwest, found mainly on warm, dry, exposed locations.

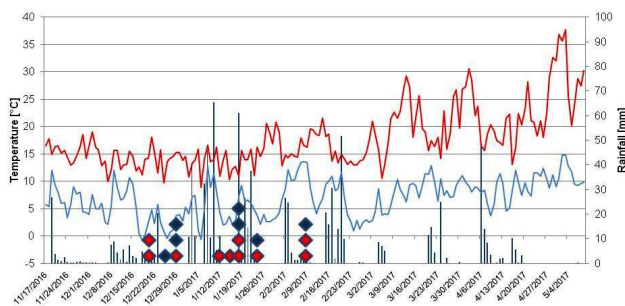
During 2018, a severe outbreak of disease was observed on chaparral plants on Mt. Tamalpais in Marin Co., with symptoms including wilting, branch dieback and occasionally plant death. Leaves and branches of several plants showed a positive reaction for *Phytophthora* spp. using immuno-strips; *P. ramorum* was detected using PCR from a manzanita stem. In addition, *Neofusicoccum australe* (Botryosphaeriaceae) was isolated from a symptomatic plant. The infested area is on a southern slope with no apparent presence of California bay laurel or tanoak. *P. cinnamomi* is also known to occur in the area, and can infect several manzanita species through the root system, causing plant death. However, *P. cinnamomi* seems to spread rather slowly through the soil.

Inoculations performed at UC Berkeley on detached leaves of *A. glandulosa* and *A. canescens* were positive, but both infection success and sporulation rates were rather low, indicating it is not likely these two species may play an important epidemiological role in the spread of SOD (M. Garbelotto, pers. com.).

### Effect of climatic factors on *P. ramorum* transmission

The transmission of *P. ramorum* inoculum from symptomatic bay laurels to healthy potted rhododendrons was tested during three rainy seasons (2016/17-2018/19) at the campus of Dominican University to gain a better understanding of the effect of environmental parameters such as rainfall on the timing and appearance of disease symptoms. Transmission occurred from late December until May, and was more common in winters with high rainfall (Figure 1). *P. ramorum* was also detected from rainwater collected from the canopy of the bay laurels (Figure 2). Potted rhododendrons were also placed near symptomatic chaparral plants on Mt. Tamalpais during winter 2018/19, but no transmission was detected on the bait plants so far.

While it is still unclear whether the observed symptoms are caused by a disease complex, and which role *P. ramorum* has in it, mounting evidence indicates that *P. ramorum* is expanding its host range from the early infestation sites in moist and shaded habitats with high densities of California bay laurels towards drier and more sun-exposed areas characterized by a chaparral-type vegetation. We're continuing the monitoring of symptoms of manzanitas in the San Francisco Bay Area, and are planning inoculation studies using potted plants at our research nursery at NORS-DUC.



**Figure 1:** Transmission of *P. ramorum* from California bay laurel to Rhododendrons during the winter 2016/17. Red diamond: symptoms detected under 'symptomatic area' of the California bay laurel; blue diamond: symptoms detected under 'asymptomatic area' of the California bay laurel.

Year	Sample	November	December	January	February	March	April	May
2016/17	Tree 1 -plating							
	Tree 1-baiting							
2017/18	Tree 1 -plating							
	Tree 1-baiting							
	Tree 2-plating							
2018/19	Tree 2-baiting							
	Tree 3-plating							
	Tree 3-baiting							
	Tree 1 -plating							
	Tree 1-baiting							
	Tree 2 -plating							
	Tree 2-baiting							
	Tree 3 -plating							
	Tree 3-baiting							

**Figure 2:** Presence of *P. ramorum* in water samples from bay laurel canopy. Water samples were collected repeatedly during three consecutive winters (2016/17; 2017/18 and 2018/19) and tested for *P. ramorum* using direct plating and BOB (Bait in bottle). Red: positive; green: negative; grey: not tested (no or very little rainfall).

### Acknowledgements

The National Ornamentals Research Site at the Dominican University of California is funded by grants from the 2008, 2014 and 2018 Farm Bills, and administrated through the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) Center for Plant Health Science and Technology (CPHST).

### Reference

Rooney-Latham, S.; Blomquist, C.L.; Williams, A.; Gunnison, E.; Pastalka, T. 2017. Identification of five new hosts of *Phytophthora ramorum* in an infested forest in California. In: Frankel, Susan J.; Harrell, Katharine M., tech. coords. Proceedings of the sudden oak death sixth science symposium. Gen. Tech. Rep. GTR-PSW-255. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 83-84.

## **Comparative Epidemiology of NA1 and EU1 *Phytophthora ramorum* Populations in Curry County, OR. Ebba Peterson<sup>1</sup>, Sarah Navarro<sup>2</sup>, Jennifer Parke<sup>1,3</sup>**

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The 2015 detection of the *Phytophthora ramorum* EU1 lineage in Oregon, USA forests poses a new threat to sudden oak death (SOD) management in Curry County. EU1 may be more aggressive and spread at a faster rate than has been observed for NA1 over the 19 years it has been managed in Oregon forests. EU1 may also infect some hosts, notably conifers, at a greater frequency. To assess any additional risk posed by EU1, we performed field surveys assessing the distribution and frequency of understory infection surrounding SOD-infested trees. We also conducted laboratory assays testing for epidemiologically relevant differences between Curry County NA1 and EU1 isolates.

To determine if the EU1-infested sites were larger upon detection, or if EU1 was infecting hosts at a greater rate, we established transects 20 m uphill, downhill and perpendicular to a confirmed, SOD-infested tanoak tree presumed to be the primary inoculum source contributing to understory infection at a site (7 sites per lineage). In 5m<sup>2</sup> blocks we recorded the presence of understory hosts and collected samples for plating in selective media to confirm infection by *P. ramorum*. Recovery of *P. ramorum* from understory vegetation declined with distance from the primary source of inoculum in both EU1 and NA1 sites. EU1 sites were the same size as NA1 sites upon detection, having similar disease incidence at a given distance from the site center (Wilcoxon rank-sum test;  $p=0.38$ ). Tanoak was abundant and was the most commonly infected host at both NA1 and EU1 sites. There was no difference between the recovery rates of either lineage for all hosts (Pearson's test for independence at  $\alpha = 0.05$ ). *P. ramorum* was not recovered from conifers.

To complement field surveys assessing differential rates of spread, we performed laboratory assays comparing traits likely to alter epidemic trajectory: aggressiveness, optimal temperatures for sporulation, and inoculum thresholds required for infection. Consistent with prior studies, Oregon EU1 isolates were, on average, more aggressive than NA1 isolates on rhododendron, bay laurel, and tanoak at 20°C. Overall, EU1 isolates sporulated at cooler temperatures than NA1 isolates, however optimal temperatures were highly dependent upon which isolate was used. While the number of sporangia produced by EU1 isolates was greater, there were fewer zoospores per sporangium. There was no difference between the two lineages in the number of zoospores needed to cause disease on all tanoak, California bay laurel, rhododendron, Japanese larch, and Douglas-fir ( $\alpha=0.05$ ).

During early stages of establishment at a site, the EU1 lineage is not causing a greater amount of disease, or disease on additional hosts, compared to the NA1 lineage in Oregon. While field studies of naturally infested sites indicate the lineages are similar at early-infestation stages, these results identify some differences that may manifest as the EU1 epidemic intensifies. Modeling of EU1 should account for epidemiological differences to optimize management efforts.

**Using citizen science and outreach education to reduce the risk of *Phytophthora ramorum* spread in Oregon forests. N. Kline<sup>1</sup>, S. Navarro<sup>2</sup>, J. LeBoldus<sup>3</sup>**

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Sudden oak death (SOD), caused by a non-native pathogen *Phytophthora ramorum* has killed hundreds of thousands of tanoak (*Notholithocarpus densiflorus*) trees in Curry County Oregon since it was first detected in 2001. With the expansion of the *Phytophthora ramorum* state quarantine in 2015, more landowners in Curry County are now under regulations to slow the spread of sudden oak death. Some landowners are under a state quarantine for the first time and in some cases are unaware of the state sudden oak death quarantine regulations. Since 2015, the European lineage (EU1) of *P. ramorum* has been detected in 19 infested areas within the SOD quarantine. This development has brought to light an increased need for outreach education of local landowners about SOD, state quarantine regulations, and the new EU1 lineage in southwestern Oregon forests. Oregon's SOD Program would greatly benefit from a coordinated outreach effort to train citizen scientists about the importance of early detection in order to slow the spread of the disease. [1] By focusing on communities along the leading edge of the disease, workshops were held to teach local residents about disease recognition, early detection methods, and effective treatment options. A citizen science project was piloted to train local resident's multiple early detection methods for SOD and coordinating landscape level sampling for new SOD infestations. Additionally, focusing on potentially resistant tanoak, we are training local residents to identify and report healthy tanoak in infested areas. This project was funded by a grant from USDA APHIS.

References:

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**Beech health decrease in Cologne Green Belts.** Cecilia Sabatini<sup>1</sup>, Oliver Menke<sup>1</sup>, Duccio Migliorini<sup>2</sup>, Nicola Luchi<sup>2</sup>, Alberto Santini<sup>2</sup>

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Beech tree (*Fagus sylvatica*) has excellent filter capacities of atmospheric pollution and it is often used for the amelioration of aerial quality in urban and peri-urban areas.

Cologne (Germany) has “Green Belts” (Kölner Grüngürtel): planned green areas, distributed in semicircle on both sides of the river Rhine, with an area of about 800 hectares, 400 of which are woodlands. Beech is the prevailing species. The area is part of an ancient military fortification zone. It was opened to the public use in 1922, even if the age of the trees reaches 140 years.

Beech trees have shown diffused diseases symptoms within the last years, consisting in bleeding lesions along the first 3 meters of the trunk and conspicuous losses of rotten branches. The phenomenon recently increased, placing considerable problems for the conservation of the Green Belts and related functions.

Local Municipality is extremely concern about this issue and it intends to undertake important efforts for stand’s restoration in the injured areas. Our team (<https://www.baumpfleger.de/>) is directly involved in the management of Kölner Grüngürtel. The work will focus mostly on surveying and monitoring of unstable trees. Together with the Institute of Sustainable Plant Protection of the Italian National Research Council of Sesto Fiorentino (IPSP-CNR) we aim to investigate this phenomenon in a wider prospective. This will consist in the accurate assessment of the responsible disease agents/abiotic factors and in the evaluation of urban forestry aspects implicated in present and future management plans. Outcomes are expected within the next months.



## INDEXES



**C** = Oral Communication – **P** = Poster

**A**

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