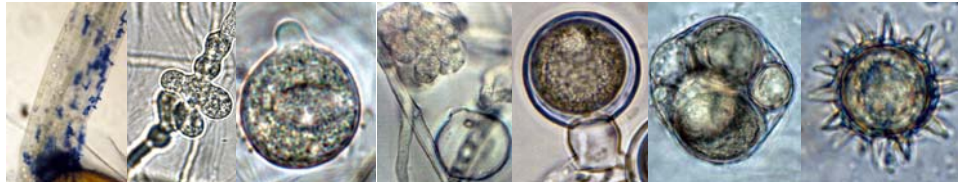
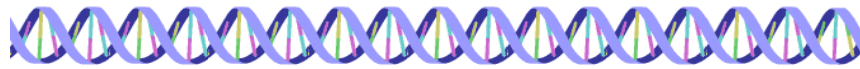
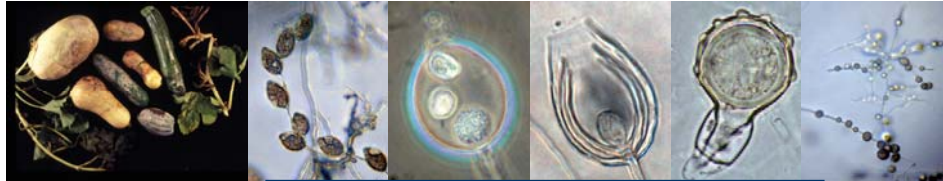

PHYTOPHTHORA/PYTHIUM and related genera **2008**

**Third International Workshop
Integration of Traditional and Modern Approaches for
Investigating the Taxonomy and Evolution**



CCACACC-TAAAAA---CTTCCACG

Turin, Italy August 23-24, 2008

Jolly Hotel Ambasciatori-Marconi Room

C.so V. Emanuele, 104 Torino Tel: +3901157521

USDA/APHIS/PPQ/PHP/PSPI Molecular Diagnostics Laboratory



**Associated with the 9th International Congress of Plant Pathology
Turin, Italy August 24-29, 2008
Lingotto Conference Centre**



Welcome Remarks

It is a great pleasure to welcome you all to the “Third International *Phytophthora*, *Pythium* and related genera workshop: Integration of Traditional and Modern Approaches for Investigating the Taxonomy and Evolution of the Oomycetes” presented in association to the 9th International Congress of Plant Pathology in Turin-Italy during August 23-24, 2008. The Program for the workshop features the contributions of the Keynote Speakers – world renown authorities in the area of Oomycetes – as well as the notable contributions of the Scientific Committee members. Participants eagerly anticipate the success of the event. I am very confident that you will enjoy the workshop, for these important genera of plant pathogens affect a significant number of important crops around the world.

My institution, the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Plant Health Programs (PHP), Plant Safeguarding and Pest Identification (PSPI) Molecular Diagnostics Laboratory (MDL), is very grateful to the Co-Chairs, Keynote Speakers, Instructors, Scientific Committee, Organizers, Collaborators, and Participants. The conference organizers would like to express our gratitude to sponsors of the workshop, including: SYNGENTA and the USDA-APHIS-PPQ for their generous support for the event. Their contributions have greatly enhanced the quality of this workshop. Our utmost appreciation and gratitude is given to Dr. Murali Bandla, Director of the USDA-APHIS-PPQ-PHP-Plant Safeguarding and Pest Identification, and Dr. Mary Palm, Director of the USDA-APHIS-PPQ-PHP-PSPI-Molecular Diagnostics Laboratory, for their support and encouragement of the achievement of this scientific event.

We are very pleased to inform you that ninety eight scientists from thirty seven countries will attend this workshop: Argentina (2), Australia (8), Austria (1), Bangladesh (1), Belgium (7), Brazil (2), Bulgaria (1), Cameron (1), Canada (5), Colombia (1), Chile (1), China (3), Egypt (2), France (4), Georgia (1), Germany (4), Ghana (1), Hungary (1), India (1), Indonesia (1), Italy (7), Japan (4), Mexico (1), Netherlands, (4), New Zealand (2), Nigeria (1), Norway (4), Oman (2), Puerto Rico (1), South Africa (2), Spain (7), Switzerland (1), Taiwan (1), Tunisia (2), UK (5), USA (8), and Venezuela (1).

Together with the Co-Chairs, Keynote Speakers, and Scientific Committee, we are very pleased to gather an outstanding group of participants who share common interests and goals, many of which belong to institutions working actively in the area of Oomycetes. Participants have indicated their excitement in receiving the contributions from colleagues bringing great experience gained from such a vast amount of research.

We hope you have an enjoyable visit to Turin-Italy -- the marvelous city that hosted the 2006 Winter Olympics, the city that hosts the Holy Shroud, as well as an extraordinary array of museums. We think that the combination of your scientific participation at the workshop and at the 9th ICPP2008, and your enjoyment of the tourist activities, will make your experiences in Turin-Italy truly memorable.

Gloria Abad, Ph.D. Chair



PHYTOPHTHORA/PYTHIUM AND RELATED GENERA 2008

**Third International *Phytophthora*, *Pythium* and related genera workshop:
Integration of Traditional and Modern Approaches for Investigating the
Taxonomy and Evolution of the Oomycetes.**

August 23-24, 2008

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Phytophthora, Pythium and related genera
August 23-24, 2008

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3rd International *Phytophthora*, *Pythium* and Related Genera Workshop

WORKSHOP PROGRAM: August 23-24, 2008

Workshop sessions will consist of one or two invited keynote presentations, each lasting 30 minutes, and up to six participant presentations, 15 minutes, each. Participants were selected to make presentations by the workshop Chairpersons, based on review of all the submitted abstracts.

Poster sessions will be displayed continuously for the entire Workshop at the facilities of the **Jolly Hotel Ambasciatori**. Special session for discussion of Posters is scheduled during the evening of Saturday 23.

Hands-off on morphological and molecular identification of *Phytophthora*, *Pythium* and related genera is scheduled for the afternoon of Sunday 24.

The following topics are scheduled for the oral presentations and posters:

- Systematics and phylogenetics
- Evolution and population genetics
- Nomenclature of present taxa and putative new species
- Morphological and molecular taxonomic methods
- Ecology, biogeography, and epidemiology
- Advances in systems for identification and diagnostics
- Integrating morphological and molecular tools for a unified phylogeny and classification



3rd International *Phytophthora*, *Pythium* and related genera workshop

PROGRAM:

SATURDAY - AUGUST 23, 2008

8:30 a.m. REGISTRATION

9:00 a.m.

Z. Gloria Abad. Introduction to the *Phytophthora*, *Pythium* and related genera workshop. USDA/ APHIS/ PPQ/ PHP/ PSPI/ Molecular Diagnostics Laboratory. USA. gloria.abad@aphis.usda.gov

SESSION: SYSTEMATICS AND PHYLOGENETICS. Moderators David Cooke and Arthur de Cock

9:15 a.m.

Clive Brasier. Keynote Speaker. How many *Phytophthora* species? History of *Phytophthora* diversity, predicting the numbers and implications for phylogeny and plant health. [C. Brasier](#). Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UNITED KINGDOM. clive.brasier@forestry.gsi.gov.uk

9:45 a.m.

Hermann Voglmayr. Keynote Speaker. Reconsidering the species problem in downy mildews – where are we now? [H. Voglmayr](#). Department of Systematic and Evolutionary Botany, University of Vienna, Rennweg 14, A-1030 Wien, AUSTRIA. hermann.voglmayr@univie.ac.at

10:15 a.m. BREAK

10:45 a.m.

André Lévesque. Keynote Speaker. Molecular phylogeny, barcoding and ecology of *Pythium* species. [C.A. Lévesque](#)^{1,2}, G.P. Robideau^{1,2}, [A.W.A.M. de Cock](#)³, N. Désaulniers¹, K. Bala^{1,2}, W. Chen^{1,2} and J.T. Tambong^{1,2}. ¹Agriculture and Agri-Food Canada, 960 Carling Ave., Ottawa, K1A 0C6, CANADA. ²Department of Biology, Carleton University, Ottawa, CANADA. ³Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, THE NETHERLANDS LevesqueCA@AGR.GC.CA

SESSION: EVOLUTION AND POPULATION GENETICS. Moderators Frank Martin and Andre Levesque

11:15 a.m.

David Cooke. Keynote Speaker. A clearing picture of *Phytophthora* evolution: from the wide-angle to the zoom lens for optimal phylogenetic focus. [D.E.L. Cooke](#). Scottish Crop Research Institute, Invergowrie, Dundee, SCOTLAND. david.cooke@scri.ac.uk

11:45 a.m.



Xuanli Ma. Races of *Phytophthora clandestina* causing subterranean clover root rot in the rainfall zones of the agricultural belt of Western Australia. *H. Li, X. Ma, P.H. Nichols, M.P. You, M.J. Barbetti and K. Sivasithamparam. School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, W.A. 6009 AUSTRALIA. xma@cyllene.uwa.edu.au*

12:00 a.m.

Isabelle De Dobbelaere. Genetic diversity of Belgian *Phytophthora ramorum* isolates. *I. De Dobbelaere, A. Vercauteren, K. Heungens and M. Maes. Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96 bus 2, 9820 Merelbeke, BELGIUM. kurt.heungens@ilvo.vlaanderen.be*

12:15 p.m. LUNCH

SESSION: NOMENCLATURE OF PRESENT TAXA AND PUTATIVE NEW SPECIES.
Moderators Arthur de Cock and Gloria Abad

1:15 p.m.

Michael Coffey. Keynote Speaker. The World Oomycetes Genetic Resource Collection (WOGRC) formerly World *Phytophthora* Collection (WPC): The history, mission, goals and projections for the future. *M. Coffey. WOGRC, University of California, Department of Plant Pathology, Riverside, CA, USA. coffey@urc.edu*

1:45 p.m.

André Lévesque. Separation of *Pythium* taxa using nuclear and mitochondrial DNA markers: Proposal of a new genus, *Phytopythium* gen. nov. *C.A. Lévesque^{1,2}, G.P. Robideau^{1,2}, Z.G. Abad³, A.W.A.M. de Cock⁴.¹Agriculture and Agri-Food Canada, 960 Carling Ave., Ottawa, K1A 0C6, CANADA. ²Department of Biology, Carleton University, Ottawa, CANADA. ³United States Department of Agriculture, USDA-APHIS-PPQ-PSPI Molecular Diagnostics Lab., Beltsville, MD, USA. ⁴Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, THE NETHERLANDS. LevesqueCA@AGR.GC.CA*

2:00 p.m.

Ross E. Beever. *Phytophthora* taxon Agathis, a threat to kauri in northern New Zealand? *R.E. Beever, S. Tsai, N.W. Waipara, I.J. Horner and T.D. Ramsfield Landcare Research, Private Bag 92170, Auckland 1142, New Zealand. BeeverR@landcareresearch.co.nz*

2:15 p.m.

Treana I. Burgess. Molecular re-evaluation of *Phytophthora* taxa collected over the past three decades from natural ecosystems in Western Australia. *M. Stukely¹, G. Hardy², D. White², J. Webster¹, J. Ciampini² and T.I. Burgess².¹DEC, Science Division, Locked Bag 104, Bentley D.C., WA 6983, AUSTRALIA. ²Centre for Phytophthora Science and Management, Murdoch University, Murdoch, WA 6150, AUSTRALIA. tburgess@murdoch.edu.au*

2:30 p.m.

Michael Coffey. Molecular Phylogeny of the Marine *Halophytophthora* Species. *M. Coffey. WOGRC, University of California, Department of Plant Pathology, Riverside, CA, USA. coffey@urc.edu*

2:45 p.m.



Motoaki Tojo. Advances in the morphological and molecular Identification of *Pythiogeton* species. [Z. G. Abad¹](#) and [M. Tojo²](#). ¹United States Department of Agriculture, USDA-APHIS-PPQ-PSPI Molecular Diagnostics Lab., Beltsville, MD, USA. ²Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Osaka 599-8531, JAPAN. tojo@plant.osakafu-u.ac.jp

3:00 p.m. BREAK

SESSION: MORPHOLOGICAL AND MOLECULAR TAXONOMIC METHODS.

Moderators Paul Tooley and Frank Martin

3:30 p.m.

Seogchan Kang. Keynote Speaker. *Phytophthora* Database: A cyberinfrastructure supporting the identification and monitoring of *Phytophthora* spp. [S. Kang¹](#), [J. Blair¹](#), [M. Coffey²](#), [D. M. Geiser¹](#), [K. Ivors³](#), [Y. Lee¹](#), [M. Mansfield¹](#), [F. Martin⁴](#), [B. Park¹](#), [J. Park⁵](#), and [M. Peiman²](#). ¹Dept. of Plant Pathology, Penn State, University Park, PA 16802, US. ² Dept. of Plant Pathology, University of California, Riverside, CA. ³Mountain Horticultural Crps Research & Extension Center, North Carolina State University, Fletcher, NC 28732. ⁴USDA-ARS, Salinas, CA 93905. ⁵School of Agriculture Biotechnology and Fungal Bioinformatics Laboratory, Seoul National University, Seoul, Korea. sxk55@psu.edu

4:00 p.m.

Lassaad Belbahri. Molecular taxonomy of recently described *Phytophthora* and *Pythium*. [L. Belbahri](#), [C. Calmin](#) and [F. Lefort](#). Plants and Pathogens Group, Institute Earth Nature and Landscape, School of Engineering of Lullier, University of Applied Sciences of Western Switzerland. 150 Route de Presinge, 1254 Jussy, SWITZERLAND. lassad.belbahri@hesge.ch

4:15 p.m.

Paloma Abad-Campos. A survey of *Phytophthora* and *Pythium* associated to export crops in Guatemala. [P. Abad-Campos¹](#), [A. Pérez-Sierra¹](#), [L.A. Álvarez¹](#), [J. Armengol¹](#), [R. López-Pineda²](#), [A. Sánchez-Pérez²](#), [E. Rodríguez-Quezada³](#) and [G. Álvarez-Valenzuela²](#). ¹ Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia. SPAIN. ²Facultad de Agronomía, Universidad San Carlos de Guatemala. GUATEMALA. ³ Laboratorio de Diagnóstico Fitosanitario, Ministerio de Agricultura, Ganadería y Alimentación. GUATEMALA. pabadcam@eaf.upv.es

4:30 p.m.

Geoffrey Denton. *Phytophthora* diversity in UK gardens. [G. Denton](#), [J. Denton](#), [I. Waghorn](#) and [B. Henricot](#). Plant Pathology Department, The Royal Horticultural Society, Wisley, Woking, Surrey. GU23 6QB UK. geoffdenton@rhs.org.uk

4:45 p.m.

Ronald French-Monar. Characterization of isolates of *Phytophthora* species isolated from pepper and cucurbits in Texas. [R.D. French-Monar¹](#), [A. Patton¹](#), [Z. G. Abad²](#). AgriLife Extension-Texas A&M, Department of Plant Pathology and Microbiology, Amarillo, TX, USA. ²United States Department of Agriculture, USDA-APHIS-PPQ-PSPI Molecular Diagnostics Lab., Beltsville, MD, USA. rdfrench@ag.tamu.edu

5:00 p.m. 6:30 p.m. Posters

6:30 p.m. 9:30 p.m. Social + posters



SUNDAY - AUGUST 24, 2008

**SESSION: ECOLOGY, BIOGEOGRAPHY, AND EPIDEMIOLOGY. Moderators
Clive Brasier and Michael Coffey**

9:00 a.m.

Paul Tooley. Keynote Speaker. Ecology, biogeography and epidemiology of the devastating *Phytophthora infestans*, *P. cinnamomi*, and *P. ramorum*. [P. Tooley](#). United States Department of Agriculture- Agricultural Research Service (USDA-ARS), Ft. Detrick, MD, USA. paul.tooley@ars.usda.gov

9:30 a.m.

Thomas Jung. Widespread *Phytophthora* infestations of nursery stock in Central Europe as major pathway of *Phytophthora* diseases of forests and semi-natural ecosystems. [T. Jung](#)¹, Jörg Schumacher², Sindy Leonhard², Günter Hartmann³, Thomas Cech⁴, Tomasz Oszako⁵, Barbara Duda⁵, Grazyna Szkuta⁶ and Leszek B.Orlikowski⁷ ¹*Phytophthora* Research and Consultancy, Thomastrasse 75, D-83098 Brannenburg, GERMANY. ²Federal Biological Research Centre for Agriculture and Forestry (BBA), Institute for Plant Protection in Forests, Messeweg 11/12, D-38104 Braunschweig, GERMANY. ³Lower Saxony Forest Research Station, Grätzelstrasse 2, D-37079 Göttingen, GERMANY. ⁴Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW), Seckendorff-Gudent-Weg 8, A-1131 Vienna, AUSTRIA. ⁵Forest Research Institute, Bitwy Warszawskiej 920 No 3, 00-973 Warsaw, POLAND. ⁶State Plant Health & Seed Inspection, Zwirki Iand Wigury 73, 87-100 Torun, POLAND. ⁷Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, POLAND. dr.t.jung@t-online.de.

9:45 a.m.

Marieka Gryzenhout. An emerging needle blight disease of *Pinus radiata* in Chile. R. Ahumada, A. Duran, B. Slippers, [M. Gryzenhout](#), B. Wingfield, A. Rotella, F. Flores and M. Wingfield Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0001, SOUTH AFRICA. marieka.gryzenhout@fabi.up.ac.za

10:00 a.m. BREAK

10:30 a.m.

Koji Kageyama. Assessment of river environment using *Pythium* species. [K. Kageyama](#), M. Senda, and H. Suga. River Basin Research Center, Gifu University, Gifu 501-1193, JAPAN. kageyama@green.gifu-u.ac.jp

10:45 a.m.

Eduardo Moralejo. Trade-offs between virulence and sporulation in *Phytophthora ramorum*. [E. Moralejo](#) and E. Descals. IMEDEA (CSIC-UIB), P.O. Box 07190, Esporles, Balearic Islands, SPAIN. vieaemr@uib.es



11:00 a.m.

Jan Nechwatal. Flooding events and rising water temperatures increase the significance of the reed pathogen *Pythium phragmitis*. [J. Nechwatal](#), A. Wielgoss and K. Mendgen. Universität Konstanz, Phytopathology, D-78457 Konstanz, GERMANY. jan.nechwatal@uni-konstanz.de

11:15 a.m.

Hemilse Palmucci and Pablo Grijalba. Status of the genera *Phytophthora* and *Pythium* in Argentina. [H.E. Palmucci](#)¹, S.M. Wolcan², [P.E. Grijalba](#)^{1,1} Cátedra de Fitopatología Facultad de Agronomía. Universidad de Buenos Aires Avenida San Martín 4453. CP 1416 Buenos Aires, Argentina ²CIC, Centro de Investigaciones de Fitopatología, UNLP. ARGENTINA. palmucci@agro.uba.ar

11:30 a.m.

Peter Scott. Pythiaceae root pathogens associated with *Eucalyptus gomphocephala* decline in Western Australia. [P.M. Scott](#), B.L. Shearer, P.A. Barber, T. Jung, T. Burgess, I.J. Colquhoun and G.E. Hardy School of Biological Science and Biotechnology, Murdoch University, Murdoch, Western Australia 6150, AUSTRALIA. Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch, Western Australia 6150, AUSTRALIA. P.Scott@murdoch.edu.au

11:45 a.m.

Joan Webber. Persistence of *Phytophthora kernoviae* and *P. ramorum* on infested sites and the impact on disease management. [Joan Webber](#). Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK. joan.webber@forestry.gsi.gov.uk

12:00 p.m. LUNCH

SESSION: ADVANCES IN SYSTEMS FOR IDENTIFICATION AND DIAGNOSIS.

Moderators Jorge Abad and Ronald French-Monar

1:00 p.m.

Frank Martin. Keynote Speaker. Advances in systems for identification and diagnosis of *Phytophthora*, *Pythium* and related genera. [F. Martin](#). United States Department of Agriculture- Agricultural Research Service (USDA-ARS), Salinas, CA, USA. Frank.Martin@ars.usda.gov

1:30 p.m.

Marko Riedel. Can nanobiotechnology help in routine diagnosis to determine *Phytophthora* species? [M. Riedel](#)¹, S. Julich², R. Möller², M. Kielpinski³, T. Henkel², S. Wagner¹, A. Breitenstein³, S. Werres¹. 1 - Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Horticulture and Forests, Messeweg 11/12, 38104 Braunschweig, GERMANY. 2 - Institute of Photonic Technology e.V., POB 100239, D-07702 Jena, GERMANY. 3 - BECIT GmbH Halle, Weinbergweg 23, D-06120 Halle/Saale, GERMANY.

Marko.Riedel@jki.bund.de; Sabine.Werres@jki.bund.de

1:45 p.m.

Peter Bonants. Quantitative multiplex detection of plant pathogens, including *Phytophthora* species, based on PRI-lock probe technology and the OpenArray platform. [P.J.M. Bonants](#), R. van Doorn, O. Mendes, R.A. van Hoof and C.D. Schoen Plant Research International BV, PO BOX 16, 6700 AA Wageningen, THE NETHERLANDS. peter.bonants@wur.nl



SESSION: INTEGRATING MORPHOLOGICAL AND MOLECULAR TOOLS FOR A UNIFIED PHYLOGENY AND CLASSIFICATION. Moderators Andre Levesque and Hermann Voglmayr

2:00 p.m.

Z. Gloria Abad. Keynote Speaker. Development of a Morphological/Molecular Lucid Key for the identification of Oomycetes: *Phytophthora*. [Z. G. Abad¹](#) and [M. Coffey²](#). ¹United States Department of Agriculture, USDA-APHIS-PPQ-PSPI Molecular Diagnostics Lab., Beltsville, MD, USA. ²WOGRC, University of California, Department of Plant Pathology, Riverside, CA, USA. gloria.abad@aphis.usda.gov

2:30 p.m.

Arthur de Cock. Keynote Speaker. *Pythium*: morphological taxonomy after the molecular revision. [A.W.A.M. de Cock](#), [Z.G.Abad](#), [C.A. Lévesque](#), [G.P. Robideau](#) and [H. Brouwer](#). Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, THE NETHERLANDS. a.decock@cbs.knaw.nl
[Locations needs some work](#)

3:00 p.m BREAK

3:30 p.m.

Michael Coffey. Past and Current Taxonomic Status of *P. cryptogea* and *P.drechleri* and Associated Species. [M. Coffey](#). WOGRC, University of California, Department of Plant Pathology, Riverside, CA, USA. coffey@urc.edu

4:00 p.m. -5:00

Hands off training for the morphological and molecular identification of *Phytophthora*, *Pythium* and related genera. INSTRUCTORS: *Phytophthora* Gloria Abad, Michael Coffey, and David Cooke. *Pythium*: Ronald French, Frank Martin, and Motoaki Tojo.

5:00 p.m.

Remembrances of the 3rd International *Phytophthora*, *Pythium* and related genera workshop and certificates.

5:30 p.m.

Close of the Event.



POSTERS

EVOLUTION AND POPULATION GENETICS

Cloning and expression of a pectin methylesterase gene from the Oomycete Plant Pathogen *Phytophthora capsici*, [B. Zh. Feng](#), Y. J. Jia and X. G. Zhang. Department of Plant Pathology, Shandong Agricultural University, Tai'an, 271018, CHINA. zhxg@sdau.edu.cn

Initial assessment of genotypic diversity of *Phytophthora ramorum* associated with Washington state ornamental nurseries. [G. Chastagner](#)¹, N. Dart², and K. Coats¹
¹Washington State University, Puyallup, WA, ²West Virginia Department of Agriculture, Charleston, WV. chastag@wsu.edu

Population Structure and Sensitivity to Phenylamides of *Phytophthora ramorum* in Spain. [A. Pérez-Sierra](#), [L.A. Álvarez](#), L. Fuster, E. Landeras, J. García-Jiménez and [P. Abad-Campos](#). Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia. SPAIN. aperesi@eaf.upv.es

Phosphite application as an explorative tool in *Eucalyptus gomphocephala* decline in Western Australia. [P.M. Scott](#), H.T. Eslick, B.L. Shearer, P.A. Barber, M.C. Calver, I.J. Colquhoun and G.E. Hardy. Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences Murdoch University, Murdoch, Western Australia 6150, AUSTRALIA. P.Scott@murdoch.edu.au

PCR-RFLP markers identify three lineages of the North American and European populations of *Phytophthora ramorum*. M. Elliott, G. Sumampong, A. Varga, [S.F. Shamoun](#), D. James, S. Masri, S.C. Brière, and N.J. Grünwald. Canadian Forest Service, Pacific Forestry Centre, Victoria, BC, Canada V8Z 1M5. CANADA SShamoun@nrcan.gc.ca

NOMENCLATURE OF PRESENT TAXA AND PUTATIVE NEW SPECIES

Morphological and molecular identification of five putative new *Phytophthora* species from the USA. [Z. G. Abad](#)¹, M. Palm¹, R. Shukla¹, S. Rice¹, J. Rascoe¹, T. Creswell², and S. Nelson³.
¹United States Department of Agriculture, USDA-APHIS-PPQ-PSPI Molecular Diagnostics Lab., Beltsville, MD, USA. ²Purdue University, West Lafayette, IN 47907, ³University of Hawaii at Manoa, Hilo, HI, USA. gloria.abad@aphis.usda.gov

Diversity of deciduous long-pedicellate *Phytophthora* isolates associated to export crops in Guatemala. [L.A. Álvarez](#)¹, [A. Pérez-Sierra](#)¹, B. Porta², S. Ramírez², G. Álvarez² and [P. Abad-Campos](#)¹.
¹Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia. SPAIN. ²Facultad de Agronomía, Universidad San Carlos de Guatemala. GUATEMALA. lualber@eaf.upv.es

A new *Pythium* species isolated from cavity spots on carrots in Norway. [M.L. Herrero](#), S.S. Klemsdal and A. Hermansen. Norwegian Institute for Agricultural and Environmental Research,



Plant Health and Plant Protection Division.. Høgskoleveien 7, 1432 Ås, NORWAY.
maria.herrero@bioforsk.no

MORPHOLOGICAL AND MOLECULAR TAXONOMIC METHODS

Morphological and molecular identification of *Phytophthora* isolates from declining forest stands in Hungary. [I. Szabó](#) and F. Lakatos. Institute of Silviculture and Forest Protection, P. O. Boks 132, University of West Hungary, H-9400 Sopron, HUNGARY. szaboi@emk.nyime.hu

Detection of *Phytophthora* and *Pythium* species on subtropical crops in Andalusia region, Spain. [P.M. Martín-Sánchez](#), T. Zea-Bonilla and [R.M. Pérez-Jiménez](#). Instituto Andaluz de Investigación y Formación Agraria (IFAPA-CICE), Centro de Churriana, Cortijo de la cruz s/n, Churriana, 29140, Málaga, SPAIN rosa.perez.jimenez.ext@juntadeandalucia.es

ECOLOGY, BIOGEOGRAPHY, AND EPIDEMIOLOGY

***Phytophthora* species associated to branch cankers on Persian lemon trees in Guatemala.** [P. Abad-Campos](#)¹, [A. Pérez-Sierra](#)¹, [L.A. Álvarez](#)¹, [R. López](#)², [L. Reyes](#)², [R. Mijangos](#)² and [G. Álvarez](#)². 1. Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia. SPAIN. 2. Facultad de Agronomía, Universidad San Carlos de Guatemala. GUATEMALA. pabadcam@eaf.upv.es

Collar canker of apple trees caused by *Phytophthora* spp. in Tunisia. [N. Boughalleb](#)^a, [M. Souli](#)^a, [L. Álvarez](#)^b, [A. Pérez-Sierra](#)^b, [J. Armengol](#)^b and [P. Abad-Campos](#)^b ^aHigher Institute of Agronomy- Chott Mariem, Biological Sciences and Protection of Plants Department , 4042 Sousse, Tunisia. ^bInstituto Agroforestal Mediterraneo, Universidad Politecnica de Valencia, Camino de Vera s/n, 46022, Valencia. SPAIN.

Occurrence of *Pythium* spp. on apple orchards in Tunisia. [M. Souli](#)^a, [N. Boughalleb](#)^a, [L. Álvarez](#)^b and [P. Abad-Campos](#)^b ^aHigher Institute of Agronomy- Chott Mariem, Biological Sciences and Protection of Plants department , 4042 Sousse, Tunisia.. ^bInstituto Agroforestal Mediterraneo, Universidad Politecnica de Valencia, Camino de Vera s/n, 46022, Valencia. SPAIN.

***Phytophthora* on carrot: evolution of species involved in ring rot disease in France.** [D. Breton](#), [M. Prunier](#) and [F. Montfort](#). INRA- UMR1099-Bio3P Centre de Rennes-Domaine de la Motte –BP 35327, F35653 Le Rheu, FRANCE. danielle.breton@rennes.inra.fr

Evaluation of certain traditional cultural practices for the management of *Phytophthora* blight of potato in organic farming system. [GKN Chhetry](#) and [H.C.Mangang](#). Department of Life Sciences, Manipur university, Canchipur, Imphal-795003, INDIA. gknc2004@yahoo.co.in

New insights in the life cycle of white tip disease (*Phytophthora porri*) in leek (*Allium porrum*). [B. Declercq](#), [N. Cap](#), [J. De Nies](#), [S. Pollet](#) and [M. Höfte](#). Laboratory of Phytopathology,



Faculty of Bioscience Engineering, Coupure Links 653, Ghent University, BE-9000 Gent, BELGIUM. Bart.Declercq@Ugent.be

Research on *Phytophthora* diseases of crops in Bangladesh. [Dey, T. K.](#) Principal Scientific Officer, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, BANGLADESH

Research on Progress of *Pythium* diseases in Bangladesh. [Dey, T. K.](#) Principal Scientific Officer, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, BANGLADESH

Residual effectiveness of fungicides in protecting rhododendron leaves from *Phytophthora ramorum*. [G. Chastagner¹](#), [A. DeBauw¹](#), [K. Riley¹](#), and [N. Dart²](#).

¹Washington State University, Puyallup, WA, ²West Virginia Department of Agriculture, Charleston, WV. chastag@wsu.edu

Phytophthora root rot in avocado in Puerto Rico. [C. Estevez de Jensen¹](#) and [H. A. Violi²](#)

¹University of Puerto Rico, Department of Crop Protection Department, Mayagüez Campus, P.O. Box 9030, Mayagüez, PR 00681-9030, USA. ²Department of Plant Pathology, Tropical Research and Education Center, University of Florida, 18905 SW 280th Street, Homestead, FL 33031, USA. cestevez@uprm.edu

Cloning and expression of a pectin methylesterase gene from the Oomycete Plant Pathogen *Phytophthora capsici*. [B. Zh. Feng](#), [Y. J. Jia](#) and [X. G. Zhang](#). Department of Plant Pathology, Shandong Agricultural University, Tai'an, 271018, CHINA. zhxg@sdau.edu.cn

Does waterlogging influence phosphite protection of *Banksia* species to *Phytophthora cinnamomi*? [D. Hüberli](#), [T. Paap](#), [K. Gower](#), [N. Long](#), [B. Dell](#) and [G.E.St.J. Hardy](#). Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Perth, WA 6150, AUSTRALIA. D.Huberli@murdoch.edu.au

Does fire influence phosphite protection of Western Australian indigenous plant species to *Phytophthora cinnamomi*? [D. Hüberli](#), [T. Paap](#), [N.A. Moore](#), [S. Barrett](#), [G. Freebury](#), [B. Dell](#) and [G.E.St.J. Hardy](#). Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Perth, WA 6150, AUSTRALIA. D.Huberli@murdoch.edu.au

Efficacy of fungicides applied to the soil for management of *Phytophthora* root and crown rot on chile peppers . [M.E. Matheron](#) and [M. Porchas](#). Yuma Agricultural Center, The University of Arizona, Yuma, Arizona, USA. matheron@ag.arizona.edu

Root and crown rots of green beans caused by several species of *Pythium* in southeast Andalucía, Spain. [Serrano¹](#), [Y.](#), [Melero-Vara²](#), [J.M.](#) and [Gómez¹](#), [J.](#) ¹Centro de Investigación y Formación Agraria "La Mojonera-La Cañada". IFAPA. 04745 Almería, Spain. ²Instituto de Agricultura Sostenible, CSIC, Apdo. 4084. 14080 Córdoba, SPAIN. cs9mevaj@uco.es

A survey for *Phytophthora* diseases in ornamental plants in Tennessee commercial nurseries. [M.T. Mmbaga](#), [L. Santamaria](#), and [R.J. Sauv e](#). Tennessee State University, Otis Floyd Nursery Res. Center, McMinnville, TN 37110. USA. mmmbaga@tnstate.edu

Phytophthora root and crown rot on fruit trees in Bulgaria. [M.B. Nakova](#). Department of Phytopathology, Agricultural University Plovdiv, 12 Mendeleev St., Plovdiv 4000, Bulgaria, tel. +359 32 654223. BULGARIA. mnakova@yahoo.com ; mnakova@au-plovdiv.bg



Monitoring of *Phytophthora* species on fruit trees in Bulgaria. [M.B. Nakova](#). Department of Phytopathology, Agricultural University Plovdiv, 12 Mendeleev St., Plovdiv 4000, Bulgaria, tel. +359 32 654 223. BULGARIA. mnakova@yahoo.com

Phosphite application as an explorative tool in *Eucalyptus gomphocephala* decline in Western Australia. [P.M. Scott](#), H.T. Eslick, B.L. Shearer, P.A. Barber, M.C. Calver, I.J. Colquhoun and G.E. Hardy. Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences Murdoch University, Murdoch, Western Australia 6150, AUSTRALIA. P.Scott@murdoch.edu.au

Genetic Diversity of Pathogenic and Nonpathogenic populations of *Phytophthora capsici* Isolated from Pepper Plants and Soil. W. X. Sun, Y. J. Jia, and [X. G. Zhang](#). Department of Plant Pathology, Shandong Agricultural University, Taian, 271018, CHINA. zhxg@sdau.edu.cn

ADVANCES IN SYSTEMS FOR IDENTIFICATION AND DIAGNOSTICS

Detection of *Phytophthora ramorum* and *P. kernoviae* using immuno assay specific monoclonal antibodies. [Avila, F.](#)¹, Chung, K.,¹ Frick, A.¹, Schoedel, B.¹, Coffey, M.², and [Z.G. Abad](#)³ ¹AGDIA Inc., Elkhart, IN, USA, ²World Phytophthora Collection, University of California, Department of Plant Pathology, Riverside, CA, USA, ³United States Department of Agriculture, USDA-APHIS-PPQ-PSPI Molecular Diagnostics Lab., Beltsville, MD, USA. favaila@agdia.com

Development of a real-time PCR assay to quantify *P.violae* and *P.sulcatum* in soil samples. [D.Breton](#), S. Morliere and F. Montfort.. INRA, UMR1099-Bio3P Centre de Rennes-Domaine de la Motte –BP35327, F35653 Le Rheu, FRANCE. danielle.breton@rennes.inra.fr

Elicitor (PiP) and Suppressor from *Phytophthora infestans* Regulate Ca²⁺-Dependent Protein Kinase (CDPK) in the Membrane of Potato. [Naotaka Furuichi](#)^{1,2*}, Kazutoshi Yokokawa¹ and Tomoo Okuta¹ ¹ Graduate School of Science and Technology, Niigata University, Niigata, JAPAN ² Faculty of Agriculture, Niigata University, Niigata 950-2181, JAPAN. nfuru@agr.niigata-u.ac.jp

Rapid identification of the pine needle and shoot pathogen *Phytophthora pinifolia* nom. prov. using species-specific PCR primers. A. Durán, B. Slippers, [M. Gryzenhout](#), R. Ahumada, B.D. Wingfield and M.J. Wingfield. Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, SOUTH AFRICA. Marieka.gryzenhout@fabi.up.ac.za

Genetic transformation of *Phytophthora ramorum* with the jelly fish GFP Gene. G. Calmin¹, M. Riedel², L. Belbahri¹, S. Wagner², S. Werres², and [F. Lefort](#)¹ ¹Plants and Pathogens Group, Institute Earth Nature and Landscape, School of Engineering of Lullier, University of Applied Sciences of Western Switzerland. 150 Route de Presinge, 1254 Jussy, SWITZERLAND. ²Institute for Plant Protection in Horticulture, Federal Biological Research Centre for Agriculture and Forestry (BBA), Messeweg 11/12, D-38104 Braunschweig, GERMANY. lassad.belbahri@hesge.ch

Molecular detection of *Phytophthora cryptogea* on *Calendula officinalis* and *Gerbera jamesonii* artificially inoculated with zoospores. [Y. Li](#), A. Garibaldi and M. L. Gullino. Centre



of Competence for the Innovation in the Agro-Environmental field, AGROINNOVA, University of Torino, via Leonardo da Vinci, 44, 10095 Grugliasco, Torino, ITALY. liyuan80416@yahoo.it

Modifications of PARP Medium Using Fluazinam, Miconazole, and Nystatin for Detection of *Pythium* spp. in Soil. [M. Tojo](#) and Y. Morita. Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Osaka 599-8531, JAPAN. tojo@plant.osakafu-u.ac.jp

Reliable discrimination of distinct *Phytophthora hedraiaandra* x *cactorum* hybrids through real-time PCR. [K. Van Poucke](#), [I. De Dobbelaere](#), K. Heungens and M. Maes. Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96 bus 2, 9820 Merelbeke, BELGIUM. kris.vanpoucke@ilvo.vlaanderen.be

3rd International Phytophthora and Pythium Workshop:



**Integration of Traditional and Modern Approaches for Investigating the Taxonomy and Evolution of
Phytophthora, *Pythium* and Related Genera**
August 23-24, 2008 – Turin, Italy in association with the 9th ICPP 2008

ABSTRACTS SUBMITTED BY AUTHORS

KEYNOTE SPEAKERS

**Development of a Morphological/Phylogenetic Lucid Key for the identification of Oomycetes:
Phytophthora.**

Z. G. Abad¹ and M. Coffey².

¹United States Department of Agriculture, USDA-APHIS-PPQ-PSPI Molecular Diagnostics Lab., Beltsville, MD, USA.

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Members of the genus *Phytophthora* occur worldwide and cause serious diseases to numerous cultivated crops, forestry, and natural vegetation. Many species of *Phytophthora* are reported causing root, crown, collar rots, wilts, leaf and stem blights, fruit and tuber rots throughout the world. The genus consists of 88 species (to the present 8/19/08) and harbors some devastating plant pathogens that have a large impact in agriculture, among them are *P. infestans*, *P. capsici*, *P. cinnamomi* and *P. ramorum*. Correct identification to species level is the first important condition to the accurate manage of the disease and to protect from dispersal of important pathogens like members of *Phytophthora* Genus around the world. In the last 10 years, twenty four species have been described on the basis of morphological and molecular supporting data. These species are in addition to the 64 morphological species presented in the *Phytophthora* treatise of Erwin and Ribeiro (1996). Powerful molecular tools have been introduced in the last ten years including sequencing analysis of the ITS, TEF 1 α , β -tubulin, Cox I and Cox II. These markers have been used for phylogenetic studies and for the validation of the new *Phytophthora* taxa reported recently. Although there have been considerable advances in the taxonomy of these organisms, the identification to species level using molecular characters remains a major challenge to plant pathologists. A great number of sequences for taxa present at GenBank are misidentified. Many species “complexes” can be observed in phylogenetic trees showing the presence of many potential cryptic species. Numerous putative new species are in progress for official description in different laboratories around the world. An interactive Morphological/Phylogenetic Lucid Key is under development. Aspects of the development of this key will be discussed.

How many *Phytophthora* species? History of *Phytophthora* diversity, predicting the numbers and implications for phylogeny and plant health

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Between De Bary's description of *P. infestans* in 1876 and the end of the 20th Century there was a steady but unremarkable rise in the number of described *Phytophthora* species to about 55. Since then there has been a dramatic increase, with almost as many species being described between 2000 and now as in the previous 125 years. This is probably due the application of modern population-based species concepts, to the application of molecular tools, to increased international trade in plants and to increased field sampling. A simple estimation approach suggests the number of extant *Phytophthora* spp. could lie between 200 and 600; and that between 140 and 540 species were still undescribed at the beginning of this century. Some of these 'missing' taxa are now being revealed - as phylotaxa - by molecular probing of the environment. The above developments have considerable implications for the plant health risks to forests and natural ecosystems. They also have implications for our interpretation of *Phytophthora* molecular phylogenies. These aspects will be discussed.

***Pythium*: morphological taxonomy after the molecular revision.**

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Taxonomy of the genus *Pythium* has traditionally been based on morphological characteristics. Species could be distinguished from each other by comparing sets of characters from vegetative and sexual reproductive structures. In the last decade many molecular studies on phylogeny and classification of *Pythium* have been published. New species descriptions almost always contain a molecular element and some species have been split up when DNA sequences showed that they were heterogeneous. Since virtually all recognized species of *Pythium* have been involved in recent DNA sequencing studies we can now evaluate the relevance of the different morphological characters. The main division of *Pythium* is based on sporangium shape and oogonium ornamentation. Sporangium type correlates well with



phylogenetic branching. Moreover, a close look at the phylogeny reveals a new sporangium type which is present only in one clade: the contiguous type. Though oogonium ornamentation is scattered all through the evolutionary tree, certain types of ornamentation are confined to single clades. One clade of *Pythium* is more closely related to *Phytophthora* than to other *Pythium* species. Species in this clade produce sporangia which are morphologically more or less similar to those in *Phytophthora*, however, the same sporangium shape occurs in some other *Pythium* species as well. Since this clade is also phylogenetically between *Pythium* and *Phytophthora*, making *Pythium* polyphyletic, we are proposing a new genus called *Phytopythium* for this clade. Many morphological characters do not correlate with phylogeny but may, nevertheless, still be useful in classification and identification. Some species show unique morphological structures.

Michael D Coffey. Keynote Speaker. The World Oomycetes Genetic Resource Collection (WOGRC) formerly World *Phytophthora* Collection (WPC): The history, mission, goals and projections for the future.

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The roots of the WPC were established in the 1960s by Professor Erwin and Professor Zentmyer from separate collections of *Phytophthora* species reflecting their separate research interests. Erwin worked mainly with pathogens of *Medicago sativa* and this led to the description and collection of different *P.megasperma*/*P. cryptogea* strains. George Zentmyer studied *P. cinnamomi* on avocado and other hosts and different morphological forms (MF1, MF3, MF4) attacking cacao. These early endeavors established a core species collection at UCR. In 1981 Professor Michael Coffey took over the curatorship of the UCR *Phytophthora* species Collection. A major development occurred in 1986 when the UC Genetic Resources Conservation Program (UCGRCP) provided funding to allow cryopreservation of the approximately 600 accessions. Over the 22 years the number of accessions in the WPC has grown to ~9500 representing over 95 species. Also represented are accessions representing the 15 described species within the marine genus *Halophytophthora*. In the last 12 years a concerted effort has been made to add a large collection of *Pythium* species to the cryostorage inventory. In the last two years this effort has grown exponentially and there are now over 900 accessions representing 97 species of *Pythium*. Also in the last two years an initial effort has been made to rescue the remnants of the Michael W. Dick Aquatic Phycomycetes Collection (APCC) now held at CABI. This collection is endangered and the rescue effort will continue. The original mission of the **World *Phytophthora* Collection (WPC)** has been broadened with the accumulation of the phylogenetically comprehensive *Pythium* Collection and will continue with the acquisition of additional genera within the Kingdom Straminipila (Dick 2001). The new name for this collection will be the **World Oomycetes Genetic Resource Collection (WOGRC)**. The future goals of the WOGRC are threefold: 1) maintenance and expansion of a worldwide collection of genera within the Kingdom Straminipila, 2) development of a DNA Bank to provide DNA for research, and 3) creation of online databases providing phenotype and genotype information on important genera.

A clearing picture of *Phytophthora* evolution: from the wide-angle to the zoom lens for optimal phylogenetic focus.

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Dramatic changes in our understanding of *Phytophthora* evolution are underway on a number of fronts. At a fundamental level we are learning more about the roots of the Oomycete branch of the tree of life. Phylogenomics is expanding our understanding of this field and comparative genomics is allowing insights into what makes *Phytophthora* a pathogen. The main branches of the *Phytophthora* tree are now established; the tips it would seem, are ever-expanding. The application of PCR-based environmental monitoring has a role to play here. At the finest resolution, individuals within populations of several *Phytophthora* species are being studied to determine the origins and pressures driving population change. Examples of each of these areas will be examined in this review paper.

***Phytophthora* Database: A cyber infrastructure supporting the identification and monitoring of *Phytophthora* spp.**

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The rapid expansion of global commerce and human travel has greatly accelerated the introduction of non-indigenous pathogens and exotic variants of indigenous ones. Given the pathogen movements across political boundaries and the interconnections among national economies, our response should also be coordinated with international partners. However, to date efforts to study and manage this threat have been fragmented, mostly regional, and limited to coping



with immediate crises. Due to their virulence and ability to spread rapidly, *Phytophthora* is one of the most destructive groups of plant pathogens. Given the global nature of *Phytophthora* problems, efforts to map and document the diversity and distribution of *Phytophthora* worldwide and to share this information are essential to significantly improve our ability to track and manage *Phytophthora*. The goal of the *Phytophthora* Database project (<http://www.phytophthoradb.org>) is to archive genotypic and phenotypic diversity of *Phytophthora* in a highly integrative cyber infrastructure that can easily be searched and updated. The database provides a number of data search, analysis and visualization tools to support identification and risk assessment of newly isolated *Phytophthora*. Geographic Information Systems tools will be incorporated to support the visualization of the distribution and change of *Phytophthora* species and their diseases across environmental, geospatial and temporal contexts. The database and associated tools can easily be adopted with minimal modification to create similar cyber infrastructures for different pathogen groups.

Molecular phylogeny, barcoding and ecology of *Pythium* species.

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The Internal Transcribed Spacer (ITS) region has been used extensively in both oomycetes and fungi for identification purposes whereas the 5' end of the Cytochrome Oxidase I gene (COI) is the GenBank approved barcode region that is being used routinely for animal identification. We have sequenced both regions for over 500 isolates of the *Pythium* strains in the CBS and DAOM collections in the Netherlands and in Canada, respectively. The 5' half of the LSU was also sequenced for at least one representative strain of each species. The three regions provided comparable phylogenies. Both ITS and COI generate enough resolution to separate the well accepted species of *Pythium*. The rate of divergence in COI and ITS are different and the most variable region out of the two is different depending on the clade, giving ITS and COI a differential resolution advantage depending on the clade. ITS has been used extensively to design assays for ecological studies but COI appears to have a similar potential. Quantitative PCR and DNA arrays have been designed to study the ecology of *Pythium* species and both approaches appear to be complementary. Recent developments in sequencing technology and oomycete genomics are opening new opportunities for molecular ecology

Advances in systems for identification and diagnosis of *Phytophthora*, *Pythium* and related genera.

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Given the wide range of morphological features these pathogens can encompass and the challenges encountered when trying to discriminate between species with overlapping characteristics, the ability to use less subject molecular criteria to assist in the efforts to accurately identify isolates to a species level has certain attractions. A variety of approaches have been used for identification of cultures, including RFLP analysis, SSCP and sequence based comparisons. The ability to rapidly and accurately identify a pathogen to a species level from infected tissue is equally important and can have significant implications when dealing with species under quarantine restrictions. A variety of techniques have been developed for species-specific detection (serological, conventional PCR, real time PCR, arrays). While much of the research effort for molecular markers has focused on using sequences from the internal transcribed spacer region of the ribosomal DNA for marker development, other loci have been more recently investigated as well (the nuclear encoded loci β -tubulin, elicitin and *Ypt1* genes as well as the mitochondrially encoded *cox1* gene or the spacer between the *cox1* and *cox2* gene). An overview of the techniques and their advantages/limitations will be discussed.

Ecology, biogeography and epidemiology of the devastating *Phytophthora infestans*, *P. cinnamomi*, and *P. ramorum*.

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The key attributes that make each of these infamous *Phytophthora* species such successful and devastating pathogens will be illustrated and discussed. Ecological and epidemiological comparisons among these pathogens will highlight the varied strategies for survival, infectivity, reproduction, and spread successfully used by each species. Specific intercontinental migration events documented using a variety of genetic markers have caused massive changes in the genetic makeup of *P. infestans* populations in recent decades. These migrations have made it possible for higher levels of sexual reproduction to occur in regions outside of Mexico, where the sexual stage was first discovered. Keys to



success for *P. infestans* include its abundant sporulation, capacity for long distance migration via wind dissemination, and persistence in surviving in infected tissue under varying conditions. *P. cinnamomi* in many ways represents the opposite extreme from *P. infestans*, with its distribution and migration potential limited by temperature, and its inability to produce large numbers of sporangia. Nonetheless, it has devastated particular ecosystems, destroying up to 75% of the native flora in some parts of Australia. *P. ramorum* when first described in the 1990s seemed to consist of discrete transatlantic populations segregated by mating type and other factors. These distinctions are being called into question with the discovery of novel genotypes in the U.S. and elsewhere. Proof of the ability of *P. ramorum* to infect and sporulate on roots of host species has opened new areas of inquiry and caused workers to reevaluate the original belief that it is strictly an above-ground pathogen. Features common to these three species which make them such successful pathogens will also be presented.

Reconsidering the species problem in downy mildews – where are we now?

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The different species concepts (morphological, biological and phylogenetic) are briefly illustrated, and their importance and impact on downy mildews systematics is given. The advantages and disadvantages of the different historical approaches of species delimitation are discussed. The main focus will be on the impetus of recent molecular phylogenetic investigations on the re-evaluation of the species concept within downy mildews. Similarly to the closely related genus *Phytophthora*, most recent investigations indicate that molecular biodiversity within many downy mildews lineages is higher than commonly thought. The molecular phylogenetic data (ITS, LSU rDNA, *cox2*) suggest a high host specificity and speciation rate, which is often not accompanied by morphological differentiation. Commonly, genetically distinct species do not show clear-cut morphological distinction, which makes morphological species determination difficult. In other cases, genetically distinct lineages previously considered conspecific were shown to differ also morphologically. Like in many fungal lineages, it becomes evident that a morphological species concept is unsatisfactory in downy mildews, as well as the commonly applied determination based on the hosts. Consequently, molecular methods are of increasing importance and more reliable for downy mildews identification. However, despite significant progress during the last years, there are also problems and gaps which shall be discussed. These include an often low number of accessions included in the molecular phylogenetic investigations, a narrow geographic range of sampling and taxonomic incompleteness. Therefore, there is a need of more detailed investigations in closely related species complexes using additional variable gene regions and a thorough taxonomic sampling to evaluate species boundaries.

PARTICIPANTS CONTRIBUTIONS

SESSION: Nomenclature of Present Taxa and Putative New Species

PRESENTATION: Oral

Advances in the morphological and molecular Identification of *Pythiogeton* species.

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Morphological and molecular characterizations are given on three putative new *Pythiogeton* species, *Pythiogeton* sp. 1 from root of Cypress (*Cupressus* sp.), *Pythiogeton* sp. 2 from root of English Ivy (*Hedera helix*) and *Pythiogeton* sp. 3 from Allyssum (*Lobularia maritima*) in North Carolina, USA. All species were characterized by the production of arachnoid mycelium, hyphae scant, narrow, aseptate, sinuous and presence of hyphal rings a characteristic that has never been reported in the genus *Pythiogeton*. The *Pythiogeton* sp. 1 and 2 had similar sporangial size and shape each other. Their sporangia were mainly terminal and often intercalary, and were spherical, globose, ellipsoide, bursiforme often curved or elongated transversely on a long. Sporangia of *Pythiogeton* sp. 3 were terminal or intercalary, and were globose or ellipsoide. All species were absent oogonium in single culture. Aspects on the production of zoospores in the Genus will be discussed. In an inoculation test performed in Petri dish, all species infected their host plant roots but had no visible symptom. The phylogenetic tree based in ITS rDNA shows that all *Pythiogeton* are a solid species and the closest are members that belong to *Pythium* than to *Phytophthora*.

SESSION: Nomenclature of Present Taxa and Putative New Species

PRESENTATION: Poster



Morphological and molecular identification of eight putative new *Phytophthora* species from the USA. Z. G. Abad¹, M. Palm¹, R. Shukla¹, S. Rice¹, J. Rascoe¹, T. Creswell², and S. Nelson³. ¹United States Department of Agriculture, USDA-APHIS-PPQ-PSPI Molecular Diagnostics Lab., Beltsville, MD, USA. ²Purdue University, West Lafayette, IN 47907, ³University of Hawaii at Manoa, Hilo, HI, USA. gloria.abad@aphis.usda.gov

A collection of 600 *Phytophthora* isolates of the first author obtained in the last ten years was evaluated by morphological and molecular characterization. Isolates are in great part obtained from samples submitted to the Plant Disease and Insect Clinic, and the formerly Plant pathogen Identification Laboratory at North Carolina State University, USA. Isolates were originally obtained in PARP – CMA and stored in water blank cultures plus hemp seeds. Characterization of species was based on the morphology of the asexual and sexual phases of isolates in V8-agar H2O cultures, and growing in V-8 + oil (respectively). Molecular identification was based on sequencing and phylogenetic analysis of the Internal Transcribed Spacer rDNA and Translation Elongation Factor regions. In addition to the presence of common species some uncommon species were identified, among them the recently described *P. bisheria* and *P. sp. glovera* (from Tobacco roots- Brazil), *P. sp. niederhauserii*, and *P. sp. kelmania*. in progress for official description. Other group of eight species with unique morphological and molecular characters has been recently identified as putative new including *Phytophthora sp. personii* (in progress for description with scientists in NC, USA and Australia), and *P. sp. caroliniana*, *P. sp. pseudobisheria*, *P. sp. catenulata*, *P. sp. proliferanta*, and *Phytophthora* spp. 1 and 2. Most of the new species in the last group have been isolated from irrigation ponds and reservoirs. Temperature analysis is in progress for the description of these new taxa. A homothallic, papillate species associated with a foliar and fruit blight of noni (*Morinda citrifolia* var. *citrifolia*) in Hawaii has been identified and named *Phytophthora sp. morindae*. Phylogenetic analysis of the ITS rDNA region and TEF positions *P. morindae* as the closest relative of *P. kernoviae*.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

***Phytophthora* species associated to branch cankers on Persian lemon trees in Guatemala**

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In the last few years, a disease in Persian lemon (*Citrus latifolia*) trees appeared in coastal zones in the southwest of Guatemala. Symptoms included visible sunken and distended lesions with gum exudation on the scaffold branches of infected trees. From these infection points, lesions progressed toward the secondary branches or to the base of the tree. As symptoms developed, individual branches and eventually the entire tree collapsed and died. Small pieces of the lesion border of affected tissues were plated into PARPBH selective medium and *Phytophthora* spp. were isolated. Based on morphological, physiological and molecular profiles, *P. parasitica* and *P. palmivora* were identified. Pathogenicity tests were carried out on detached shoots and trunk of three years-old Persian lemon trees. A 5-mm wound was made with a cork borer and a 5-mm block of the agar culture was placed under the bark and sealed with Parafilm. Results showed that both *Phytophthora* species were pathogenic, but *P. palmivora* was more aggressive than *P. parasitica*. There are not previous reports on the presence and distribution of these *Phytophthora* species in the country and the described symptoms are unusual in the major citrus growing-areas around the world. This disease could become a constraint in the production of Citrus in Guatemala.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Oral

A Survey of *Phytophthora* and *Pythium* associated to export crops in Guatemala

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Guatemala is turning to the production of new crops destined for export and for rapidly growing urban markets within the country. Diseases caused by Oomycetes have been detected with increasing incidence in these crops in Guatemala. Surveys of ornamental, vegetable and fruit crops were carried out during 2007. Isolations were made from soil, roots, stems and leaves of symptomatic plants on selective medium for Oomycetes. *Phytophthora* isolates were identified based on colony morphology, mycelial characteristics, cardinal growth temperatures, and production, morphology, and



dimensions of sporangia, oogonia, and antheridia. The ITS region of the isolates was amplified with the primers ITS4 and ITS6, sequenced and compared with sequences available in the EMBL/GenBank database. Six different *Phytophthora* species were identified associated to different crops: *P. capsici*, *P. cinnamomi*, *P. citrophthora*, *P. palmivora*, *P. parasitica*, and *P. tropicalis*. *Pythium* species were also isolated and identified based on the sequence of the ITS region: *P. cucurbitacearum*, *P. splendens*, *P. sylvaticum*, and *P. ultimum*. Furthermore, there were some *Pythium* isolates which ITS sequences were nearly identical to undescribed *Pythium* species.

SESSION: Nomenclature of present taxa and putative new species

PRESENTATION: Poster

Diversity of deciduous long-pedicellate *Phytophthora* isolates associated to export crops in Guatemala

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In the central region of Guatemala, in several crops were observed decline and root rot symptoms under field and greenhouse conditions. In 2007, surveys were conducted to establish the causal agents. Long-pedicellate *Phytophthora* strains were isolated from pepper, carnation, rose and golden pothos. Morphological characters such as length: wide (L:W) ratio of sporangia, presence or absence of chlamydozoospores and physiological profiles such as growth at 35 °C, were used to differentiate *P. capsici* and *P. tropicalis*, according to the statement of Aragaki and Uchida (2001). Sequencing of the ITS region was not useful to differentiate both *Phytophthora* species. Pathogenicity tests were performed by inoculating roots of pepper and leaves of golden pothos with isolates obtained from the different crops surveyed. Results showed isolates with papillate and semi-papillate sporangia, most of them did not formed chlamydozoospores and presented a L:W ratio more than 1,8:1. Isolates were heterothallic with similar proportion of both mating types. Isolates from pepper and golden pothos identified as *P. capsici* and *P. tropicalis* did not showed differences of virulence between them on the different hosts, conversely to the observations of Aragaki and Uchida (2001), which indicated a weak response or no virulence in pepper for *P. tropicalis* isolates. Our results are not in agreement with this previous study on *P. capsici* and *P. tropicalis*. More studies should be conducted to establish more accurate distinctions between long-pedicellate isolates of *Phytophthora*.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Oral?

***Phytophthora* taxon Agathis, a threat to kauri in northern New Zealand?**

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Kauri (*Agathis australis*, Araucariaceae) is a dominant tree of lowland forests in northern New Zealand. Giant individual trees, which can reach over 4.5m in trunk diameter and exceed 1000 years age, are cultural icons. *Phytophthora* taxon Agathis (PTA) was first recorded (as *P. heveae*) in 1974 associated with a collar rot, causing large bleeding lesions near the ground, yellowing foliage and frequent tree death. Our ITS sequence studies of PTA (GenBank EF067922) show it belongs with, but is distinct from, *P. heveae* in ITS clade 5 of Cooke et al. Phylogenetic analysis indicate it is closely related to *P. katsurae*, but it differs from it morphologically in its rugose, as distinct from bullate, oogonia. PTA may be introduced to New Zealand, but too few isolates are as yet available to determine whether its genetic variability provides support for this hypothesis. Recent surveys have found collar rot is now present throughout most of the natural range of kauri, affecting trees of all ages. Pathogenicity tests show PTA is highly pathogenic to kauri, but suggest it is not pathogenic, or only slightly so, to a range of species that occur in kauri ecosystems. We propose that collar rot caused by PTA is an emerging disease caused by an introduced pathogen which is spreading from widespread disease foci. It poses a threat to kauri, both at the individual icon level and at the population level, with flow-on effects to kauri ecosystems.

SESSION: Advances in systems for identification and diagnostics

PRESENTATION: Oral



Quantitative multiplex detection of (plant) pathogens, including *Phytophthora* species, based on PRI-lock probe technology and the OpenArray platform.

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A concept is described for quantitative multiplex detection of (plant) pathogens, including several *Phytophthora* species, in which several pathogens will be detected real-time in one sample. After the extraction of genomic DNA we introduce a ligation step with target specific probes. In this step we make use of PRI-lock probes, linear oligonucleotides in which the 5' and 3' ends of the probes will hybridize in immediate juxtaposition on a DNA or RNA target strand and can be covalently joined by a DNA ligase, converting the probe to a circular molecule only when both end segments correctly recognize nearby target segments. Ligation reactions permit easy differentiation among similar target sequence variants, as mismatched probes are poor substrates for ligases. After ligation, circularized probes can be PCR amplified in each well of the BioTrove OpenArray platform. This platform uses wells with a volume of only 30 nanoliter. On one slide 1024 reactions can be followed real-time on the NT-Cycler of BioTrove. In this way quantitative data can be obtained. Target specific set of primers are preloaded in each well, if the corresponding sequences have been included in the non-target complementary segment of the PRI-lock probes. The orientation of the primers is chosen in such a way that only originally circularized PRI-lock probes will be amplified.

PRI-lock probes combined with the OpenArray platform are promising for multiplex DNA and RNA diagnostic analyses, since the targeted sequences and the PCR amplifiable target probes are independent. The use of pre-selected codes in the PRI-lock probes makes universal PCR conditions and universal TaqMan detection assay possible and is easily modifiable and extendable to include other, even newly-emerged pathogens. The OpenArray platform of BioTrove offers also applications in the quantitative multiplex analysis of gene expression.

Examples and possibilities of this new technology will be presented and discussed.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Abstract 1:

Collar canker of apple trees caused by *Phytophthora* spp. in Tunisia

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Considerable losses of apple trees have been observed in the major apple-growing areas of Tunisia. Samples were collected from 23 orchards and isolations and pathogenicity tests were conducted to determine the aetiology of a serious canker disease. The surveys were conducted in two periods, in spring and autumn 2006-2007. Affected trees showed cankers on the scion and the rootstock.

Phytophthora nicotianae (syn. *P. parasitica*) was identified based on its morphological and molecular profiles.

Pathogenicity tests were performed *in vitro* and *in situ* on the more frequently cultivated apple varieties in Tunisia and MM106 rootstock. The virulence was determined by measurement of canker area after 7 and 42 days for *in vitro* and *in situ* inoculation tests, respectively. *In vitro*, MM106 rootstock revealed to be the more susceptible to all tested isolates of *P. nicotianae*, and Anna and Lorka varieties seemed to be the most resistant. *In situ*, the virulence of *P. nicotianae* was confirmed on all tested varieties. Furthermore, pathogenicity tests demonstrated that Lorka and Meski were the most susceptible and resistant varieties, respectively.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Abstract 2:

Occurrence of *Pythium* spp. on apple orchards in Tunisia

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In a survey of *Phytophthora* species associated with apple trees canker in Tunisia, five *Pythium* isolates were found, which were characterized with molecular techniques and morphological pictures for *Pythium* spp. These isolates were collected in 10 from the 23 surveyed orchards, in spring and autumn. In spring, only *Pythium indigoferae* and *P. irregulare* were found. Whereas, in autumn the *Pythium* species found were: *P. rostratifingens*, *P. indigoferae*, *P. irregulare*, *P. undulatum* and *P. sterilum*. *P. indigoferae* and *P. sterilum* were found only in orchards soils. Isolations were done on modified PARBPH selective agar and then transferred to PDA for colony pattern description, while V8 juice agar was used for morphological description.

Pathogenicity tests were performed *in vitro* and *in situ* on the more frequently cultivated apple varieties in Tunisia and MM106 rootstock. The excised-twig assay was used in these experiments. The virulence of *Pythium* species was determined by the measurement of canker area after inoculation.

In vitro, results showed that *P. rostratifingens* was the less virulent species on different apple varieties. MM106 rootstock was susceptible to all tested isolates. Anna and Lorka varieties seemed to be the most resistant. Field inoculations demonstrated that Lorka and Meski were the most susceptible and resistant varieties, respectively.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Abstract 1:

Phytophthora on carrot: evolution of species involved in ring rot disease in France.

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The ring rot disease is one of the most severe diseases during the winter period of carrot production in France. The symptoms are observed on the mature root in the field during autumn and winter: a vitreous spot appears on the root and spreads transversally to draw a well delimited ring spot. Although this disease was a serious problem until the end of the 80's, it was almost not observed in the carrot fields from 1990 to 2001. *Phytophthora megasperma* used to be the primary pathogen but for the past six years, the disease has been increasing and another species of *Phytophthora*, has also been isolated. In 2004, 2005 and 2006, carrots samples were analysed in order to establish relative frequencies of both species: *P.megasperma* is still present (10%) but it has been supplanted by *Phytophthora sp* (79%). Biological characterisations show significant differences between isolates from both species. Morphological and molecular results such as RFLP on ITS, show that *Phytophthora sp* is close to *P.porri* and *P.brassicae*. Phylogenetic analysis on complete ITS sequences confirms this closeness but it appears that the carrot isolates form a different cluster. The hypothesis of a putative new species is now supposed. The shift inside the genus *Phytophthora* of species involved in the carrot ring rot disease in France is now clear. This situation has consequences on disease epidemiology as well as crop protection and must be taken in account for the research and development of new way of crop protection.

SESSION: Advances in systems for identification and diagnostics

PRESENTATION: Poster

Abstract 2:

Development of a real-time PCR assay to quantify *P.violae* and *P.sulcatum* in soil samples.

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The *Pythium* are among the most important soil-borne pathogens of carrot in France and all over the world. The major and most reported disease caused by these fungi is cavity-spot, with two most often associated species *P.violae* and *P.sulcatum*. A real-time PCR assay has been developed in order to have a rapid and accurate tool to evaluate the density of inoculum of the two species in carrot field soils. Variable regions of the ITS of the nuclear ribosomal DNA of both species were used to design species-specific primers and probes ; a standard curve was generated for each species using dilutions scales of cloning DNA and a real time PCR protocol using TaqMan chemistry was developed. Sensitivity tests as well as specificity test using DNA of pure culture of different Oomycetes isolates were conducted. The results show that the method allows the DNA detection until 10fg/µl and a good specificity for each primers and probe set is demonstrated. Extractions of DNA from 3 different soils, artificially infested respectively with *P.violae* and *P.sulcatum*, were performed and DNA was amplified using the TaqMan real time PCR. Quantification of *P.violae* and *P.sulcatum* is effective and different amount of *P.violae* and *P.sulcatum* DNA in the soils are obtained. The presence of inhibitors of the PCR in the 3 soils was studied in order to evaluate the accuracy of the quantification; different results are obtained depending the type of soil. Despite its limitation, this molecular technique for the quantification of the two *Pythium* species will be a useful tool for future epidemiological studies.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster



Molecular re-evaluation of *Phytophthora* taxa collected over the past three decades from natural ecosystems in Western Australia

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Phytophthora cinnamomi has had a huge impact on natural ecosystems in Western Australia. For 29 years the extent of the disease in native forests has been mapped based on large-scale aerial photography, with validation of observations involving the routine testing of soil and root samples for the presence of the pathogen. In addition to *P. cinnamomi*, six other morphological species have been reported from native ecosystems in Western Australia: *P. citricola*, *P. megasperma*, *P. cryptogea*, *P. drechsleri*, *P. nicotianae* and *P. boehmeriae*. Within the collection there were many isolates that were difficult to identify based on morphology and as more *Phytophthora* species have been described with similar morphology it was realised that molecular identification of some of the morphological species was required. Thus, the internal transcribed spacer (ITS) region of the rDNA gene has been amplified and sequence data compared to that of known species. Based on phylogenetic analysis, nine potentially new and undescribed taxa can be distinguished. In addition *P. inundata*, *P. gonapodyides*, and *P. sp. asparagi* and *P. sp. niederhauseria* were identified based on sequence data. Several of the new species are morphologically indistinguishable from known species (eg *P. citricola*, *P. drechsleri*, *P. megasperma*). In some cases the new taxa are indeed most closely related to the known species (eg P.sp. 4 and *P. citricola*). However, the DNA sequences of other new taxa show that they are not closely related to the morphologically similar species (eg P.sp. 3 and *P. drechsleri*, P.sp. 9 and *P. megasperma*). Most of the new species have been associated with dying *Banksia* spp. whilst P.sp. 2 and P.sp. 4 have also been isolated from *Eucalyptus marginata* (jarrah). Some species (eg P.spp. 3, 6 and 7) appear to have limited distribution, whilst others (eg P.sp.4) are more widespread. Further work is planned to describe the new taxa and to test their pathogenicity.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Residual effectiveness of fungicides in protecting rhododendron leaves from *Phytophthora ramorum*.

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Over 20 fungicides have been tested in the last 3 years to determine their residual effectiveness in protecting Rhododendron x 'Nova Zembla' foliage from *P. ramorum*. Following application, leaves were periodically collected from fungicide-treated and untreated container-grown rhododendron plants for up to 16 weeks. Detached leaves were inoculated with suspensions of zoospores from an NA1 lineage rhododendron isolate by pipetting three 10- μ l drops of zoospore suspension onto the lower leaf surface on each side of the leaf midrib. The leaf tissue was injured beneath 3 drops on one side of the leaf midrib using an insect pin. The tissue beneath the drops on the other side of the leaf was left unwounded. Checks included inoculated and non-inoculated leaves from untreated plants that had been sprayed with water. Leaves were then incubated for 7 days at 19C. Fungicide efficacy was quantified by measuring the areas of the resulting leaf spots using ASSESS. No disease developed on any of the non-inoculated checks. The size of the leaf spots on fungicide-treated leaves was compared to the size of leaf spots that developed on the inoculated check leaves after each inoculation test. Results indicate that residues of some fungicides, such as captan, had very limited residual activity. On the other hand, residues of other fungicides such as cyazofamid significantly reduced disease development up to 12 weeks after application. Overall, the residual effectiveness of fungicides was greater on unwounded leaves. Isolations from fungicide treated leaves indicated that none of the fungicide had any affect on the recover of the pathogen from symptomatic tissue. Isolations from asymptomatic inoculated leaf tissue indicated that mefenoxam and pyraclostrobin may suppress symptom development.

SESSION: Evolution and Population genetics

PRESENTATION: Poster

Initial assessment of genotypic diversity of *Phytophthora ramorum* associated with Washington state ornamental nurseries

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Isolates (117 total) of *P. ramorum* were obtained with help of the Washington State Department of Agriculture from 18 nurseries, two streams, a retention pond, and one landscape site in Washington and genotyped using 4 previously developed microsatellite markers (Prospero et al., 2007). Three previously described lineages, known as EU1, NA1 and NA2 (Ivors, 2007), were detected. The NA1 lineage was the most common, occurring in 16 nurseries, both streams, the pond, and at the “landscape” site. The NA2 lineage was detected in four nurseries, while the EU1 lineage was detected at a single nursery. Both the NA1 and EU1 lineages were isolated from different branches on the same rhododendron plant. DNA fingerprinting identified four unique genotypes among the NA1 lineage isolates. High heterozygosity coupled with the clonal population structure, suggests that *P. ramorum* has not undergone sexual recombination in Washington State. High levels of genotypic diversity observed at three “repeat positive” nurseries and apparent lack of sexual recombination suggests multiple introduction events have occurred at Washington nurseries. The genotyping results have also provided some insights on potential sources of inoculum that infested the two streams. In one instance, isolates obtained from an infested stream matched isolates associated with an infested nursery on the stream. In the other instance, the isolate from the stream may have come from a retention pond with the same genotype located between the stream and a positive nursery. An isolate from the landscape site and the nursery it came from also have the same genotype that was detected in the second stream. Although neither the landscape site nor the nursery is located within the stream watershed, this situation indicates the potential that the infestation may have come from an infected plant planted along the stream.

SESSION: Ecology, Biogeography, and Epidemiology
PRESENTATION: Poster

Evaluation of certain traditional cultural practices for the management of *Phytophthora* blight of potato in organic farming system

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Traditional farming system locally called “Jhum cultivation” is basically a slash and burn system of agriculture which is self sufficient and organic by default, practiced mostly by tribal farmers of North-East region of India since generations. Among other crops, potato is an important cash crop of the system but get infected by *Phytophthora infestans*. Being an organic system of cultivation, Control of late blight of potato cultivated as summer crop in the hill slopes is usually done through a series of cultural practices viz; fresh jhuming, fallowing fresh jhum fields for different periods(10,5,2year,etc),following sequential cropping pattern,augmenting sowing dates, soil amendments with available local resources, spacing between plants, localized burning of plant-soil mixed debris, application of improvised compost, application of botanical extracts etc .Fresh jhuming , fallow land of longer durations, compost application around the planting pit and early sowing ,etc minimizes disease severity effectively in comparison to other cultural practices. Balanced soil health status enriched with rich microbial activities and evading the favourable environmental conditions for the epidemic outbreak of the disease have shown to be the main factors responsible against the minimization of *Phytophthora* infection. Although the approaches were eco-friendly, limitation of virgin forest for fresh jhuming, increased population pressure on arable land and influence of modern agriculture were the constraints in the commercial production of *Phytophthora* free potatoes in the hill regions using these potential traditional organic practices. While maintaining the organic character of the system,an alternative method using sequential cropping technique with disease free seeds of potential crop in jhum converted dry land farming through proper soil amendments with available local resources have been identified which checks the *Phytophthora* blight to some degrees. This may be due to organic decay of crop plant residues enhanced by repeated reshuffling & exposure of soil to hot sunshine coupled with the application of botanical extracts. As these observation were based on the study of singly potato variety, there are number of local potato varieties under cultivation which need to be screened for effective control of the disease in view of the existence of **physiological specialization** in *Phytophthora infestans*

SESSION: Nomenclature of Present Taxa and Putative New Species
PRESENTATION: Oral

Michael D Coffey. Molecular Phylogeny of the Marine *Halophytophthora* Species (a work in progress). *M.D.Coffey. University of California, Department of Plant Pathology and Microbiology, Riverside, CA 92521, USA. coffey@ucr.edu*

The WOGRC contains a unique and comprehensive collection of marine oomycetes, specifically *Halophytophthora* species. Currently, there 15 described species including the two varieties *spinosa* and *lobata* of *H. spinosa*. In this work we have utilized several different phylogenetically informative regions to begin to create a molecular phylogeny of the genus. Initially, we have utilized the ITS region of the nuclear ribosomal DNA to create a phylogenetic tree that can



also be utilized to compare with *Phytophthora* and *Pythium* species. In addition, we have generated sequence data for β -tubulin and the large subunit ribosomal DNA (LSU) and the barcode region of the mitochondrial cytochrome c oxidase subunit I (COI). These individual trees will be compared with that for ITS and some preliminary results will be discussed.

SESSION: Integrating morphological and molecular tools for a unified phylogeny and classification
PRESENTATION: Poster

PRESENTATION: Oral

Michael D Coffey. Past and Current Taxonomic Status of *P. cryptogea* and *P. drechsleri* and Associated Species (a work in progress). [M.D.Coffey](mailto:coffey@ucr.edu). University of California, Department of Plant Pathology and Microbiology, Riverside, CA 92521, USA. coffey@ucr.edu

The controversy surrounding the description of *P. cryptogea* and *P. drechsleri* continues until the present day. The separation of the two species either by their ability to grow at 35C or through differences in sporangial morphology has proven controversial. In addition, other species such as *P. melonis* (syn *P. sinensis*) and *P. cajani* have been described that further complicate the picture. In 1991 a study based on morphology, isozymes and mtDNA banding patterns was published. It utilized 123 accessions described as either *P. cryptogea* or *P. drechsleri*. These 123 accessions could be divided into 10 phylogenetic groups (A-K) including A containing the authentic isolate of *P. drechsleri* and B containing the types of *P. cryptogea* (and *P. erythroseptica*). In the current work in progress we have examined 242 accessions utilizing ITS region of the nuclear ribosomal DNA to create a phylogenetic tree. Focusing for the moment mainly on the previously identified groups A and B we have generated additional phylogenetic trees for the large subunit ribosomal DNA (LSU) and the barcode region of the mitochondrial cytochrome c oxidase subunit I (COI). These individual trees will be compared with that for ITS and some preliminary results will be discussed.

SESSION: Advances in systems for identification and diagnostics
PRESENTATION: Oral

New insights in the life cycle of white tip disease (*Phytophthora porri*) in leek (*Allium porrum*)

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One of the major diseases in leek is *Phytophthora porri*. *P. porri* causes major yield and quality losses in cold and humid conditions, so mainly in autumn and winter. On 1 September 2005, a project of the Ghent University together with some vegetable research stations started. The goal of the project is to develop a prediction model for *P. porri* which will help farmers to spray at a more adequate moment. To effectively control the pathogen, there is a need to clearly understand the epidemiology of *P. porri*. From September 2005 up till now, the infestation of leek by *P. porri* has been monitored in different fields. It is known that *P. porri* survives the crop free period by forming oospores which constitute the source of primary inoculum. It is generally believed that oospores initiate infection when they are splashed on leek plants in a rain shower during the growing season. Our results indicate that oospores of *P. porri* germinate in the soil during periods of heavy rain and form sporangia, which release zoospores in puddles on the soil. There is a strong possibility that the primary infection of *P. porri* is not caused by splash dispersal of the oospores, but by splash dispersal of these zoospores. Interestingly, oospores are able to germinate *in vitro* when soil solution was added instead of sterile distilled water. So it is possible that the germination of oospores and subsequently the forming of zoospores is triggered by soil solution or minerals in the soil.

SESSION: Advances in systems for identification and diagnostics
PRESENTATION: Poster

Genetic diversity of Belgian *Phytophthora ramorum* isolates.

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Phytophthora ramorum is a relatively recently described quarantine *Phytophthora* species that causes extensive mortality of selected oak trees at the west coast of the USA, the so-called "Sudden Oak Death". Only a few forest or park trees have been found infected by *P. ramorum* in Europe, where the pathogen is mainly present in nurseries, especially on *Rhododendron*, *Viburnum*, and *Camellia*. It is suspected that *P. ramorum* was recently introduced in Europe and therefore possesses only a limited amount of genetic diversity. To verify this hypothesis and to develop molecular markers that would aid in the study of the dispersion of the pathogen, we have screened 80 *P. ramorum*



isolates with AFLP and SSLP. The isolates originated from Flanders and were mainly selected based on differences in isolation year and location. Using the AFLP method with five primer combinations, 13 polymorphic fragments were identified. These markers identified only 8 isolates that differed from the main genotype by one to three polymorphisms. For SSLP, a total of 132 candidate polymorphic microsattellites were prescreened using 10 isolates belonging to different EU genotypes. Finally, six primer pairs were selected and used for screening the 80 isolates. These revealed 10 isolates that differed from the main genotype, two of which were also genetically distinct based on the AFLP analysis. The overall level of genetic diversity within these isolates of *P. ramorum* would indeed appear to be limited, indicating a recent dispersion of the pathogen. Several new molecular markers were found, but these mainly identified genotypes that differed from the main EU lineage by single mutation events.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

***Phytophthora* diversity in UK gardens**

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Phytophthora is a common cause of death of a range of herbaceous and woody plants in gardens. Over the last 10 years the Plant Pathology department at the Royal Horticultural Society has diagnosed over 1000 enquiries with *Phytophthora* from more than 185 genera. The most common hosts affected are *Taxus*, *Rubus*, *Rhododendron*, *Prunus* and *Viburnum* with *Taxus* accounting for over 20% of the cases. Confirmation of the pathogen has traditionally been via apple baiting and then water float for sporangial production. Alternative diagnostic methods of a commercial lateral flow device and a nested PCR, based on the ITS region, are being compared to traditional baiting for their efficacy with soil and/or plant samples. Initial findings indicate that the lateral flow and nested PCR systems detect *Phytophthora* at higher frequency from symptomatic plants compared to apple baiting. Through baiting we recovered *P. cactorum*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. gonapodyides* and *P. sp. niederhauserii*. With the nested PCR we detected a wider range of *Phytophthora* species and additionally identified *P. alni*, *P. cambivora*, *P. hibernalis*, *P. megasperma*, *P. porri*, *P. quercina* and *P. syringae*. Sequence results also identified *Pythium* species from dying plants in association with *Phytophthora* or alone, indicating their possible involvement in root death. Species commonly identified are *Pythium attrantheridium*, *Py. heterothallicum*, *Py. intermedium*, *Py. irregulare* and *Py. sylvaticum*. Future work will investigate their role in causing diseases on ornamentals.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

***Phytophthora* root rot in avocado in Puerto Rico**

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Avocado (*Persea americana* Mill.) is the fourth most important fruit crop in Puerto Rico. In the traditional production areas avocado yield has decreased between 70% and 88%. *Phytophthora* root rot (PRR) appears to be responsible for these declines and disease symptoms are especially severe in poorly drained soils. *Phytophthora cinnamomi* (Pc) and several other *Phytophthora* species have been isolated from avocado groves, including *Phytophthora palmivora*, *Phytophthora hevea* and an unidentified *Phytophthora* species. All identified species are known to cause disease in avocado. *P. cinnamomi*, *P. palmivora* and the unidentified *Phytophthora* sp. were used in pathogenicity assays involving several avocado cultivars. Disease severity (DS) was based on a percent root necrosis scale. The scale ranged from 1 to 5 where 1 = 0%, 2 = 25%; 3 = 50%; 4 = 75% and 5 = 100% root necrosis. *Phytophthora cinnamomi* isolate ISA-LP-1S1-05 was highly pathogenic on Wilson Popenoe (DS= 5), causing severe root rot and plant death. Relative to the Wilson Popenoe, Pc caused less severe PRR in Buttler (DS= 4.3) and Donaldson (DS= 3.6). *Phytophthora palmivora* also caused PRR in Wilson Popenoe (DS= 2.3), Buttler (DS= 2.3) and in Donaldson (DS= 3.3) but disease severity was considerably less than that caused by Pc. *Phytophthora* sp. isolate (JD-EEA-IPM-06) caused severe PRR in Wilson Popenoe (DS= 5) and moderate PRR in Donaldson (DS= 2.6). *Phytophthora* was recovered from all the inoculated plants. Our results imply that future breeding efforts should be expanded to include additional *Phytophthora* species.

SESSION: Advances in systems for identification and diagnostics

PRESENTATION: Poster



Cloning and expression of a pectin methylesterase gene from the Oomycete Plant Pathogen *Phytophthora capsici*

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Phytophthora capsici is a plant pathogen that causes severe diseases in a wide variety of crops. Pectin methylesterase (PMEs) play a major role in degradation pectin in plant cell walls. A *pme* gene (*pcipg6*) were isolated from a highly virulent *P. capsici* isolate with high PMEs activity. They are all encoded a polypeptide of 348 amino acid residues with a predicted molecular mass of 38 kDa. *Pcpme6* has a signal peptide with 20-amino acid and 4 N-glycosylation sites. We explored the functions of *Pcpme6* based on its number of N-glycosylation sites. Heterologous expression of *Pcpme6* in *Pichia pastoris* produced a protein about 50 kDa (PMEC) that was not corresponded to the mass of this protein. The PMEC exhibited high activity toward citrus pectin and pepper pectin, and PMEs activity varied in PMEC treated pepper leaves was consistent with symptom development in pepper leaves. These results suggested that PMEC might also contribute to cell wall death and variation in pathogen virulence on the host. Western blot, RT-PCR and northern blot analysis of *Pcpme6* expression in the host showed that *Pcpme6* was highly expressed during interaction of *P. capsici* with pepper host, so *Pcpme6* might be involved in the infection process. All of these results were confirmed by the presence of symptoms in pepper leaves inoculated with PMEC compared with plants inoculated with a zoospore suspension.

SESSION: Morphological and Molecular Taxonomic Methods

PRESENTATION: Oral

Characterization and molecular identification of *Phytophthora* spp. associated with Phytophthora blight of vegetables in Texas.

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Phytophthora blight of pumpkin, squash, watermelon, and pepper (*Capsicum* spp.) has increased in importance in many production areas of the United States in recent years. This disease, caused by *Phytophthora capsici*, has been reported in Texas for over 60 years. During the past two years, the disease was observed on chile pepper, pumpkin, and winter squash in the Texas High Plains (NW Texas) and on watermelon in the Lower Rio Grande Valley (S. Texas). A preliminary characterization was done on 11 isolates obtained from NW Texas and isolated from diseased chile pepper (9), pumpkin (1), and winter squash (1). Three isolates obtained from S. Texas, isolated from chile pepper (1) and watermelon (2), were also characterized. All isolates were heterothallic, pathogenic on pepper, and sensitive to mefenoxam. Several isolates from pepper, however, grew at a much faster rate on clarified 20% V-8 juice agar plates than the isolates from pumpkin or squash. The internal transcribed spacer (ITS) region of the rDNA was amplified, sequenced, and compared to sequences reported for *Phytophthora* spp. Based on phylogenetic analysis, the isolates from watermelon, squash, and chile pepper (S. Texas) group with *P. capsici*. All but one isolate from chile pepper from NW Texas group with *P. mexicana*. One isolate from chile pepper and pumpkin group separately to one another and do not cluster with *P. capsici* or *P. mexicana*. Each one of the two potentially represents a new species of *Phytophthora*. Further characterization will allow for a better understanding of the role that each species or potential species plays in Phytophthora blight of vegetable crops in Texas.

SESSION: Advances in systems for identification and diagnostics

PRESENTATION: Poster

Elicitor (PiP) and Suppressor from *Phytophthora infestans* Regulate Ca²⁺-Dependent Protein Kinase (CDPK) in the Membrane of Potato

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The relationship between the effect of Ca²⁺-dependent protein kinase (CDPK) domain-III peptide-antibodies (Abs) raised against CDPK from *Arabidopsis thaliana* on the hypersensitive response of plant cells is a challenging one: The effect of elicitor (PiP) and suppressor from *Phytophthora infestans* on the kinase activity of membrane protein (MP) from potato (R₁ gene) was investigated. Stimulation of the kinase activity of the MP with CDPK-Abs but not with pre-immune sera was assumed to be caused by the interaction with CDPK suggesting the presence of the kinase in MP. It was assumed that interaction of kinase domain-III peptide-Abs with CDPK in MP might have disengaged the active site of CDPK resulting in an increase in the kinase activity of MP. The PiP and suppressor from *P. infestans* caused inhibition of the kinase activity of MP containing CDPK-Abs. It was suggested that PiP and suppressor might have interacted with the active site resulting in the inhibition of the CDPK activity of MP. We suggest that PiP and suppressor may interact with CDPK of MP initiating the signal, which, in the case of PiP, leads to the occurrence and/or the inhibition of hypersensitive response.

SESSION: Advances in systems for identification and diagnostics

PRESENTATION: Poster

Abstract 1:

Rapid identification of the pine needle and shoot pathogen *Phytophthora pinifolia* nom. prov. using species-specific PCR primers

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Phytophthora pinifolia nom. prov., is the probable causal agent of the *Pinus radiata* needle shoot disease in Chile referred to as “Daño foliar del Pino” (DFP). This is one of the most serious diseases of plantation pines in the world and tools to work with the pathogen effectively are required. Identification of species of *Phytophthora* is time-consuming and labour-intensive and requires comprehensive knowledge of the organisms. Molecular markers can be valuable to reduce the time and cost needed to identify *Phytophthora* species, especially when they can be applied to identify these organisms directly from plant material. The aim of this study was to develop species specific primers for the *Ypt1* gene, to identify *P. pinifolia* in culture and to detect the pathogen *in situ* in pine needles. The primers were designed by analysing highly variable regions of the *Ypt1* gene available for several *Phytophthora* species. When tested on DNA extracted from cultures of *P. pinifolia* and six other related *Phytophthora* spp., only DNA of *P. pinifolia* was amplified. Total DNA extracted from symptomatic and asymptomatic needles was amplified with the specific primers designed for the *Ypt1* gene. Initial PCR reactions, followed by an additional nested PCR, yielded single bands for symptomatic needles that were confirmed with sequencing to represent *P. pinifolia*. No amplicons were observed for DNA extractions from asymptomatic needles. The results provide irrefutable evidence of the pathogen within host tissue and an effective technique to detect *P. pinifolia* in monitoring and quarantine programmes.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Oral

Abstract 2:

An emerging needle blight disease of *Pinus radiata* in Chile

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A new and serious needle blight disease appeared and spread rapidly on *Pinus radiata* in the Arauco and Valdivia Provinces of Chile since 2003. This disease known in Chile as Daño Foliar del Pino (DFP) now affects an area of about 60 000 ha with different levels of intensity. Infection is closely associated with the onset of rain at the end of the summer season. Needles from the previous season's growth are infected on the lamina and die rapidly, typically falling from the trees at the end of the winter period. Particularly on young trees, copious amounts of resin form at the needle bases, reflecting a strong reaction by the stems of trees to exclude infection. Although cankers can develop in the cambium at the bases of infected needles, these appear to be limited in extent and infections do not appear to enter the wood. Only *P. radiata* trees have been affected by DFP and other *Pinus* species, like *P. pinaster* in the affected area remain healthy. Isolations from infected needles on selective media have consistently yielded a *Phytophthora* sp. with non-papillate sporangia, which are caducous and typical of aerial *Phytophthora* spp. DNA sequence and morphological comparisons have shown that the fungus represents an undescribed species, which is currently being described as *Phytophthora pinifolia* prov. nom. Research is underway to understand the life cycle of *P. pinifolia* and to develop appropriate management strategies to reduce its impact on the forestry activity of Chile.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Oral



Widespread *Phytophthora* infestations of nursery stock in Central Europe as major pathway of *Phytophthora* diseases of forests and semi-natural ecosystems

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Between 2001 and 2007 extensive nursery surveys were carried out across Germany, Austria and Poland. In Northern Germany beech, oak and maple fields of 14 nurseries were investigated and 54% were found infested by *P. cactorum* (23%), *P. syringae* (23%) and *P. cambivora* (15%). Other *Phytophthora* spp. were isolated infrequently. In Lower Saxony, Northwestern Germany beech fields were surveyed in 6 nurseries and 5 were found infested by *P. cactorum*, *P. cambivora*, *P. citricola*, *P. gonapodyides* (each 2 nurseries) and *P. pseudosyringae* (1 nursery). In Bavaria, Southern Germany the beech fields of all 9 nurseries tested were infested by a range of 8 *Phytophthora* species with *P. citricola*, *P. cactorum* (each 7 nurseries) and *P. cambivora* (5 nurseries) being most widespread. In Southern and Western Germany all oak fields (*Q. robur*, *Q. rubra*, *Q. petraea*) of the 8 tested nurseries were infested by *Phytophthora* spp. *P. quercina*, *P. citricola* (each 5 nurseries) and *P. cactorum* (4 nurseries) were most common. In Poland beech, ash, maple, oak, fir, spruce, pine and larch fields in 40 forest nurseries were tested, and a range of 5 *Phytophthora* species, mainly *P. citricola*, *P. cactorum* and *P. cinnamomi*, were regularly found in 26 nurseries. Alder fields were investigated in Bavaria, in Eastern Germany, in Poland and in Austria with the specific purpose of detecting *P. alni* which is responsible for the epidemic alder mortality across Europe. In Bavaria *P. alni* was recovered from rootstocks of alders from 3 out of 4 nurseries which regularly bought in alder plants for resale, but not in rootstocks from four nurseries that grew their own alders from seed. In addition, *P. cambivora*, *P. cactorum*, *P. gonapodyides* and *P. taxon* 'Pgchlamydo' (each 37.5%), *P. megasperma* (50%) and *P. citricola* (62.5%) were isolated. In Brandenburg *P. alni* was found in 5 out of 10 nurseries. In addition, *P. cambivora*, *P. cactorum* and *P. syringae* were recovered. In both countries the infested nurseries used water from infested water courses for irrigation. As a result alders in Bavaria and Brandenburg were produced according to a code of good practice. Control isolations showed that *P. alni* but not the other *Phytophthora* spp. could be eliminated. *P. alni* was also found in several nurseries in Austria and Poland. Extensive field studies in young forest and amenity plantations in Southern and Northwestern Germany, and in more than 3000 alder and more than 200 mature beech, oak, lime and maple stands across Germany demonstrate the ubiquitous involvement of *Phytophthora* species in the devastating broadleaf tree declines, and the role of infested nursery stock as a major pathway of *Phytophthora* diseases of trees. The implications of our results for the nursery and the forest industries are discussed.

SESSION: Nomenclature of present taxa and putative new species

PRESENTATION: Poster

A new *Pythium* species isolated from cavity spots on carrots in Norway.

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In 1996, a survey on cavity spot started in Norway. Among the *Pythium* species isolated from the lesions a pathogenic *Pythium* was found that could not be identified neither by morphological nor by molecular methods (ITS sequencing). This *Pythium* sp. was repeatedly detected using specific primers developed for this purpose and in many cases isolated from cavity spots lesions. The isolates from the new species do not produce zoospores. Oogonia are smooth walled and mostly intercalary with an average size about 20µm. Oospores are aplerotic with an average diameter approximately 17 µm and wall less than 2 µm thick. Antheridia are mostly monoclinal with a sort stalk or sessile, but declinal antheridia are also common. The modal number of antheridia per oogonium is one or two. Some differences are observed between the isolates studied. Some isolates have a daily growth rate of ca 23 mm on PCA at 25 °C while others have a growth rate around 28 mm. The isolates that grow slower have a tendency to produce one antheridium per oogonium while the isolates with higher growth rates normally produce two. These two groups could also be differentiated based on their ITS sequences which showed 97.8% identity in the 955 base pair rDNA fragment. By using the developed PCR primers it is possible to detect isolates that do not produce the sexual stage in single cultures. These isolates have the same ITS sequence and a similar growth rate and pattern than the fast growing fertile ones.



SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Abstract 1:

Does waterlogging influence phosphite protection of *Banksia* species to *Phytophthora cinnamomi*?

D. Hüberli, T. Paap, K. Gower, N. Long, B. Dell and G.E.St.J. Hardy

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Parts of the southwest of Western Australia are subjected to periodic flooding events that are also devastated by Phytophthora dieback disease caused by *P. cinnamomi*. Phosphite has been shown to be effective in controlling this pathogen. Waterlogging induces multiple physiological dysfunctions in plants, but it is unknown whether waterlogging alters the uptake, distribution and efficacy of phosphite in controlling *P. cinnamomi*. Waterlogging trials were conducted in the greenhouse using *Banksia attenuata* and *B. baxteri*. The response of these plants and subsequent recovery from waterlogging was examined. A phosphite spray treatment was applied pre- and post- waterlogging of either 3 or 14 days duration. Leaf gas exchange, leaf water potentials, lesion development and phosphite concentrations in leaf, stem and root tissue were monitored 1 week, 1 month and 4 months after the phosphite treatment. For the 1 week harvest when phosphite was applied pre-waterlogging, phosphite in plant tissue were at similar levels for each species and were not affected by waterlogging. But lesions on *B. baxteri* stems were not reduced in treated plants as they were for *B. attenuata*. Photosynthesis and water potentials were reduced for waterlogged *B. attenuata*, but had no impact on waterlogged *B. baxteri*. Leaf water potentials, leaf gas exchange, lesion lengths on inoculated stems, and phosphite concentration in leaves, stems, and roots measured at different time periods after waterlogging will be presented.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Abstract 2:

Does fire influence phosphite protection of Western Australian indigenous plant species to *Phytophthora cinnamomi*?

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Large areas of indigenous forests, *Banksia* woodlands and heathlands in Australia are devastated by Phytophthora dieback disease caused by *P. cinnamomi*. Phosphite has been shown to be effective in controlling this pathogen on a wide range of plant species across different families. Although fire is a regular event in the Australian landscape and plays key roles in ecosystem processes, nothing is known about the relative uptake of phosphite by shoots pre- and post-fire or how fire may alter the redistribution and persistence of phosphite within woody plants. *Adenanthos cuneatus* (resprouter), *Banksia attenuata* (resprouter) and *B. baueri* (reseeder) are all susceptible to *P. cinnamomi* and are responsive to phosphite treatment. These species were selected within four plots in an area of the Stirling Range National Park that was scheduled for a fuel-reduction burn in November 2006. Treatments of the plots were: 1) phosphite spray without fire, 2) phosphite spray with fire, 3) no phosphite spray without fire, and 4) no phosphite spray with fire. A phosphite treatment was applied either 6 weeks pre-fire or 9 months post-fire when all resprouter species had sufficient foliage. Leaf water potentials, leaf gas exchange, lesion lengths on inoculated stems, and phosphite concentration in leaves, stems, lignotubers and roots measured periodically throughout the experiment will be presented.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Assessment of river environment using *Pythium* species

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Although animals and plants have been widely used as an indicator in an environment assessment, studies on the use of microorganisms are still limited. Microorganisms may be a good indicator for an environment assessment, since they



are able to respond more quickly to environmental changes. *Pythium* is a worldwide-distributed genus, consisting of saprophytes, animal and plant pathogens, and mycoparasites. Therefore, we investigated the feasibility of *Pythium* species to be used as an indicator for an assessment of river environment. Soil samples were collected from Japanese pampas grass colonies in river basins of three rivers, Nagara, Kiso and Chikugo Rivers, in Japan. Soil dilution method was applied to isolate *Pythium* species on *Pythium* selective medium. Twenty species and five groups were isolated from the three rivers. Most of the species except for *P. irregulare* did not show any trend in their distribution. Population density of *P. irregulare* was higher in downstream of all three rivers. While soil texture, pH and C/N ratio of soil did not influence the population density of *P. irregulare*, the density was positively correlated to the area of farmland and negatively to the degree of naturalness. The results suggest that *P. irregulare* might be a useful indicator for evaluation of the impact level of agricultural activity in river.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Research on *Phytophthora* diseases of crops in Bangladesh

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Abstract

Diseases caused by *Phytophthora* are a very common and widespread constraint for crop production. Most important species are *P. infestans*, *P. parasitica* and *P. palmivora*. *Phytophthora infestans* is a common visitor in Bangladesh, causing severe damage of potato and tomato. It causes yield reduction ranging from 25.0 to 57.8%. In potato prevalence of 20 races of *P. infestans* has been detected in the country. Potato varieties Raja, Dheera and BARI TPS-1 showed tolerant reaction to late blight. More than 25 fungicides have been recommended against the disease. Metalaxyl resistant strain of *P. infestans* has been detected in the country. Spraying both contact and systemic fungicides in proper time showed most effective in controlling the disease. *Phytophthora parasitica* possess wide host range and specially limiting factor for seedling production in seedbed, gummosis disease in lemon and bud rot of coconut. Solarization for seed bed diseases and fungicides for foliage disease have been recommended. A good number of fungicides have been registered for controlling leaf blight of aroids (*P. colocasiae*), leaf rot of betelvine (*P. palmivora*) have been suggested to combat the disease.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Research on Progress of *Pythium* diseases in Bangladesh

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Abstract

The genus *Pythium* is one of the most devastating soil borne diseases in Bangladesh. It possess wide host range. The most common genera are: *P. aphanidermatum*, *P. debaryanum* and *P. butleri*. Soil solarization (45 days), sawdust burning and soil amendment with poultry refuse and oilcake found promising in controlling damping off disease of vegetable crops in seed bed. *Pythium aphanidermatum* causing rhizome rot is alarming for ginger production. Application of antagonist *Trichoderma harzianum* not worked well against the disease. Integration of soil organic amendment (mustard oil cake or poultry refuse) with fungicidal drenching markedly reduced rhizome rot of ginger. Stem rot of papaya (*P. aphanidermatum*) also a common threat for papaya production. In panikachu (*Colocasia esculenta*) the fungus seriously affect on curd, causing rotting of internal tissue resulting death of plants.

SESSION: Morphological and molecular taxonomic methods

PRESENTATION: Oral

Abstract 1:

Molecular taxonomy of recently described *Phytophthora* and *Pythium*

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In the frame of environmental monitoring of managed and natural ecosystems for *Phytophthora* and *Pythium* diseases, new species, clearly distinct from previously described taxa have been observed. Following conventional morphological description, molecular characterization has been applied and supported unambiguously the status of new species of these findings. *Pythium sterilum* and *Pythium spiculum* were found in France, Spain and Poland in soils of decline forest stands and vineyards. *Pythium mercuriale* was discovered in France, Poland, South Africa and Spain in diseased *Vitis vinifera*, *Macadamia integrifolia*, roots lesions on *Quercus* spp. and soils of decline forest stands and vineyards. *Pythium recalitrans* was collected from diseased collected from grapevine roots in South Africa and roots of common beet (*Beta vulgaris*) in Spain. *Phytophthora polonica* was retrieved in numerous occasions from declining forest soils from alder stands in Poland. *Phytophthora sylvatica* (ex *Phytophthora* taxon forest soil) and *Phytophthora hungarica* were found in soils of declining alder stands and diseased alder roots. In the course of these studies, molecular diagnostics tools have been developed and include a set of oomycete specific amplification primers, specific real time PCR protocols for *P. alni* and *P. ramorum*, as well as a DNA chip for *Pythium* and *Phytophthora* species detection and diagnostics. The oomycete specific primer set was used to develop important tools for oomycete environmental survey and diagnostic: Automated Ribosomal Intergenic Spacer Analysis (ARISA) and single tube nested PCR.

SESSION: Advances in systems for identification and diagnostics???? Or other session

PRESENTATION: Poster

Abstract 2:

Genetic transformation of *Phytophthora ramorum* with the jelly fish GFP Gene

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The important quarantine organism *Phytophthora ramorum* has been dramatically increasing its host range in the past years and represents a threat to environment, plant nursery activities and commercial trade. Many studies concerning *P. ramorum* focus on these issues but still very little is known about the latency period of the pathogen. Such information would be very important when sampling and analyzing potentially infected plant material. In order to know where and how to search in asymptomatic plants, a tool for following and understanding the infection process would be necessary. We attempted to develop such a tool by establishing a reliable method for the stable genetic transformation of *P. ramorum* isolates. The transferred genes were the marker gene nptII for resistance to genitcin and the target gene GFP. The first and most important step in this protocol was to develop a stable system to produce protoplasts from *P. ramorum* tissue. The next step was the transformation of protoplasts with plasmids containing marker gene and target gene, following an improved polyethylene glycol (PEG)-mediated protocol of protoplasts transformation. After transformation, protoplasts were cultivated on a selective medium and allowed to regenerate mycelium. The selected transformants were checked for integration and expression of transferred genes by PCR amplification and the use of anti-GFP antibodies. About forty-three different transformed isolates were produced and then tested for GFP fluorescence, GFP expression and GFP gene integration. They were further tested for their infection potential on Rhododendron plants.

SESSION: Nomenclature of Present Taxa and Putative New Species

PRESENTATION: Oral

Separation of *Pythium* taxa using nuclear and mitochondrial DNA markers: Proposal of a new genus, *Phytophythium* gen. nov.

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Pythium boreale, *P. ostracodes*, *P. oedochilum*, *P. chamaeophyon*, *P. helicoides*, *P. cucurbitacearum*, *P. vexans*, and *P. indigoferae* belong to what is currently classified as clade K of *Pythium* species. This clade shares morphological characteristics of both *Pythium* and *Phytophthora* species. Like in *Phytophthora*, the sporangia of species in this group can be ovoid, often with a papillum, and they proliferate internally in most species. Zoospore development on the other hand, occurs in a vesicle outside the sporangium as is the case in *Pythium*. Elicitin-like protein similar to *Phytophthora*



have been found in some species from this group. On a molecular level, the separation of oomycete taxa of the two main orders Saprolegniales and Peronosporales, using Large Subunit (28S) ribosomal and Cytochrome Oxidase I (COI) mitochondrial DNA sequences, can be demonstrated. Within Peronosporales, a polyphyletic origin of the genus *Pythium* is apparent. Previous studies have identified this molecular polyphyly and proposed a re-evaluation of the genus with particular reference to species of *Pythium* Clade K. Analysis of 28S and COI molecular data shows strong support for the separation of Clade K from both *Pythium* and *Phytophthora*, leading to the proposal of a new genus description for *Phytopythium* gen. nov.

SESSION: Morphological and Molecular Taxonomic Methods
PRESENTATION: Oral

Molecular detection of *Phytophthora cryptogea* on *Calendula officinalis* and *Gerbera jamesonii* artificially inoculated with zoospores

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A conventional PCR and a SYBR Green real time PCR assays for the detection and quantification of *Phytophthora cryptogea*, an economically important pathogen, have been developed and tested using *Ypt1* gene specific primers. A protocol for *Phytophthora cryptogea* zoospores production was optimized. Five different concentrations of *Phytophthora cryptogea* zoospores (5×10^5 , 5×10^4 , 5×10^3 , 5×10^2 , 5×10^1 zoospores/ml) were used as inoculum on pot marigold (*Calendula officinalis*) and gerbera (*Gerbera jamesonii*) plants. Conventional PCR was able to detect the pathogen in artificially inoculated symptomless pot marigold (collected 12 days after pathogen inoculation) and gerbera plants (after 8 days), with the suspension of 5×10^5 , 5×10^4 , 5×10^3 *P. cryptogea* zoospores/ml. Real time PCR showed the possibility to detect the pathogen in artificially inoculated symptomless pot marigold (collected 8 days after pathogen inoculation) and gerbera plants (after 4 days), with the suspension of 5×10^5 , 5×10^4 zoospores/ml. Real time PCR was more sensitive than conventional PCR since it enabled detection of the pathogen 4 days before conventional PCR and 6 days before the appearance of symptoms both on pot marigold and gerbera plants. The molecular detection methods developed in this study could be a valid complement to traditional methods and should assist in setting up monitoring programmes for ornamental crops threatened by *P. cryptogea*.

SESSION: Ecology, Biogeography, and Epidemiology
PRESENTATION: Oral

Races of *Phytophthora clandestina* causing subterranean clover root rot in the rainfall zones of the agricultural belt of Western Australia

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Phytophthora clandestina is an important pathogen of annual pasture legumes across southern Australia, especially subterranean clover (*Trifolium subterraneum*) on which it is the most important root rot pathogen. *P. clandestina* had been found to only occur in Australia, with the majority of its distribution and most diversity of races found in the 700-1000 mm rainfall zones in the south-west of Western Australia. The most promising and economic approach to manage this disease is through host resistance. However, this appeared to break down and/or was lost over time, suggesting the rapid development of new races of the pathogen in response to field deployment of various host resistances. Our recent work, screening isolates of *P. clandestina* across subterranean clover host differentials to characterize races, identified 10 races with varying degrees of pathogenicity on the differential host plant cultivars. Races 173 and 177 were found to be widely distributed and were the most common in Western Australia, together constituting 80% of the isolates characterized. While resistance in subterranean clover against some races was easy to identify, it was less readily found against other races. One race in particular, race 177, was the most virulent of the races across the subterranean clover genotypes tested and no resistance to this race has been identified to date. Hence, studies were undertaken to locate sources of tolerance/resistance to race 177, among newly available subterranean clover germplasm

SESSION: Ecology, Biogeography, and Epidemiology
PRESENTATION: Poster

Efficacy of fungicides applied to the soil for management of *Phytophthora* root and crown rot on chile peppers

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The oomycete pathogen *Phytophthora capsici* can cause extensive losses in pepper plantings. Studies were initiated to evaluate and compare several new and existing fungicides for their ability to protect pepper plants from *Phytophthora* crown and root rot when applied to the soil. In 2005 and 2006, soil was collected in the field from within the root zone of chile pepper plants infected with *P. capsici*. Five parts of this field soil was thoroughly mixed with 2 parts sand in a large container, then dispensed into a series of 0.5 liter capacity plastic pots. A bell pepper seedling (approximately 8 cm tall) was transplanted into the soil within each pot, after which the soil in each container was drenched with 200 ml of a solution containing one of the chemical treatments. Plants were maintained in a greenhouse for approximately 2 months. Additional applications of materials to the soil were made after 1-month in 2005 and after 3- and 6-weeks in 2006. Average survival time for pepper plants grown in infested soil not treated with a fungicide was 5 and 29 days in 2005 and 2006, respectively. On the other hand, in both years the survival of plants in soil treated with cyazofamid, fluazinam, fluopicolide, dimethomorph, mandipropamid, and fenamidone + propamocarb did not differ significantly from plants grown in soil not containing *P. capsici*. These active ingredients were all effective tools for management of root and crown rot on pepper plants when applied as a soil drench in these trials.

SESSION: Ecology, Biogeography, and Epidemiology
PRESENTATION: Poster

Root and crown rots of green beans caused by several species of *Pythium* in southeast Andalucía, Spain

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Green beans (*Phaseolus vulgaris*) are extensively grown in plastic-houses of southeastern Spain, where important crop damage is caused by diseases affecting roots and basal stems of adult plants. Five species of *Pythium*, i.e. *P. aphanidermatum*, *P. irregulare*, *P. myriotylum*, *P. ultimum* var. *ultimum*, and the recently described *P. solare*, were associated to these symptoms. However, *P. aphanidermatum*, *P. myriotylum*, and *P. solare* are among the most virulent pathogens causing root necrosis, stem necrotic streaks, wilt and high levels of plant death, in contrast with *P. irregulare* and *P. ultimum* var. *ultimum*, which showed much lower virulence, with symptoms restricted to root necrosis. The severity of infections caused by *Pythium* spp. depends on the species involved and of the temperature regimes during the different crop periods. Thus, higher temperatures (25-35°C) occurring mainly in spring-summer crops are more favourable to *P. aphanidermatum* and, specially, to *P. myriotylum*, whereas *P. solare* is very severe to crops grown in fall or in spring, with moderate temperatures (20-30°C). Therefore, disease levels achieved in artificially inoculated plants of the highly susceptible cv. Emerite were usually heterogeneous, when *P. solare* killed most of the plants the damage by *P. aphanidermatum* and by *P. myriotylum* was not severe and viceversa. *P. irregulare* and *P. ultimum* var. *ultimum* required low temperatures for infection and the root necrotic symptoms were only observed under these conditions.

SESSION: Ecology, Biogeography, and Epidemiology
PRESENTATION: Poster

A survey for *Phytophthora* diseases in ornamental plants in Tennessee commercial nurseries

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Phytophthora diseases are important in field and container-grown trees in nurseries and landscape settings. A survey for *Phytophthora* diseases in Tennessee commercial nurseries evaluated different tree species and isolated *Phytophthora* spp. and other pathogens that cause *Phytophthora*-like symptoms. During cool weather (18-23 C), some container-grown dogwood trees (*Cornus kousa* and *C. florida*) developed blight symptoms starting on the upper foliage with a sudden appearance of light brown to red-brown leaf blight and tip dieback. Over time, new growth masked the initial infection, but some affected plants died later in the season or in the following year during bud-break. Similar symptoms in field-grown dogwoods resulted in sporadic plant mortality. Four fungi, *Phytophthora* spp., *F. oxysporum*, *F. solani*, and *Pestalotiopsis* sp. were isolated from symptomatic leaves and stem tips. Pathogenicity tests with *Phytophthora* spp., *F. oxysporum* and *Pestalotiopsis* sp reproduced the initial disease symptoms in dogwood seedlings killing test plants in 14-18 days. Disease development from *F. solani* was much slower, killing test plants in 30 days. *Phytophthora* sp., *F. oxysporum* and *Pestalotiopsis* sp. were also isolated from diverse trees including maple, Cyprus, juniper and arborvitae, but pathogenicity tests have not been completed. Our observations suggest that *F. oxysporum*, *F. solani* and *Pestalotiopsis* sp., may be involved in disease complexes with *Phytophthora*. These pathogens are not



known to be as destructive on trees as *Phytophthora spp.*, but understanding their role in plant mortality will benefit disease management.

Trade-offs between virulence and sporulation in *Phytophthora ramorum*

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The plant pathogen *Phytophthora ramorum* exhibits a broad virulence, defined here as host range, comprising angiosperms, gymnosperms and ferns of diverse biogeographic origin. Despite such pathogenic trends, it has not become widespread in natural ecosystems as was initially expected. We here provide evidence of trade-offs between virulence and sporangial production as a possible clue explaining why this pathogen has not become pandemic. Detached leaves of plant members of Sub-Mediterranean, Mediterranean and Macaronesian laurel forests were inoculated with zoospores and the lesion area and sporangial production calculated seven days after the inoculation. We also compared its colony growth rate and sporangial capacity in carrot agar, a suitable culture medium for this purpose. Lesion areas varied widely between plant species from large lesions almost reaching the colony growth rate of *P. ramorum* in carrot agar to small lesions. Sporangial production was usually about 1000 times lower on inoculated plants than on carrot agar. Such a reduced sporulation, together with a short-distance dispersal of sporangia by rain-splash mechanisms, constrains the spread of *P. ramorum* to favourable environmental, such as those found in nurseries (host density dependent/ within-host transmission), or where mild rainy climates enhance between-hosts transmission within the plant community. The behaviour of *P. ramorum* in our pathogenic tests tends to fit with that expected for a multiple-host plant pathogen in its native habitat: **coevolution would have favoured the selection for a broader virulence at the expense of an average lower aggressiveness and sporangial production on their main hosts.**

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Abstract 1:

Phytophthora root and crown rot on fruit trees in Bulgaria

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In the period after year 2000 symptoms of new, unknown disease causing dead of single or group of trees have been found on apple, cherries and almonds in Bulgaria. Laboratory analyses of pathogenic fungi that have been isolated proved that they belong to genus *Phytophthora*. Prevailing specie was *Phytophthora cactorum*. In vitro tests have been done about *P. cactorum* and *P. citrophthora* utilization of different C, P, N, and S sources. Different N sources have more influence on mycelia growth of *P. citrophthora* than on *P. cactorum*. Added amino acids suppress mycelia growth of *P. citrophthora*. Carbon sources are utilized well by *P. cactorum*, and sacharose and maltose have better effect on mycelia growth of *P. citrophthora*. $MgSO_4 \cdot 7H_2O$ is preferred sulphur source for both fungi, as well L-cysteine and L-methionine only for *P. cactorum*. Physiological changes as carbon/sugar, protein and dry matter content have been studied, as well N, P, K quantities in infected and healthy cherry trees. In infected woody tissues changes in protein and sugar content is more significant, and also in Ca and Mg. Differences in amino acids content have been studied in apple rootstocks, and breeding activity in cherry and quince rootstocks, healthy and inoculated. Two months after infection changes have been registered in photosynthesis and transpiration. Changes in aminoacids content are more significant in woody tissues than in leaves.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Abstract 2:

Monitoring of *Phytophthora* species on fruit trees in Bulgaria

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During the period 1999-2000 on fruit trees (2-4 years old apple and cherries) in Plovdiv region new type of symptoms have been found after heavy rains and damping of the trees. On some plants during July leaves become reddish and latter in the season affected leaves fall off. Diseased trees dye back 10 to 50% same or the following season. Infected



tissues have been used for isolating causal agents on PDA and selective PARP media, or by applying “trap cultures” protocol on young green apple fruits /Granny smith/. Most of the fungi that have been isolated belong to genus *Phytophthora*. Pathogenicity of the isolates have been tested on green apple fruits or on 1 year old apple rootstocks.

In the period 2000-2007 monitoring of young fruit gardens in South West, Central and South East part of Bulgaria have been carried out, including apple, cherries, almond, peach orchards and nurseries. In some nurseries disease spread is up to 2%, and in orchards can reach up to 10%. Samples from plants with symptoms have been taken and isolates have been done. Most of isolates have been of fungi from genus *Phytophthora*. Based on morphological and cultural characteristics (sporangiospores, oogonia and anteridia, oospores, etc.) and temperature requirements following species have been identified: *Phytophthora cactorum*, *P. citrophthora*, *P. drechsleri*, hybrid specie and *Pythium*. Morphology identification will be followed by application of molecular tools for studding the samples. Laboratory studies for the effect of temperature of mycelia growth point out that most the isolates can grow between 5-6 to 30 °C, optimum - 16-18 to 26 °C. Only 3 strains develop up to 35°C, and one growth well. Optimal pH for mycelia is between 6.05 and 8.95. Aiming control of disease spread plot tests have been carried for the resistance of some rootstocks to *P. cactorum*. At the end of the season a good level of resistance have shown M 9, M29C, SP 80, Gisela, GF 677, MAXMA 14. Experiments will continue during 2008.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Oral

Flooding events and rising water temperatures increase the significance of the reed pathogen

Pythium phragmitis

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Pythium species are economically significant soilborne plant pathogens with worldwide distribution, causing seedling damping-off or root rot diseases. *Pythium phragmitis* is a newly described pathogen of common reed (*Phragmites australis*), widespread in the reed-belt of Lake Constance, Germany, and other European freshwater lakes. It is highly aggressive towards reed leaves and seedlings, but obviously does not affect roots. In the context of ‘reed decline’ phenomena, *P. phragmitis* infection of reed inundated during flooding events may be of particular significance. We could show that flooding itself is not necessarily detrimental for reed plants, while in the presence of the pathogen, most submerged leaves and plants were killed within several weeks. Clipped plants did not show regrowth in the *Pythium* infested treatments. Significant losses in vigour and assimilating leaf area of reeds could thus be the result of *Pythium* infection rather than of flooding alone. We suggest that the combination of extended flooding and the presence of *P. phragmitis* might considerably contribute to ‘reed decline’ at Lake Constance. In parallel, we could show that pathogenicity and spread of this species are considerably favoured by rising temperatures. Since an increase in average water temperature has been forecast for Lake Constance, we propose that *P. phragmitis* could be an important factor in the dieback of reed stands, likely to be promoted by predicted climate change phenomena.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Oral

Status of the genera *Phytophthora* and *Pythium* in Argentina

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The genera *Phytophthora* and *Pythium* include important pathogens affecting a wide range of hosts of economic value. The knowledge of these species and its populations allow a better management of the diseases. In order to have a more comprehensive vision of these Straminipiles, a review and an updated report of recent progress in this matter in Argentina was carried out. Information was taken from printed and on line resources (<http://web5.silverplatter.com/webspirs>) such as CAB and Biological Abstract data bases, Proceedings of national and international Scientific Meetings; Bulletins from National Institutions and Universities, periodical Journals, books, and the Phytopathological Atlas from Argentina (www.fitopatoatlas.org.ar). As a result of this review, the information was analyzed and categorized, thus updating the number of species of both genera, their geographical distribution, hosts affected, races, severity, economic damages, sp. nov. described, researched topics. Different conclusions were reached permitting a clearer interpretation of present and future situation and state of the research on these genera in Argentina. All these information will give grounds for a future Survey of Oomycetes of *Phytophthora* and *Pythium* in Argentina.



SESSION: Morphological and molecular taxonomic methods

PRESENTATION: Poster

Detection of *Phytophthora* and *Pythium* species on subtropical crops in Andalusia region, Spain

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The coastal region of Andalusia is the most important subtropical fruit producing area of Europe, with the avocado (*Persea americana*), cherimoya (*Anona squamosa*) and mango (*Mangifera indica*) as the most significant crops. However, these crop trees suffer notable diseases, which could affect production and limit their development. Amongst the diseases caused by oomycetes, the *Phytophthora* root rot caused by *Phytophthora cinnamomi* is without doubt the most serious disease of the avocado, limiting the avocado industry in nearly every country where it is grown. Actually, this destructive invader has been isolated from more than two hundred different fields in this area during the last years. Nevertheless, other *Phytophthora* and *Pythium* species may infect these crops. In this work, we present results of several *Phytophthora* and *Pythium* species that have been recently described in Andalusia infecting avocado and mango trees. In these studies, morphological and molecular characterizations were both performed to attain the species identification. *P. citricola* was isolated from mango roots and has been described as a new pathogen to this crop. *P. capsici* and *P. cactorum* were isolated from avocado roots and fruits respectively and their pathogenicity demonstrated. Finally, *Pythium vexans* was frequently isolated from avocado roots and soils and its harmful capability to avocado was established. Moreover, co-infection of avocado trees with *P. cinnamomi* and *Py. vexans* was recorded. Our findings are important because the presence of *Phytophthora* and *Pythium* species in this subtropical area is hazardous not only to mango and avocado but also to other susceptible hosts.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Population Structure and Sensitivity to Phenylamides of *Phytophthora ramorum* in Spain

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Phytophthora ramorum was first identified in Spain on *Rhododendron* spp. in 2002. Since then surveys have been carried out to detect this pathogen which has been found on nurseries and garden centres. It was detected for the first time in a public garden in 2007. A total of 89 isolates of *P. ramorum* from different geographic areas in Spain were obtained from *Rhododendron*, *Viburnum* and *Camellia*. The isolates were identified as *P. ramorum* based on morphological features: colony morphology, growth rate, cardinal growth temperatures, and production, morphology, and dimensions of sporangia, oogonia, and antheridia. The isolates were paired with A1 and A2 tester strains of *P. cryptogea* on carrot piece agar (CPA), and sexual structures were only induced by *P. cryptogea* A2 tester strain. Inter Simple Sequence Repeat (ISSR) were analyzed to assess genetic diversity using four dinucleotide and six trinucleotide ISSR primers and no polymorphism was identified among genotypes. Our results suggest that *P. ramorum* population in Spain is constituted by clonal descendants of likely a single introduction of the pathogen. All the isolates were assayed for sensitivity to phenylamides fungicides (metalaxyl and mefenoxam). Among isolates from all locations, 89.9% were classified as sensitive, 2.2 % as intermediate, and 7.9 % were resistant to metalaxyl, and most of them (94.4 %) resulted sensitive to mefenoxam. Only isolates obtained from *Camellia* sp. in 2007 were tolerant to these fungicides.

SESSION: Advances in Systems for Identification and Diagnostics

PRESENTATION: Poster

Can nanobiotechnology help in routine diagnosis to determine *Phytophthora* species?

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Determination of *Phytophthora* species with the help of morphological traits presupposes a microbiological laboratory, experienced personnel and time. Using recent molecular techniques like real time PCR and sequencing reduces the time consumption but nevertheless requires experienced personnel and expensive molecular equipment and chemicals. Especially for diagnosis of quarantine organisms reliable and easy to handle techniques which give results within a



short time are demanded. Within a three year project a diagnostic technique will be developed which combines a chip based PCR system with an electrical DNA detection chip. A stationary chip with integrated microstructured heaters and temperature sensors has been developed for amplification of specific *Phytophthora* DNA fragments. On-chip micro array technologies to detect labelled DNA fragments will be combined with microfluidic systems. This combination of nanobiotechnological elements with automated DNA amplification and analysis enables a significantly shortened diagnosis time and the reduction of labour input and costs per sample.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Oral

Abstract 1:

Pythiaceus root pathogens associated with *Eucalyptus gomphocephala* decline in Western Australia

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Eucalyptus gomphocephala is a keystone canopy species endemic to a narrow (5-10 km wide) coastal strip approximately 300 km in length in south-west Western Australia. *E. gomphocephala* is undergoing a significant decline that was first identified as a spot decline in 1994 and now occurs throughout large sections of its remnant distribution within Yalgorup National Park, in some areas resulting in 100% mortality. Multiple factors, including soil-borne pathogens, have been identified as possibly contributing to the decline. Less fine roots are associated with trees on declining sites compared to those on healthy sites. Foliar analysis indicates that declining trees have lower concentrations of some micronutrients, including zinc, which uptake is typically impaired by fine feeder root loss. A range of Pythiaceus microorganisms have been isolated from declining roots, including a new isolation of a yet described *Phytophthora* species. The *Phytophthora* isolates appear morphologically similar to the *Phytophthora citricola* holotype although are distinct using molecular analysis of the internal transcribe region. **The exact phylogeny of the new *Phytophthora* isolates is being determined using sequence analysis of other gene regions.** These isolates may be contributing to the loss of fine roots. Glasshouse trials are currently underway to determine whether these isolates are indeed pathogenic.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Abstract 2:

Phosphite application as an explorative tool in *Eucalyptus gomphocephala* decline in Western Australia

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Eucalyptus gomphocephala is a mediterranean forest canopy species endemic to a narrow (5-10 km wide) coastal strip approximately 300 km in length in south-west Western Australia. *E. gomphocephala* is undergoing a significant decline that was first identified as a spot decline in 1994 and now occurs throughout large sections of its remnant distribution within Yalgorup National Park, in some areas resulting in 100% mortality. The reduction of this keystone species represents a significant modification to the associated ecosystem. Modifications to hydrology, fire regimes, entomological pressures, and fungal and Pythiaceus soil pathogens have been identified as possibly contributing to the decline syndrome. The potential of phosphite (phosphonate), nutrient and insecticide treatments to reverse the decline in tree health was assessed as (a) a method for controlling the decline and (b) a method for diagnosing possible causal agents. Phosphite has been successfully used to control *Phytophthora* and Pythiaceus soil pathogens by inducing a host defense response within the plant. Stem injection of declining *Eucalyptus gomphocephala* in the present study has resulted in improved canopy health and vigor, indicating that *Phytophthora* and/or other Pythiaceus microorganisms may be playing a role in the decline. The impact of phosphite application on nutrient uptake and fine feeder root concentration was also assessed.

SESSION: Advances in Systems for Identification and Diagnostics



PRESENTATION: Poster

PCR-RFLP markers identify three lineages of the North American and European populations of *Phytophthora ramorum*

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Phytophthora ramorum, causal agent of Sudden Oak Death, has a wide host range and is found in the northern hemisphere. It is thought to be introduced to North America and Europe, but its origin is unknown. It has three major clonal lineages and two mating types. Sexual reproduction can only occur when both mating types are present in the same location. In most cases, these mating types have been restricted to different continents. The European lineage (EU1, mostly A1 mating type) has been consistently found in Europe, and occasionally in North American nurseries. The North American lineages (NA1 and NA2, all A2 mating type) have not been found in Europe at present. All molecular tests currently available for detecting *P. ramorum* do so at the species level. In tests that use the ITS region, cross-reaction with other closely related species such as *P. hibernalis*, *P. foliorum*, or *P. lateralis* can occur. Regions in the mitochondrial gene *Cox1* are different among *P. ramorum* lineages and mitochondrial genotyping of the North American and European populations seems to be sufficient to differentiate between mating types, since the EU1 lineage is mostly A1 and both NA1 and NA2 lineages are A2. *Phytophthora ramorum* isolates can be identified to lineage using PCR-RFLP of the *Cox1* gene, first using *Apo1* to separate *P. ramorum* from other species and EU1 from North American populations, and then *Ava1* to distinguish between NA1 and NA2 genotypes. However, *P. foliorum* had the same mtDNA genotype as *P. ramorum* NA1 isolates

SESSION: Integrating morphological and molecular tools for a unified phylogeny and classification

PRESENTATION: Poster

Morphological and molecular identification of *Phytophthora* isolates from declining forest stands in Hungary

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Since 1999 occurrence and impact of *Phytophthora* species have been investigated in declining forest stands in Hungary. *Phytophthora* was isolated from soil samples taken from around the symptomatic trees by baiting with *Prunus laurocerasus* leaves. The isolates were identified on morphology and by ITS sequences of rDNA. Six species were determined and furthermore four haplotypes were delimited representing undescribed taxa. The isolates belong to four major clada of the phylogenetic tree built on the ITS sequences. *P. cactorum* (clade I) occurs in declining *Juglans nigra* stands. *P. citricola* (clade II) was found in large numbers in *J. nigra*, less frequently in *Alnus glutinosa*, *Quercus petraea* and *Q. cerris*.

P. gonapodyides, *P. inunctata*, *P. megasperma* and the four undefined haplotypes (clade VI), as well as *P. alni* (clade VII) occur in *Alnus glutinosa* stands in wet lowland forests. High intraspecific variation was detected among the isolates of *P. citricola* (4 haplotypes) and *P. gonapodyides* (5 haplotypes). The pathogenicity tests showed high aggressivity of *P. citricola* and *P. cactorum* in *J. nigra* and of *P. alni* in *A. glutinosa*.

SESSION: Advances in systems for identification and diagnostics

PRESENTATION: Poster

Modifications of PARP Medium Using Fluazinam, Miconazole, and Nystatin for Detection of *Pythium* spp. in Soil

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The standard *Pythium* selective medium PARP [pimaricin + ampicillin + rifampicin + pentachloronitrobenzene (PCNB) agar], was modified by replacing PCNB and pimaricin with other antifungal agents. Several antifungal agents such as fluazinam, miconazole, 2,4,5,6-tetrachloroisophthalonitrile (TPN), iminoctadine triacetate, tolclofos-methyl, captan, and nystatin, were initially screened for effects on *Pythium* growth. Based on these results, the following three media were developed: PARF (pimaricin + ampicillin + rifampicin + fluazinam agar), NARF (nystatin + ampicillin + rifampicin + fluazinam agar), and NARM (nystatin + ampicillin + rifampicin + miconazole agar). New media were comparable with PARP on yield of naturally occurring *Pythium* species from two different types of soil using the soil-dilution plating technique. PARF and NARF were significantly better than PARP on inhibition of non-pythiaceous



microbes on the soil-dilution-plates, but were significantly lower than PARP on the rate of mycelial growth of six of eight isolates belonging to seven species of *Pythium*. NARM was equivalent to PARP on inhibition of non-pythiaceous microbes except for *Fusarium oxysporum*, and was significantly better than PARP on rate of mycelial growth of five of eight isolates of *Pythium*.

SESSION: Advances in systems for identification and diagnostics

PRESENTATION: Poster

Reliable discrimination of distinct *Phytophthora hedraiaandra* x *cactorum* hybrids through real-time PCR

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Several *Phytophthora* isolates were obtained during a survey of *Phytophthora* spp. at Rhododendron nurseries. Species typing was initially carried out via PCR-RFLP of the rDNA ITS region. Several isolates showed restriction patterns identical to those of *P. cactorum* (AluI-alternative)/*P. hedraiaandra*. The ITS1 region of three isolates was sequenced after cloning. For one isolate, it was identical to the *P. cactorum* ITS1 sequence, for the other two to the *P. hedraiaandra* ITS1 sequence. Because we could not exclude that these isolates were hybrids of *P. cactorum* and *P. hedraiaandra*, they were further analyzed through direct sequencing of the PCR-amplified ITS region. In hybrid species, double peaks should be present at the polymorphic positions. A faster method is the restriction of the PCR-amplified ITS1 region by *Hph*I, which is discriminative for *P. cactorum* and *P. hedraiaandra*. Both methods confirmed equally well that several isolates were indeed hybrids and also revealed the presence of different proportions of parental ITS copies. To determine these proportions, we designed ITS-based real-time quantitative PCR (qPCR) primers for *P. cactorum* and for *P. hedraiaandra*. Surprisingly, some isolates formerly identified as non-hybrids were actually hybrids, but with a relatively low ITS copy number of one parent. Therefore, only qPCR enabled unambiguous distinction between hybrids and parental species. In contrast to the other methods, it also allowed a clear discrimination between four different hybrid types.

SESSION: Ecology, Biogeography, and Epidemiology

PRESENTATION: Poster

Persistence of *Phytophthora kernoviae* and *P. ramorum* on infested sites and the impact on disease management

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Phytophthora ramorum and *P. kernoviae* are considered to be two of several invasive tree Phytophthoras that have recently arrived in the UK. Both have established to some extent, primarily in the south west of England, and are found in both planted woodland-gardens and woodlands where rhododendrons (mainly *Rhododendron ponticum*) dominate. Foliage of rhododendron is generally highly susceptible to infection by both these aerial Phytophthoras and supports abundant sporulation. There is little doubt that this host has played a key part in the spread of both these pathogens in the natural environment and subsequent infection of trees. While *P. ramorum* is mainly found primarily in woodland-gardens that are tourist venues in Cornwall, southwest England, *P. kernoviae* is found more often in mixed woodlands with rhododendron as an understorey component. A key part of efforts to eradicate and contain both pathogens therefore centers on the removal of infected foliar hosts, particularly rhododendron because it appears to be the primary inoculum source on most outbreak sites. However, studies at both *P. ramorum* and *P. kernoviae* infested sites show that both pathogens may persist for many months, even years, in infected rhododendron foliage that is incorporated into the litter layer of infested woodlands. This suggests that removal of infected rhododendron is only the first stage in any attempt to eradicate these pathogens, as they have the potential to persist for extended periods and infect any re-growth of rhododendron that occurs later

SESSION: Evolution and Population genetics

PRESENTATION: Poster

Genetic Diversity of Pathogenic and Nonpathogenic populations of *Phytophthora capsici* Isolated from Pepper Plants and Soil

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Thirty-six *Phytophthora capsici* strains and one *Phytophthora parasitica* strain were evaluated for pathogenicity on pepper (*Capsicum annuum* L.) plants. The strains represent a range of geographic locations and were collected primarily from pepper stems or roots of plants with symptoms of blight, and from soil. Among the 36 *P. capsici* strains, 13 and 23 were non pathogenic (NP) and pathogenic to pepper, respectively. Genetic diversity was assessed by sequence analysis of the rDNA internal transcribed spacers (ITS1 and ITS2) and the 5.8S rDNA gene, and by RAPD analysis. The strains grouped into two ITS clusters. All pathogenic strains clustered in one group, consisting of four subgroups. The NP *P. capsici* strains resolved into two groups, in which 84.6% of NP strains were in ITS group II, and 15.4% of the NP strains were in ITS group I. Pathogenic and nonpathogenic strains also separated into different clusters based on RAPD data, although two of the NP strains grouped with pathogenic strains. The population of pathogenic strains was less diverse than that of the NP strains. No relationship was established between the genetic profiles of pathogenicity and the geographic origin of the host plant.

