7th meeting IUFRO

WORKING PARTY 7.02.09

***Phytophthora* in Forests & Natural Ecosystems**

PROGRAM/ ABSTRACTS

10th · 14th November 2014
Esquel, Chubut. Patagonia Argentina

The 7th Meeting of the International Union of Forest Research Organizations

IUFRO Working Party 7-02-09

Phytophthora in Forests & Natural Ecosystems

9th - 14th November 2014

Esquel, Argentina

IUFRO WORKING PARTY 7.02.09

***PHYTOPHTHORA* IN FORESTS & NATURAL ECOSYSTEMS**

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7th meeting IUFRO WORKING PARTY 7.02.09
Phytophthora in Forests & Natural Ecosystems

10th · 14th November 2014

Esquel, Chubut. Patagonia Argentina

Organized by

Universidad Nacional de la Patagonia San Juan Bosco

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Centro de Investigación y Extensión Forestal Andino Patagónico

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*Mari Mari Peñi!**

Dear friends and colleagues:

We are delighted to welcome you to the VII Meeting of IUFRO Working Party and to our hometown! It is an honor for us that Argentina, and particularly far-away Patagonia, has been chosen to be the venue for this Congress which, for the first time, takes place in South America.

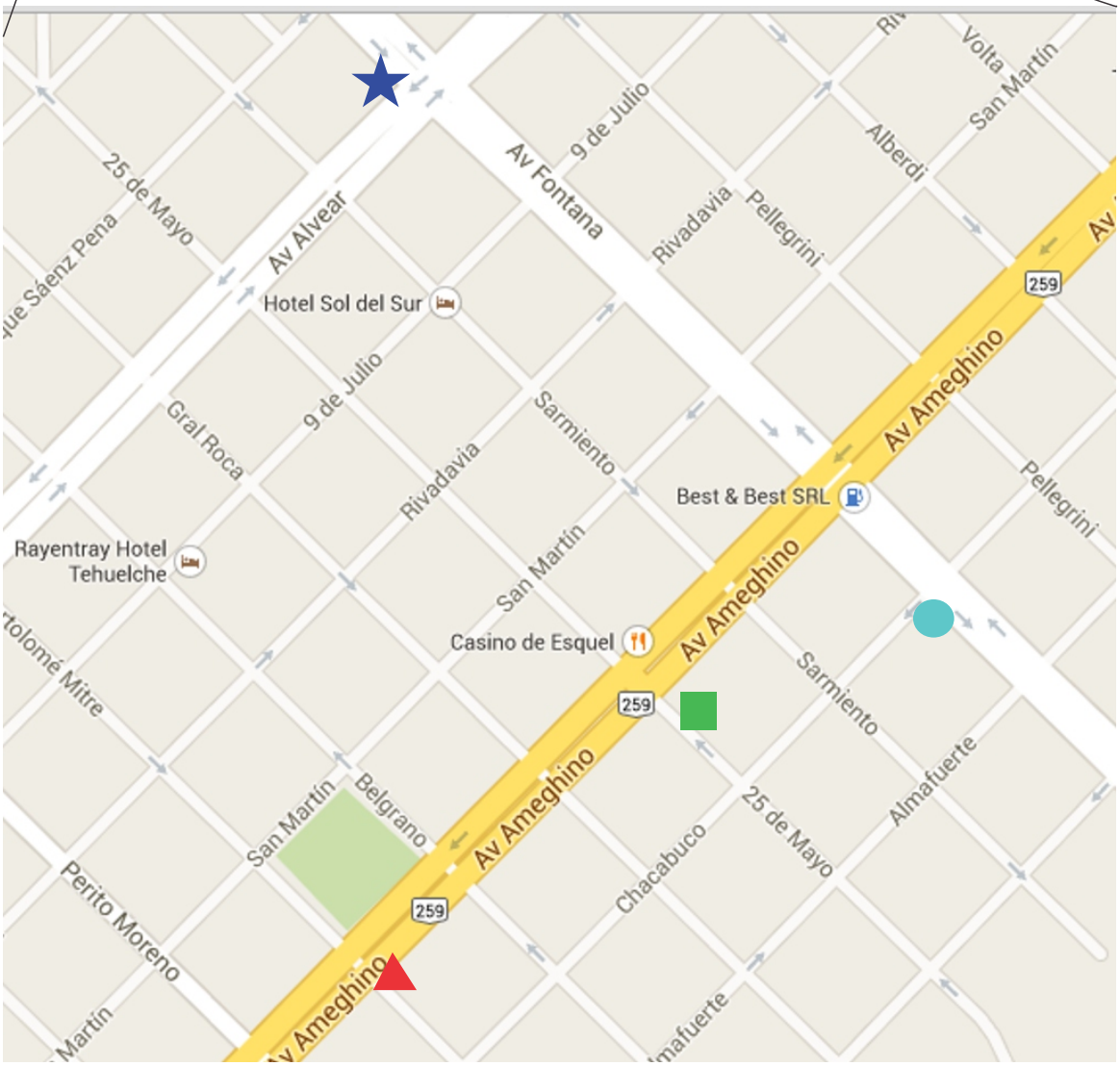
Esquel, head of the Futaleufú Department, is located in the Andean Region of Chubut Province. This small and young city, founded in 1906, has mostly grown through immigration from many places of Argentina and other countries. The local native populations were the Mapuches ("earth people"). Their descendents keep their culture alive either adapted to city life or living in the field, as part of Nature, as their ancestors used to do. The magnificent natural environment and our cultural identity make Patagonia a unique place to visit.

We would like this forum to be a fructiferous meeting in academic as well as social aspects, and that it provides an opportunity for scientific discussion and for making new friends.

We hope you feel warmly welcome and that you take back with you the best memories of Patagonia. It remains for us only to say: ¡Bienvenidos! Welcome! Willkommen! Bem-vindos! Bienvenue! 欢迎! Vítejte! Fáilte! خوش! Benvenuti! Nau mai! Velkommen! Välkommen! Chào mừng!

Organizing Committee / Comité Organizador
7th IUFRO Working Party 7.02.09
Phytophthora in Forests and Natural Ecosystems

**Mari Mari Peñi: "Welcome Friends" in Mapudungun, native Mapuche language.*



Melipal Cultural Center (IUFRO CONGRESS PLACE)
Sur Sur Hotel
Plaza Hotel
IUFRO lunch place

PROGRAM

Sunday 9th November

17:00	Accreditation of attendees
20:30	Opening ceremony
21:00	Welcome reception

Monday 10th November

08:30	15'	**Welcome and Conference Opening** Alina Greslebin and Everett Hansen
		Session 1 Taxonomy **Co-Chairs: Z. G. Abad and E. Hansen**
08:50	20'	Six new *Phytophthora* species from ITS Clade 7a including two sexually functional heterothallic hybrid species detected in natural ecosystems in Taiwan *T. Jung, M. Horta Jung, B. Scanu, A. Pérez-Sierra, T. Chang, P. Abad-Campos, M. Léon, G. M. Kovács, C. Husson and J. Bakonyi*
09:10	20'	The Taxonomy of *Phytophthora*: What is done and what is needed for the correct identification and diagnostics of species in the Genus *Z. G. Abad*
09:30	20'	A new multi-locus phylogeny for the genus *Phytophthora* *X. Yang , B. M. Tyler and C. Hong*
		Poster session of session 1: Taxonomy **Co-Chairs: Z. G. Abad and E. Hansen**
09:50	5'	Introducing…*Phytophthora chlamydospora* (née P. taxon Pgchlamydo), *P. obrutafolium* (née P. taxon oaksoil), and P. "himalsylva-like" (née P. taxon ceanothus) *E. Hansen , N. Grunwald , C. Brasier , P. Reeser , L. Sims and W. Sutton*
	5'	Genetic, morphological and physiological characters of the plant pathogen *Phytophthora cactorum* and its hybrids *M. Pánek and M. Tomšovský*
10:10	30'	**Coffee break**

10:40	30'	What is a species? The challenge of *Phytophthora* *E. Hansen*
11:10	15'	**Chair's review of preceding presentations** *Z. G. Abad and E. Hansen*
11:25	20'	**Session Discussion**
		Session 2: Role of *Phytophthora* species in emergent diseases **Co-Chairs: J. F. Webber and M. E. Sánchez**
11:45	10'	Pathogenicity of *Phytophthora* species isolated from declining European blackberry (*Rubus anglocandicans*) in natural ecosystems of South Western Australia *S. Aghighi, T. I. Burgess, J. K. Scott, M. Calver and G. E. St. J. Hardy*
11:55	10'	*Phytophthora pseudosyringae* found on bilberries in Norway *V. Talgø, M. L .Herrero, M. B. Brurberg, L. Kitchingman and G. M. Strømeng*.
12:05	10'	The comparative pathogenicity of two *Phytophthora ramorum* lineages, EU1 and EU2, on a range of hosts *A. McCracken, L. M. Quinn, M. A. Wilson and J. F. Webber*
12:15	10'	Involvement of *Phytophthora* species in beech decline in Lower Austria *T. L. Cech, T. Jung, T. Corcobado Sanchez and A. Daxer*
12:25	5´	**Questions and Discussion**
12:30	01:30	**Lunch**
		Session 2 (continued): Role of *Phytophthora* species in emergent diseases **Co-Chairs: J. F. Webber and M. E. Sánchez**
14:00	10'	*Phytophthora pluvialis* on Douglas-fir in Oregon, USA. *P. Reeser, W. Sutton, J. Laine and E. Hansen*
14:10	10'	Identification and pathogenicity of oomycetes causing root disease on wild olives *M. González, M. E. Sánchez, A. Pérez-Sierra and M. S. Serrano*

14:20 10' Unexpected discovery of *Phytophthora siskiyouensis* in the UK

A. Perez-Sierra, S. Sancisi-Frey, M. Kalantarzadeh and C. Brasier

14:30 10' Mycelial growth and pathogenicity of *Phytophthora cinnamomi* Rands

E. A. Gutierrez Rodriguez, R. C. Panizzi, A. B. G. Martins and R. A. Andrade

14:40 10' The role of *Phytophthora* in the decline of *Corymbia calophylla* (marri), a dominant and widespread tree species in southwest Western Australia

T. Paap, L. Croeser, T. I. Burgess and G. E. St. J. Hardy

14:50 10' *Phytophthora austrocedrae* emerges as a serious threat to juniper (*Juniperus communis*) in Britain.

S. Green, M. Elliot, A. Armstrong and S. J. Hendry

15:00 10' Evolutionary epidemiology of *Phytophthora austrocedrae* on juniper in Great Britain and the Northern Hemisphere

J. Assmann , S. Green and P. Sharp

15:10 10' *Phytophthora pseudosyringae* associated to the mortality of *Nothofagus* forests of central-southern Chile.

S. N. Fajardo , A. Figueredo , S. Valenzuela and E. Sanfuentes

15:20 10' *Phytophthora kernoviae* detection in *Drimys winteri* (Winter‟s Bark) forest of southern Chile.

E. A. Sanfuentes , S. N. Fajardo , M. A. Sabag , E. Hansen and M. G. González

15:30 10' **Questions and Discussion**

15:40 30 **Coffee break**

16:10 10' Pathogenicity tests of *Phytophthora* species on *Agathis australis*

I. J. Horner and E. G. Hough

16:20 10' Aerial stem cankers associated with *Phytophthora syringae* on *Fraxinus*: how and why?

J. Webber , B. Wylder , A. Harris and C. Brasier

16:30 10' Multiple Phytophthoras associated with larch (*Larix*) in Britain.

S. Sancisi-Frey and J. Webber

16:40 10' Aerial dieback on *Thuja* caused by *Phytophthora lateralis*.

A. Schlenzig, S. Clark and R. Campbell

16:50 10' *Phytophthora* disease on alder (*Alnus* spp.) in Norway.

G. M. Strømeng , M. B. Brurberg , M. L. Herrero , W. Couanon , A. Stensvand , I. Børja and V. Talgø

17:00 10' First report of *Phytophthora* sp. on *Epipremnum aureum* in Mexico.

M. Díaz-Celaya , A. L. Mora-Dañino , S. P. Fernández-Pavía , G. Rodríguez-Alvarado and K. Lamour

17:10 15' **Chair's review of preceding presentations**

J. F. Webber and M. E. Sánchez

17:25 15' **Session Discussion**

Tuesday 11th November

Session 3: Tools for *Phytophthora* surveys

Co-Chairs: A. Pérez-Sierra and D. E. L. Cooke

8:30 15' Fishing for *Phytophthora* 2.0

S. Català, A. Puértolas, S. Larregla, A. Pérez-Sierra and P. Abad-Campos

8:45 15' Exploring hidden *Phytophthora* via amplicon Pyrosequencing using eDNA from soil and water

S. Català, R. Arenas, P. Abad-Campos and A. Pérez-Sierra

9:00 15' Next Generation Sequencing reveals unexplored *Phytophthora* diversity in Australian soils.

T. Burgess, S. Català, D. White and G. Hardy

9:15 15' Molecular Tools for the PCR detection of *Phytophthora austrocedri.*

Z. G. Abad, K. J. Owens, J. C. Bienapfl, S. Green and M. K. Nakhla

9:30 15' Tools for rapid characterization of *Phytophthora infestans* and *Phytophthora ramorum* using real-time PCR and microsatellites from genomic resources.

G. J. Bilodeau, M. Gagnon, C. A. Lévesque, L. Kawchuk, C. P. Wijekoon, N. Feau, M. Bergeron, N. J. Grünwald, C. M. Brasier, J. F. Webber and R. C. Hamelin

		Poster session of session 3: Tools for *Phytophthora* surveys **Co-Chairs: A. Pérez-Sierra and D. E. L. Cooke**
9:45	5'	Detection of *Phytophthora* species on different woody species in nurseries *D. Migliorini, E. Tondini, N. Luchi, L. Ghelardini, P. Capretti and A. Santini*
9:50	5'	Determining an optimal sequence identity threshold value for *Phytophthora* spp. retrieval from environmental data. *S. Català, A. Puértolas, A. Pérez-Sierra and P. Abad-Campos*
9:55	5'	Development of new Real-Time specific assays for the detection of *Phytophthora* species in Holm Oak calcareous forests *S. Català, M. Berbegal, A. Pérez-Sierra and P. Abad-Campos*
10:00	5'	Evaluation of Illumina MiSeq as a new tool for the detection of *Phytophthora* species *C. Morales-Rodríguez, W. Oßwald and F. Fleischmann*
10:05	5'	Metagenomic analysis of *Phytophthora* diversity in nurseries of potted ornamental species *M. L. Prigigallo, A. Abdelfattah, S. O. Cacciola, D. E. L. Cooke and L. Schena*
10:10	5'	Citizen Science Helps Predict Risk of Emerging Infectious Disease *R. K. Meentemeyer, J. B. Vogler, and M. Garbelotto*
10:15	5'	The detection and quantification of four *Phytophthora* species in soil in the UK *M. Elliot, S. Green*
10:20	30'	**Coffee break**
10:50	30'	Whither the species? *Phytophthora* taxa, MOTUs and barcodes in the world of metagenomics *D. E. L. Cooke, M. I. Prigigallo, A. Abdelfattah, L. Schena, E. Randall and J. N. Squires*
11:20	10'	**Chair's review of preceding presentations** *A. Pérez-Sierra and D. E. L. Cooke*
11:30	20'	**Session Discussion**

		Poster session of session 4: Surveys and new records **Co-Chairs: M. Horta Jung and T. Jung**
11:50	5'	*Phytophthora* species in forest streams in Nyingchi, Tibet Autonomous Region and Ganzi, Sichuan Province, China *W. Huai, E. M. Hansen, W. Zhao, G. Tian and Y.Yao*
11:55	5'	Distribution and impact of *Phytophthora* species on alder (*Alnus spp.*) in Southern Sweden *M. A. Redondo, J. Boberg, C. Olsson and J. Oliva*
12:00	5'	Discovering *Phytophthora* species in the laurel forest in Tenerife and La Gomera islands (Canary Islands, Spain) *S. Català*, *A. Pérez-Sierra*, *C. Rodríguez Padrón* , *F. Siverio de la Rosa and P. Abad-Campos*
12:05	5'	Invasive pathogens in Austrian forests: preliminary planning within the European project "Responses of European forests and society to invasive pathogens (RESIPATH)" *T. Corcobado Sanchez, T. L. Cech, C. Huettler*, *M. Brandstetter*, *A. Daxer and T. Majek*
12:10	5'	Oomycetes survey in Northern Norway *M. L. Herrero, L. Sundheim*, *A. M. Brevik*, *M. Tojo and M. B. Brurberg*
12:15	5'	*Phytophthora* survey in a beech forest in Norway *K. H. Telfer*, *V. Talgø*, *M. Herrero*, *M. B. Brurberg and A. Stensvand*
12:20	5'	Maps of *Austrocedrus chilensis* forests affected by dieback *C. I. Núñez, A. Pérez and C. Raponi*
12:20	5'	Alternatives for detection of *Phytophthora cinnamomi* in commercial subtrate *E. A. Gutierrez Rodriguez*, *M. Panizzi Penariol*, *M. C. Ohya*, *R. C. Panizzi* and *R. A. Andrade*
12:25	5'	Species of *Phytophthora* on rhododendrons in Argentina *P. E. Grijalba and H. E. Palmucci*
12:30	5'	Status of the genus *Phytophthora* in Argentina *H. E. Palmucci and S. M. Wolcan*
12:35	01:25	**Lunch**

		Session 4: Surveys and new records **Co-Chairs: M. Horta Jung and T. Jung**
14:00	15'	*Phytophthora* spp. invasions in european post-communist economies – the example of the Czech Republic *K. Černý, M. Hejná and M. Mrázková*
14:15	15'	Survey of Oomycetes found in western Washington streams *M. Elliott, G. Chastagner, K. Coats, G. Dermott and L. Rollins*
14:30	15'	Diversity of *Phytophthora* species in forests, forest nurseries and riparian ecosystems of Portugal. *M. Horta Jung, A. Cravador, C. Maia and T. Jung*
14:45	15'	Diversity of *Phytophthora* species in the oak forests of Southwest China *W. Huai, E. M. Hansen, W. Zhao, G. Tian and Y. Yao*
15:00	15'	*Phytophthora* species associated to Holm oak decline in western Spain *B. Mora-Sala, R. Moliner, T. Corcobado, A. Solla and P. Abad-Campos*
15:15	15'	Multiple new and invasive alien *Phytophthora* taxa from Mediterranean maquis ecosystems in Italy *B. Scanu, B. T. Linaldeddu, A. Deidda, L. Maddau, A. Franceschini and T. Jung*
15:30	30'	**Coffee break**
16:00	15'	*Phytophthora* detections in native plant nurseries and restorations sites in California. *S. Rooney Latham, C. Blomquist, T. Swiecki, E. Bernhardt, E. Natesan and S. J. Frankel*
16:15	15'	*Phytophthora* - an emerging threat to plantation forestry in Vietnam. *T. Q. Pham, D. N. Quynh, T. Burgess and B. Dell*
16:30	30'	Diversity and impact of *Phytophthora* spp. in natural ecosystems of Taiwan. *T. Jung, T. Chang, A. Pérez-Sierra, K. Hsueh, C. Fu, P. Abad-Campos, M. Léon and M. Horta Jung*
17:00	10'	**Chair's review of preceding presentations** *M. Horta Jung and T. Jung*
17:10	20'	**Session Discussion**

Wednesday 12th November

	Field Trip
08:30	Departure from Hotels.
09:30	First stop near to Nant y Fall cascades to see Mal del Ciprés at landscape scale.
09:45	Arrival to Nant y Fall protected area and visit to a monitoring plot in an affected area: symptoms and ecophysiology of the disease. *Alina Greslebin and María Laura Vélez*.
10:00	Visit to the protected area to see natural native flora and the cascades.
11:00	Visit to a panoramic point to observe the magnitude of the disease in the Valley: spread and progression of the disease.
11:30	Departure from Nant y Fall to Los Alerces National Park
12:30	**Lunch** (Asado in Los Alerces National Park)
14:00	The history of "Mal del Ciprés". *Mario Rajchenberg*
14:20	Other species threatened by *Phytophthora austrocedri. María Laura Vélez*
14:30	Situation of *Phytophthora austrocedri* in UK. *Sarah Green*
14:50	Mapping Mal del Ciprés. *Cecilia Nuñez*
15:00	Ride to see Mal del Ciprés and other forest diseases as well as beautiful views of the National Park. *Alina Greslebin*
17:30	Discussion
18:00	Departure from National Park to Esquel
19:00	Arrival to Esquel

Thursday 13th November

		Session 5: Biology & Genetics **Co-Chairs: A. M. Vettraino and M. Garbelotto**
08:30	15'	Patterns and processes of emergence in the genus *Phytophthora*. *N. J. Grünwald*
08:45	15'	Visualisation of early infection by *Phytophthora* “taxon Agathis” in the

		roots of 2-year old kauri *Agathis australis* plants. *S. E. Bellgard, S. E. Williams , C. Probst , M. Padamsee , N. Anand and T. Lebel*
09:00	15'	*Phytophthora ramorum*: Study of the lineage EU2 / EU1 in Ireland *L. de la Mata Saez, C. Fleming and A. McCracken*
09:15	15'	Basic and applied research into *Phytophthora ramorum* in Ireland: the PHYTOFOR project. *R. O'Hanlon , J. Choiseul , H. Grogan and J. M. Brennan*
09:30	15'	Lineage, phenotype and environment factors influencing the *Phytophthora ramorum* epidemic on larch. *J. Webber, A. Harris and C. Brasier*
09:45	15'	Investigation of the tree pathogen, *Phytophthora lateralis*, newly discovered in Northern Ireland. *L. M. Quinn, A. R. McCracken, L. R. Cooke, D. J. Studholme and M. J. Larkin*
10:00	30'	**Coffee break**
		Poster session of session 5: Biology & Genetics **Co-Chairs: A. M. Vettraino and M. Garbelotto**
10:30	5'	Molecular and morphological data shows two consistent lineages in *Phytophthora plurivora* strains isolated from streams in northern Spain. *A. Puértolas, S. Català, A. Pérez-Sierra and P. Abad-Campos*
10:35	5'	*Phytophthora ramorum*: differences in the gene expression during infection in the lineages EU1/EU2. *L. de la Mata Saez, C. Fleming and A. McCracken*
10:40	5'	Comparative fitness of European lineages of *Phytophthora ramorum*. *A. Harris, B. Scanu and J. Webber*
10:45	5'	Genetic variation in *Phytophthora lateralis* lineages by analysis of microsatellite profiles. *A. Vannini , C. M. Brasier , E. M. Hansen , S. Green , C. Robin , J. F. Webber , A. Tomassini , N. Bruni and A. M. Vettraino*
10:50	5'	Genotypic variability of *Phytophthora cinnamomi* mating type A1 in native forests of Taiwan. *A. Vannini , C. M. Brasier , A. Tomassini , V. Forlenza , N. Bruni and A. M. Vettraino*

10:55	5"	Biology of *Phytophthora* species in aquatic ecosystems. *C. Hong , P. Kong , P. A. Richardson , S. R. Ghimire , G. W. Moorman and J. D. Lea-Cox*
11:00	30'	Phenotypic, genotypic, genetic, genomic, analyses of plant pathogens and their application in plant pathology *M. Garbelotto*
11:30	10'	**Chair's review of preceding presentations** *A. M. Vettraino and M. Garbelotto*
11:40	20'	**Session Discussion**
12:00	01:30	**Lunch**

Session 6: Ecology

Co-Chairs: S. J. Frankel and A. Vannini

13:30	30'	Landscape heterogeneity and features are associated to the impact of Ink disease in chestnut orchards in Italy. *A. Vannini, G. Natili and A. M. Vettraino*
14:00	15'	Ecology and pathology of *Phytophthora nemorosa, P. pseudosyringae*, and other ITS clade 3 species in forests in western Oregon. *W. Sutton, P. Reeser and E. Hansen*
14:15	15'	Maternal effects mediate the resistance of *Quercus ilex* to *Phytophthora cinnamomi.* *A. Solla, J. Hernández, T. Corcobado and E. Cubera*
14:30	15'	Long term impact of *Phytophthora alni* on an alder riparian stand. *B. Marçais, C. Husson and Z. Nagy*
14:45	15'	The interplay among human, biotic and abiotic factors explains quick *Phytophthora cinnamomi* spreading and tree decline in a Mediterranean Biosphere Reserve. *L. V. García , P. De Vita , M. S. Serrano , C. Ramo , J. S. Cara , M. R. Écija and M. E. Sánchez*
15:00	30'	**Coffee break**

		Poster Session of session 6: Ecology **Co-Chairs: S. J. Frankel and A. Vannini**
15:30	5'	Climate change can affect the impact of *Phytophthora alni* subsp. *alni*. *K. Černý, N. Filipová and V. Strnadová*
15:35	5'	Economical losses caused by *Phytophthora alni* in riparian stands. Typological study of Vltava River basin (Czech Republic). *K. Černý, V. Strnadová, L. Fedusi , Š. Gabrielov , Z. Haňáčkov, L. Havrdová, M. Hejná, M. Mrázková, K. Novotná, V. Pešková, P. Štochlová and D. Romportl*
15:40	5'	Factors affecting *Phytophthora alni* distribution in State Forests of the Czech Republic. *K. Černý, V. Strnadová, D. Romportl, M. Mrázková, L. Havrdová and V. Pešková*
15:45	5'	Identification of *Phytophthora alni* subspecies in riparian stands in the Czech Republic. *M. Tomšovský, P. Štěpánková, V. Strnadová, P. Hanáček and K. Černý*
15:50	5'	Community structures of root-rotting *Phytophthora* species affecting *Abies* in U.S. christmas tree farms & screening true fir for resistance to *Phytophthora* root rot *K. M. McKeever and G. Chastagner*
15:55	5'	Assessing the risk of chestnut ink disease spreading using TOPMODEL. *A. M. Vettraino , T. Mazzetto , N. Bruni , A. Tomassini , A. Petroselli and A. Vannini*
16:00	5'	Influence of multiple stress sources on cork oak seedling susceptibility to *Phytophthora cinnamomi*. *O. Gutiérrez-Hernández, L. V. García Fernández, P. de Vita, M. S. Serrano, C. Ramo, E. Gutiérrez, P. Ríos, I. Pérez-Ramos, L. Gómez-Aparicio and M. E. Sánchez*
16:05	10'	**Chair's review of preceding presentations** *S. J. Frankel and A. Vannini*
16:15	20'	**Session Discussion**
20:00		**Congress Dinner**

Friday 14th November

		Session 7: Ecophysiology and Physiopathogenicity **Co-Chairs: M. L. Vélez and F. Fleischmann**
08:30	15'	Screening *Quercus ilex* for tolerance to water stress and *Phytophthora cinnamomi*. *T. Corcobado, E. Pérez, B. Krajnc, A. Martos, A. Pérez, E. Cubera, L. Nuñez, M. Horta Jung, A. M. Vettraino and A. Solla*
08:45	15'	The spatial and temporal spread of *Phytophthora alni* subsp. *alni* in alder bark tissue – an ecophysiological study. *H. Pfanz, J. Mombour, C. Wittmann, F. Fleischmann and W. Oßwald*
09:00	15'	Diterpene resin profile of *Austrocedrus chilensis* affected by *Phytophthora austrocedri*. *V. Olate , M. L. Vélez , A. G. Greslebin and G. Schmeda-Hirschmann*
09:15	15'	Effect of cinnamomins on *Phytophthora cinnamomi* biomass growth and on the oxidative burst in infected *Quercus suber* roots. *G. Ebadzad , J. Martins and A. Cravador*
09:30	15'	De Novo Assembly of *Phlomis purpurea* Transcriptome challenged with *Phytophthora cinnamomi*. *A. Baldé, A. Cravador, D. Neves and M. S. Pais*
09:45	15'	Screening of Asian oak species for potential resistance to *Phytophthora cinnamomi*. *M. Horta Jung, C. Maia, T. Chang, K. Hsueh and T. Jung*
10:00	30'	**Coffe break**
		Poster Session of session 7: Ecophysiology and Physiopathogenicity **Co-Chairs: M. L. Vélez and F. Fleischmann**
10:30	5'	Spectral measurements for detecting *Phytophthora*-related stress in *Corymbia calophylla* (marri). *L. Croeser, T. Burgess, G. Hardy, T. Paap and M. Andrew*
10:35	5'	Age-related susceptibility of *Eucalyptus* spp. to *Phytophthora boodjera* nom. prov. *A. Simamora, M. Stukely, G. Hardy and T.Burgess*
10:40	5'	Histopathology of *Phytophthora austrocedri* in *Austrocedrus chilensis*. *O. Troncoso, A. G. Greslebin and M. L. Vélez*

10:45	5'	RNAseq reveals different defense responses of *Quercus robur* microcuttings against *Phytophthora quercina* during root and shoot flush. *F. Fleischmann, O. Angay, S. Recht , L. Feldhahn, M. Tarkka, S. Hermann and T. Grams*
10:50	30'	Recent advances in understanding *Phytophthora*-woody plant interactions *F. Fleischmann*
11:20	10'	**Chair's review of preceding presentations** *M. L. Vélez and F. Fleischmann*
11:30	20'	**Session Discussion**

Poster Session of session 8: Management & Control

Co-Chairs: E. M. Goheen and G. Hardy

12:00	5'	Phosphite for control of kauri dieback: forest efficacy trials. *I. J. Horner and E. G. Hough*
12:05	5'	Epidemiology of *Phytophthora boodjera* nom. prov., a damping-off pathogen in tree production nurseries in Western Australia. *A. Simamora , T. Paap , M. Stukely , G. Hardy and T. Burgess*
12:10	5'	Chemicals for management of red needle cast in *Pinus radiata* plantations in New Zealand: efficacy and persistence of phosphite and other fungicides. *C. Rolando, N. Williams and M. Bader*
12:15	5'	*In vitro* control of *Phytophthora cinnamomi* with *Brassica* pellet. *C. Morales-Rodríguez , A. Vannini and A.M. Vettraino*
12:20	5'	Wooden vectors of *Phytophthora ramorum*: Are Douglas-fir logs a risk? *J. M. Hulbert, J. J. Morrell and E. M. Hansen*
12:25	5'	Polyacrylamide and Movement of *Phytophthora ramorum* in Irrigation Water. *S. Tjosvold, D. Chambers, S. Koike and M. Cahn*
12:30	5'	Introduction and spread of *Phytophthora ramorum* in Northern Ireland, UK. *A. McCracken , J. Finlay and S. Morwood*
12:35	5'	Surveillance and management of Kauri Dieback in New Zealand. *N. Waipara and T. Beauchamp*

12:40	01:30	**Lunch**

		Session 8: Management & control **Co-Chairs: E. M. Goheen and G. Hardy**
14:15	30'	Challenges associated with the management of *Phytophthora* diseases in Australia and the importance of community engagement for success. *G. Hardy, B. Dunstan, T. Paap and T. Burgess*
14:45	15'	Continued Monitoring of Sudden Oak Death Treatments in Oregon Tanoak Forests. *E. M. Goheen , A. Kanaskie , E. Hansen , P. Reeser and W. Sutton*
15:00	15'	Sudden Oak Death: Intensification and Spread in Oregon Forests. *A. Kanaskie, R. Rhatigan, R. Wiese, J. Laine, E. M. Goheen, E. Hansen, P. Reeser and W. Sutton*
15:15	15'	Approaching 15 years of research on SOD control. *M. Garbelotto, D. Schmidt, S. Schechter, P. Croucher and C. Hayden*
15:30	15'	Eradication of *Phytophthora cinnamomi* from infested *Eucalyptus marginata* (jarrah) forest during large scale mining operations. *B. Dunstan, J. Gyeltshen, A. Vettraino, V. Stokes, T. Burgess and G. Hardy*
15:45	15'	Potential Impacts of the Revised APHIS *Phytophthora ramorum* Domestic Quarantine Regulatory Requirements on the Spread of this Exotic Pathogen within Washington State. *G. Chastagner and M.Elliott*
16:00	15'	Enabling technologies to combat *Phytophthora* diseases. *N. M. Williams, R. L. McDougal, P. Scott, E. Telfer, L.J. MacDonald, N. Graham and A. Wagner*
16:00	30'	**Coffee break**
16:30	15'	Searching for *Phlomis purpurea* metabolites with anti-*Phytophthora cinnamomi* activity. *D. Neves, C. Maia, S. Durães, M. Horta, O. Holdenrieder and A. Cravador*
16:45	15'	Screening of biofumigants against *Phytophthora cinnamomi* root disease. *P. Ríos, M. S. Serrano, A. Pérez-Sierra, A. de Haro and M. E. Sánchez*

17:00 10' **Chair's review of preceding presentations**

E. M. Goheen and G. Hardy

17:10 20' **Session Discussion**

17:30 01:00 **Closing discussion. Situation of *Phytophthora* in forests and Natural Ecosystems in the continents: what is being done and what is needed.**

30' **Business meeting IUFRO Working Party 7.02.09**

E. Hansen

19:00 15' **Conference closing**

E. M. Goheen and G. Hardy

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Session 1

Taxonomy

What is a species? The challenge of *Phytophthora*

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There are two related questions here. First, the fundamental evolutionary question: How do we interpret the biological species concept for organisms like *Phytophthora*, that live “alternative lifestyles?” And second, the very practical question: How do we identify species of *Phytophthora*? A species is a population of individuals, or a group of populations, with the same evolutionary trajectory. They have a "recent" common ancestor, and they share the same adaptations to their environment. Evolution is an ongoing process - it should be no surprise that some species have fuzzy edges. The fundamental biological process of evolution is influenced in our time by human actions, but it began before us and will continue after we are gone. The challenge of naming and identifying species, however, is a uniquely human problem. We make (and change) the taxonomic rules. Ultimately, a species description is a hypothesis, to be tested, accepted, or rejected by our colleagues. So are there rules for *Phytophthora*? Should there be? Can there be? We will address these questions with examples highlighting the biological challenges presented by hybridization, clonality, and sexuality, and the practical problems of nomenclature, molecular markers and statistics, and quarantine regulations.

Six new *Phytophthora* species from ITS Clade 7a including two sexually functional heterothallic hybrid species detected in natural ecosystems in Taiwan

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During a survey of *Phytophthora* diversity in natural ecosystems in Taiwan a swarm of six new species was detected. Multigene phylogeny demonstrated that they belong to ITS Clade 7a with *P. alni*, *P. cambivora*, *P. europaea*, *P. fragariae* and *P. rubi* being their closest relatives. Despite sharing general morphological characters all six new species differed from related species and from each other by a unique combination of characters including the breeding system, oogonial abortion rates, the proportion of ornamented oogonia, ratios of paragynous and amphigynous antheridia, size of sporangia and oogonia, cardinal temperatures and growth rates. Three homothallic species, TW sp.01, TW sp.02 and TW sp. 05, were isolated from rhizosphere soil of *Quercus glandulifera*, *Q. tarokoensis*, *Castanopsis carlesii*, *Chamaecyparis formosensis* and *Araucaria cunninghamii* in different forest stands using leaves of *Quercus variabilis* and *Castanopsis indica* as baits. The other three species, TWsp.04, TWsp.08 and TWsp.09, which were exclusively detected in three rivers using leaves of *Citrus sinensis* and *Q. variabilis* as *in-situ* baits, were heterothallic. All TWsp.08 and TWsp.09 isolates belonged to the A2 mating type while isolates of TWsp.04 represented both mating types. Abortion rates of oogonia from extensive mating tests were consistently according to Mendelian ratios (4-33%) which was unexpected as the highly polymorphic sequences of the nuclear ß-tubulin, HSP, TRP, ASF and RAS genes demonstrate that two heterothallic species are hybrids. Phylogenetic analyses of ß-tubulin, HSP, TRP, ASF and RAS clones and of cox1 and NADH gene sequences were performed to elucidate the nature of the parental species and clarify whether or not the hybrids might have been involved in the hybridizations that created *P. alni* ssp. *multiformis* and *P. alni* ssp. *alni*. Pathogenicity trials on *Quercus suber* seedlings indicate that all six new species might be a potential threat to European forests.

The Taxonomy of *Phytophthora*: What is done and what is needed for the correct identification and diagnostics of species in the Genus

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The genus *Phytophthora* contains 141 spp., many of which cause significant economic impact to crops, ornamentals, and forests. Many species are globally recognized of regulatory concern including 29 spp. at the USA (Schwartzburg et al, 2009). As of 1999, 59 spp. were described based on morphology, and at present 82 additional spp. have been described based on morphological/molecular characters of nuclear and mitochondrial genes. A great number of species are expected to be described in the near future thanks to the availability of powerful molecular tools and extensive international surveys associated to *Phytophthora* spp. of concern (i.e. *P. ramorum* and *P. kernoviae*). Although **c**onsiderable progress has been made in identification, phylogenies, and diagnostics, the work is still challenging due to omissions of the types in taxonomic publications and culture collections (i.e. Herb. IMI) and numerous misidentifications submitted to NCBI. The problems are exacerbated especially when dealing with species "complexes." The importance of the rules for nomenclature stated at the "International Code of Nomenclature for algae, fungi and plants" is many times overlooked and the *Nomen invalidum* (i.e. *P. asparagi*, *P. hydropathica*, and *P. katsurae*) are not known in many instances. Many of the types of the species described in the early 1900's have been lost and there is a need to create neotypes or epitypes. CPHST-BL is pioneering the "Revision of the Taxonomy of *Phytophthora*" and the implementation of the "Online Identification Tools of *Phytophthora*: Lucid Key, Tabular Key and Sequencing Analysis" based on the type isolates with the intention to provide correct information for identification of species in the genus. Establishing proper nomenclature and making correct identification of species in the genus will enhance diagnostic systems significantly and will facilitate the understanding and management of the pathogens of concern.

A new multi-locus phylogeny for the genus *Phytophthora*

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The genus *Phytophthora* includes many destructive pathogens that attack a huge number of agriculturally and ecologically important plants. Investigation into its phylogeny is critical to understanding of the evolutionary history and formulating sustainable disease management strategies. The current 10-clade framework was established in 2007 based on seven nuclear genetic markers, covering approximately 80 species [1]. Thereafter, a number of new species have been isolated and named with many from forests and natural ecosystems. The total number of species in this genus now is about 130. Among the new species is *P. stricta* that does not belong to any known ITS clade [2]. In this study, seven phylogenetically-informative genes were sequenced for *P. stricta* and a vast majority of other recently-described species. These sequencing included many ex-type and authentic cultures from domestic and international Phytophthora research and extension communities. An array of phylogenetic analysis methods is being employed to develop a new phylogeny for this genus. This study helps understand the evolutionary relationships among and within groups while providing the signature sequences of known species to assist their identification in this rapidly-expanding genus.

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Poster 1

Introducing…*Phytophthora chlamydospora* (née P. taxon Pgchlamydo), *P. obrutafolium* (née P. taxon oaksoil), and P. "himalsylva-like" (née P. taxon ceanothus)

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Three new species of *Phytophthora* are illustrated, each with a morphological and phylogenetic description and taxonomic history. Available information on distribution and pathogenicity is summarized. *Phytophthora chlamydosporum*, clade 6, is common in many streams and riparian soils around the world. *P. obrutafolium,* also clade 6, is the sterile twin sister species to the homothallic *P. bilorbang.* P. "himalsylva-like", clade 2, is known as a horticultural pathogen allied with *P. citrophthora*. Perhaps it will have a proper name by meeting time.

Poster 2

Genetic, morphological and physiological characters of the plant patogen *Phytophthora cactorum* and its hybrids

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Phytophthora cactorum is homothalic species placed in Cooks *Phytophthora* group I. Its host spectrum includes more than 200 plant species growing worldwide in the temperate climatic zone. *P. cactorum* can form two hybrids: *P. × serendipita (P. cactorum × P. hedraiandra)* and *P. × pelgrandis (P. cactorum × P. nicotianae)*. Hybridization events are possible way how to extend or change the host spectrum and improve pathogenicity of *Phytophthora*. During this work *P. cactorum* and both its hybrids were examined to compare morphological, physiological and genetic differences. The isolates included in the study were isolated from 27 different host species in twelve European countries. The characters of isolates were determined using i) light microscopy for morphological characters, ii) cardinal temperatures measurement iii) the RAMS PCR fingerprinting method to determine genetic differences, iv) the sequencing analysis of selected isolates using ITS, *cox*I and *Pheca* DNA region. According to RAMS results, all isolates were divided in three main lineages, one includes exclusivelly *P. ×pelgrandis*, and the other ones grouped both *P. cactorum* and *P. ×serendipita* grouped together according to their geographical origin. Very similar results were obtained also in cardinal temperatures measurement, again without differences between *P. cactorum* and *P. serendipita*. According to morphological characters, only *P. pelgrandis* was distinguishable from the other species. The sequence analysis showed different levels of heterozygosity for ITS and *Pheca* nuclear gene regions in *P. ×serendipita* and *P. ×pelgrandis,* those regions included heterozygous sites inherited from both parent species. The sequence analysis of mitochondrial *cox* I gene revealed that in *P. ×serendipita* this locus can be inherited from either parental species, *P. cactorum* or *P. hedraiandra*. Currently we continue in identificaion of the hybrids and population structure of *P. cactorum* and its hybrids in Europe using AFLP method.

7th meeting IUFRO WORKING PARTY 7.02.09 Phytophthora in Forests & Natural Ecosystems

10th · 14th November 2014

Esquel, Chubut. Patagonia Argentina

Session 2

Role of *Phytophthora* species in emergent diseases

Pathogenicity of *Phytophthora* species isolated from declining European blackberry (*Rubus anglocandicans*) in natural ecosystems of South Western Australia

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European blackberry is a species complex within the *Rubus fruticosus* L. aggregate [1] and is one of the 20 Weeds of National Significance in Australia. Blackberry is the most widespread and abundant *Rubus* species in Western Australia (WA). A disease recorded as "blackberry decline" was first observed in some blackberry sites in WA in 2006. A disease survey was conducted in the Manjimup-Pemberton region along the Warren and Donnelly river catchments in WA between 2010 and 2012 [2]. *Phytophthora amnicola*, *P. bilorbang* [3], *P. cryptogea, P. inundata, P. litoralis, P. multivora, P.* taxon personii*, P. thermophila,* and a *P. thermophila-amnicola* hybrid were recovered from declining and adjacent decline-free sites, as well as from streams and rivers. *P. cinnamomi* was isolated from dying *Banksia* species from two non-decline sites. Of these ten species, *P. bilorbang* and *P. cryptogea* appeared to be more pathogenic than the others in underbark inoculations using excised stems (primocanes) and *in planta* primocane inoculations in blackberry growing wild in native forest stands. In glasshouse trials, *P. bilorbang* and *P. cryptogea* were both confirmed to be pathogens of blackberry, and when co-inoculated disease impact was more severe, indicating a synergistic response. It was concluded that blackberry decline is a complex syndrome and *Phytophthora* species and in particular *P. bilorbang* and *P. cryptogea* together with temporary inundation are major biotic and abiotic factors, respectively contributing to blackberry decline [2].

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Phytophthora pseudosyringae found on bilberries in Norway

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In 2012, *Phytophthora pseudosyringae* was found on bilberries (*Vaccinium myrtillus*) in a natural environment on a small peninsula in lake Farris in Vestfold county. There were two circular patches, each with a diameter of 2 to 3 meters, consisting of dead plants in the center and chlorotic plants along the edge. Damaged, but still living plants displayed a dark brown discoloring on sections of the stems with a distinct boundary towards green tissue. Stem pieces including boundaries between healthy and necrotic tissue were rinsed in tap water and plated on selective agar for *Phytophthora* (PARPH). Emerging colonies were subcultured on V8 to observe morphological features. A selected culture was identified by ITS-sequencing of the rDNA to *P. pseudosyringae.* This pathogen has not been found in association with any plant species in Norway prior to this finding, but was previously detected once by baiting with rhododendron leaves in a stream in the south-western part of the country. We do not know how the pathogen was spread to the area. Since bilberry is a very common forest plant in Norway and an important feeding plant for wild-life, we are concerned about the spread of *Phytophthora* in such areas.

The comparative pathogenicity of two *Phytophthora ramorum* lineages, EU1 and EU2, on a range of hosts

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Phytophthora ramorum, the causative agent of Sudden Oak Death (SOD), is a lethal pathogen of oak and Bay laurel in North America. However, in Europe its primary hosts include rhododendron, larch and vaccinium (bilberry). The pathogen is known to have four distinct evolutionary lineages; NA1, NA2, EU1 and EU2. The North American *P. ramorum* population is predominantly comprised of NA1 and NA2 lineages, whilst EU1 and EU2 lineages occur within Europe. EU2 is the most recent of the four lineages to be identified and is found exclusively within a relatively small area, in south-western Scotland and in the east of Northern Ireland. To understand the implications of the occurrence of this newly identified *P. ramorum* lineage, EU2, we compared the relative pathogenicity of EU1 and EU2 on a range of hosts. Wound-inoculations of the bark of 11 tree species, including Japanese, European and Hybrid Larch, sessile oak, beech and noble fir, with six EU1 and six EU2 isolates revealed that EU2 isolates generated larger lesion areas on all three larch species, compared with EU1. However, when detached rhododendron leaves were inoculated with all 12 isolates, EU1 generated larger lesions compared with EU2. This could indicate that EU2 is specifically adapted to infection and spread in larch, which has implications for the management of *P. ramorum* in areas where EU2 is present.

Involvement of *Phytophthora* species in beech decline in Lower Austria

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In Lower Austria, studies were conducted in 43 forest stands and 6 urban sites to analyse the relations between root pathogens, crown condition and stem cankers of European beech (*Fagus sylvatica*). 24 forest stands and 3 urban sites were found infested by these pathogens. Among the six species found *P. cambivora* and *P. plurivora* were most common. From one beech stand a yet undescribed *Phytophthora* taxon was isolated which is closely related to *P. quercina*. The quarantine species *P. ramorum* and *P. kernoviae* were not recorded. The symptoms caused by *Phytophthora* on beech comprise extensive fine root losses, death of large parts of the root system, lesions on above-ground parts of the roots and the stem, and as a consequence thinning and dieback of the crowns. Disease symptoms were found in all stand types and all regions of Lower Austria with presence of beech. Although fine root damage and mycorrhization are correlated to the crown thinning the whole phenomenon is not exclusively related to *Phytophthora*-infestation of the soil. The *Phytophthora*-lesions on superficial roots and stems are colonized by several secondary fungal species, which commonly cause rot and consequently a destabilisation of the trees. Among those, honey fungus (*Armillaria* sp.) and Carbon cushion (*Hypoxylon deustum*) were most frequent. Damaged trees were concentrated on sites subject to water logging and also in the vicinity of forest roads which, especially if having been paved with soil material from urban areas, most likely acted as pathway for the introduction of *Phytophthora*. Since the precipitation showed an increase in intensity and duration during the preceding years, an increase in inoculum of *Phytophthora* in the soil can be assumed reaching thresholds able to cause decline of stands; in particular if the fine root losses interact with succeeding periods of drought stress.

Phytophthora pluvialis on Douglas-fir in Oregon, USA

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Phytophthora pluvialis was originally isolated from baited soil, streams, and raintraps, and from tanoak stem lesions in Oregon as early as 2002. It was later associated with a needle cast of Douglas-fir (*Pseudotsuga menziesii*) in New Zealand. Subsequently, we began studies to explore the relationship between *P. pluvialis* and Douglas-fir in Oregon. Detached Douglas-fir twigs were inoculated with zoospores of *P. pluvialis* to measure sporulation potential. Baited raintraps were placed under stands of Douglas-fir in the southern coastal region, in the central Coast Ranges, and in the central Willamette Valley. Douglas-fir seedlings were exposed to natural inoculum in stands where raintraps yielded *P. pluvialis* in Rhododendron leaf baits. *P. pluvialis* was found to sporulate on detached Douglas-fir needles within two weeks of inoculation. We detected *P. pluvialis* in baited raintraps at two coastal sites and in one central Coast Range site during March and April of 2013 and 2014. Douglas-fir trap plants showed needles with yellow-green mottling. *P. pluvialis* was re-isolated from surface-disinfested symptomatic and asymptomatic needles, and an association with specific symptoms was not established.

Identification and pathogenicity of oomycetes causing root disease on wild olives

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From the beginning of 90'ş, wilting and death of cultivated olive trees (*Olea europaea*) caused by *Phytophthora* spp. (*P. megasperma* and *P. inundata*), are common in southern Spain. Recently, a similar root rot has been detected in a protected wild-olive woodland of high ecological value (Dehesa de Abajo, Seville, Spain). In this natural forest, two sampling trials of roots and soil were done on 25 wild-olives in spring and autumn 2013. For each sample, feeder root segments were directly plated on NARPH; and olive leaves baits were prepared for soil samples. Isolates obtained were morphologically identified and confirmed by analysis of their ITS regions. *Pythium spiculum* (48% in spring and 76% in autumn), *Phytophthora cryptogea* A1 (76% and 44%) and *P. megasperma* (0 and 4%) were consistently isolated from sampled trees.
For pathogenicity tests, isolates from each species were grown separately *(Py. spiculum* and *P. megasperma*) or in dual culture (*P. cryptogea* A1 × A2 tester) for 21 days in Petri dishes containing carrot broth. After this time, the mycelium obtained was washed, added to sterile water, shaken and adjusted to 2.2×10^4 oospores × ml^{-1}. One year-old wild olives were infected by adding to the rootball 50 ml of oospore suspensions. Ten plants (replicates) per oomycete species were inoculated and transferred to plastic pots, each one containing 2 L of fertilized peat. Uninoculated plants were exposed to the same conditions than inoculated ones. All the plants were incubated in an acclimatized greenhouse and severity of foliar symptoms was weekly evaluated on a 0-4 scale (0 = 0-10% symptomatic leaves, 4 = total wilt). Root symptoms were assessed at the end of the experiments following a similar 0-4 scale for root necrosis. All the three oomycetes were consistently reisolated from infected roots and their pathogenicity on wild-olives demonstrated. Both *Phytophthora* species resulted highly virulent, in contrast with *Pythium*.

Unexpected discovery of *Phytophthora siskiyouensis* in the UK

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Dieback of alder caused by *Phytophthora alni* was first recorded in the UK 20 years ago. It is now widespread over many river systems and is routinely isolated from symptomatic native *Alnus glutinosa*. During a recent visit to a mixed alder and ash site in the county of Dorset in south west England typical symptoms of „alder Phytophthora" were apparent on a number of the alders, including bleeding basal lesions and some crown dieback. The area was planted in the late 1990s with a mixture of ash, introduced European *Alnus incana* and oak. Alder symptoms were first noticed 2 years ago and assumed to be due to *P. alni*. However, following isolations from basal lesions in November 2013 the morphological features and ITS sequences of the isolates identified them as *Phytophthora siskiyouensis*. Subsequently *P. siskiyouensis* was obtained from alder bark, root and soil samples collected from the site. Previously *P. siskiyouensis* has been recorded from urban *A. glutinosa* in Melbourne eastern Australia, native streams and forest trees (*Umbellularia californica* and *Notholithocarpus densiflorus*) in south west Oregon and urban *A. cordata* and *A. rhombifolia* in southern California. This is apparently the first time the pathogen has been found affecting *A. incana* and the first record from Europe. It seems likely that *P. siskiyouensis* was introduced onto the site with the planting stock. Many trees are affected. Although the affected trees had been planted 15-20 years ago alder planting continued on the site until 2000. Some of the younger trees appear to have been killed by the infection, others survive with active symptoms. Work is underway using potted saplings (i) to satisfy Kochs Postulates and (ii) to compare the risk posed by *P. siskiyouensis* to some other European and North American alder species.

Mycelial growth and pathogenicity of *Phytophthora cinnamomi* Rands

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Production of Avocado (*Persea americana*), among other fruits, is considered an alternative to the establishment of agroforestry systems, such as ecosystems conservation strategy. However, incidence of *Phytophthora cinnamomi* (Oomycete) has been a constant limitation in avocado crop production worldwide, due to the limited availability of tolerant rootstocks and the high cost of clonal seedlings. In a preliminary study of seedling tolerance, seeds pollinated to colonization by *P. cinnamomi*, two isolates were tested LRS 21/88 and LRS 22/93, donated by the *Agencia Paulista de Tecnologia Agropecuaria* - APTA (Brazil), and two methods of inoculation in seedlings of the susceptible selection "Ouro verde". Both evaluated the mycelial growth of the three isolates in four culture mediums: lima bean agar (ML), carrot agar (CA), V8 (Cambell) and potato dextrose agar (PDA). The three isolates were tested in both light conditions (10 $\mu m.m^2. s^{-1}$) and in the dark. Results of pathogenicity among the isolates tested after fifteen days suggest that the LRS 21/88 was more pathogenic, developing lesions that averaged 2,33 cm^2 from an initial injury method, and averaging 0,50 cm^2 without initial injury method, prior to inoculation. Already in mycelial growth after four days of testing, an interaction was found between the test variables; the effect of the medium depends on lighting and isolation. Independent of the test factors, the ML medium, in general, induced higher development of mycelium. However, the media V8 and CA, resulted in lower observed growth and greater formation of hyphall swelling, typicall in *P. cinnamomi*. These are partial results and other studies are being developed in parallel regarding protein expression in avocado seedlings in response to *P. cinnamomi* and photosynthetic behavior of the fluorescence ratio and chlorophyll in plants inoculated.

The role of *Phytophthora* in the decline of *Corymbia calophylla* (marri), a dominant and widespread tree species in southwest Western Australia

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Corymbia calophylla (marri), a keystone tree species in the majority of woodlands and forests in the southwest of Western Australia, is suffering a major decline syndrome associated with the canker fungal pathogen *Quambalaria coyrecup.* Evidence suggests *Q. coyrecup* is endemic, however, mortality attributed to the canker pathogen has increased since the 1970s with disease incidence and severity much greater in anthropogenically disturbed areas, suggesting there are additional biotic and abiotic predisposing factors. The current study investigated the role of *Phytophthora* species in marri decline. An extensive survey was undertaken across the marri range, an area of approximately 70 000 km^2. Within this region, 62 sites were assessed for canker disease presence, and soil samples collected for *Phytophthora* detection. *Phytophthora* species were recovered from more than half the sites, with up to three species present at a single location. A total of six *Phytophthora* species, including *P. boodjera* prov. nom., *P. cinnamomi, P. cryptogea, P. elongata, P. multivora* and the previously undescribed *Phytophthora* sp. *calophyllaphile* prov. nom. (a species closely related to *P. quercina*), were isolated from the roots and rhizosphere of healthy and diseased marri. The pathogenicity of these species towards marri seedlings was tested in glasshouse experiments, with isolates of *P. cinnamomi* and *P. multivora* significantly reducing root health and mass. The results of these experiments and their implications for marri health will be discussed in detail.

Phytophthora austrocedrae emerges as a serious threat to juniper (*Juniperus communis*) in Britain

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From 2011-2013, *Phytophthora austrocedrae* was isolated from diseased *Juniperus communis* exhibiting dieback and mortality at eight geographically separate sites in Scotland and northern England. The pathogen was also confirmed present either by standard PCR and sequencing of the ITS locus or by real-time PCR on symptomatic *J. communis* at a further eleven sites in northern Britain. Out of 167 *J. communis* sampled across the nineteen sites, 154 had foliage dieback over all or part of the crown as a result of basal lesions originating in the root system and extending up the stem, killing phloem and cambial tissues. Thirteen sampled trees had aerial branch lesions or discrete stem lesions with no apparent connection to the base of the tree. At thirteen sites, dieback was concentrated in areas of poor drainage and /or alongside streams and other watercourses. In artificial inoculation experiments, *P. austrocedrae* caused rapidly extending stem and root lesions on *J. communis* and was re-isolated from these lesions. Lesions also developed on *Chamaecyparis lawsoniana* and *Chamaecyparis nootkatensis* but the pathogen was not re-isolated. All *P. austrocedrae* isolates obtained from *J. communis* in Britain shared 100% identity across the ITS locus but were distinctat one position on the alignment from *P. austrocedrae* isolates collected in Argentina from diseased *Austrocedrus chilensis*. This study provides clear evidence that *P. austrocedrae* is a primary pathogen of *J. communis* and now presents a significant threat to this species in Britain. Pathways for the emergence of *P. austrocedrae* in Britain, and the possible ways in which the pathogen may have spread within the country, will be discussed.

Evolutionary epidemiology of *Phytophthora austrocedrae* on juniper in Great Britain and the Northern Hemisphere

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The oomycete forest pathogen *Phytophthora austrocedrae* is causing large-scale dieback of *Austrocedrus chilensis* in Argentina, where it was first described in 2007. It was not known to occur anywhere else until 2011, when it was found in Great Britain causing mortality in the common juniper (*Juniperus communis*). Genetic differences between British and Argentinian isolates have been observed, making an introduction of *P. austrocedrae* from Argentina unlikely. So far, little is known about the pathogen and its ecology in the Northern Hemisphere and a three and a half year PhD project has begun in order to shed light on its evolution and epidemiology. *J. communis* populations will be surveyed in Europe and in North America, to determine the wider distribution of the pathogen within the broad boreo-temperate host range. Samples will be collected from soil and trees for isolation and molecular analysis. The ecology of *P. austrocedrae* will be studied via surveys of asymptomatic and symptomatic juniper sites with regard to environmental factors, such as climatic conditions and distribution of waterways. *In vitro* experiments will be carried out to determine the growth conditions (temperature and pH) required by *P. austrocedrae* for the completion of its life cycle. Finally, the population genetics and evolutionary history of the pathogen will be studied using microsatellite markers and phylogenetic analyses based on multiple loci (ITS, coxI, coxII among others) to elucidate the intriguing distribution of the pathogen.

Phytophthora pseudosyringae associated to the mortality of *Nothofagus* forests of central-southern Chile

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In recent years, mortality has been observed in several species of *Nothofagus* in Chile, especially in the Los Andes mountains. In 2009, partial defoliation and bleeding cankers was detected on *Nothofagus obliqua* trees located in Nahuelbuta coast ranges of southern central Chile. From stem cankers and litter samples around the symptomatic trees were collected and processed directly in CARN selective media (CMA, ampicillin, rifampicin and ampicillin) and indirectly by apple baits. The obtained isolates were identified with the use of morphological keys and the Ypt 1 DNA sequence, which permitted the identification of all the isolates as *Phytophthora pseudosyringae.* Currently, *N. obliqua* and *N. procera* plants were inoculated at the stem, using mycelial plugs and the roots, with zoospore suspension, under controlled conditions. Positive results in the inoculation assays will be able to confirm pathogenicity of the isolates on these *Nothofagus* species. Considering the low genetic variability of *P. psuedosyringae* in Europe and North America, and causing disease in *N. procera* and *N. obliqua* trees growing in gardens in the UK, its center of origin could be in South America.

Phytophthora kernoviae detection in *Drimys winteri* (Winter's Bark) forest of southern Chile

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Partial defoliation and necrotic leaves were found in winter"s bark (*Drimys winteri*) forest located in southern Chile. Soil and litter collected from symptomatic trees were baited. The isolates obtained were identified with the use of morphological and molecular techniques. Pathogenicity tests were carried out inoculating detached *D. winteri* leaves and plants using collected isolates, under controlled conditions. Morphological characteristics and the Ypt1 DNA sequence of the isolates, led to the identification of the species *Phytophthora kernoviae*. The pathogenicity of the isolates was confirmed on *D. winteri* leaves and plants, causing similar foliar symptoms observed on trees. This is the first detection of *P. kernoviae* in South American native forest.

Pathogenicity tests of *Phytophthora* species on *Agathis australis*

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Phytophthora taxon Agathis (PTA) is a devastating pathogen of iconic kauri (*Agathis australis*) in New Zealand. Soil surveys to detect PTA, targeting sites with kauri trees showing disease symptoms, detected a number of other *Phytophthora* species (Waipara et al. 2013). *P. cinnamomi*, *P. multivora* and *P. cryptogea* were particularly common. *In vitro* and glasshouse studies were carried out to determine the relative pathogenicity of these four species, prior to investigating potential interactions among these species in the field. When excised leaves were inoculated with colonized agar plugs, all four *Phytophthora* species produced lesions. Lesion advance was significantly slower with *P. cinnamomi*, *P. multivora* and *P. cryptogea* (<1 mm/day) than with PTA (>3 mm/day). Similar results were obtained with inoculated excised twigs, with average lesion advance of 0.04, 0.49, 0.17 and 4.0 mm/day for *P. cinnamomi*, *P. multivora*, *P. cryptogea* and PTA, respectively. The growth rate of PTA through live kauri twig tissue was similar to that on V8 agar. Potted 2-year-old kauri seedlings were trunk-inoculated. Small lesions (mostly <10 mm over 4 months) appeared with *Phytophthora cinnamomi*, *P. multivora* or *P. cryptogea*, no trees died, and plant growth was suppressed only slightly. When PTA-inoculated, lesions spread rapidly, trunks were girdled, and all trees died within 4-6 weeks. All kauri seedlings died within 10 weeks when soil was inoculated with PTA. Feeder root damage occurred following soil inoculation with *P. cinnamomi*, *P. multivora* or *P. cryptogea*, and the respective *Phytophthora* species were readily isolated from root lesions, but there were no plant deaths. Results suggest that PTA is a highly aggressive pathogen on kauri while relatively, the other three species are weaker pathogens.

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Aerial stem cankers associated with *Phytophthora syringae* on *Fraxinus*: how and why?

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Phytophthora syringae is known for causing foliar and twig blights of shrubs such as *Syringa* and *Kalmia* and lesions on fruits such as apple and *Pyracantha,* but not as a pathogen of forest trees. Curiously for an aerial pathogen it has non-caducous sporangia. Recently reports of dieback in young plantation European ash, *Fraxinus excelsior,* came from several sites in southern England. At one mixed plantation of <1 hectare in Dorset, over100 trees exhibited sunken bleeding aerial stem lesions, bark cracking and crown dieback. The trees were 15-20 years old with a diameter of 10-15 cm. The stem lesions were mostly 2-3 m above ground level, lenticular and several cm long. Isolations from the lesion margins readily yielded *Phytophthora syringae* as identified by morphological criteria including semi papillate non-caducous sporangia, supported by ITS sequence. At another similar site leaflet necroses were also observed and again yielded *P. syringae.* However in Dorset regular summer rainwater samples were negative for *P. syringae* in PCR testing i.e. no evidence was obtained for that canopy sporulation the source of inoculum for the stem lesions. This appears to be a new host record (Kochs postulates not yet completed) and the first record of *P. syringae* associated with aerial lesions on a forest tree. It is notable that both *Fraxinus* and *Syringa* belongs to the Oleaceae. Early descriptions of *P. syringae* on lilac emphasised its role as a foliar pathogen, with sporangia forming on the surfaces of infected leaves. Regarding *P. syringae* on ash, outstanding questions include (i) How does foliar infection occur if sporangia are non caducous? (ii) How does infection of ash stems occur? (iii) Why has *P. syringae* infection of ash not been observed previously in UK or Europe? (iv) Is this another new UK tree disease outbreak resulting from imported plants?

Multiple Phytophthoras associated with larch (*Larix)* in Britain

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Since the first findings in 2009 of *Phytophthora ramorum* killing plantation grown larch in the UK (mainly Japanese larch – *Larix kaempferi*), thousands of larch samples have come into Forest Research laboratories for diagnosis. If *P. ramorum* is confirmed, woodland owners must fell affected trees as part of the management of *P. ramorum* as a quarantine plant pathogen. Therefore, correct identification is critical. Occasionally larch samples with resinous lesions and bark cankers consistent with those of *P. ramorum* give a strong positive with a *Phytophthora* Lateral Flow Device (LFD) test (Pocket Diagnostics®) but *P. ramorum* cannot be confirmed with the real-time PCR assay developed to detect it in larch samples. This raises the possibility that other *Phytophthora* species regularly infect larch bark but have not been detected previously. This may be due in part, to the difficulty of isolating *Phytophthora* from infected larch tissue. Also, the symptoms caused by other Phytophthoras may be relatively localised and easy to overlook in mature trees. In 2010-11, attempts were made to isolate from ~200 samples of Japanese larch bark which had fresh, necrotic phloem lesions and gave *Phytophthora* positive LFD tests. Of those, 28% yielded *P. ramorum* but ~3% produced cultures of two other *Phytophthora* spp – *P. pseudosyringae* and *P. gonapodyides.* Inoculation of all three species into Japanese and European larch bark demonstrated they were able to attack and colonise healthy phloem tissue, thereby satisfying Koch"s Postulates. The lesions caused by *P. pseudosyringae* and *P. gonapodyides* were significantly smaller than those incited by *P. ramorum*. Under natural conditions the lesions caused by *P. pseudosyringae* and *P. gonapodyides* usually occur on branches at least 2-3 m above ground level and are discrete aerial infections. This raises the possibility that they also infect and sporulate on larch needles (as *P. ramorum* does) thereby providing the inoculum for the aerial bark infections.

Aerial dieback on *Thuja* caused by *Phytophthora lateralis*

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In February 2011, Scottish plant biosecurity inspectors found three potted *Thuja occidentalis* cv. „Emeraude" infected with *Phytophthora lateralis* in a nursery, originally imported from France [1]. The pathogen was isolated from the foliage; no symptoms were observed on root collar or roots. This was the first record of *P. lateralis* infecting *Thuja.* During garden surveys in January 2014, the inspectors discovered a more than 50 years old *Thuja plicata* as part of a hedge row with aerial dieback and again *P. lateralis* was isolated from the foliage and no symptoms were present on bark or root collar. Two dead semi-mature Lawson's cypress trees (*Chamaecyparis lawsoniana*) were present in the same garden and assumed to be the source of the outbreak. Both obtained isolates were tested for their pathogenicity on their respective hosts and re-isolated, completing Koch's postulates. The ITS and COXII sequences revealed that both isolates belonged to the American „Pacific Northwest" lineage of the pathogen [2]. Six different genera of conifers including nine species and 16 different cultivars were tested for their foliage susceptibility to *P. lateralis.*

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Phytophthora disease on alder (*Alnus spp.*) in Norway

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In 2012, conspicuous dark, bleeding cankers were observed on trunks of dying trees in natural stands of grey alder (*Alnus incana*) along the shore of lake Årungen in Akershus county in Norway. *Phytophthora alni*, which has been killing alder in Europe during the past two decades, was suspected to cause the disease. Work was initiated to identify the causal agent and assess the damage. A survey in the alder population around the lake, revealed that out of approximately 6000 examined trees, nearly 200 showed typical symptoms on the trunk. At the sites where the damage was most severe, 20 % of the trees showed symptoms. Samples for isolation of potential pathogens were collected from the leading edge of canker wounds. The samples were rinsed in tap water and small pieces were plated on selective medium for *Phytophthora* (PARPH). After a few days at room temperature, colonies emerged from the tissue samples. Subcultures on V8 agar were identified to *P. alni* ssp. *uniformis* by PCR using three primer pairs to identify the subspecies. This is the first time *P. alni* has been found in Norway. The same subspecies was also isolated from symptomatic grey and black alder (*A. glutinosa*) by a pond located approximately 1 km from the lake. Symptoms observed on alder at other locations indicate that the disease may be widespread in south-eastern Norway. By spring 2014, we do not know if there are other subspecies of *P. alni* present in Norway, but a survey of *Phytophthora* on alder will take place in a larger region later this year to investigate distribution and the possible presence of other *P. alni* subspecies.

First report of *Phytophthora sp.* on *Epipremnum aureum* in Mexico

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The state of Michoacan has an important nursery industry that is actively growing. Wilting plants of *Epipremnum aureum* (commonly known as golden pothos) were observed in nurseries located in Michoacan, Mexico. Diseased tissue was plated out on NARPH selective media. The oomycete *Phytophthora* was consistently isolated. Morphology, mating type, sensitivity to mefenoxam, growth at 35°C and sequences of ITS, 60S and Cox I and II, were determined in one isolate. Sporangia were spherical, broadly ellipsoid or obovoid with one papilla (occasionally two papillae), and deciduous with a long pedicel. The isolates were heterothallic, and oogonia with amphigynous antheridia were observed in pairings with A1 and A2 isolates of *P. capsici*. The isolate was A2, sensitive to mefenoxam and grew at 35°C. The ITS and 60S sequences showed 99% and 100% similarity with *P. capsici* respectively. Cox II sequence showed 100% similiraty with *P. tropicalis* therefore, it appears to be intermediate between these species. No amplification was obtained with the Cox I primers. Pathogenicity tests were carried out with 90 days old rooted plants, placed in glass containers with 40 mL of distilled sterile water. The plants were inoculated with a suspension of 10,000 zoospores per mL and maintained at 25°C in the laboratory. Symptoms were observed after 7 days with 100% mortality at 10 days. The isolate was none virulent on inoculated pepper plants in tests performed in a greenhouse. To our knowledge, this is the first report of *Phytophthora* sp. on *Epipremnum aureum* in Mexico.

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Phytophthora in Forests & Natural Ecosystems

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Session 3

Tools for *Phytophthora* surveys

Whither the species? *Phytophthora* taxa, MOTUs and barcodes in the world of metagenomics

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The study of ecosystem diversity is being transformed by high throughput sequencing technology that allows an unparalleled depth of sampling of DNA barcode sequences. The opportunities are great at this fascinating interface of pathology, ecology, taxonomy and molecular biology but there are pitfalls. It is important to consider the potential for bias at all steps in the process from sampling through to data analysis. Increasingly *Phytophthora* diversity is being examined by metabarcoding of the PCR-amplified rDNA ITS regions from soil, water or plant sample DNA. We have applied baiting and isolation, cloning and Sanger sequencing and Illumina MiSeq analysis to a time-series of filtered water samples from several Scottish streams within a UK-wide sampling network. These data, the literature and other presentations in this session will be explored in a critical analysis of the field. A specific focus will be placed on exploring the range of species and their boundaries, the potential for species quantification and possible benefits of the technology for plant health legislation.

Fishing for *Phytophthora* 2.0

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Isolation of *Phytophthora* species from water sources is common and worldwide known. Usually, the most common methods used are baiting *in situ* and filtering of water. Both methods are successful for the isolation of aquatic species included in clade 6 like *P. gonapodyides*, *P. megasperma*, *P.* taxon *PgChlamydo* or *P. lacustris.* However, only a few species from other clades are isolated using these methods due to the relative low inoculum available. Therefore, a study was performed to compare the number of *Phytophthora* species isolated using baits and the number of *Phytophthora* species detected using pyrosequencing technology. A total of 16 forest streams from northern Spain were selected for the study. Water samples were filtered *in situ* and the filters were used for isolation and for pyrosequencing studies. Additionally, baits (rhododendron leaves and carnations petals) were placed in the same streams and collected after one week. Isolation from baits were performed using selective media and amplicon pyrosequencing was performed from each sample. Results compared data among culturing of *Phytophthora* species from filters and baits, and those species detected by pyrosequencing.

Exploring hidden *Phytophthora* via amplicon Pyrosequencing using eDNA from soil and water

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Phytophthora is one of the most important and aggressive plant pathogens in agriculture and forestry. Early detection and identification of its pathways are of high importance to minimize the threat that they pose to natural ecosystems. Therefore, a new improved method for its detection in environmental samples is proposed. eDNA was extracted from soil and water from rivers and streams from *Fagus sylvatica* and *Abies alba* forests, and *Chamaecyparis lawsoniana* and *Pseudotsuga menziesii* plantations in the north of Spain (Irati Forest and Villanua). A *Phytophthora*-specific amplicon pyrosequening based on the barcoding target ITS1 was applied. Different score coverage threshold values were tested for optimal *Phytophthora* species separation. Clustering at 99 % was the best criteria to separate most of the *Phytophthora* species. Of the total of 37 *Phytophthora* species detected in the environmental samples, 24 were known to science (*P. lacustris*, *P. gonapodyides*, *P. syringae*, *P. hedraiandra*, *P. cambivora*, *P*. taxon *PgChlamydo*, *P. alni* subsp. *uniformis*, *P. cactorum*, *P. pseudosyringae*, *P. porri*, *P. gallica*, *P. asparagi*, *P. megasperma*, *P. cryptogea*, *P. gregata/gibbosa*, *P. europaea*, *P. lactucae*, *P. niederhauserii*, *P. drechsleri*, *P. inundata*, *P. psychrophila*, *P. plurivora*, *P. trifolii* and *P. quercina*) and 14 were unknown to science. Thirteen of the unknown species were detected in rivers and streams revealing that water environments could represent important pathways and a potential source for pathogen discovery. Pyrosequencing of soil samples revealed low *Phytophthora* diversity (14 species) in comparison with the 35 species detected in water samples, representing the 95 % of the total *Phytophthora* community. Water eDNA pyrosequencing proved to be a valuable method for the detection of *Phytophthora* species in natural ecosystems.

Next Generation Sequencing reveals unexplored *Phytophthora* diversity in Australian soils

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The Vegetation Health Survey (VHS) at the Department of the Environment, Western Australia has a *Phytophthora* collection extending back to 1979. Isolates in this collection have been recovered during routine monitoring on natural ecosystems in Western Australia for the presence of *Phytophthora cinnamomi.* Through molecular re-evaluation of this collection we have subsequently described 11 new Phytophthora species and the diseases associated with them and additional descriptions are underway. Elsewhere in Australia, however, there is extremely limited information on *Phytophthora* diversity within natural ecosystems. Using modern molecular techniques such as Next Generation Sequencing, it is possible to determine *Phytophthora* species diversity from environmental soil samples. In this study, DNA was extracted from soils obtained from 700 locations around Australia. ITS1 amplicons were generated using *Phytophthora* specific primers (Scibetta et al. 2012) adapted for NGS by Santi Català and sequenced on a Roche Junior GS platform. For 50 samples roots and rhizosphere soil were extracted separately. Results reveal an astonishing diversity, several new species and very different species profiles when comparing roots and rhizosphere soil from the same location. Species described and known only from Western Australia have an Australia-wide distribution raising intriguing questions in regards to origin and movement of species.

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Molecular Tools for the PCR detection of *Phytophthora austrocedri*

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Phytophthora austrocedri (*Phytophthora austrocedrae*, orthographic variant) was described in 2007 in Argentina causing dieback on Chilean incense cedar in Patagonia. The pathogen was found in 2010 in UK causing a serious decline of native juniper and in Scotland in 2011 on Lawson"s cypress and Nootka cypress. Recent surveys of juniper woodland in northern England and Scotland conducted by CFCC since November are showing that the pathogen is pretty widespread. There is indication that the pathogen was present in Germany around 2001 according to Julius Kühn-Institute Datasheet for the pathogen. Further concern for this pathogen is due to its potential to be disseminated through shipments of contaminated nursery stock. *P. austrocedri* is ranked # 21 in the list of 29 *Phytophthora* spp. of concern for the USA published by CPHST- Plant Epidemiology and Risk Analysis in 2009. Due to the importance of the pathogen denoted by the recent discoveries and its inclusion in the Cooperative Agricultural Pest Survey List (2014), we consider *P. austrocedri* as a priority for USDA regulatory programs. In order to facilitate the efforts in pest detection of *P. austrocedri,* a multiplex conventional PCR was developed using species-specific primers for the 60S Ribosomal protein L10 60SL10_for/60S gene (L10) and paired with the plant gene NADH dehydrogenase subunit 5 mRNA (nad5) as the internal control. In addition a real-time PCR assay that targets the Internal Transcribed Spacer rDNA region (ITS) published by the CFCC in Scotland was validated and paired with the cytochrome oxidase gene (COX) to detect host plant DNA in a multiplex assay. Both methods have been tested with DNA from cultures and environmental samples from the UK provided by the CFCC. In addition, both methods are under evaluation for cross-reactivity with other *Phytophthora* species in clade 8d, as well as other Oomycetes and fungal pathogens.

Tools for rapid characterization of *Phytophthora infestans* and *Phytophthora ramorum* using real-time PCR and microsatellites from genomic resources

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Oomycete pathogens such as *Phytophthora infestans* (Mont.) de Bary and *Phytophthora ramorum* Werres De Cock & Man in't Veld cause a devastating impacts worldwide. More DNA-based tools are needed to identify and characterize these species, where some genotypes/lineages may be more problematic than others. For example, some *P. infestans* strains are more resistant to fungicides and have preferred hosts. Mining the full genome sequences of these two organisms allowed development of markers for intraspecific genotyping. Our first objective was the development of Allele Specific Oligonucleotide-PCR (ASO-PCR) assays using real-time PCR to differentiate Canadian strains of *P. infestans* and the four lineages of *P. ramorum* (NA1, NA2, EU1 and EU2). The *P. infestans* genome revealed several regions containing SNPs within genes and in flanking sequences of microsatellite loci. Nine ASO-PCR assays were developed from these SNPs, allowing the unambiguous identification of the five dominant *P. infestans* Canadian genotypes from the most recent outbreaks. Two new ASO-PCR assays were developed in a gene coding for cellulose binding elicitor lectin (CBEL). Combined with two existing assays within the same gene region, it is now possible to identify all four lineages of *P. ramorum*, including the recently discovered EU2 lineage. Our second objective was to develop microsatellite markers to evaluate *P. ramorum* genetic diversity mostly within the NA2 lineage, where fewer markers are currently available. Analysis of the genome of the NA2 *P. ramorum* lineage revealed microsatellite loci that reveal polymorphism within this lineage. Previous markers were biased toward NA1 and did not reveal the level of polymorphism discovered using these new microsatellites. These DNA-based tools will contribute to the available genomic toolbox to assess the genetic diversity of these oomycete pathogens, from the species to the intra-lineage level.

Poster 3

Detection of *Phytophthora* species on different woody species in nurseries

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Among the diseases affecting plants in nurseries, root rots are one of the most serious, especially those caused by *Phytophthora* species. Since this class of pathogens are difficult to isolate, and they may remain alive in the soil for long time before causing any symptom, the availability of an early detection tool would be crucial to reduce the risk of spread of these pathogens. Aim of this work was to develop a real time PCR assay to detect and quantify *Phytophthora* spp. from plant, water and soil samples collected in nurseries in Tuscany (Italy). The sensitivity and specificity of an assay based on real time PCR make possible the detection of small quantities of *Phytophthora* DNA in soil and plant samples before symptoms occurrence. Potted plants, soil and water samples were collected from nurseries. Isolation on selective media and DNA extraction were performed from leaves, roots and soil of both symptomatic and asymptomatic plant samples. A genus-specific Taqman probe was designed and tested by qPCR assay to quantify *Phytophthora* DNA in samples. After isolation on selective medium, about 40% of the plant samples resulted positive to *Phytophthora* spp., while *Phytophthora* was not isolated from water and compost. The Taqman MGB probe was much more efficient and revealed the presence of *Phytophthora* in about 85% of the samples analyzed. Also water and compost samples resulted positive to *Phytophthora* after qPCR. Eight species of *Phytophthora* were isolated, which were characterised by different frequency and host specificity. Host plants differed for the number and set of *Phytophthora* species that could colonize them. Consistent results about host susceptibility were obtained by classical isolation and qPCR assay. The quantity of DNA of *Phytophthora* did not differ between symptomatic and asymptomatic plant samples, although the frequency of *Phytophthora* species could slightly differ between classes.

Poster 4

Determining an optimal sequence identity threshold value for *Phytophthora* spp. retrieval from environmental data

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Generation of molecular data from environmental samples via DNA or amplicon massive sequencing becomes an easy and fast process in genomics and metagenomics analysis. The increasing number of new technologies allow the easy data generation, but bioinformatics still representing a critical step and a bottleneck in metagenomics analysis. One of the key steps in the data analysis for species identification purposes is the clustering of the reads based on their identity. Clustering parameters will define the species community composition and its reliability by reducing the risk of creating false MOTUs (Molecular Operational Taxonomic Units). An equimolecular mix composed with the DNA of eight pure cultures of *Phytophthora* species was used as control, and pyrosequenced using a nested PCR for library generation. Different identity threshold values were tested for MOTU clustering with 6.698 sequences obtained from the DNA mixture and with a custom-curated database including 146 ITS1 sequences of described and new *Phytophthora* taxa. Clustering at 100% of the reference sequence database, or at 99.5%, separated the higher number of *Phytophthora* species. However, applying this barcoding threshold to control data generated an exponential increase of MOTUs (mainly composed by singletons) due to the presence of sequencing and homopolymer errors. Furthermore it was not possible to separate some species (10%) in the custom-curated database using 100% of score coverage threshold (ITS1 taxonomic limitations). The cut-off value of 99% was the lower value able to separate all the species in the mix. Applying a cut-off value of 99% guarantee an optimal species separation and reduce the risk of false MOTUs, with minimum loss of data.

Poster 5

Development of new Real-Time specific assays for the detection of *Phytophthora* species in Holm Oak calcareous forests

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Oak decline in non-calcareous soils in south-western Spain has been associated with *Phytophthora cinnamomi* for decades. However, other *Phytophthora* species such as *P. quercina* and *P. psychrophila* have been associated with *Quercus* decline in the eastern part of Spain where calcareous soils are predominant. With the aim of investigating the involvement of *Phytophthora* spp. in oak decline in eastern Spain, two forests in different geographical areas (Alcoi and Vallivana) were selected as sampling sites. Both forests are similar in altitude, soil and vegetation composition and are located 230 km apart. Soil samples were analyzed in parallel by isolation using baiting methods and by amplicon massive sequencing. Results showed that one of the most frequent species detected in both sampling sites was *P. quercina*, although cultures were only obtained from Alcoi"s holm oak forest. Pyrosequencing showed a very similar *Phytophthora* species composition in both areas. Furthermore, an uncultured *Phytophthora* taxa (named provisionally *Phytophthora* taxon *ballota*) was the dominant species, followed by *P. quercina*. Considering the difficulty in the isolation of *Phytophthora* taxon *ballota,* new Real-Time specific assays based in the ITS1 region were developed for the detection of this new taxa and *P. quercina* in environmental samples from oak declined areas. Taqman assays were tested on soil samples and on *Phytophthora* pure cultures. Results revealed the coexistence of both species in most of the samples, with the predominance of *P.* taxon *ballota* in terms of the amount of DNA available. Quantitation assays were high congruent with pyrosequencing results (relative number of reads per species). In order to evaluate the implication of different *Phytophthora* spp. in oak decline in eastern-Spain a new Real-Time specific detection protocol is proposed.

Poster 6

Evaluation of Illumina MiSeq as a new tool for the detection of *Phytophthora* species

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Next-generation sequencing (NGS) applied to metagenomic offers the opportunity to obviate most of the limitations of biological detection. Recent papers have been published on the application of pyrosequencing assay to detect and identify fungal and oomycetes communities in forest ecosystems. Different NGS platforms have been developed which are available for the metagenomic analysis of natural communities of microrganisms. The 454 GS-FLX amplicon pyrosequencing method has been employed successfully for describing Phytophthoras in environmental chestnut soil samples (Vannini *et al*., 2013). The MiSeq (Illumina) platform based on the existing Solexa, present the highest throughput per run and the lowest error rates compared with 454 GS (Loman et al., 2012). Moreover the Miseq workflow has the fewest manual steps as template amplification is done directly on the instrument without manual intervention; consequently the simplicity of workflow and the running cost are lower. However, the Miseq delivered shorter read lengths than the 454 GS (Loman et al., 2012). The aim of the work was to evaluate the accuracy of MiSeq in describing a *Pythiaceae* community. A laboratory experiment was designed with a mixture of DNA from various *Pythiaceae* simulating a community of species in an environmental sample. The precision in terms of detection and identification of taxa, and the risks of false Molecular Operational Taxonomic Units will be discussed. Furthermore natural soil samples have been collected where the *Phytiaceae* community will be described by mean of biological detection (baiting used as gold) compared to MiSeq analysis.

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Poster 7

Metagenomic analysis of *Phytophthora* diversity in nurseries of potted ornamental species

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The genetic diversity of *Phytophthora* was investigated in soil and root samples of potted ornamental and fruit tree plants collected in nurseries located in Apulia and Calabria (Southern Italy) using metagenomic approaches based on *Phytophthora* genus specific primers. PCR amplicons containing the ITS1 region of the rDNA were sequenced using both a conventional cloning and Sanger sequencing approach and a 454 pyrosequencing protocol. All sequences were accurately analyzed with an appropriate software and used as barcode for species identification utilizing a validated ITS database. The cloning/Sanger sequencing approach enabled the identification of nine different *Phytophthora taxa* (*P. nicotianae, P. citrophthora, P. meadii, P. cinnamomi, P. parvispora, P. cambivora, P. niederhauserii, P. taxon Pgchlamido,* and *P. lateralis*), 3 phylotypes associated to "species complexes" (*P. citricola*, *P. cryptogea* and *P. pseudosyringae*) and three other phylotypes considered as unknown or non well identified *Phytophthora taxa*. The 454 pyrosequencing confirmed above results and provided a higher levels of accuracy enabling the detection of four additional species (*P. cactorum*, *P. psycrophila*, *P. palmivora* and *P. ramorum*) and a general higher level of diversity (number of detected genotypes) within analyzed samples. Data of the present study indicate the use of genus specific primers combined with next generation sequencing approaches as valuable tools to investigate *Phytophthora* diversity in different environments and pathosystems. Furthermore, the large number of genotypes and *Phytophthora taxa* detected in a limited geographic area confirms a primary role of nurseries in favoring the diffusion and the evolution of *Phytophthora* species.

Poster 8

Citizen Science Helps Predict Risk of Emerging Infectious Disease

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Citizen science holds great potential for advancing spatial prediction of biological invasions by providing inexpensive location-based, time series data of unprecedented quantity and distribution. In 2008, we developed a citizen science program to detect the spread of the emerging forest disease Sudden Oak Death (SOD) across the metropolitan region of the San Francisco Bay Area in California, including under-sampled habitat within urban areas and along the wildland-urban interface. Each year, our "SOD Blitz" program used crowdsourcing methods to encourage citizens to collect leaf tissue symptomatic of this disease and submit it to our lab for molecular diagnosis. Results are made public through the internet each year, they are added to the disease distribution database known as SODmap (available also through the App SODmap mobile) and they helped scientists identify critical elements correlated to disease spread, generating the strongest predictive model yet known.

Poster 9

The detection and quantification of four *Phytophthora* species in soil in the UK

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A number of newly described *Phytophthora* species have been discovered in the UK over the past decade infecting a wide range of host species. Little is known about the role of soil in the epidemiology of these pathogens including the role of humans and animals in the spread of soil both within and between infected sites. We describe methods for the detection and quantification of four *Phytophthora* species in soil; *Phytophthora ramorum*, *P. kernoviae*, *P. lateralis* and *P. austrocedrae*. These methods will lead to a better understanding of *Phytophthora* disease spread and inoculum persistence in soil.

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Session 4

Surveys and new records

Diversity and impact of *Phytophthora* spp. in natural ecosystems of Taiwan

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In spring and autumn 2013 a survey of Phytophthora diversity was performed in 22 natural forest stands and 25 rivers in subtropical and tropical regions of Taiwan with altitudes ranging from 6 to 2287 m asl. In total 144 soil samples were taken from the rhizosphere of 40 tree species. Using leaves of *Q. variabilis*, *C. indica*, *Citrus sinensis* and other species as baits 12 known species, four designated taxa and 17 unknown species of *Phytophthora* have been isolated: *P. cinnamomi* (Pc, 4 haplotypes), *P. citrophthora, P. cryptogea, P. europaea, P. heveae, P. katsurae, P. palmivora, P. parvispora, P. plurivora*, *P.* t. „PgChlamydo", 3 new species related to *P. botryosa*, 2 new species related to *P. meadii*, and 6 new species from ITS Clade 7a from 97 soil samples (67.4%) of 33 tree species (82.5%); *P. capensis, P. cinnamomi, P. citrophthora, P. insolita, P. parvispora, P. tropicalis, P.* t. „PgChlamydo", *P.* t. „Ceanothus", *P.* t. „insolita-like", *P.* t. „Kunnunara", *P.* t. „Kunnunara-like", *P.* t. „Peru 4", *P.* t. „Peru 4-like", *P.* t. „forestsoil-like", and another 2 new Clade 9 species from 20 rivers (80%). Most Phytophthora species were not associated with disease symptoms. The A1 mating type of Pc was widespread in most mountain and lowland forests and was rarely associated with disease symptoms. In contrast, the distribution of the A2 mating type was much more limited and always associated with often severe decline of different forest types. In one declining rainforest A1, A2, homothallic A2 and sterile Pc isolates were found. It is concluded that the A1 mating type is native to Taiwan while the A2 mating type is a recently introduced invasive pathogen, indicating that probably in the pleistocene the A1 and A2 mating types got geographically separated.

Phytophthora spp. invasions in European post-communist economies – the example of the Czech Republic

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Phytophthora spp. belong among the most important pathogens of ornamental and forest woody plants in Europe. Many of them are considered to be alien or cryptogenic. The number of their introductions depends on the globalization and level of imports of goods. That´s why Central- and East European economies were partially protected from invasions before collapse of Eastern block. The diversity and distribution of *Phytophthora* spp. pathogenic to woody plants in the Czech Republic support this claim, because up to one half of *Phytophthora* spp. described in the area could be probably introduced after coup d´état in 1989. The intensive investigation of *Phytophthora* spp. diversity in different environments (from ornamental nurseries, gardening centres to forest stands) started in 2006. Hundreds of *Phytophthora* isolates were collected, determined, preserved and their distribution was analysed. There were found 21 taxa belonging to *Phytophthora*. Only 4 taxa are probably native to Europe (19 %: *P. gallica*, *Phytophthora lacustris*, *P*. taxon oaksoil, *P. polonica*). The other 17 taxa (81 %) are probably alien or cryptogenic. The distribution analysis of them shows that 6 taxa (29 %) are more or less regularly distributed in natural stands of the area, thus their introductions are probably of older date (*P. plurivora*, *Pau*, *P. gonapodyides*, *P. cambivora*) or their natural spread is extraordinarily effective (*Paa*, *P. multivora*). The 11 other species (52 %) are regularly distributed in anthropogenic environments and only some of them are distributed very occasionally in highly invasible riparian stands. Moreover, the distribution of highly pathogenic *P. cinnamomi*, *P. citrophthora*, *P. cryptogea*, *P. palmivora* and *P. ramorum* is limited only to ornamental nurseries, gardening centres and ornamental plantings.

Survey of Oomycetes found in western Washington streams

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Information on what Oomycete pathogens are present in a waterway is of interest to growers of horticultural, forest, and food crops, especially if a quarantine organism such as *Phytophthora ramorum* is found. Since 2003 *P. ramorum* has been detected in over 50 ornamental plant nurseries in Washington State. Stream monitoring by state agencies has resulted in the detection of this exotic pathogen in nine streams, three ditches, and two rivers outside of nurseries since 2006. In all cases, streams have tested positive for *P. ramorum* in subsequent years after the first detection. Stream monitoring using baiting and culturing methods designed to detect *P. ramorum* was carried out in spring 2011 in the Puget Sound region of western Washington. Ten streams representing a variety of habitats were sampled for six two-week baiting intervals. Much of the sample collection and isolation of Oomycetes was done by volunteers and students as part of an outreach program. Oomycetes were isolated and characterized by morphological methods and by DNA sequence analysis of the ITS region of the rDNA. 276 isolates of *Phytophthora, Pythium, Halophytophthora*, and other Oomycete genera were examined. Some putative new species of *Phytophthora* and *Pythium* were identified for further study. Several Oomycetes having a worldwide distribution were found in Washington streams. The ecology and importance of these species with relation to *P. ramorum* is discussed. In addition to providing preliminary information about the Oomycete species present in western Washington streams, this study was an opportunity to educate the public about the importance of these organisms and their effects on ecosystems. This was done by enlisting the aid of students and volunteers from the community.

Diversity of *Phytophthora* species in forests, forest nurseries and riparian ecosystems of Portugal

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In Portugal, the involvement of *Phytophthora cinnamomi* in the decline of *Quercus ilex* and *Q. suber* is notorious but the dimension of the risk posed by other *Phytophthora* spp. to both natural ecosystems and forest nurseries is unknown. In the framework of the European BiodivERsA Project RESIPATH (Responses of European Forests and Society to Invasive Pathogens) a national-wide, two-years *Phytophthora* survey in natural ecosystems and forest nurseries in Portugal is being conducted in close cooperation with the Instituto da Conservação da Natureza e das Florestas (national authority responsible to propose, monitor and ensure the implementation of policies on nature conservation and forestry). This survey aims to study the diversity of both known and as yet unknown *Phytophthora* species in Portugal and clarify whether they are endemic or of exotic origin. For potentially non-native *Phytophthora* species morphological and physiological studies and pathogenicity to common European tree species will be tested to evaluate their invasive potential. In addition, in cooperation with the Direção Geral de Alimentação e Veterinária (national authority responsible for the design, implementation and evaluation of policies for plant protection and plant health), freshly arrived consignments of plants-for-planting from overseas will be tested for *Phytophthora* infestations at ports of entry to verify this potentially important pathway into Europe. First results will be presented and discussed in terms of the actual or potential threat to the natural biodiversity of the Portuguese ecosystems.

Diversity of *Phytophthora* species in the oak forests of Southwest China

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Phytophthora species are best known as destructive pathogens of agricultural crops or invasive pathogens destroying forests and natural ecosystems. Prior to 2005, little was known about the presence of *Phytophthora* in forest in China. From 2005 to 2011, surveys of *Phytophthora* spp. in the oak forests of southwest China were conducted to obtain an overview of the species that inhabit these forests. Twelve stands plus 12 associated streams in four regions or were surveyed. A total of 421 isolates of *Phytophthora* spp. were recovered from baited leaves using standard baiting techniques, and 11 taxa including eight known species (*P. borealis*, *P. cryptogea, P. gonapodyides*, *P. gregata*, *P. lacustris*, *P. megasperma*, *P. plurivora*, *P. syringae*), the known but as yet unnamed *P.* taxon PgChlamydo, and two previously unrecognized species, *Phytophthora* sp.1 and *P.* sp.2., were identified based on morphological features and ITS and *cox* I sequence data. *Phytophthora* species residing in ITS Clade 6 were most abundant overall, but only *P.* taxon PgChlamydo were found in all four regions. The abundant *P. gonapodyides* and *P. borealis* were present in three regions, and the three rare speices, *P. gregata*, *P. lacustris* and *P. megasperma* were recovered only in one region. Phylogenetic analysis revealed that two novel heterothallic species can be distinguished. The new taxa appear to have limited distribution and definite pathogenicity. *P. syringae* was isolated for the first time from stream water and soil in two regions. The occurrence of *Phytophthora* species in forests in southwest China and their pathogenicity toward some plants highlights once again the urgent need to investigate the potential risk and impact of both native and previously unknown introduced forest Phytophthoras.

Phytophthora species associated to Holm oak decline in western Spain

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The oak-rangeland „dehesa" ecosystem plays an important economic, ecological and social role in south-western Europe. The decline of cork and holm oak trees and the absence of natural regeneration are the main concern of this valuable ecosystem. The decline syndrome can develop in a few months or several years and it has been explained by several concomitant factors of abiotic and biotic nature. Among these factors, *Phytophthora cinnamomi* is considered the main cause of decline because its aggressive behaviour on the feeder roots of the oaks. However, its presence has not been always confirmed in the affected stands. To overcome traditional *Phytophthora* isolation difficulties, the aim of this work was to apply molecular methods to study *Phytophthora* diversity. Surveys were conducted in Extremadura region (western of Spain) in five adult holm oak stands and thirteen regeneration zones. In each sampling site, soil and root samples were collected from declining and non-declining holm oaks (*Quercus ilex*). The study compares three different approaches: (i) traditional isolation methods consisting of roots in contact with selective media, and soil isolation using apples and leaf baits, (ii) 454-pyrosequencing analysis of root and soil samples with tagged amplicons specific for *Phytophthora*, and (iii) TaqMan real-time PCR of root and soil samples using a *P. cinnamomi* specific probe.

Multiple new and invasive alien *Phytophthora* taxa from Mediterranean maquis ecosystems in Italy

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The Mediterranean basin is recognized as a global biodiversity hotspot accounting for more than 25,000 plant species that represent almost 10% of the world's vascular flora. In particular, the maquis vegetation on Mediterranean islands and islets constitutes an important resource of the Mediterranean plant diversity due to its high rate of endemism accounting for 4.3% of all plant species worldwide. Since 2009, a severe and widespread dieback and mortality of *Quercus ilex* trees and several other plant species of the Mediterranean maquis has been observed in the National Park of La Maddalena archipelago (northeast Sardinia, Italy). Infected plants showed severe decline symptoms and a significant reduction of natural regeneration. First studies revealed the involvement of the highly invasive *Phytophthora cinnamomi* and several other fungal pathogens. Subsequent detailed research led to a better understanding of these epidemic showing that the aetiology is more complex than initially assumed and that multiple other *Phytophthora* spp. are also involved, some of them unknown to science. A total of 13 *Phytophthora* species were isolated from roots and soil samples collected from symptomatic trees and shrubs such as *Arbutus unedo*, *Asparagus albus*, *Juniperus phoenicea*, *J. oxycedrus*, *Pistacia lentiscus* and *Q. ilex*. Based on morphological characters, growth–temperature relations and sequence analysis of the ITS and *cox*1 gene regions, the isolates were identified as: *P. asparagi*, *P. bilorbang*, *P. cinnamomi*, *P. cryptogea*, *P. gonapodyides*, *P. melonis*, *P. nicotianae*, *P. parvispora*, *P. psychrophila*, *P. quercina*, *P. syringae* and two informally designated taxa, *P. aplerotica* prov. nom. and *P. ornamentata* prov. nom., both within ITS Clade 6. Studies are currently underway to formally describe the new species in conjunction with large scale pathogenicity trials to confirm Koch's postulates for the new host/*Phytophthora* associations.

Phytophthora detections in native plant nurseries and restorations sites in California

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Phytophthora tentaculata was recovered from sticky monkey flower (*Mimulus aurantiacus*) at a California native plant nursery in 2012, which was the first detection of *P. tentaculata* in the USA (Rooney-Latham and Blomquist 2014). *Phytophthora tentaculata* is listed as a threat to nurseries and forests in United States federal New Pest Response Guidelines (APHIS 2010). In the first half of 2014, the pathogen was detected in both sticky monkey flower and coffeeberry (*Frangula californica*) at additional native plant nurseries in different counties that have no reported connection to the nursery with the initial detection. In addition, *P. tentaculata* was recovered from outplanted toyon (*Heteromeles arbutifolia)* and sticky monkey flower at native plant restoration sites. In the latter species, *P. tentaculata* was recovered from declining plants growing at the site for over a year. The detection of *P. tentaculata* and *P. cactorum* in nursery stock planted at restoration sites in Alameda County prompted additional investigations into both symptomatic and asymptomatic plant material at several source nurseries and multiple recently-planted restoration sites. Numerous *Phytophthora* species were recovered, some from rushes and sedges, plant species typically not considered to be *Phytophthora* hosts. While some of the *Phytophthora* species detected are common in California in nurseries and cultivated landscapes (e.g., *P. cactorum, P. cambivora, P. cryptogea, P. megasperma*) other species are relatively uncommon or not previously documented in California (e.g., *P. tentaculata, P. quercetorum, P. inundata, P. plurivora*). The wide diversity of *Phytophthora* species recovered from plants grown at these native plant nurseries and their relative abundance raises concern that native plant nurseries serve as a source of *Phytophthora* introductions in restoration sites in California. These recent *Phytophthora* detections will be examined as a case study of the potential for the spread of *Phytophthora* species in native plant nurseries and restoration plantings.

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Phytophthora - an emerging threat to plantation forestry in Vietnam

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The impact of diseases caused by a number of *Phytophthora* species has been well documented in Vietnam but the focus until now has been exclusively on horticultural plants and some annual crops, including pepper, fruit trees, taro and potato. In 2012, *Phytophthora* was isolated for the first time from the rhizosphere soil of severely declining *Acacia mangium* plantations in Tuyen Quang province. Initial isolates were identified as *P. cinnamomi* and these isolates were shown to cause root rot and stem lesions in *A. mangium* seedlings. Since then, a program was initiated to assess whether *Phytophthora* was present and causing damage in plantations, hedge orchards and nurseries. So far, a range of highly pathogenic *Phytophthora* and *Phytopythium* isolates have been obtained. *Phytophthora cinnamomi, P. parvispora* and a new species most closely related to *P. elongata* were the most frequently isolated species. The distribution and potential threat from *Phytophthora* spp. to plantations and other forest species in Vietnam will be discussed. Management strategies to manage disease outbreaks and to reduce the spread of *Phytophthora* will be considered.

Poster 10

Phytophthora species in forest streams in Nyingchi, Tibet Autonomous Region and Ganzi, Sichuan Province, China

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Phytophthora species were surveyed by placing bait leaves in selected streams at six sites during June to October in the year 2006, 2010 and 2011 in rhododendron-oak forests in south-east Tibet and west Sichuan Province, China. A total of 202 isolates of *Phytophthora* spp. were recovered from 120 baited leaf samples. Five *Phytophthora* species were identified by observation of morphological features and ITS1-5.8S-ITS2 rDNA sequence analysis. The five taxa included one well-known species *P. gonapodyides*, three recently described species *P. borealis*, *P. lacustris* and *P. plurivora*, and one named but as yet undescribed taxa, *P.* taxon PgChlamydo. The most numerous species, *P. gonapodyides*, the second most abundant species, *P. borealis*, and the third most numerous species, *P.* taxon PgChlamydo, were all recovered at four sites, while the other two species were found only at the same one site in Sichuan Province. Phylogenetic analysis showed that the isolates belonged to two ITS clades, one species including 17 isolates in clade 2 and four species including 185 isolates in clade 6. The relatively rich of *Phytophthora* species and genetic diversity in the species based on ITS gene were examined, and interpreted in light of the various environments from which they were isolated.

Poster 11

Distribution and impact of *Phytophthora* species on alder (*Alnus* spp.) in Southern Sweden

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Invasive pathogens of the genus *Phytophthora* threaten forest ecosystems globally. One of the most significant examples is the damage associated to the hybrid *Phytophthora alni,* an alien pathogen infecting and causing dieback of alders *(Alnus* spp*)*. In Sweden, damages attributed to alder *Phytophthora* were first identified in the 90s, but since then, scarce research has been carried out to determine the extent of the spread and damages caused by this pathogen. This study aimed to determine the distribution and impact of *Phytophthora* species infecting alders (*Alnus glutinosa* and *Alnus incana*) in Sweden. A systematic survey was performed on the sixteen major river systems of southern Sweden and *Phytophthora* colonies were isolated from alder tissue. The recovered isolates were typed according to morphological features and using molecular markers. In each plot, an estimation of the damages was obtained by measuring defoliation, dieback or chrorosis symptoms on ten dominant trees. *Phytophthora*-infected alders were recorded in 28% of the 176 assessed plots. We isolated two subspecies of *P. alni: P. alni* subsp. *uniformis* (Pau) and *P. alni* subsp. *alni* (Paa). In addition, *Phytopthora plurivora* were also isolated from alder tissue. In total, 115 *Phytophthora* isolates were recovered. Estimation of damages showed that the percentage of trees with healthy crowns in plots with *P. alni* presence was lower than in plots without the pathogen. *Phytophthora alni* appeared to be widespread in southern Sweden and preliminary results showed that the distribution of both subspecies seems to be correlated with winter temperature. Pau was present across the whole range of temperatures of the host while Paa was located in areas with milder winter temperatures. The presence of both *P. alni* and *P. plurivora* in Southern Sweden could threaten other riverbank ecosystems thus further monitoring is required.

Poster 12

Discovering *Phytophthora* species in the laurel forest in Tenerife and La Gomera islands (Canary Islands, Spain)

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A survey in the laurel forest was performed in three different sites in Tenerife (Anaga Rural Park, Corona Forestal Park and Teno Rural Park) and in La Gomera (Garajonay National Park) during January 2013. Water and soil samples were collected and DNA was extracted using E.Z.N.A. and Zymo DNA kits respectively. Amplicon library generation was performed using a nested PCR with *Phytophthora*-specific primers. A total of 100.095 sequences were obtained from a single pyrosequencing run and used to assess *Phytophthora* species diversity. In total, 24 *Phytophthora* species were detected, 22 species (*P. gonapodyides*, *P. megasperma*, *P*. taxon *PgChlamydo*, *P. gregata*, *P.* taxon *oaksoil*, *P. lacustris*, *P*. taxon *walnut*, *P. asparagi*, *P. cryptogea*, *P. drechsleri*, *P. syringae*, *P. plurivora*, *P. multivora*, *P. quercetorum*, *P. cactorum* and *Phytophthora* sp1, sp2, sp3, sp4, sp5, sp6 and sp7) were found in Tenerife and 11 species (*P. gonapodyides*, *P. megasperma*, *P.* taxon *PgChlamydo*, *P*. taxon *oaksoil*, *P. hydropathica*, *P. europea*, *P. quercetorum* and *Phytophthora* sp2, sp5, sp6 and sp7) were detected in La Gomera. Seven of these *Phytophthora* species are new to science. *Phytophthora* taxon *walnut* was detected in a water sample from Tenerife (Anaga Rural Park), with a total of 65 sequences, which could represent a new world location of this species. Fifteen of the species identified by pyrosequencing are also commonly detected in mainland Spain. This study showed that Macaronesian Islands could be considered as a hotspot for genus *Phytophthora* research due to the isolation and species evolution with their hosts.

Poster 13

Invasive pathogens in Austrian forests: preliminary planning within the European project "Responses of European forests and society to invasive pathogens (RESIPATH)"

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The ongoing European project "Responses of European forests and society to invasive pathogens (RESIPATH)" within the BiodivERsa network includes the collaboration of 14 countries and comprises five work packages: (WP1) Long term sustainability of tree species affected by invasive pathogens and framework for impact assessment; (WP2) Understanding the mechanisms involved in adaptation of forest tree populations to new pathogens; (WP3) Mechanisms of hybridisation in Europe; (WP4) Detection and early warning of fungal and oomycete pathogens and WP5: Public perception on impact of invasive pathogens. The Austrian project part aims to study the impact of both host and pathogen population demographics and evolution, to develop detection systems and to better understand the public perception. The Austrian project part will focus on the following pathogens that threaten these tree species: *Ophiostoma novo-ulmi* in elms (*Ulmus* spp.); *Chalara fraxinea* in ash (*Fraxinus* spp.); *Phytophthora alni* in alder (*Alnus* spp.) and *Erysiphe alphitoides* in oak (*Quercus* spp). In WP1 the long-term sustainability of ash and alder populations affected by invasive pathogens will be assessed by calculating the base-rate mortality. In WP2 it will be tested whether the timing of mortality (affecting seedlings versus mature trees) has a crucial influence on the capacity of tree populations to adapt to new invaders. It is also intended to provide a validated early detection system of potentially harmful and invasive pathogens within WP4. In WP5 it is aimed to evaluate the inter-correlations between impact of invasive pathogens, mass media coverage and public awareness. Preliminary results will be shown.

Oomycetes survey in Northern Norway

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In 2012, we started a survey of oomycetes in natural habits in Norway as part of a national biodiversity project. We sampled in two areas north of the Arctic Circle in continental Norway (Finnmark and Nordland counties) and in the Svalbard archipelago. We collected mostly mosses in Svalbard and grass and mosses in continental Norway. Furthermore, we baited with grass and rhododendron leaves in streams, lakes and brackish water in Finnmark and Nordland. The isolates obtained were identified by ITS sequencing. Preliminary results show that most of the species recovered belong to in the genus *Pythium*, but *Phytophthora,Halophytophthora* and *Saprolegnia* were also found. In Svalbard *Pythium polare* is the most common *Pythium* species distributed across a wide area of the largest island, Spitsbergen [1]. In Finnmark we detected *P. volutum, P. undulatum, P. pyrilobum*, *P. monospermun*, *P. intermedium*, *P. attrantheridium*, *Phytophthora gonapodyides*, *Ph. lacustris, Saprolegnia parasitica*, *S. ferax, S. hypogina* and *S. australis.* In Nordland we isolated *P. anandrum*, *P. pachycaule*, *P. dissimile, P. intermedium, P. angustatum* and *Ph. gonapodyides.* All identifications were based on ITS sequence similarity of 99% or more to isolates in GenBank. In addition, we discovered several isolates that did not match previously described species, from all three locations in our survey.

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Phytophthora survey in a beech forest in Norway

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In 2011, *Phytophthora* canker was observed in a beech tree (*Fagus sylvatica*) forest in the city of Larvik in Vestfold county. During 2012, trees with a circumference above 20 cm were surveyed for *Phytophthora* symptoms. A total of 49 beech trees had *Phytophthora* symptoms. Samples from the leading edge on selected diseased trees where collected and isolations were carried out on an artificial *Phytophthora* specific media (PARPH). To search for *Phytophthora* spp. in the water in the forest, rhododendron leaves were used as bait in open ditches. Isolates were identified by DNA analysis (ITS sequencing of the rDNA).Two localities had denser concentration of diseased trees than the rest of the forest, with16 of 329 trees (4,9 %) and 12 of 680 trees (1,8 %) showing *Phytophthora* symptoms. The remaining diseased trees were mainly situated close to frequently used paths. DNA analysis of isolates resulted in *P. cambivora* from examined trees and *P. plurivora and P. gonapodyides* from ditch water. Both *P. cambivora* and *P. plurivora* are well known pathogens on beech in European forests.

Poster 16

Maps of *Austrocedrus chilensis* forests affected by dieback

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Forests of the monotypic native conifer *Austrocedrus chilensis* are affected with the dieback caused by *Phytophthora austrocedrae*. However, no regional maps were available, even when these are fundamental to understand the dynamics of the disease. Therefore, we collected information of the spatial distribution of affected populations under the jurisdiction of the National Parks Administration. Using existing maps of *Austrocedrus chilensis* distribution (1:250.000, INTA-APN, 2005) information collected in the field was set in a grid of 2x2 km (according to geographical coordinates) indicating areas with or without symptoms of dieback, or with mortality of *Austrocedrus chilensis*, in the four National Parks where the species is present: Lanin, Nahuel Huapi, Lago Puelo and Los Alerces. Sectors with proven pathogen presence are also shown (tests by CIEFAP laboratory). To gather information from such a vast area we involved key informants (rangers, researchers, technicians, etc.) by developing a method to standardize the sampling and further monitoring. The presence of dieback signs is discontinuous and isolated, ranging from a few individuals to large clumps. In Lanin NP the percentage of forest area with dieback was *ca*. 20%, while in Nahuel Huapi NP was *ca*. 57%, being eastern forests (lower rainfall) less affected. Lago Puelo NP had the lowest percentage of affected forest (11%), however, most of the species distribution is outside the Park and not included in this survey. The higher percentage of affected forest area was found in Los Alerces NP, where *ca*. 87% of the forest showed symptoms of decay or large clumps of dead trees. As the methodology induces to overestimate percentages, further monitoring, with more detail, is required.
This work provided a regional sight of the dieback presence and allowed establishing bases for regional monitoring and diagnosis, which helps to make decisions related to the conservation and management of *Austrocedrus chilensis.*

Poster 17

Alternatives for detection of *Phytophthora cinnamomi* in commercial substrate

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Different materials can be used as bait for diagnosis of the presence of pathogens in different environments such as water, soil and plant tissue. Specifically, to *Phytophthora sp*., several sources have been referenced, including leaf explants of *Camellia Japonica*, *Eucalyptus cinerea, Rhododendron catawbiense, Citrus lemon*, rose petals, fruits, cotyledons, and others. In Brazil, for commercial production of certified fruit plants, substrate among other things, should be free of pathogens. In order to test alternative for baiting of *Phytophthora cinnamomi* 5 materials as bait (carrot, avocado leaves, red rose petals, champagne rose petals and cellophane) were compared under light conditions (35 ± µm.m2 . s-1) and in the dark at constant temperature (± 21 ° C) room for 48 h. In a completely randomized design, the unit with ten replicates consisted of a plastic container with 10 g of substrate previously inoculated with *P. cinnamomi* (LRS 21/88 donated by *Agencia Paulista de Tecnología Agropecuaria* - Brazil - APTA) immersed in 30 mL of deionized water stelilized and a fragment of about 1 cm^2 of each material used as bait. This evaluation of the effectiveness of bait to capture the oomycete was taken from a scale (0, 1-5, 6-10, 11-15, 16 sporangia). As a result, the factors tested, both as light as in the dark there was formation of sporangia. In relation to materials used as bait, in cellophane did not find the presence of sporangia, however, the rose petals was observed increased amount of sporangia and avocado leaves sporangia observed in lower density. In the case of carrot that, unlike other materials did not remain on the water surface, there were more elongated sporangiophores. These are partial results and other studies are being developed in parallel with the expression of related proteins in plants and seeds of avocado for their photosynthetic behavior of chlorophyll and chlorophyll content of plants inoculated with *P. cinnamomi.*

Species of *Phytophthora* on rhododendrons in Argentina

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Since 2011 affected plants growing in gardens and nurseries near Buenos Aires have been surveyed. Samples of plant tissue with typical oomycetes disease symptoms have been collected and examined. On rhododendrons two *Phytophthora* species were consistently isolated from symptom-bearing leaf tissues and roots on PARBH medium. Sporangia were produced abundantly in non-sterile soil extract. In Isolation 1, most of the sporangia were semi-papillate and ovoid, limoniform, ellipsoid or obpyriform; chlamydospores were not observed. Isolates were homothallic with plerotic oospores, 22.9 ± 1.9 µm and paragynous antheridia. The optimum growth temperature was 25 ± 1°C on V8A and the maximum growth temperature was 32 ± 1°C. The ITS1 and the *B-Tubulin* genes were amplified. Isolation 2: sporangia without papilla, ovoid, ellipsoidal, obpyriform, terminal, 45-25 µm, internal proliferation. The isolate produced spherical, terminal or intercalary chlamydospores, in clusters on short side stalks. This isolate was heterothallic (no sexual structures were formed). Cardinal temperatures were 5 (24-28) > 35 °C. The ITS gene was amplified too. The rDNA sequences obtained from both isolates were compared with sequences deposited at the GeneBank, using the Basic Local Alignment Search Tool (BLAST) program. Both sequences from Isolation 1 proved to be identical to *Phytophthora multivora* ex-type CBS 124.094 (FJ237517). The sequence from Isolation 2 was identified as *Phytophthora cinnamomi,* showed 99,74% homology with NFJ801806, culture type. These species were determined on the basis of their morphological, cultural and molecular characteristics. *Azalea, Viburnum tinus* and *Photinia fraseri* are *Phytophthora multivora* potential hosts because they were infected on artificial inoculations but were not found during this survey on natural infections.

Poster 19

Status of the genus *Phytophthora* in Argentina

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The genus *Phytophthora* includes important pathogens affecting a wide range of hosts causing severe losses on the crops. The knowledge of these species allows a better management of the diseases. In the past century Frezzi studied and summarized the information about *Phytophthora* in Argentina. Since then no update of this matter was performed. In order to have a more comprehensive vision of this genus, a review and an updated report of recent progress in this matter were carried out. Information was taken from printed and on line resources. As a result, the information was analyzed and categorized, thus updating the number of species of *Phytophthora*, its geographical distribution, the affected hosts and symptoms, percentage of host-pathogen relation, distribution maps, citing the first report on each localities and the first references of molecular studies. According to this searching the first species reported by Spegazzini was *Phytophthora infestans* (Mont.) de Bary in 1901, infecting *Solanum tuberosum* in Buenos Aires province. Until 1977, 17 species were identified. Since then, between 2005 -2012, 5 species of were found affecting important crops, nursery crops and trees growing in natural ecosystems. To date 22 *Phytophthora* spp have been reported on 222 hosts-pathogen relations and 5 species were isolated from soil or water. *P citrophthora* and *P nicotianae* are the species that affect the greatest number of hosts. The main species affecting tress are *P. cinnamomi* and *P. citrophthora*. The most recently identified species were *P. austrocedrae* associated with *Austrocedrus chilensis* mortality in Patagonia; *P. lacustris* on *Pyrus communis* as a postharvest fruit pathogen in Northern Patagonia, *P.* taxon *kelmania* on *Gerbera jamesonii* and *P. multivora* on *Rhododendron* sp. in surroundings Buenos Aires city. The review provides information that allows interpreting more clearly the current and future status of investigations concerning the genus *Phytophthora* in Argentina.

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Phytophthora in Forests & Natural Ecosystems

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Esquel, Chubut. Patagonia Argentina

Session 5

Biology & Genetics

Phenotypic, genotypic, genetic, genomic, analyses of plant pathogens and their application in plant pathology

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Time, reproductive isolation, drift and adaptation are the main forces shaping all living species including Phytophthoras. It is well understood that individual histories at the long-term scale shape species diversity, however even short-term histories may have evolutionary implications. In fact, it has been recently discovered that different hosts with their different chemical environments may trigger substantial and permanent changes in the structure of genomes leading to phenotypic changes. The broader concept of phenotypic differentiation includes traits such as virulence levels, mating type, growth rates at different temperatures, sporulation and transmission rates and thus it is key to the understanding of diseases. In this session, a variety of papers address the issue of diversity emphasizing that genetically and phenotypically different groups, even if morphologically undistinguishable, may each represent substantially different threat. The distribution of distinct groups may also provide insights on the spread pathways of microbes, and possibly help us identify their area of origin. Bayesian theory and alternative model testing now provide a much more robust analytical approach to understand spread patterns both at the geographic and at the topographical level. Population genetics help us understand the reproductive mode, the evolutionary potential, and the migration potential of microbes whose life stories are for obvious reasons hard to monitor by direct observation. Genetic epidemiology, based on repeated population genetics analyses in time has surfaced as the best tool to reconstruct the actual epidemiology of a disease. Equally powerful is the range of -omic approaches through which we may understand how pathogens respond to defense mechanisms of the host, to changing climate and to new environments, and to control strategies. In my talk I will cover the studies presented using genetic markers to identify diversity within species, and the experiments aimed at defining the phenotypes that may be associated with diverse groups. I will then present a synthesis of genetic epidemiology studies performed in California, and finally will briefly introduce a couple of transcriptomic and genomic projects with great potential.

Patterns and processes of emergence in the genus *Phytophthora*

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Phytophthora pathogens continue to emerge and reemerge globally. The recent availability of whole genome sequences for *P. sojae, P. ramorum* and *P. infestans* as well as several draft genomes of other taxa have provided novel tools for inferring the evolutionary history and dissecting processes involved in emergence of new linages, strains or species of *Phytophthora*. This talk will assess what we have learned about the genetic and evolutionary mechanisms that explain patterns of emergence. What is the importance of effectors such as RxLR and crinkler genes? Which processes such as migration mutation, gene flow, hybridization, and recombination are important in shaping patterns observed for the genus? What have we learned from extensively studied model pathogens such as *P. ramorum* and *P. infestans* relative to other *Phytophthora* species? Many questions remain unresolved but the recent population genetic and genomic tools have already provided novel insights into specific processes involved in emergence. All of these approaches are helpful in developing informed disease management systems.

Visualisation of early infection by *Phytophthora* "taxon Agathis" in the roots of 2-year old kauri *Agathis australis* plants

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Phytophthora "taxon Agathis" (PTA) is the causal agent of a root- and collar-rot of kauri *Agathis australis* in the northern forests of New Zealand. The host range is restricted to kauri, and it is considered that early infection is facilitated though the fine roots. We aimed to document the infection biology of PTA into the roots of 2-year old kauri plants over a 20 day time course, using light microscopy of plant material that had been cleared (using potassium hydroxide) and stained (Trypan Blue in 1:1:1 lacto-glycerol solution). The light micrography was validated by SEM of replicated sub-samples. Presence of PTA as the causal agent was confirmed through the complementary use of PTA-specific FISH assay. Haustoria-like organs were produced by 5 days in cortical cells of fine roots. As the infection progressed (20 d.a.i), the haustoria also developed revealing a thickened haustorial matrix. Survival propagules (oospores, thin-walled chlamydospores, and stromata) were observed in these artificially infected plants. Intra-cellular hyphae appeared to be encased in lignotubers as, "digitate", protuberances within the cortical cells. PTA chlamydospores and stromata have not been observed before in pure axenic culture. The ability of the pathogen to grow as an endophytic biotroph is putatively indicated by the presence of haustoria. Oospores (produced during the necrophytic-stage) could play a part in the persistence of the pathogen, residing in infected, and degrading, root tissues.

Phytophthora ramorum: Study of the lineage EU2 / EU1 in Ireland

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Phytophthora ramorum is a very pathogenic Oomycete which has caused a great impact in the ecosystem and economy in the last two decades. It was first reported affecting larch in the UK in 2003. This pathogen can infect a wide range of hosts including larch, beech and rhododendron. Four different lineages have been described to date: NA1 and NA2 in North America and EU1 and EU2 in Europe. The aim of this project is to study the Irish population of *P. ramorum*, focusing in the lineage EU2, which seems to be very specifically located in Northern Ireland and the South-West coast of Scotland. Over 300 isolates were studied using RFLP and microsatellite markers (SSR). The result of the study showed that the majority of the Northern Irish population is EU2 (89%), EU1 was observed in small areas where infected plants had been imported from other nurseries. The population of the Republic of Ireland was 100% EU1, as in the rest of Europe. The results of the SSR analyses were processed with software specialized in Population Genetics, resulting in Phylogenetic trees that group the population according the genetic variation.

Basic and applied research into *Phytophthora ramorum* in Ireland: the PHYTOFOR project

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Phytophthora ramorum is an invasive pathogen affecting woody plant and tree species in the wild in Ireland and Britain. In the Republic of Ireland (ROI), only the EU1 lineage of *P. ramorum* is present, while Northern Ireland (NI) has both the EU1 and EU2 lineages present. As part of the PHYTOFOR project, we have carried out experiments to phenotypically characterize the Irish *P. ramorum* populations in respect to those world-wide (including all four lineages: EU1, EU2, NA1, NA2). Radial growth rate and also *in-vitro* pathogenicity on detached Rhododendron leaves at five temperatures have been investigated. Host range susceptibility trials on 7 tree species are under investigation. Significant differences have been found in the radial growth rate of all four lineages of *P. ramorum* across five tested temperatures. There was no difference between EU1 and EU2 in terms of their pathogenicity on detached Rhododendron leaves at 20°C. Tests of the phenotypic lineage discrimination method according to Franceschini et al. (2013) were also carried out, and this information was compared with the molecular lineage determination. Studies on the *in-vitro* fungicide sensitivity and on mating type characterization are underway. The PHYTOFOR project also investigates the survival and spread of the disease in previously infected, and since felled, forest stands in Ireland. Monthly plot visits since August 2013 to rain water traps, bait plants, water baits and soil samples have found pathogen presence in three of the six plots. One of the plots still has standing Japanese larch trees, and this plot has had consistently positive rain water traps in September, October and November 2013. Almost all of the positive samples came from rain water traps, with infected beech (non-transmissive host) also found positive at one plot.

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Lineage, phenotype and environment factors influencing the *Phytophthora ramorum* epidemic on larch

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Between 2003 and 2008 in woodlands in the UK, *Phytophthora ramorum* mainly affected understorey rhododendron. The number of reported tree infections remained low at <100 and largely comprised native beech (*Fagus sylvatica*) and non-native oak species such as *Quercus cerris*. In 2009, *P. ramorum* was unexpectedly found to have spread to plantation grown Japanese larch (*Larix kaempferi*), causing increasingly heavy mortality, and endangering other plant and trees species due to the prolific sporulation on infected larch needles. Rain trap data indicates that with naturally infected larch, sporulation levels peak in October just before and during needle loss, although lab data suggest that sporulation may also occur on larch foliage in spring and summer. Between 2010 and the end of 2013, the combined area of affected larch in England, Scotland and Wales had risen from 2,000 ha to more than 10,000 ha. Many millions of trees have been felled to curtail sporulation and limit pathogen spread. Heavy crown symptoms in 2013 have been partly ascribed to the cool, wet summer and autumn conditions of 2012 considered conducive to sporulation and dispersal of *P. ramorum*. The behaviour of the recently characterised EU2 lineage may also account for exceptionally enhanced disease levels in Scotland, as it is a much more effective coloniser of Japanese larch bark than the EU1 lineage. However, there may be fitness trade-offs between growth rates, sporulation potential and the ability to colonise bark. This is under investigation. It also appears that the more aggressive colonising ability of the EU2, compared with the EU1, may be specific to larch and not replicated in other hosts. Currently, evidence suggests that the ranges of the EU1 and EU2 lineages do not overlap but they are rapidly converging in south west Scotland. This raises the likelihood of mixed lineage populations of *P. ramorum* affecting larch forests with further potential consequences.

Investigation of the tree pathogen, *Phytophthora lateralis*, newly discovered in Northern Ireland

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Phytophthora lateralis is genetically most closely related to *Phytophthora ramorum*, however, it has a limited host range, lethally infecting Lawson cypress [Port-Orford-cedar, *Chamaecyparis lawsoniana* (A. Murr.) Parl]. *P. lateralis* has been prevalent in Pacific North-Western USA since the 1920s, but was undetected in Europe until 1996 when it was isolated from Lawson cypress in France. *P. lateralis* was first detected in the UK, in Scotland in 2011 and was subsequently found in Northern Ireland later in the same year. The pathogen is invasive and spreads by the dissemination of motile zoospores in waterways and by the movement of infested soil and plant material. Characterisation of isolates from Northern Ireland has revealed a largely clonal population. Nevertheless, genetic sequencing of three isolates from two geographically distinct sites has revealed single nucleotide polymorphisms (SNPs), with fewer SNPs present in isolates from the same site. Subtle differences in phenotype, such as sporulation capacity, have also been observed. It is hoped that this information will provide some indication of the extent of new introductions of the pathogen into Northern Ireland and may also enhance the understanding of the epidemiology of emerging plant pathogens within the British Isles.

Poster 20

Molecular and morphological data shows two consistent lineages in *Phytophthora plurivora* strains isolated from streams in northern Spain

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Phytophthora plurivora, included in the *P. citricola* complex, was recently described as new species based on its morphology, physiological and molecular characters. Recently, isolated strains from natural waterways in northern Spain showed a high morphological colony variability in pure cultures at PDA medium. The isolates were subjected to temperature assays, morphological studies and phylogenetic analysis based on five different molecular markers: ITS, HSP90, Elongation Factor 1-α and the mitochondrial genes COX II and NADH. The result of these assays revealed differences between isolates of *P. plurivora*. Temperature assays showed two distinct patterns of growth at the optimal temperature. Statistical analyses of sporangia and oogonia measurements showed significant differences (p-value 0.5 and 0.1). Phylogenetic analysis based on Maximum Likelihood showed two consistent lineages, which corresponded with the same groups obtained in the temperature assays, revealing *P. plurivora* as a possible species complex.

Poster 21

Phytophthora ramorum: differences in the gene expression during infection in the lineages EU1/EU2

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Phytophthora ramorum is an exotic fungus-like organism that has been affecting a wide range of woody hosts since it was first reported in North America in 1993 and in Europe in 2001. There are four lineages of *P. ramorum*: NA1, NA2, EU1 and EU2. EU2 seems to be unique as it is only located in Northern Ireland and the South-West coast of Scotland. Japanese and European larch trees were inoculated with two different isolates of the pathogen, which had different pathogenicity as shown in an artificially inoculated trial, and RNA was extracted from the lesson. The transcriptome was sequenced in order to observe the differences in the expression of the genes involved in the process of infection. Differences in pathogenicity were observed between EU1 and EU2 at a gene expression level.

Poster 22

Comparative fitness of European lineages of *Phytophthora ramorum*

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Until recently the population structure of *Phytophthora ramorum* was known to consist of three largely clonal evolutionary lineages, with only the EU1 present in the UK and wider Europe. However, a fourth evolutionary lineage of *P. ramorum*, the EU2, was discovered in 2012 and can be distinguished from the other lineages (EU1, NA1 and NA2) both genetically and phenotypically. The EU1 and EU2 are both present in the UK, although the EU1 is much more widespread. Both cause mortality to plantation grown larch (mainly Japanese larch – *Larix kaempferi* which accounts for 6% of all forest cover in Britain) and this is now suffering heavy disease levels as a result of *P. ramorum*. To understand if the EU2 could pose an increased threat to forests, and in particular to larch, the ability of both European lineages to attack bark (phloem) tissue of mature larch (*L. kaempferi* and *L. decidua*), oak (*Quercus robur*) and beech (*Fagus sylvatica*) was evaluated. In addition, the susceptibility of Japanese larch and European larch saplings was tested against EU1 and EU2 isolates at two incubation temperatures, 10^{o}C and 20^{o}C. Out of the four tree species, the ranking of bark susceptibility (from most to least) was Japanese larch> European larch> beech>oak, but on average EU2 isolates produced markedly larger lesions in the bark of Japanese larch and European larch compared with EU1 isolates. With sapling material, the same pattern emerged of increased susceptibility of Japanese larch to the EU2 lineage at 20^{o}C, although it was striking that even 10^{o}C both lineages were capable of causing significant damage to both larch species after just 7 days incubation. With rhododendron foliage however, there were no consistent differences in the amount of necrosis caused by the two lineages, suggesting that the increased threat posed by the EU2 may be unique to larch.

Poster 23

Genetic variation in *Phytophthora lateralis* lineages by analysis of microsatellite profiles

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Phytophthora lateralis is one of the most destructive of introduced Phytophthoras. First isolated from roots and collar of dying ornamental cedars (*Chamaecyparis* spp.) in North America, it has been recently discovered in Europe. In 2007 *P. lateralis* were recovered from soil beneath an old growth *Chamaecyparis obtuse* cloud forest in Taiwan in the absence of visible host symptoms: Taiwan probably lay within the natural range of this pathogen (Brasier *et al.*, 2010). During an expedition in Taiwan in 2010 a larger number of isolates were collected from necrotic foliage and soil samples (Webber *et al.,* 2012). The comparison of phenotypic and genotypic traits of Taiwan population with provenances from Pacific Northwest (PNW), France, United Kingdom, Netherlands and Northern Ireland evidenced for four phenotypically and genotypically distinct lineages in *P. lateralis* (Brasier *et al.,* 2012). Furthermore, sequencing of five polymorphic loci among representative isolates demonstrated that these populations were well-supported phylogenetic units. To strengthen the phylogenetic data this study aims to analyze microsatellite (SSR) profiles of representative isolates belonging to the lineages defined in previous studies. A total of 29 primers pairs, including newly developed in this work, have been tested. The clustering of *P. lateralis* isolates analyzed based on the only polymorphic primer pairs is described and discussed.

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Poster 24

Genotypic variability of *Phytophthora cinnamomi* mating type A1 in native forests of Taiwan

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Phytophthora cinnamomi is an important plant pathogen with a wide host range and worldwide distribution. Its presence in the native forests of geographical areas distant from each other poses doubts about the real center of origin of this species, and there are studies that lead us to collocate the origin in some Asian regions, especially Papua New Guinea. In this area, in fact, *P. cinnamomi* is located in the natural environment without specific impact to native flora and showing high genetic variability in the population of the dominant mating type A1. The island of Taiwan has been indicated as an alternative center of origin of this species. *P. cinnamomi* is also widely present in the natural forests of the island mainly with the A1 mating type and without visible impact to native vegetation. No data are however available on the genotypic diversity of the resident populations. In this study, isolates of *P. cinnamomi* identified between 2008 and 2010 in two forest areas of the island of Taiwan (Chilan and TaipingSan) were compared with the Australian population A1 in terms of morphological, molecular, and of the optimum growth, in order to assess the genetic and phenotypic variability. The presence of microsatellite markers (SSR) was examined using 12 pairs of primers, 4 of which developed within this work. Among tested primers, 4 primer pairs were polymorphic and used to examine the level of genotypic diversity. Isolates from TaipinShan and Australia showed a similar genetic structure, confirming the possibility of the introduction of *P. cinnamomi* in Australia from Taiwan. The mitochondrial loci at a DNA sequence level have been also evaluated as marker for their ability to differentiate mitochondrial haplotypes of *P. cinnamomi*. Comparison of genetic and biometric data of the micro and macro structures is reported.

Biology of *Phytophthora* species in aquatic ecosystems

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Phytophthora species were added to the list of water moulds back in 1944 [1]. Over the past 70 years, a total of approximately 60 species have been detected from natural waterways and agricultural irrigation systems [2]. However, little is known about their biology in aquatic ecosystems [3]. This is a major gap in our knowledge about this important genus and its members. To fill this knowledge gap, we have conducted three lines of studies: 1. Determining the diversity and population dynamics of *Phytophthora* species along water path in an irrigation reservoir with irrigation runoff entrance in one side while exit and pump house locating in the opposite side. 2. Continuously monitoring water quality including temperature, pH, dissolved oxygen, oxidation-reduction potential, electrical conductivity, salinity, total dissolved solids, turbidity, and chlorophyll *a* in the same reservoir. 3. Assessing zoosporic responses to pH, electrical conductivity and dissolved oxygen stresses of selected *Phytophthora* species in a simulated aquatic system. The data from these studies do not support the conventional wisdom that all *Phytophthora* species are water moulds. Some *Phytophthora* species may be a resident of aquatic ecosystems, while others may be merely a transient or even terrestrial. These data are crucial to developing sustainable management strategies for Phytophthora pathogens in agricultural water reservoirs and crop health locally and plant biosecurity globally. They also help understand the diversity and population dynamics of these species in natural aquatic ecosystems where water quality does not fluctuate as dramatically and frequently as in agricultural reservoirs.

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Session 6

Ecology

Landscape heterogeneity and features are associated to the impact of Ink disease in chestnut orchards in Italy

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Spread of forest Phytophthoras diseases has been frequently associated to landscape heterogeneity and presence of natural water drainages and forest roads. Aim of the present study was to investigate the interaction between the nets of forest roads and natural water drainages with the spread of ink disease over a large chestnut area in Central Italy. To achieve such objective, remote sensing techniques and GIS applications have been integrated. Presence of ink disease foci have been highlighted through the visual interpretation of high resolution spectral images. A confusion matrix has been elaborated to validate the data; accuracy in ink disease foci identification was 86.1%, while no specific decline was identified with 98.1% accuracy. Informative layers for roads and natural water drainages have been overlapped with the ink disease foci map. A significant association have been found between the presence of ink disease foci and roads (Spearman $r = 0.69$ $P \leq 0{,}0001$), and water drainages density (Spearman $r = 0.63$ $P < 0{,}0001$). Number of intersections between roads and water drainages was also significantly associated to the presence of infection foci (Pearson $r = 0.68$ $P \leq 0{,}0001$). These results support the results of studies carried out in smaller areas in Central Italy and are in accordance with other experiences considering other forest Phytophthoras epidemics.

Ecology and pathology of *Phytophthora nemorosa, P. pseudosyringae,* and other ITS clade 3 species in forests in western Oregon

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We explore the population structure, pathology, and epidemiology of a group of closely related species: *Phytophthora nemorosa, P. pseudosyringae, P. pluvialis, P. psychrophila*, and to a lesser extent *P. ilicis*. All are in ITS clade 3. These species form a tight phylogenetic cluster and are readily separated from each other by small but consistent differences in ITS and COXI sequences. All are homothallic. They have slow to moderate growth at cool to moderate temperatures. All produce more or less caducous semi-papillate sporangia. They are pathogenic, with apparent canopy infection. The species appear to have distinct host "preferences" despite holding several hosts in common. They appear to be reproductively isolated even though their host ranges overlap and they are sympatric in Oregon. The wide distribution of *P. nemorosa, P. pseudosyringe,* and *P. pluvialis* in western Oregon, their scattered and generally low incidence, and their relatively non-aggressive pathogenic behavior suggest that they are indigenous here. The species diversity of *Phytophthora* in coastal Oregon and California is striking. Thirty or so species have been tallied, in part the result of the concentration of "phytophtherologists" in the region and the extensive sampling that has taken place, but for ecological and geographic reasons as well.

Maternal effects mediate the resistance of *Quercus ilex* to *Phytophthora cinnamomi*

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The resistance of trees to diseases is increasingly recognised as being impacted by maternal effects, given that environmental conditions experienced by parent (mother) trees affect stress tolerance in offspring. We hypothesised that environmental maternal effects may mediate the resistance of *Quercus ilex* seedlings to *Phytophthora cinnamomi*. In December 2010, acorns from 15 declining *Q. ilex* trees infected with *P. cinnamomi* and from 15 non-declining trees free of infection were collected and weighted (40 acorns per tree). Pots (0.3 l vol) containing peat and sand were used and distributed in a randomized block design (N = 1200). In September 2011 seedlings were inoculated with *P. cinnamomi*. Acorns from non-declining trees were heavier than acorns from declining trees (4.4 and 4.2 g, respectively), and germination rates and dates did not differ significantly between acorns from declining and non-declining trees. Mortality rates, however, were lower and slower in seedlings from declining-infected trees in comparison to seedlings from healthy trees. The more resistant seedlings to *P. cinnamomi* were those collected from the more declining stand. Results indicate a transgenerational induction of resistance, possibly through epigenetic mechanisms non-dependant to acorn weight.

Long term impact of *Phytophthora alni* on an alder riparian stand

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The evolution of riparian alder stands was monitored for 12 years on the Sarre river en eastern France between 2 villages (about 3000 alders on 4km of river). The recruitment of new seedlings, crown status, mortality and diameter growth of the trees were recorder annually. In 2010, the possibility of natural selection for resistance to P. alni in the stands was investigated. 39 healthy alders were cloned for further characterization, half of them present in 2002 as seedlings and having been under *P. alni* selective pressure since and half of them just recruited in the study in 2010. Ramets of these genotypes were inoculated with *P. alni* in 2013 in greenhouse conditions. The disease was very active in the stands with very high seedlings mortality rate, but much more progressive decline for larger trees. However, despite that, the total alder basal area increased slightly over the study period. The evolution of seedling recruitment showed a dramatic decrease over the 12 years that could not be explained by a canopy closure and increased competition. Finally, the alder genotype which had been exposed to *P. alni* selective pressure showed less infection after inoculation in greenhouse compared to those not exposed indicting that natural selection for resistance is occurring in the stands. The results are discussed for future of alder riparian stands in the area.

The interplay among human, biotic and abiotic factors explains quick *Phytophthora cinnamomi* spreading and tree decline in a Mediterranean Biosphere Reserve

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Allien invasion is one of the main threats to biodiversity and ecosystem integrity.In this work we analyse a case study in a Biosphere Reserve (Doñana National Park), located at SW Spain, which includes both aquatic and terrestrial endangered ecosystems. We monitored soil for *Phytophthora cinnamomi* presence under both symptomatic and asymptomatic centenarian oaks along 7 years (2008-2014). Before 2008 there were no reports about the pathogen. In 2008/09 several infected trees were detected in an area recently aforested with seedlings grown in uncertified nurseries. An unusual climate event occured in 2010: late winter/early spring rainfall rates exceeded all previous records, extending the period with flooding/high soil moisture towards warmer months. Infestation frequency increased from ~20% to >70% of the surveyed trees. In 2014, the isolation frequency in soil raised to 99,7%. Spore densities in the infested soils quickly increased and, in 2013, it exceeded on average the infection threshold experimentally determined. On the other hand, pathogen dissemination and root infection have complex interactions with soil chemistry. Soil changes caused by tree nesting wading bird drops affected to different stages of the pathogen cycle in a different way. For example, soft levels of soil contamination stimulated both sporangial production and chlamidospore germination, while high contamination levels inhibited both processes, but stimulated chlamidospore production. Root infection was stimulated by soil contamination, although zoospore production was not influenced. Since nesting birds occupy a significant fraction of the oaks, they will probably have a significant effect on pathogen progression. Unfortunately, neither individual (trunk injection based) treatments for the big centenarian oaks, nor prohibition of afforestation with uncertified seedlings have been adopted by managers, despite the recommendations of an international panel of experts. Therefore, the inaction of forest protection authorities also results as an important factor of the ecological consequences derived from *P. cinammomi* expansion.

Poster 26

Climate change can affect the impact of *Phytophthora alni* subsp. *alni*

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The sensitivity of alder pathogen *Phytophthora alni* subsp. *alni* (Paa) to low temperatures should be supposed because of absence of resting structures and from other reasons. This assumption was confirmed with the help of series of investigations. During the field works (Central Europe) it was found out, that the pathogen survival in trees differed according to winter temperature course. The pathogen survival was highly limited after standard winter (avg. temperature –1.96°C), whereas after extremely mild winter (2.54°C) without deep frosts (such winters are predicted by IPCC to be frequent in future in the area) the survival was ca 10× more effective. Moreover, thickness of covering tissues and exposure to the most heated quadrant of stem girth enhanced the pathogen survival. During *in vitro* test, the effect of low temperatures and frost duration on Paa viability was investigated. Paa isolates were incubated at different temperatures (from –0.1 to –10.0 °C) and frost durations (0 – 28 days) and significant influence of both factors was identified. The failure time analysis showed that pathogen survival significantly decreased after 4-days-incubation at –7.5 °C and completely died after 2 days at –10.0 °C. Following incubation test (trunk segments of a black alder tree were cut out in several distances from collar to top, infected with Paa and incubated for 3 days at –7.5°C) verified the sensitivity of the pathogen to deep frost. Under thin tissues (upto 8 mm) the pathogen died within 3 days, whereas under thicker tissues it survived at different rate (0.1 – 0.8). The significant regression (r=0.76) of pathogen survival on thickness of covering tissues was identified. The results proved the pathogen to be very sensitive to heavy frost. The temperature limit (–7.5°C and below) occurs regularly in Central Europe in January. The climate change characterized by the increase in the lowest winter temperatures (as hypothesized by IPCC) poses a significant risk for alder population in the area.

Poster 27

Economical losses caused by *Phytophthora alni* in riparian stands. Typological study of Vltava River basin (Czech Republic)

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In recent years *Phytophthora alni* subsp. *alni* (Paa) causes heavy but heterogeneously distributed losses in European alder riparian stands. It is necessary for river authorities to have basic information on economical losses caused by Paa in different landscape types. The information about pathogen distribution and ecology was summarized and the most important environmental factors influencing the pathogen distribution and disease impact (density of river system, density of forest alder plantings, vertical heterogeneity and temperature) were selected and used in a statistical model. The Vltava River basin (VRb) is highly affected by the pathogen and served us as a model area. It was divided by rectangular grid (2.5 × 2.5 km), the average values of variables were computed for all quadrates using GIS. The quadrates were clustered into 6 groups according to their environmental similarity. Detection of Paa distribution and evaluation of losses were started in randomly selected quadrates in 2013. The economical losses in alder stands were computed according to applicable regulations evaluating the price of trees [1] and the cost of necessary works in affected alder stands – removing of dead and highly diseased trees and planting of more resistant trees [2]. After the first year of investigation it should be stated the following. The pathogen was identified in ca 70% of investigated squares. The average economical losses exceeded 1700 €/100 m of affected alder riparian stand. The most affected landscape types were flat landscapes in middle altitudes, South Bohemian (pond) basins and valleys of broad rivers in low altitudes. The less affected landscapes were varied uplands with relatively sparse water systems, whereas mountain landscapes with high vertical heterogeneity (and cold climate) and vice versa dry and warm landscapes with low frequency of alder plantations were the least affected ones. The study is ongoing.

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Poster 28

Factors affecting *Phytophthora alni* distribution in State Forests of the Czech Republic

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Phytophthora alni is the most important pathogen of European alders. Besides riparian stands, the pathogen causes important losses in forests where the pathogen was introduced with planting material [1]. *P. alni* distribution in forest stands was investigated with cooperation with State Forests in 2013. The information on distribution of phytophthora root and collar rot of alder was collected from more than 840 stands covering the whole area of the Czech Republic. The determination of primary sources of infection in landscape was carried out in highly forested area covering ca 420 km^2 on the border between Central and Southern Bohemia. The presence of *P. alni* was searched in 13 forest nurseries. Among others, it was found out that phytophthora alder disease was identified in 53 % of forest stands. The multiple regression analysis ($p < 0.01$) showed that the disease presence was positively affected by the presence of watercourse and alder biomass (standing timber stock) and negatively by altitude. The presence of watercourses was identified in 78% of diseased stands and significant positive regression of disease presence to watercourse width was identified. Moreover, median of age of affected stands was 59 years (the difference in age of affected and healthy stands was not found). During the searching of infection sources in landscape there were identified 32 presumptive primary sources of infection: 30 fish farming ponds and 2 forest alder stands. It should be stated that the natural spread of the pathogen dominates in the area due to 1) relation of the pathogen presence to watercourses, 2) the high age and biomass volume of infected stands, 3) fish farming ponds as a sources of inoculum in landscape and 4) evidently low frequency of *P. alni* in nurseries.

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Poster 29

Identification of *Phytophthora alni* subspecies in riparian stands in the Czech Republic

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In the Czech Republic, *Phytophthora alni* was first confirmed in 2001 and the pathogen has been quickly spreading and occupying almost the whole area of the country. The pathogen attacks *Alnus glutinosa* or *A. incana* to a lesser extent and causes considerable losses of alder trees along hundreds of kilometres of riverbanks. The aim of our work was to perform the identification of *P. alni* isolates at the subspecific level using PCR and to determine the frequencies and distribution of particular subspecies. The allele-specific PCR primers focused on allele diversity of orthologs of *ASF*-like, *TRP*1, *RAS-Ypt*, and *GPA*1 genes were selected for identification. Eighty-eight per cent of the 59 analysed isolates belonged to *P. alni* ssp. *alni* while 12% were *P. alni* ssp. *uniformis. P. alni* ssp. *multiformis* has not been recorded in the country till now. The two subspecies differed in distribution. *P. alni* ssp. *alni* dominated in riparian stands along broader rivers in lowlands and the results confirmed the more effective spreading of *P. alni* ssp. *alni* based on its higher aggressiveness and ecological advantage. *P. alni* ssp. *uniformis* was acquired rather from riparian stands of small watercourses at higher altitudes. The insular distribution of *P. alni* ssp. *uniformis* may represent the remains of its former occurrence. Therefore, *P. alni* ssp. *uniformis* may be an previously introduced subspecies suppressed by the more aggressive related taxon.

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Poster 30

Community structures of root-rotting *Phytophthora* species affecting *Abies* in U.S. christmas tree farms & screening true fir for resistance to *Phytophthora* root rot

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True fir trees in the genus *Abies* are common hosts of various root–rotting *Phytophthora* species. Losses due to root disease can significantly affect bareroot conifer nurseries and Christmas tree plantations. There are limited methods available to control *Phytophthora*, but practicality may vary depending on field topography, crop maturity, and available capital. For these reasons, investigation of a marker-assisted selection system for identifying fir trees that can resist *Phytophthora* infection is justified to help alleviate current losses. Our research is intended to provide information on the host-parasite interactions between true fir and *Phytophthora* species, with the ultimate goal of facilitating the development and implementation of molecular markers associated with resistance. Main objectives include construction of an isolate collection of *Phytophthora* species affecting fir roots, assessment of relative virulences among isolates from different geographic regions, and screening of true fir seedlings for resistance to *Phytophthora*. The compiling of *Phytophthora* isolates from fir roots has provided information on the community structures and habitats of soilborne *Phytophthoras* from widely differing geographical regions within the U.S. A subset of isolates will comprise the inoculum that will be used in a subsequent phenotype screening for resistance in fir. Prior to utilization as inoculum for the screening project, virulence testing will facilitate the selection of the three most virulent genotypes from each of the four most commonly-occurring *Phytophthora* species from the U.S. collection. Resistance phenotype screening of seven true fir species will be performed to provide information about which species of fir can generally be regarded as resistant to PRR and whether there are individual genotypes *within* the different fir species that are more resistant than others. This information will be used to facilitate a future genomics project to identify molecular marker patterns that are common among resistant fir species and/or genotypes within species.

Poster 31

Assessing the risk of chestnut ink disease spreading using TOPMODEL

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Ink disease is one of the most destructive diseases of sweet chestnut (*Castanea satival,* Mill.). This is currently under strong recrudescence and, for some areas in Italy, is responsible of environmental emergencies. The causal agents of the disease are *Phytophthora cambivora* and, the less present in Italy, *P. cinnamomi*, which spread through flowing water during mild winters. The gradual increase of temperatures and the different distribution and intensity of rainfall, due to climate changes, affect the distribution areas of pathogens furthering the more aggressive and polyphagous *P. cinnamomi*. The containment of the disease in the forest is mainly based on integrated pest management systems, under which the prevention and the rapid identification of areas at risk play a crucial role. For this scope, in this study we applied the TOPMODEL hydrogeological model for identifying the areas affected by chestnut Ink disease in an area in the municipality of Allumiere (Rome, Italy). During a 2 years period (2009-2010), *P. cinnamomi*, in association with other species such as *P. cactorum* and *P. plurivora*, has been consistently isolated in that area. In this study we report on the results of the spatial and temporal evolution of the disease in relation to hydrogeomorphological variables, such as the potential saturation of the soil, that is spatially affected by the topography, and the depth of the ground water respect to the soil surface, that is influenced by the precipitation time series.

Poster 32

Influence of multiple stress sources on cork oak seedling susceptibility to *Phytophthora cinnamomi*

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Some sclerophilous species of the genus *Quercus* (namely *Q. ilex* and *Q. suber*) are of paramount socio-economic and ecological importance in Spain and Portugal. Two main threats to their long-term survival are currently recognized. Firstly, invasive soil-borne pathogens, particularly *Phytophthora cinnamomi,* which kill myriads of trees every year. Secondly, the potential longterm response of these key species to extended stress derived from climate change. A temperature increase, together with a decrease of annual rainfall is expected at the end of the century, which means increased drought periods and extended stress conditions for plants. Related to the combined effects of stress and pathogens on plants, a critical question is whether increased stress is able to induce a weakening of trees and facilitate root infections. Alternatively, it can be hypothesized that, as long as the conditions will be favorable for the pathogen, the previous stress-history of a susceptible host is not especially relevant for the progression of the disease. We tested these hypotheses in a greenhouse experiment where *Q. suber* seedlings were submitted to two water regimes (current and a dryer scenario of 30% reduced water inputs) and three levels of soluble salts (physiological drought). After 150 days, plant performance was evaluated and transferred to new pots inoculated with resting spores of *P. cinnamomi.* Pots were subjected to periodical soil flooding and assessed weekly for crown symptoms. After 6 weeks plants were assessed for root necrosis. We found that both water and salt stresses significantly affected plant performance, but no significant differences in *Phytophthora* symptom severity were found among plants subjected to different intensity of both stresses after they were infected. That is, there was no evidence of stress-facilitated pathogen infections. In contrast, our results support the idea that the pathogen equally infect and kill healthy than stressed seedlings.

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Session 7

Ecophysiology & Physiopathogenicity

Recent advances in understanding *Phytophthora*-woody plant interactions

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Within the last decade the so called "-*omics*"-approaches– such as gen*omics*, transcript*omics*, prote*omics* and metabol*omics* – found their way into life sciences, due to newly developed techniques allowing high throughput of samples and data. With some offset in time, these approaches are now also used for research on *Phytophthora*-plant interactions. First comparative genome analyses uncovered an unexpected large arsenal of effector genes in *P. ramorum* (Tyler et al, 2006). Right now, genome sequencing of up to 150 *Phytophthora* isolates is ongoing, including many species pathogenic on woody hosts. The rapid development of microarrays and of *next generation sequencing* technologies enabled the study of a broad spectrum of differentially expressed genes during pathogenesis, both of the host and the pathogen, deepening our understanding of these interactions on the molecular level. The development of modern mass spectrometric techniques allows the rapid identification of proteins and metabolites, the real players in plant-pathogen interactions. All these techniques generate a so far unknown amount of data, challenging the field of bioinformatics to reliably identify relevant information. The recent literature on these "-*omics*"-approaches on *Phytophthora*-woody plant interactions will be summarized and their possible implications on the control of *Phytophthora* diseases will be discussed.

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Screening *Quercus ilex* for tolerance to water stress and *Phytophthora cinnamomi*

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The health of forests in south-western Europe is conditioned by global change through direct rise in average temperature and variability of climate, with potential to increase the occurrence of severe droughts. In consequence, programs focused on breeding trees for resistance to pathogens should screen to drought stress too. The tolerance of 16 populations of *Quercus ilex* from five Mediterranean countries (France, Italy, Morocco, Portugal and Spain) to water stress and *Phytophthora cinnamomi* was assessed. The experiment included seedlings grown under greenhouse conditions and following a full randomized block design (12 mother trees per population; 20 seedlings per mother tree). During their first vegetative period, half of seedlings were submitted to a severe water stress treatment. During the second vegetative period, all plants were inoculated with *P. cinnamomi*. Plant mortality after the water stress treatment ranged from 0 to 40% and varied significantly between populations. Mortality after inoculations was about 10% higher in water stressed plants in comparison to non-water stressed plants. Survival time of seedlings varied significantly between populations and mother trees, being generally more resistant the populations from arid areas than the populations from semiarid and suhumid areas. Seed mass did not influence the tolerance of seedlings to water stress and *P. cinnamomi*. From about 3.300 *Q. ilex* seedlings tested, the most vigorous 100 plants were selected for further assessments. Breeding for adaptation to new climatic environments will be discussed.

The spatial and temporal spread of *Phytophthora alni* subsp. *alni* in alder bark tissue – an ecophysiological study

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The impact of alder *Phytophthora (P. alni subsp. alni*) on corticular photosynthetic metabolism via measurements of chlorophyll fluorescence was explored. Ten weeks after stem-base inoculation the pathogen induced a sharp reduction of maximum (Fv/Fm) and effective quantum yield of PSII (ΔF/Fm‟) within the visually detectable stem lesion. Observations of the axial as well as radial spread of the pathogen revealed that near to the point of inoculation and in the whole center of the tongue-shaped stem lesion Fv/Fm and ΔF/Fm‟ of the cortex chlorenchym decreased to almost zero, indicating tissue necrosis. Thereby, low values of Fv/Fm and ΔF/Fm‟ was also found in some pre-symptomatic regions beyond the visibly stem lesion. On the opposite substantial photosynthetic activity was found in uninvaded parts of the inoculated trees and in the control. These stem parts showed a marked light-adapted quantum efficiency of PSII as well as marked electron transport rates (ETR) in there bark tissues. Thus, corticular photosynthesis stayed unaffected in these stem parts supporting stem carbon balance. Additional chlorophyll fluorescence measurements in the field further illustrated that stem infection with *Phytophthora alni subsp. alni* and the effect on the bark tissues is not only highly heterogeneous but also underlies very quick temporal changes, due to a rapid destruction of the photosynthetic apparatus by the pathogen.

Diterpene resin profile of *Austrocedrus chilensis affected by Phytophthora austrocedri*

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The exudation of resins is considered as one of the most important defense mechanisms in conifers. The resins have the ability to protect against pathogenic agents, insects, and other types of damage. The resin composition of the Cupressaceae *Austrocedrus chilensis* (known as "ciprés de la cordillera") has been studied recently, but there is not information about the variability and properties of resins from different individuals, as well as in different functional conditions. This work considered the response of *Austrocedrus chilensis* against the pathogenic agent *Phytophthora austrocedri,* which is responsible for the devastating disease commonly named as "mal del ciprés". The study included the profiling of resin from healthy, naturally infected and artificially inoculated trees of *A. chilensis* and the possible detection of antifungal activity of the resin against *P. austrocedri.* Resin samples were collected in Los Alerces National Park, Argentina. The chemical analysis of the resins was carried out by GC-MS and ^{1}H-NMR. To obtain semi-purified fractions of resin, preparative TLC of representative resin samples were performed. The antifungal activity assays were conducted in Petri dishes using tomato agar as culture medium amended with the different fractions of resin. The resin profiling showed differences between healthy and infected or artificially inoculated *A. chilensis* trees. Artificially inoculated and naturally infected trees showed the same profile. The preparative TLC gave nine fractions of semi-pure compounds. After a comparison by TLC, GC-MS and NMR analysis, five of the nine fractions were evaluated to analyze possible antifungal activity. Two fractions significantly inhibited the pathogen mycelial growth. The resin profile components, as well the description of composition of the fractions and their antifungal activity will be discussed.

Effect of cinnamomins on *Phytophthora cinnamomi* biomass growth and on the oxidative burst in infected *Quercus suber* roots

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In previous work we have evaluated the effect of elicitins (cryptogein, capsicein, α-cinnamomin and β-cinnamomin) on the infection of *Fagaceae (Quercus suber, Q. ilex, Castanea sativa*) by *Phytophthora cinnamomi* and shown, namely through histological and ultra-structural studies that they trigger defence reactions against the pathogen, contradicting the belief that with the exception of Nicotiana, most plant species lack the capacity to respond to elicitins. In this study, we applied two other approaches to evaluate the effect of cinnamomins, on the infection process of cork oak roots by *P. cinnamomi*: quantification of pathogen biomass and measurement of ROS and antioxidant enzymes during initial responses of *Q. suber* roots. Genomic DNA was extracted from infected roots and DNA levels of the pathogen measured as the ratio between the amount of pathogen DNA and an internal plasmid DNA using quantitative PCR. A strong reduction of *P. cinnamomi* biomass in α- and β-cinnamomin-treated (0.5 μM) roots was observed in the first 24h. The production of reactive oxygen species (ROS) H_2O_2 and $O_2^{\bullet-}$ was measured at 6, 12, 18, 24, 36 and 48h and POD, SOD and CAT activities were determined during the time course of infection with *P. cinnamomi* mycelia in 2-month-old roots before and after α-cinnamomin treatment. A significant higher production of ROS in elicitin and non-elicitin treated roots in interaction with *P. cinnamomi* in comparison to controls was observed, as well as an earlier increase in H_2O_2 production but lower when compared with plants treated with *P. cinnamomi* alone. A lower level of $O_2^{\bullet-}$ in elicitin pre-treated plants was observed as compared with those inoculated without elicitin treatment. Activities of antioxidant enzymes upon challenge with *P. cinnamomi* either pretreated or not with α-cinnamomin, increased when compared to controls. In roots pretreated with α-cinnamomin, enzymatic activities increased as compared with those in elicitin non-treated plants. The overall results confirm elicitins trigger defence responses in *Q. suber* against *P. cinnamomi*.

De Novo Assembly of *Phlomis purpurea* Transcriptome challenged with *Phytophthora cinnamomi*

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Phlomis purpurea is a perennial evergreen shrub that grows spontaneously in Mediterranean ecosystems of south Iberian Peninsula. It can be found in Algarve, Portugal, in *Quercus suber* (cork oak) and *Quercus ilex* (holm oak) stand habitats severely infested by *Phytophthora cinnamomi.* It is resistant to *P. cinnamomi*, since this pathogen did not cause any visible symptoms and was never isolated from infested roots. Noticeably, *P. cinnamomi* hyphae are unable to penetrate beyond the surface layer of the root epidermis. The molecular mechanisms underlying defence responses are unknown in the *Phlomis* genus. Characterizing changes triggered by the pathogen in gene expression in the resistant plant would provide insights into, when and where each gene is expressed and would offer a glimpse at the strategy used by *Phlomis* to oppose *Phytophthora*. We used high-throughput deep sequencing technology to profile the *P. purpurea* transcriptome using the Illumina HiSeq™ 2000 platform and Flash and CLC assemblers for *de novo* assembly of transcriptomic data. The comparison of differential gene expression profiles was conducted between different cDNA libraries from *P. purpurea* plants challenged with *P. cinnamomi* at six post inoculation time points (0, 6, 12, 24, 48 and 72h). Out of 48,711 total annotated genes displayed the highest homology to genes from plants, 1,558 were down regulated and 3,755 were up regulated. The differential expression patterns among libraries revealed that the largest differences in expression occurred in the interval between 6 and 24h. Between 24h and 48h post-inoculation no significant difference expression was found, but between 48 and 72h occurred a slight difference in expression. Moreover, a large number of specifically plant fungi interaction transcripts, were differentially expressed. To assess the reliability of our sequencing-based approach in identifying *Phytophthora*-responsive genes, we monitored the expression of candidate differential expressed genes by qPCR for 10 candidates. The expression profiles of eight candidates were in agreement with the predictions from the IIlumina sequencing results.

Screening of Asian oak species for potential resistance to *Phytophthora cinnamomi*

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In the past 20 years various studies have demonstrated the involvement of *Phytophthora cinnamomi* and, to a lesser extent, other *Phytophthora* species in the widespread complex declines of *Quercus* suber and *Q. ilex* in Portugal, Spain and the southern parts of France and Italy. The progressive fine root losses by *P. cinnamomi* interact with droughts causing a slow chronic decline. Prolonged droughts and collar infections by *P. cinnamomi* after heavy unseasonal rain can cause rapid and dramatic mortality. Given the modelling projections of a warming climate with an increasing frequency of heavy rain events and prolonged droughts a further intensification of *Phytophthora* activity and root losses is most likely which in turn will enhance the vulnerability of the affected ecosystems to the climatic extremes. On the long-term, increasing the genetic resistance to *P. cinnamomi* in *Q. suber* and *Q. ilex* seems to be the most promising management approach for stabilising the Mediterranean oak ecosystems against the interaction between *Phytophthora* and climatic extremes. Several evidences point to the hypothesis of Southeast Asia as the center of origin of both mating types of *P. cinnamomi*. High levels of sympatric resistance to *P. cinnamomi* might be expected in co-evolved *Quercus* spp. from Southeast Asia as compared to the high susceptibility of the European *Q. suber and Q. ilex*. In an ongoing research project Asian oak species are being screened for potential resistance to *Phytophthora cinnamomi*. Soil infestation trials with six oak species from Taiwan and the analysis of the transcriptome of infected and healthy roots of the two most promising oak species are in progress. The results obtained in these experiments will be presented and discussed. The finding of resistant oak species could be the basis for future breeding programmes, the development of molecular markers for screening European oak populations for resistance to *P. cinnamomi* and for developing resistant varieties of susceptible species by genetic engineering.

Poster 33

Spectral measurements for detecting *Phytophthora*-related stress in *Corymbia calophylla* (marri)

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Corymbia calophylla (marri) is a keystone species in the forests and woodlands of south-west Western Australia. Since the 1970's widespread marri mortality has been reported and various factors have been cited as the cause of this decline. In this study we investigated the pathogenic effect of *Phytophthora* root infection to evaluate its potential role in marri decline. Field surveys were conducted to determine the extent of *Phytophthora* infection on marri. Soil and root samples from the rhizosphere of declining marri, from both remnant and natural sites, were collected and baited to recover *Phytophthora* species. The recovered *Phytophthora* species were used in pathogenicity trials. Hyperspectral remote sensing measurements, sensitive to leaf chemical and functional traits (especially related to foliar pigment and water content), and stomatal conductance measurements of plant function, in addition to estimates of above- and below-ground biomass, were taken during the trials to develop indicators of *Phytophthora*-related stress in marri. Five *Phytophthora* species were isolated from marri, *P. cinnamomi, P. cryptogea, P. elongata, P. multivora and P. calophyllaphile* prov. nom. These varied in their pathogenicity to marri. *P. cinnamomi* was the most pathogenic species whilst some isolates of *P. multivora* stimulated root and shoot growth on marri compared to the control plants. Stomatal conductance measurements correlated with the results of the pathogenicity trials, as did a number of spectral indices. The Normalised Difference Vegetation Index (NDVI) and Simple Ratio Index (SRI), general indicators of "greenness" and vegetation condition, and the Anthocyanin Reflectance Index 1 (ARI1), indicator of auxiliary pigment content were closely related to marri response to *Phytophthora* infection. Conductance and greenness measurements were decreased and pigment content was increased by the *P. cinnamomi* infection. Non-destructive spectral measurements taken regularly throughout the trial reveal time-courses of marri decline and may provide early-warnings of infection. More experimental work is underway, including dual inoculation with *Phytophthora* and *Quambalaria coyrecup*, the cause of marri canker disease.

Age-related susceptibility of *Eucalyptus* spp. to *Phytophthora boodjera* nom prov.

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Since 2011 damping-off and mortality of *Eucalyptus* seedlings in Western Australian (WA) nurseries has been observed. The casual agent of this disease was identified as *Phytophthora boodjera* prov. nom based on a combination of morphology and a multi-gene phylogeny. This study evaluated the age-related susceptibility of five species of *Eucalyptus* (*E. polybractea*, *E. kochii* subsp. *plenissima*, *E. kochii* subsp. *borealis*, *E. loxophleba* subsp. *lissophloia*, and two seedlots of *E. loxophleba* subsp. *gratiae*) to six isolates of *P. boodjera* and three isolates of *P. arenaria* in pasteurised washed river sand-infestation pot trials. *P. cinnamomi* was included for comparison. *Eucalyptus* spp. were inoculated with all *Phytophthora* isolates at 0, 2, 4, 12 and 100 weeks post-germination. The following measurements were included in data sets where applicable: number of seedlings germinated, height of seedlings, root length and dry root weight.
Susceptibility of *Eucalyptus* spp. to all *Phytophthora* isolates decreased with age. When *Eucalyptus* seedlings were inoculated with *Phytophthora* at sowing (0 week), less than 5% germination occurred compared with 100% for the controls. Damping-off and mortality occurred for the 2 week-old seedlings, and on average, the root length of any living inoculated seedling was 19-59% shorter than those of the non-inoculated controls. *P. boodjera* caused the greatest reduction in root length. Conversely, no *Eucalyptus* seedlings died in pots inoculated after 12 or 100 weeks. However, the height and dry root weight of seedlings treated with *Phytophthora* were different significantly less than that of the controls. For example, when seedlings inoculated at 12 weeks were harvested at 24 weeks, dry root weight of seedlings inoculated with *P. boodjera*, *P. arenaria* and *P. cinnamomi* were 42.8, 20.6, 23.1% smaller than controls, respectively. Variability in susceptibility was observed between eucalypts species tested; *E. kochii* subsp. *plenissima* was more susceptible than other eucalypts. The *Eucalyptus* spp. tested are more susceptible to *P. boodjera* prov. nom (and other *Phytophthora* spp. tested) pre- and post-germination than at later growth stages.

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Histopathology of *Phytophthora austrocedri* in *Austrocedrus chilensis*

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Austrocedrus chilensis (D. Don) Pic. Ser. & Bizzarri (mountain cypress), Cupressaceae, suffers a disease caused by *Phytophthora austrocedi* that leads the trees to death. It has been speculated that trees are killed by extensive death of bark and cambium tissues and by the disruption of phloem transport but the pathogen mechanisms are not totally elucidated yet. The histopathology of *A. chilensis* disease is being studied to understand the pathogenic mechanisms of *P. austrocedri* as well as the tree defense mechanisms. Necrotic and healthy tissues, of naturally and artificially infected adult trees, were studied in order to assess the effects of the pathogen on the phloem and xylem. Portions of tissues from the advancing, medium and old areas of necrotic lesions, as well as from healthy areas at least 60-80 cm above lesion, were sliced into transverse, tangential and radial sections of 15 µm using a microtome and observed in a light microscope. Oospores of *P. austrocedri* were observed in affected phloem, especially in resin pockets. Hyphae were observed in phloem and xylem. In the xylem hyphae grow through rays, and pass from rays to tracheids through the cross-field pitting, and from one tracheid to another through the pits. Crossing hyphae filled the torus of the pit completely and consequently blocked the pit. Affected xylem showed the formation of trabeculae, single or double and frequently aligned, that were absent in healthy xylem. The trabeculae might appear as a response of the tree against the presence of the pathogen and could also contribute to the decrease of hydraulic conductivity observed in affected trees. Formation of traumatic resin ducts in the phloem associated to necrotic lesions was also observed. The resin ducts were much more abundant and bigger than the normal resin ducts of healthy phloem and can fuse to form resin pockets. Thus, these structures are related to the profuse resination produced associated to the advancing zone of the lesion that is assumed as a defense mechanisms of the tree.

Poster 36

RNAseq reveals different defense responses of *Quercus robur* microcuttings against *Phytophthora quercina* during root and shoot flush

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Within the joint research project "TrophinOak", we analyze multitrophic interactions of *Quercus robur* micro-cuttings with respect of the rhythmic growth of oak. Our research team focuses on interactions with the root pathogen *Phytophthora quercina.* In addition, we compare the effects of the ectomycorrhizal (EM) fungus *Piloderma croceum,* as an additional interacting partner. It turned out, that infestation of roots with *P. quercina* is positively correlated with the concentration of non-structural carbohydrates (NSC) in oak roots (Angay et al., 2014). NSC concentrations were strongly influenced by flush status of microcuttings, and mycorrhization further accentuated this flush dependent shift without protecting roots against the pathogen. To elucidate the processes in oak roots after infection with *P. quercina* on the molecular level, we performed a transcriptomic approach using RNAseq as described by Tarkka et al. (2013). It turned out that more than 4,000 contigs were differentially expressed in lateral roots, when root and shoot flush, respectively, were compared. However, only a low number of contigs (about 80 in root flush and 40 in shoot flush, respectively) were differentially expressed upon *Phytophthora* infection, with almost no overlap in differential expression patterns between flush stages. Moreover, contigs related to pathogen defense were hardly addressed indicating that *P. quercina* might suppress defense response in oak roots in a similar way as it has been described for *Fagus sylvatica* and *P. plurivora* (Schlink, 2009, 2010).

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Session 8

Management & Control

Challenges associated with the management of *Phytophthora* diseases in Australia and the importance of community engagement for success

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Phytophthora cinnamomi is listed as a „Key Threatening Process to Australia"s Biodiversity" by the Commonwealth Government, consequently there is a national threat abatement plan (TAP) in place. The TAP establishes a national framework to guide and coordinate Australia"s response to *P. cinnamomi*. It sets out the actions necessary to abate impacts of this key threatening process, and identifies the research, management and other actions needed in Australia"s response to this pathogen. The success of this TAP depends on a high level of cooperation between all key stakeholders. We will discuss how different stakeholders have met the challenges of identifying and mapping the pathogen across the landscape, taking into account other *Phytophthora* species, global change, other environmental priorities, the need for prioritising areas that are „protectable" over the next 50-100 years and the importance for societal engagement to ensure uptake. A number of case studies from the Commonwealth, State, non-government organisations, „friends of groups" and industry will be provided to show how different stakeholders have engaged in attempts to meet the objectives of the TAP. We will discuss how research can help guide and invigorate different stakeholders in the process of managing and containing the spread and impacts of this pathogen across different landscapes. Lastly, examples of the importance of working with community to ensure uptake of processes and proceedures will be highlighted, together with the associated challenges.

Continued Monitoring of Sudden Oak Death Treatments in Oregon Tanoak Forests

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Phytophthora ramorum, the cause of sudden oak death, was first identified in tanoak forests in coastal Southwest Oregon in 2001. Since that time, treatments using a combination of herbicides, cutting, and burning affected and exposed vegetation have been done to initially, eradicate the pathogen, and more recently, slow the spread of the disease. Monitoring done in 2010 of treatments completed through 2008 showed that 63 percent of treatment plots were negative for *P. ramorum* in sampled soil and vegetation, 25 percent of plots were positive for *P. ramorum* in sampled soil only, 7 percent were positive in both soil and vegetation, and five percent of monitored treatment plots were positive for the pathogen in vegetation only. *P. ramorum* hosts persisted, at some level, on all treatment plots. Our current monitoring effort is focused on two questions: Does *P. ramorum* recur or persist in sprouting and seedling vegetation?, and Does *P. ramorum* spread plant to plant on sites where it recurs? These concepts are of particular interest in that we have treated sites within a larger geographic area where treatment no longer occurs. Work is ongoing to revisit monitoring plots previously assessed, tally vegetation cover, and sample symptomatic vegetation for *P. ramorum*. Results will be available and reported at the meeting.

Sudden Oak Death: Intensification and Spread in Oregon Forests

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Sudden Oak Death, caused by *Phytophthora ramorum,* is lethal to tanoak (*Notholithocarpus densiflorus*) and threatens the species throughout its range in Oregon. Since July 2001, an interagency team has been attempting to eradicate and slow spread of disease through a program of early detection and destruction of infected and nearby host plants. Because of increasing disease, high eradication costs and limited funding, not all infested sites are being treated equally. Highest priority for treatment are sites located at or beyond the leading edge of the infestation or near the quarantine boundary. Within a 145 km^2 area near the center of the quarantine area most sites are not treated and the disease has been allowed to intensify and spread. In this report we describe the rate of disease intensification and spread in terms of tanoak canopy mortality in areas with and without eradication treatments.

Approaching 15 years of research on SOD control

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In California, SOD is mostly spread by infectious tanoaks and bay laurels, while high mortality is observed in tanoak and coast live oak stands. Intensive surveys and repeated measures analyses indicate that density of bays and tanoak will determine final mortality rates. While thinning of both species is an obvious disease control approach, we suggest that knowledge of natural disease tolerance and infectivity of individual trees during thinning operations is key in maximizing outcomes. We also show that phosphonate treatments increase disease tolerance in oaks and tanoaks. However, the genetic and physiological mechanisms of action of phosphonates and mechanisms leading to natural disease tolerance are still imperfectly known. We used a transcriptomic approach to compare these two tolerance mechanisms: results identified several genic pathways that are up- and down-regulated in both types of tolerance, and show that the two mechanisms, although both quantitative and resulting in a comparable tolerant phenotype, are almost completely not overlapping.

Eradication of *Phytophthora cinnamomi* from infested *Eucalyptus marginata* (jarrah) forest during large scale mining operations

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Phytophthora cinnamomi is widespread throughout the *Eucalyptus marginata* (jarrah) forest in the south-west of Western Australia. Alcoa of Australia Ltd. mine bauxite in both infested and uninfested forest and rehabilitates 350-400 ha of mined forest annually. *P. cinnamomi* is unevenly distributed throughout the areas to be mined, with infested areas adjacent to non-infested areas. In order to minimize the spread of *P. cinnamomi* from infested to non-infested areas, detailed mapping of infestations is used to design detailed hygiene plans. As a result of eradication trials reported at previous IUFRO meetings, we now believe it is feasible to return infested mine pits to pathogen-free post mining. In addition, it will allow strict hygiene practices to be relaxed along haul roads, as these can be made pathogen-free post-mining. This will result in substantial savings in transport costs as *Phytophthora* free road building material will not need to be sourced. As *P. cinnamomi* is a poor saprotroph eradication methods are based on ensuring haul roads, topsoil, overburden (soil horizons not suitable for bauxite extraction) stockpiles and sumps (drainage points designed to collect water running off roads) are kept plant-free for 2-3 years. Where necessary metham sodium and other fumigants will be used. We will report on (1) the fallow, herbicide, fumigation methods being used to eradicate the pathogen, (2) the traditional and molecular genetic approaches being used to monitor the effectiveness of treatments to kill all survival stages (chlamydospores, zoospores, oospores, and stromata), and (3) approaches to containment that will ensure the pathogen is not inadvertently spread during the 2-3 year fallow period prior to revegetation. Successful outcomes will allow many hundreds of hectares of previously *Phytophthora* infested forest to be returned to a pathogen-free forest post-mining.

Potential Impacts of the Revised APHIS *Phytophthora ramorum* Domestic Quarantine Regulatory Requirements on the Spread of this Exotic Pathogen within Washington State

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Since 2003, *Phytophthora ramorum* have been detected in over 50 ornamental plant nurseries in Washington State. Stream monitoring by state agencies has resulted in the detection of this exotic pathogen in about a dozen waterways in six western Washington counties since 2006. Genotype analysis indicates that the NA1, NA2, and EU1 clonal lineages of *P. ramorum* are present in nursery and waterways. In all cases, streams have tested positive for *P. ramorum* in subsequent years after the first detection. Although *P. ramorum* has not been detected in a forest landscape in Washington State, in the spring of 2009, infested ditch water resulted in the infection of salal (*Gaultheria shallon*) plants by the NA2 lineage along the perimeter of a positive nursery in Pierce County. Composite soil samples collected from along the ditch were also positive in 2010. In addition, positive soil has also been detected at 3 trace forward sites where infected plants from a nursery in Thurston County had been planted in urban landscape sites. Effective March 31, 2014, the USDA Animal and Plant Health Inspection Service (APHIS) revised regulatory requirements relating to the interstate movement of host nursery stock from nurseries located in *P. ramorum* regulated and quarantine areas in California, Oregon, and Washington went into effect. The impact of these revisions on the number of nurseries being inspected in Washington and its potential impact on the spread of *P. ramorum* within the state will be discussed.

Enabling technologies to combat *Phytophthora* diseases

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New Zealand"s conservation, forestry and horticultural tree estates are all impacted by *Phytophthora* species. Of particular note are *Phytophthora pluvialis*, causal agent of red needle cast in *Pinus radiata*, *Phytophthora* taxon Agathis which is causing severe disease in a native tree species, *Agathis australis* (kauri), and *Phytophthora cactorum* which has a long-standing record causing collar rot in apple. These three host trees are central in a Scion led, six-year collaborative research programme with significant support from sector groups, to address the biosecurity threat of *Phytophthora* species to New Zealand"s forestry, agriculture and natural ecosystems. The genetic, metabolomic and histological host-pathogen interactions will be investigated between each of these host species and eight species of *Phytophthora* with biosecurity relevance to New Zealand (*P. cactorum, P. cinnamomi, P. kernoviae, P. multivora, P. pinifolia, P. pluvialis* and *P. ramorum*). Through this multi-host-pathogen model we are assessing the potential for utilizing genetic, gene expression and/or metabolite signatures for tree breeding, improving disease management and advancing current knowledge of *Phytophthora*-tree interactions. Initial work has focused on screening established breeding lines of *Pinus radiata* for RNC resistance, assessing cross resistance to the other species of *Phytophthora,* and contrasting the timing of infection by each species of *Phytophthora*. The project model and results from the first year of this six year program will be presented and discussed.

Searching for *Phlomis purpurea* metabolites with anti-*Phytophthora cinnamomi* activity

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We recently reported that *Phlomis purpurea* root extracts (PRE) inhibit *Phytophthora cinnamomi* mycelial growth and chlamydospore and zoospore germination, and protect susceptible hosts from infection. The plant reduces the inoculum potential of *P. cinnamomi* in the soil, suggesting it has the potential to reduce disease spread.These findings prompted us to search for metabolites responsible for this activity or/and produced upon challenge with the pathogen. HPLC analysis of PRE allowed the identification of a fraction with anti-*P. cinnamomi* activity. Fractionation by preparative chromatography resulted in the isolation, crystallization and preliminary X-ray diffraction of a compound also characterized by ESI/MS/MS, IR, H and C NMR (DEPT, correlation NMR spectrometry-COSY, TOCSY, HMBC and Multiplicity Edited HSQC), revealing a novel triterpenoid structure, probably a triterpenoid saponin. *P. purpurea* metabolites produced constitutively and upon challenge with *P. cinnamomi* were quantified using a LC-MS system, according to established standard workflows.Root exudates were analysed by GC-MS after derivatization. Two and half-month-old *P. purpurea* seedlings were challenged with P. cinnamomi zoospores. The samples for analysis consisted of roots of 10 plants inoculated with the pathogen at 6 time points (0h, 6h, 12h, 18h, 24h and 72h), controls and root exudates of the same plants at the same time points. Five replicates were performed. The material from the plants at each time point was pooled for each independent replicate (11 pools x 5 replicates). Roots and leaves were extracted with MeOH. Lipids and slightly polar metabolites were separated using reversed phase chromatography. The exudates were also collected and filter sterilised at each time point, immediately submersed in liquid nitrogen and kept at -80ºC. Data analysis will target the already known anti-fungal compounds and explore as well the data set for new potential compounds of interest.

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Screening of biofumigants against *Phytophthora cinnamomi* root disease

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Root rot caused by *Phytophthora cinnamomi* is one of the most destructive diseases affecting many woody hosts and specially *Quercus* spp. in rangeland ecosystems in Southern Iberia. Biofumigation is a potential control method suitable to be used in these seminatural ecosystems. Then, we studied its effect on the viability of *P. cinnamomi* chlamydospores in the soil and their ability to cause root disease. Volatiles derived from different cruciferous plants (*Brassica carinata, B. juncea, B. napus, Eruca sativa* and *Lepidium sativum*) with different glucosinolate (GSL) profiles (analyzed by HPLC) were tested on natural soil artificially infested with water suspensions of *P. cinnamomi* chlamydospores (final concentration 650 cfu×g^{-1}). Fresh plant material were collected, lyophilized and placed at the bottom of 250 ml containers, and then rehydrated before the infested soil was added. All containers were closed in order to avoid loss of volatiles. Chlamydospore viability was evaluated after 1, 4 or 8 days of incubation (22 ºC day – 18 ºC night) by counting the number of cfu growing in selective NARPH medium. After the incubation period, pre-germinated *Lupinus luteus* seeds (40 per soil treatment) were planted in plastic containers containing the infested soil. Plant mortality was evaluated daily. Biofumigant plants rich in the aliphatic GSL Sinigrin (*B. carinata* and *B. juncea*) significantly decreased the viability of *P. cinnamomi* chlamydospores in the soil, as well as the mortality of *L. luteus.* Biofumigants without Sinigrin did not show any effect on inoculum viability nor on *L. luteus* mortality. This work marks a criterion for the selection of potential biofumigant plants to be used in rangeland ecosystems, based on their richness in 2-propenyl glucosinolate (Sinigrin).

Poster 37

Phosphite for control of kauri dieback: forest efficacy trials

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Kauri dieback, caused by *Phytophthora* taxon Agathis (PTA) threatens the health and survival of kauri (*Agathis australis*) trees in New Zealand. In January 2012, trials were established in four kauri forest sites severely affected by PTA, to determine the potential of phosphite (phosphorous acid) as a control tool. Trial trees ranged from 40 to 120 cm girth and all 160 trees showed symptoms of PTA infection (canopy thinning/dieback and/or lower trunk lesions) at the start of the trial. Photographs for future comparisons were taken of all canopies and at cardinal points around the base of each trunk. Baseline assessments of each tree included canopy disease rating, trunk lesion dimensions and lesion activity (recent bleeding/oozing). To ensure a balance of disease severities across treatments, trees were grouped into disease severity classes and then randomly assigned to the various treatments. Phosphite (Agrifos®600) at concentrations of either 7.5% or 20% was injected (20 ml at 20-cm intervals around the trunk) using Chemjet® stem injectors. Control trees were left untreated. After one year, half the previously injected trees were re-injected, in all cases with 7.5% phosphite. Phytotoxicity symptoms (leaf yellowing, browning or leaf/twig abscission) were noted in some PA-injected trees, particularly where the 20% concentration was used. In assessments made 2 years after initial treatment, canopy health and vigour was similar to or slightly worse than that noted initially in most trial trees, with no obvious differences between treatments. However, treatment differences were detected in the activity of trunk lesions. Averaged across sites, many more lesions remained active (expressing ooze, continued expansion) in untreated trees (46%) than in phosphite-treated trees (1.5%). Average lesion expansion after two years was 6.5 cm in untreated and 0.4 cm in PA-treated trees. There were no obvious differences in lesion activity/expansion among the different phosphite rates or regimes.

Poster 38

Epidemiology of *Phytophthora boodjera* nom. prov.; a damping-off pathogen in tree production nurseries in Western Australia

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The recently emerged plant pathogen *P. boodjera* prov. nom is responsible for damping-off and mortality of *Eucalyptus* seedlings in Western Australian (WA) nurseries. It emerged in 2011, in a nursery producing mostly eucalypt seedlings for restoration purposes in agricultural land. Symptoms included mortality or stunting of growth that was often not observed until seedlings reached the 4-6 true leaf stage. Extensive sampling on-site detected *P. boodjera* under benches, in drainage outlets, in tree shelterbelts, in used seedling trays and in the nursery lawn. Prior to seeding in November 2012, stringent hygiene was applied and to prevent contamination of potting mix, trays were boiled and machinery separated. Testing of potting mix and trays returned negative results for *P. boodjera* and yet it still reappeared in January 2013. Additionally, *P. boodjera* was also found in other nurseries after an up-regulation of the sampling regime. Studies are currently being undertaken to determine the epidemiology of this pathogen. Recent epidemiological studies have shown that: (a) *P. boodjera* can be reisolated from used seedling trays but not from sterilised or pasteurised trays, (b) when used seedling trays were seeded with various hosts only the *Eucalypts* become infected and died, (c) even though increased mortality of seedlings was observed along the drip lines on benches, the infection process does not require excess water, and (d) *P. boodjera* was not present in seed, fungal gnats or dust collected from the site. In the 2013 season, all trays containing potting mix were pasteurised and no symptoms developed in any seedlings although the pathogen is known to be persisting on site. This suggests that good hygiene coupled with pasteurisation prevented disease development. It also implies that the potting mix itself or on-site contamination of the potting mix are the most likely sources of *P. boodjera* inoculum in the trays.

Poster 39

Chemicals for management of red needle cast in *Pinus radiata* plantations in New Zealand: efficacy and persistence of phosphite and other fungicides

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Red needle cast (RNC), a foliar disease of *Pinus radiata* D. Don caused by *Phytophthora pluvialis* Reeser, has the potential to cause up to 38% growth loss in severely infected mature *P. radiata* plantations in New Zealand (Dick et al., 2014). A cost-effective, chemical control strategy is needed to provide a short-term management option for control of severe outbreaks of RNC in existing *P. radiata* forests. Phosphite, a fungicide known to be effective against diseases caused by *Phytophthora's*, is the primary active ingredient currently being investigated for its potential to manage RNC in *P. radiata* plantations in New Zealand. However, the efficacy of other fungicides is also being investigated. The results of controlled trials to determine the efficacy and persistence of phosphite, copper, metalaxyl-M and two disinfectants will be discussed together with the progress that needs to be made before an operational control strategy for control of RNC can be deployed. These results are discussed in context with the challenges that are faced in developing chemical control strategies for a new forestry disease of high economic importance. The importance of close collaboration between epidemiological research and pesticide application science for developing cost effective control is discussed.

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Poster 40

In vitro control of *Phytophthora cinnamomi* with *Brassica* pellet

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Phytophthora cinnamomi is the causal agent of serous epidemic in forest and natural ecosystems. Its control in the open field can only be done through integrated pest management protocols, often expensive and difficult to implement. The biofumigation, widely used in horticulture, could be a viable and effective alternative to the use of chemicals in agroforestry systems, given its easy use and low cost. In this paper we report the results of *in vitro* analysis of the inhibiting effect of the pellet of brassicas (BioFence®, Triumph Italy) on the growth of *P. cinnamomi.* The effect of treatments has been evaluated under different experimental conditions. Four biofumigant concentrations (5, 10, 20 and 40 mg; 40% humidity) at four different temperatures (15°C, 20°C, 25°C and 30°C) were tested *vs* an isolate of *P. cinnamomi* from *Castanea sativa* (Allumiere, Italy). The efficacy of treatment is strongly influenced by the ambient temperature; even at the temperature favourable to the development of the disease, 25°C. The Biofence, when was applied at a dose of 40 mg showed a fungicidal effect.

Poster 41

Wooden vectors of *Phytophthora ramorum:* Are Douglas-fir logs a risk?

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Sudden oak death, ramorum leaf blight, ramorum shoot dieback, and sudden larch death are diseases caused by *Phytophthora ramorum*. These diseases are examples of the consequences of the global trade of plant material. Globalization and increases in worldwide trade have unequivocally resulted in a higher frequency of exotic plant pathogen invasions and biosecurity challenges. Pathogens of the genus *Phytophthora* are exceptionally well suited to spread within the trade of plant material because of the production of resting spores and their benign behavior in their native range due to coevolution with the associated plant community. However, once introduced to new environments, susceptible hosts, or possibly even through sexual or somatic hybridization, these species can cause dramatic epidemics. In some areas of the sudden oak death epidemic in Oregon and California, Douglas-fir is the primary commercial species. Douglas-fir is listed as a host for *P. ramorum* because foliar infection has been observed under high inoculum pressure. Current regulations require foliage to be removed prior to transport outside of quarantined areas. Although it is not recognized as a bole host, a study was conducted to investigate the risk of spreading *P. ramorum* within Douglas-fir logs. The ability of *P. ramorum* to grow and survive within Douglas-fir sapwood and bark pieces was investigated in conjunction with developing methods to mitigate the risk of accidentally disseminating *P. ramorum* within the trade of Douglas-fir logs. Douglas-fir sapwood wafers were inoculated to investigate whether *P. ramorum* is capable of growing into the sapwood of Douglas-fir logs, and subsequently, the wafers were treated to determine whether the borate, disodium octaborate tetrahydrate, is effective at controlling *P. ramorum* within the sapwood of Douglas-fir logs.

Poster 42

Polyacrylamide and Movement of *Phytophthora ramorum* in Irrigation Water

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Water molds, including *Phytophthora ramorum* are high risk pathogens in the nursery industry. They affect the viability of the nursery industry, create serious quarantine concerns and, in many cases, threaten the health of susceptible natural ecosystems. Linear anionic polyacrylamide (PAM) is used in mitigating sediment runoff and other water quality issues in field crops [1] and was demonstrated to reduce transport of microorganisms associated with animal waste over and through soil [2]. A novel method of applying PAM directly in potting soils was tested to determine its effect on movement and viability of *P. ramorum* propagules in pot-leachate and runoff water. This method may limit plant disease and reduce infested water from entering recycling ponds and propagules from moving off site into natural water bodies.

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Poster 43

Introduction and spread of *Phytophthora ramorum* in Northern Ireland, UK

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Phytophthora ramorum was detected in Northern Ireland on plants, almost exclusively Rhododendron spp., in trade from 2002 – 2007 when in August it was found in Rhododendron plants in a number of gardens. The first record of *P. ramorum* on Japanese larch (*Larix kaempferi)* was in July 2010 on a mature larch stand on the Antrim Plateau in the east of the Province. Infected stands, plus larch trees in at least a 250 m buffer zones, were felled immediately and processed under strict biosecurity measures. Since that time each spring and late summer an aerial survey has been carried out of larch stands, symptomatic trees are identified and after confirmative diagnosis, symptomatic and buffer trees are felled. To date around 1,000 ha from a total larch area in N. Ireland of approximately 5,500 ha has been required to be felled due to the disease. In spite of the eradication and containment policy the pathogen has continued to spread north, south and west. It is possible that when trees are felled in response to symptom development that this is happening behind the actual disease front. The aerial survey often identifies stands with a small number of infected trees and this would appear to be the pattern in areas of low infection. On other occasions widespread symptom development occurs very rapidly. At Castelewellan Forest the aerial survey did not detect any symptoms in September 2012 while when the survey was conducted in spring 2013, over 90 ha of Japanese larch was severely damaged.

Poster 44

Surveillance and management of Kauri Dieback in New Zealand.

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Kauri dieback caused by *Phytophthora* „taxon Agathis" (PTA), has been prioritised as an emerging biosecurity threat to kauri (*Agathis australis*) and the kauri forest ecosystem of northern New Zealand. PTA is a water and soilborne pathogen that infects kauri through its roots. Post-infection symptoms of kauri dieback include; root rot, a collar rot resulting in large basal trunk lesions, canopy defoliation and death. All size and age classes of kauri have been confirmed as being susceptible to infection and death. In 2008, PTA was declared an unwanted organism and a national kauri dieback management programme was initiated. Standardised survey methods were developed to undertake national surveillance of prioritised forests to determine the distribution of PTA in New Zealand. Survey sites were prioritised to kauri areas with; high conservation value; containing culturally significant or iconic trees, or representing the natural geographic range of kauri. A risk assessment to determine current vectors and potential historic pathways of disease spread led to surveys and sampling also being undertaken in rural fragments containing remnant kauri, historic kauri plantations and nurseries, and at sites of high soil disturbance. Detection of PTA from soil and tissue was achieved by the development of specific baiting and isolation methods. Both aerial and ground based surveys were undertaken to locate symptomatic trees as was a passive surveillance programme whereby the public reported symptomatic trees for inspection and diagnosis. Since 2009 an adaptive management programme to contain disease spread and protect healthy kauri has also been implemented across kauri land. Management methods include; hygiene measures to reduce soilborne spread, vector control, upgrading visitor walking tracks and closing public access to some high value kauri areas. Long term monitoring is underway to assess efficacy of these management methods.

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