

### PHYTOPHTHORA/PYTHIUM/DOWNY MILDEWS and related genera 2018 Sixth International Workshop

Oomycetes in the era of Plant Health in A Global Economy



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Workshop organized by the ISPP – SMC Oomycetes to celebrate the 50th anniversary of ISPP

> Boston, Massachusetts, USA July 28, 2008

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OOMYCETES WORKSHOP CELEBRATING 50TH ANNYVERSARY OF JSPP - JULY 28, 2018

#### WELCOME REMARKS

It is a great pleasure to welcome all of you to the "6th International Oomycetes Workshop: *Phytophthora, Pythium*, Downy Mildews and related genera: Oomycetes in the era of Plant Health in A Global Economy". This workshop is organized to celebrate the "50th anniversary of the International Society of Plant Pathology" and presented in association to the 11th International Congress of Plant Pathology in Boston, Massachusetts, USA. The Program for the workshop features the contributions of 28 Speakers – world renowned authorities in the area of Oomycetes. At least eighty five participants, in addition to the speakers, moderators and collaborators, 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.

As the Chair of the ISPP Subject Matter Committee (SMC), along with the Co-Chairs Marco Thines (Germany) and David Cooke (UK) as well as the 35 members of this SMC, I extend a warm welcome to the keynote speakers, collaborators, and participants of the event. We are very pleased to inform you that ninety eight scientists from twenty five countries from all continents of the world are going to participate at the event. 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 world authorities in the Oomycetes who are not only bringing their great experiences gained from such a vast amount of research, but also many of them presenting their experiences with a great number of collaborators.

We are highly honored to have the presence of Dr. Mary Palm, President of the American Phytopathological Society to make to "Opening Remarks" and to have Dr. Greg Johnson, President of the International Society of Plant Pathology presenting the "Closing Remarks". These important visits to our event in conjunction with the great number of world authorities present (two of them participating remotely from Germany and Australia), plus the enthusiasm of the co-organizers, moderators and participants make our celebration for the "50th anniversary of the International Society of Plant Pathology" being recognized in our "6<sup>th</sup> International Oomycetes Workshop" truly memorable.

Gloria Abad, Ph.D. Chair of the ISPP-SMC Oomycetes

### 6th International Oomycetes Workshop: *Phytophthora*, *Pythium*, Downy Mildews and related genera

Oomycetes in the era of Plant Health in A Global Economy

Workshop organized to celebrate the 50th anniversary of ISPP

#### WORKSHOP DESCRIPTION AND OBJECTIVES

The Irish Potato Famine of 1845 marked human history, and the description of the agent triggering it, *Phytophthora infestans*, in 1876 marked the beginning of modern Plant Pathology. This Oomycetes Workshop will be presented to salute and celebrate our science in the 50th anniversary of the ISPP. Topics will include taxonomy and nomenclature, identification and diagnostics, population diversity, genetics and genomics, enhancing resistance, management and control, trade regulations, sustainable production, and microbiomics. Authorities will be keynote speakers and contributions from oral and poster presentations are encouraged to participants. The "IDphy: Molecular and Morphological Identification of *Phytophthora* Based on the Types," an online resource with Lucid Key, and Tabular Key that was developed to facilitate accurate and robust identification of species using type specimens will also be demonstrated.

#### **PRESENTED BY**

Subject Matter Committee (SMC) of the Oomycetes of the International Society of Plant Pathology (ISPP) and its 35 members from 14 countries. Refer to: <u>http://www.isppweb.org/smc\_10.asp</u>

Chair Gloria Abad (USA) Co-chair Marco Thines (Germany) Co-chair David Cooke (UK)

#### WORKSHOP CHAIR AND ORGANIZER

Gloria Abad (USA)

#### WORKSHOP SCIENTIFIC COMMITTEE

Marco Thines (Germany), David Cooke (UK), Frank Martin (USA), Guillaume Bilodeau (Canada), Lina Quesada (USA), JoAnne Crouch (USA), Pallem Chowdappa (India), Santa Olga Cacciola (Italy), Ronald French (USA), John Bienapfl (USA), Yazmín Rivera (USA), Carla Garzon (USA), Pablo Grijalba (Argentina), Chris Spies (South Africa)

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#### **APS Sponsoring Committees**

Diagnostics Mycology Plant Pathogen and Disease Detection Regulatory Plant Pathology Office of International Programs APS-Caribbean Division

#### **International Patronage**

Italian Phytopathological Society (SIPaV) Latin American Association of Phytopathology (ALF) Phytopathological Society of Nigeria (PSN)

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#### AGENDA

#### 6th International Oomycetes Workshop: *Phytophthora, Pythium*, Downy Mildews and related genera

Satellite Meeting, Saturday, July 28th, 2018 John B. Hynes Veterans Memorial Convention Center – Room 206

#### 7:00 – 8:00 Registrations and posters set up.

**8:00 – 8:05 am** Welcoming Remarks by **Mary Palm** (USA), President of the American Phytopathological Society (APS).

**8:05 – 8:15 am** Introduction to the workshop by **Gloria Abad** (USA) Chair and organizer of the workshop.

#### **SESSION #1**

#### **8:15 – 9:00 am Taxonomy and Nomenclature: Past, present, and future.**

Moderator: Hai Nguyen (Canada)

• **8:15 – 8:30 am. Gloria Abad** (USA) **Topic:** Status of the Taxonomy of *Phytophthora* and related genera: Why the Ex-types Matter?.

• **8:30 – 8:45 am. Marco Thines** (Germany). **Topic:** Status of the Taxonomy of Downy Mildews.

• **8:45** – **9:00 am. Carla Garzon** (USA), Gloria Abad (USA), and Jin-Hsing Wang (Taiwan). **Topic:** Taxonomy of *Pythium, Phytopythium,* and *Pythiogeton* associated to plants and aspects on the aggressive vs. the moderately, low and non-aggressive species.

#### **SESSION #2**

# 9:00 -10:00 am Identification and diagnostics: From the traditional tools to the Innovative Genomic Sequencing Technologies (IGST) including NGS and TGS.

#### Moderator: Guillaume Bilodeau (Canada)

• **9:00 – 9:15 am. Steven Jeffers** (USA). **Topic:** Isolation and identification of *Phytophthora* species the "old-fashioned" way.

• 9:15 – 9:30 am. David Cooke (UK), Eva Randall, Beatrix Clark, Peter Thorpe, Leighton Pritchard, Tim Pettit, Frederickson-Matika Debbie, Sarah Green (UK). Topic: The validation of eDNA barcoding in the study of Phytophthora diversity for plant health testing and natural ecosystem surveillance.

• 9:30 – 9:45 am. Frank Martin (USA). Topic: Mitochondrial Genomics: a systematic method for development of loci for diagnostics, taxonomy, phylogenetics and metagenomic studies.

9:45 – 10:00 am. Richard Hamelin (Canada) Topic: BioSurveillance of Forest Alien Enemies (BioSAFE): developing the next generation of genomics tools.

#### **10:00 - 10:30 am Break with posters view.**

#### SESSION #3

**10:30 – 12:00 pm Diversity in cultivated and natural environments: The global spread of pathogens.** 

#### **Moderator: Ronald French** (USA)

 10:30 – 10:45 am. Santa Olga Cacciola (Italy), Bruno Scanu, Leonardo Schena, Italy), David E.L. Cooke (UK). Topic: The Diversity and Ecology of *Phytophthora* in Europe

• **10:45 – 11:00 am. Koji Kageyama** (Japan). **Topic:** "*Phytophthora* and other Oomycetes diversity in cultivated and natural environments in Japan".

11:00 – 11:15 am. Pablo Grijalba, Grijalba P., Palmucci H., Greslebin A., Vélez M. L.,
Irribarren J. (Argentina); Gonçalves D., Costamilán L. (Brazil); Restrepo S. (Colombia);
Ahumada R. (Chile); Aragón L. (Perú); Stewart S. (Uruguay). Topic: "*Phytophthora* and other
Oomycetes diversity in cultivated and natural environments in Latin America region"

• **11:15 – 11:30 am. Guillaume Bilodeau** (Canada), Gloria Abad (USA), John Bienapfl (USA), Yazmin Rivera (USA), Sylvia Fernandez (Mexico). **Topic:** "*Phytophthora*, Downy Mildews and other Oomycetes diversity in cultivated and natural environments in North America region"

11:30 – 11:45 am. Treena Burgess (Australia), Thomas Giles, et al. Topic:
"*Phytophthora* and other Oomycetes diversity in cultivated and natural environments in Oceania"
11:45 – 12:00 pm. Marco Thines (Germany) et al. Topic: Diversity of Downy Mildews

in cultivated and natural environments: The global spread of pathogens.

#### 12:00 – 1:00 pm Lunch Break with posters view.

#### **SESSION #4**

**1:00 – 1:45 pm Management and control: Advanced IPM-tools, improved biocontrol, enhancing resistance, sustainable production, agricultural systems for the future.** 

#### Moderator: Nick Grünwald (USA)

• **1:00 – 1:15 pm. David Shew (USA). Topic:** Quantifying Adaptation to Host Resistance in *Phytopthora nicotianae.* 

• **1:15 – 1:30 pm. Lina Quesada** (USA), Alamgir Rahman (USA), Frank Martin (USA), Tim Miles (USA) **Topic:** Tracking a cucurbit killer: developing bio-surveillance tools for improved management of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*.

• **1:30 – 1:45 pm. Aleš Lebeda,** Eva Křístková, Božena Sedláková (Czech Republic). **Topic:** Pathotypes and races of *Pseudoperonospora cubensis* – great challenge for science and cucurbit breeding.

• 1:45 – 2:00 pm. Mohamed Khan (USA) Topic: Managing *Aphanomyces* on Sugar Beet.

#### SESSION #5

# □ 2:00 – 2:45 pm Plant pathogenic Oomycetes of concern: Trade regulations, advanced monitoring and certification.

Moderator: Yazmin Rivera (USA)

• **2:00 – 2:15 pm. Jo Anne Crouch** (USA), Nicholas Le Blanc (USA), Catalina Salgado-Salazar (USA). **Topic:** Emerging downy mildews: The same old pathogens or new enemies?

• **2:15 – 2:30 pm. Susan J. Frankel** (USA). Topic: An Update on *Phytophthora ramorum*, *P. tentaculata* and other species of regulatory concern in the USA.

• **2:30 – 2:45 pm. Sarah Green (FR), David Cooke(JHI)**, Mariella Marzano (FR), Peter Thorpe (JHI), Mike Dunn (FR), Debbie Frederickson-Matika (FR), Beatrix Clark (JHI), Mhairi Clark (FR), Tim Pettitt (UW), Eva Randall (JHI), Leighton Pritchard (JHI), Alexandra Schlenzig (SASA), Jane Barbrook (APHA) (ALL UK). Topic: *Phytophthora* in plant nursery settings, engaging with stakeholders and early initiatives on accreditation in UK.

#### **SESSION #6**

## **2:45 – 3:15 pm Plant pathogenic Oomycetes: The era of genomics and microbiomics.**

#### Moderator: David Cooke (U.K.)

• 2:45 – 3:00 pm. Brett Tyler (USA) Topic: Genomics and adaptation in Oomycetes

• **3:00 – 3:15 pm. Richard Michelmore** (USA), Kyle Fletcher (USA), Frank Martin (USA), Juliana Gil (USA), Lin Zhang (USA), Kelsey Wood (USA), Aubrey Kenefick (USA), Fe Dela Cueva (Philippines), Doug Luster (USA), Yazmin Rivera (USA), Clint Magill (USA), Rajan Sharma (India), Steve Klosterman (USA), Yan Zhang (USA), Gloria Abad (USA), Sebastian Reyes-Chin-Wo (USA). **Topic:** Comparative Genomics of Downy Mildews.

**3:15 – 4:00 pm Break with posters view.** 

#### SESSION #7

□ 4:00 – 5:00 pm New technologies for Oomycete research: looking to the future.

#### Moderator: Frank Martin (USA)

• **4:00 – 4:15 pm. Yazmín Rivera** (USA), Gloria Abad (USA), John Bienapfl (USA), Jin-Hsing Huang (Taiwan), et al. **Topic**: New tools for the diagnostics and identification of Oomycetes. Yazmin Rivera, Gloria Abad, John Bienapfl, Marco Galvez, Jin-Hsing Huang (Taiwan).

• **4:15 – 4:30 pm. Jimmy Botella,** M. G. Mason; Y.P. Zou; M. Trau; P.J. Blackall (Australia) **Topic:** Nanotech+Molecular Biology+Electronics: Combining complicated technologies to simplify your life.

• **4:30 – 4:45 pm. James Brayer** (UK-USA ONT). **Topic:** Portable Sequencing for Disease Detection, Diagnosis, Discrimination, and Discovery in Biological areas.

• **4:45** – 5:00 **pm.** Shankar K. Shakya, **Niklaus J. Grünwald**, Jerry Weiland, Valerie Fieland, Brian J. Knaus (USA), Marilia Horta Jung, Cristiana Maia (Czech Republic), André Drenth, David I. Guest, Edward C.Y. Liew, Coline Crane (Australia), Bruno Scanu (Italy), Thomas Jung (Czech Republic). **Topic:** Analysis of global populations of *Phytophthora cinnamomi* suggests presence of two dominant clonal lineages and evidence of sex in Southeast Asia.

**5:00 – 5:15 pm. Gloria Abad (USA)**, Treena Burgess (Australia), John Bienapfl (USA), Amanda Redford (USA), Michael Coffey (USA) and Leandra Knight (USA). **Topic:** IDphy: Molecular and Morphological Identification of *Phytophthora* Based on the Types.

#### **5:15 – 6:00 pm Discussion, certificates, memories.**

**5:15** – **6:00 pm** Closing Remarks (any time) by **Greg Johnson** (Australia), President of the International Society of Plant Pathology (ISPP).



Greg Johnson, ISPP President

(Closing Speaker) Dr. Greg Johnson (Australia) is the 2013-2018 - President, International Society for Plant Pathology (ISPP).

Greg Johnson specializes in research & development in tropical & subtropical agriculture with emphasis horticultural crops, plant pathology & development co-operation. 1995-2006 - Program Manager with the Australian Centre for International Agricultural Research (ACIAR), managing postharvest & supply chain projects. 2006-present - Founder, Horticulture 4 Development, a consultancy in horticulture & plant pathology.

#### **Speakers and Abstracts**

6th International Oomycetes Workshop: Phytophthora, Pythium, Downy Mildews and related genera	
Schedule for the Satellite Meeting, Saturday, July 28th, 2018	
8:00 – 8:15 am.	Mary Palm. Welcoming remarks
	Gloria Abad (USA). Introduction to the workshop.



Mary Palm, President of APS

(Opening Speaker) Dr. Mary Palm (USA) is the 2018 – President of the American Phytopathological Society.

Mary Palm has spent her career in regulatory diagnostics of plant pathogens, as National Mycologist with USDA APHIS Plant Protection and Quarantine from 1984-2005, Lab Director of the PPQ Molecular Diagnostics Laboratory in Beltsville MD from 2005-2011, and Assistant Director of PPQ National Identification Services in the Riverdale, Maryland headquarters. In December 2013 she took leadership of the newly established HLB Multiagency Coordination Group, which includes federal, state and industry representatives. In May 2016 she became Director of the Pest Management staff. She was President of the Mycological Society of America.

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8:15 – 9:00 am. SESSION: Taxonomy and Nomenclature: Past, present, and future.
Moderator: Hai Nguyen, Research Scientist, Agriculture and Agri-Food Canada, 960 Carling Ave, Ottawa, ON, K1A 0C6, 1-
613-759-1579, Hai.Nguyen(at)agr.gc.ca. Zoosporic fungi and Oomycetes biosystematics and genomics.

8:15 – 8:30 am. Topic: Status of the Taxonomy of Phytophthora and related genera: Why the Ex-types Matter?



**Speaker: Gloria Abad (USA)** is a Senior Plant Pathologist at the USDA APHIS PPQ S&T Beltsville Laboratory and Adjunct Professor at Pennsylvania State University. She is an expert in oomycetes with an emphasis in *Phytophthora* taxonomy and nomenclature. She is the Chair of the International Society of Plant Pathology, Subject Matter Committee of Oomycetes established in August 2016.

Phytophthora in the Kingdon Stramenopila with 182 species (until May 2018) contains a high number of species causing diseases of high economic impact around the world. Many species are of biosecurity concern in different countries. Accurate identification of species is fundamental not only for disease management, but also for the implementation of regulatory measures to prevent pathogen spread. Given the swift increase in international trade, rapid responses based on accurate pathogen identification are critical for protecting agriculture and natural ecosystems from the spread of devastating diseases. Even when considerable progress has been made in the area of taxonomy in the last 15 years with the introduction of robust molecular phylogenies there is still a high concern on the proliferation of misidentified sequences at the GenBank. In order to encourage the correct identification of species in the Genus we are pioneering the use of the Ex-types and well authenticated specimens as the "Anchor Points" to high quality identification and diagnostics systems. We are implementing well curated "database" for sequencing and phylogenetic analysis. In this topic we are going to present the Phylogeny of the 161 culturable species (until May 2018) which includes data of 139 Ex-types and 29 well authenticated specimens. We are going to provide also answers to the question: Why the Ex-types matter? presenting the cases of Phytophthora citricola, P. cryptogea/P. dreschleri/P. erythroseptica, P. boehemeriae and P. richardiae.

#### 8:30 - 8:45 am. Topic: Taxonomy and classification of obligate biotrophic oomycetes. Marco Thines (Germany)



**Speaker: Marco Thines (Germany)** Professor, Biodiversity and Climate Research Centre (BiK-F) and the Goethe University Frankfurt am Main, Department of Biological Sciences. His research is centered on the evolutionary ecology of plant pathogens, in particular obligate biotrophic oomycetes. He and his group are studying a broad range of subjects related to this, including functional genetics, genomics, molecular plant-pathogen interactions, trait evolution, taxonomy, evolutionary ecology, and ecological modelling.

The classification of obligate biotrophic oomycetes has changed drastically since the first molecular phylogenetic studies became available at the turn of the century. It was realised that white blister rusts are fundamentally different from downy mildews, and as a consequence, the former were transferred to their own order, Albuginales. Because of morphological and cytological differences, as well as huge genetic distance, two new genera were described – Wilsoniana, with asymmetrical sporangia, parasitic to caryophyllids and Pustula, with a distinctly annullate sporangia, parasitic to asterids. In the downy mildews, the genera Benua and Paraperonospora were confirmed as distinct genera, while Bremiella was merged with Plasmopara. In addition, it was found that no genus includes hosts in both grasses and dicots and that the largest genera, Peronospora and Plasmopara are polyphyletic. Together with some other new discoveries, this led to the description of several new genera, including Baobabopsis, Eraphthora, Graminivora, Hyaloperonospora, Novotelnova, Perofascia, Plasmoverna, Poakatesthia, Protobremia, and Viennotia. In addition, it was found that the downy mildews are embedded within a paraphyletic Phytophthora, which will ultimately lead to a splitting of Phytophthora into 4-5 genera. Additional changes in classification over the past 20 years

8:45 - 9:00 am. Taxonomy of Pythium, Phytopythium, and Pythiogeton associated to plants and aspects on the aggressive vs. the moderately, low and non-aggressive species. Carla D. Garzon, Department of Entomology and Plant Pathology, Oklahoma State University (USA); Gloria Abad, USDA-APHIS-PPQ-Center for Plant Health Science & Technology, CPHST Beltsville Laboratory (USA); Chris Spies, ARC-Plant Health and Protection, Stellenbosch (South Africa), and J.-H. Huang, Taiwan Agricultural Research Institute (Taiwan).



**Speaker: Carla D. Garzon, Ph.D. (USA)** Associate Professor of Plant Pathology. Department of Entomology and Plant Pathology, Oklahoma State University. 127 Noble Research Center; PH: 405-744-9947; carla.garzon(at)okstate.edu. Dr. Garzon's research focuses on population biology, phylogenetics, molecular and bioinformatics diagnostics of oomycetes and fungi, with emphasis on causal agents of soilborne diseases.

**Abstract:** *Pythium* Pringsheim is a polyphyletic genus that includes more than 200 oomycete species with diverse ecological niches. Phylogenetic revisions of *Pythium* divided this group into 5 genera, based on sporangium morphology and DNA sequence analyses of traditional molecular barcodes. Nonetheless, important clades were not supported and the phylogenetic relationships of the new genera and related taxa were still poorly defined. The discovery of new phylogenetic informative markers helped to support these important genera and provided support to separate formerly *Pythium* clades into two orders, Pythiales and Peronosporales. This presentation will discuss current perspectives on the taxonomy of *Pythium* sensu lato, *Phytophythium*, *Pythiogeton* and closely related taxa, including *Phytophthora* and *Langena*, among others. These oomycete groups include plant pathogens of economic importance, hence aspects on the aggressive, moderately aggressive and non-aggressive behavior of these species will be discussed. Finally, we will discuss current challenges to resolve phylogenetic relationships among clades and to define boundaries between closely related species, and the opportunities offered by newly developed next-generation-sequencing technology, bioinformatic tools, and phylogenomic analyses.

• 9:00 -10:00 am Identification and diagnostics: From the traditional tools to the Innovative Genomic Sequencing Technologies (IGST) including NGS and TGS.

**Moderator: Guillaume Bilodeau** (Canada), CFIA-ACIA Canadian Food Inspection Agency, Research Scientist and Adjunct Professor Universite Laval, Quebec, Canada

9:00 – 9:15 am. Topic: Isolation and identification of Phytophthora species: Back to the basics.... Steven Jeffers (USA) Clemson University, Dept. of Plant and Environmental Sciences, Clemson, SC.



**Speaker: Steven N. Jeffers, (USA)** Professor & Extension Specialist, Clemson University, Dept. of Plant and Environmental Sciences, Clemson, SC 29634 USA; 864-656-7157, sjffrs(at)clemson.edu Dr. Jeffers works on diseases of ornamental crops and trees in nurseries, greenhouses, landscapes, and natural ecosystems. Currently, his research focusses on Phytophthora root rot on American chestnut and lavender. Before coming to Clemson University, he worked on Phytophthora diseases on apples, cherries, cranberries, and other small fruits. He has worked on Phytophthora diseases for over 35 years—primarily on isolation and identification of the pathogens and etiology and management of the diseases they cause.

**Abstract:** In recent years, there have been many new methods and procedures for isolating and identifying species of Phytophthora from plants, soil, and water—particularly from samples collected in natural ecosystems. Researchers often use the terms isolation, detection, and recovery synonymously or interchangeably; however, based on years of experience in the field, I propose that isolation is a two-step process—involving recovering the pathogen from a substrate and then detecting the pathogen that was recovered. Once the pathogen has been isolated, it is important that it is accurately identified, which usually involves some type of molecular characterization (e.g., DNA sequencing). Even though molecular identification methods have become very efficient and effective, molecular identities should be verified and confirmed by examining common morphological characters—e.g., sporangia, sporangiophores, chlamydospores, hyphal swellings, and oospores with antheridia.

**9:15-9:30 am. Topic:** The validation of eDNA barcoding in the study of Phytophthora diversity for plant health testing and natural ecosystem surveillance. David E.L. Cooke1, Eva Randall1, Beatrix Clark1, Peter Thorpe1, Leighton Pritchard1, Tim Pettit2, Frederickson-Matika Debbie3, Sarah Green3. 1The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK, 2University of Worcester, Jenny Lind Building, 1013 Henwick Grove, Worcester, WR2 6AJ, UK, 3Forest Research, Northern Research Station, Roslin, Midlothian, EH25 9SY, UK.

david.cooke(at)hutton.ac.uk



**Speaker: David Cooke, (UK)** Senior Pathologist, The James Hutton Institute Since 1994 he has been at the James Hutton Institute carrying out research into Phytophthora species on crops, forests, nursery plants and natural ecosystems. Much of David's current work is on Phytophthora detection and population genetics with research into genetics allowing a fuller understanding of the pathogen and the diseases it causes. David is a highly cited expert in his field, he has written more than 70 publications and contributed to descriptions of many new species of Phytophthora. His research interests include: evolution and population genetics of the genus Phytophthora; detection, management and ecology of Phytophthora species in crops, horticulture, forests and natural ecosystems.

**Abstract:** Plant health protocols are based on known listed species but monitoring is increasing our awareness of a potential reservoir of novel undescribed Phytophthora taxa. It is important to understand the spatial and temporal distribution of such known and unknown Phytophthora species to advance our understanding of pathogen ecology and impact and for the accurate interpretation of plant biosecurity protocols. We have combined in situ water filtration, a generic Phytophthora PCR test based on the rDNA ITS1 region and high-throughput sequencing technology to examine Phytophthora diversity in samples of environmental DNA (eDNA). We have been applying this method to the study of Phytophthora diversity in natural ecosystems as well as planting material and irrigation water in nursery production systems. The method is proving valuable but technical challenges and questions remain. The downstream computational biology pipeline to process the data must be validated and based on a robust database of reference sequences that copes with 'fuzziness' and overlap around species boundaries. We report our findings on the application of the method to replicated samples from UK nurseries and Scottish soil and stream networks including an evaluation of the reliability and reproducibility of different stages of the process (sampling, PCR and sequencing). Such validation is critical to an objective measure of the benefits of the technology for plant health legislation and ecosystem surveillance.

9:30 – 9:45 am. Topic: Mitochondrial Genomics: a systematic method for development of loci for diagnostics, taxonomy, phylogenetics and metagenomic studies. Dr. Frank N. Martin, Research Plant Pathologist, USDA-ARS 1636 East Alisal St. Salinas, CA 93905, (831) 755-2873. Frank.martin(at)ars.usda.gov



#### Speaker: Frank Martin (USA)

Frank has been a Research Plant Pathologist with USDA-ARS in Salinas, CA, since 1996 and was with the Plant Pathology Department at the University of Florida for 10 years prior to this. His research has focused on the ecology, biology, detection, identification, and phylogeny of the genera Pythium and Phytophthora.

**Abstract:** Having a systematic approach for designing amplification primers for loci that are useful for taxonomic, phylogenetic, population, and metagenomic studies, as well as diagnostic assays, would enhance research oomycete opportunities. To address this need, the mitochondrial genomes of over 550 isolates representing 155 taxa for a range of Oomycetes were assembled and comparative genomics conducted. Gene order differences compared to plant and Eumycotan fungal are useful for development of specific primers for amplification of loci from a wide range of taxa (including downy mildews) and design of diagnostic assays. A single assay capable of detecting Phytophthora at a genus and species-specific level was validated with data supporting development of species-specific TaqMan probes for 89% of the genus. Similar types of assays are in development for Pythium, Aphanomyces and several downy mildews. Unique putative open reading frames also are useful for design of species-specific diagnostic assays for detection and quantification. Polymorphic regions of the genome have also been useful for identification of loci to characterize mitochondrial haplotypes for population studies.

9:45 - 10:00 am. Topic: BioSurveillance of Forest Alien Enemies (BioSAFE): developing the next generation of genomics tools.



**Speaker: Richard C. Hamelin, (Canada)** Professor Forest Pathology, Faculty of Forestry, The University of British Columbia, Vancouver, BC and Faculté de Foresterie et Géomatique, Université Laval, Québec, QC; richard.hamelin(at)ubc.ca. Dr. Hamelin works on forest diseases and the use of genomics to further our understading of pathogen biology and epidemiology and develop new tools for biosurveillance.

The world's forests face unprecedented threats from invasive insects and pathogens that can cause large irreversible damage to the ecosystems. This threatens the capacity to provide long-term fibre supply and ecosystem services that range from carbon storage, nutrient cycling, water and air purification, soil preservation and maintenance of wildlife habitat. The key to reduce this threat is via vigilant biosurveillance to increase preparedness and facilitate early interventions. PCR-based detection and metabarcoding have already revolutionized our ability to detect and identify oomycetes and other microbial pathogens. However, these approaches use only a small portion of the genomes of the targeted organisms. The availability of whole genomes for a large number of Oomycetes makes it possible to develop tools that will take advantage of whole genomes and identify informative genomic regions that can provide information about sub-specific variants, presence of hybridization and level of pathogenicity. The BioSAFE (BioSurveillance of Forest Alien Enemies) project is developing a pipeline to generate genomic tools that will provide accurate identification of pests and pathogens, including forest Phytophthoras, and assign outbreak or survey samples to putative sources to identify pathways of spread and assess risk based on traits that impact the outbreak outcome.

10:00 - 10:30 am Break with posters view.

• 10:30 – 12:00 pm Diversity in cultivated and natural environments: The global spread of pathogens.

Moderator: Ronald French (USA), USDA-APHIS-PPQ-FO-Plant Germplasm Quarantine Program

10:30 – 10:45 am. Topic: The Diversity and Ecology of Phytophthora in Europe. Santa Olga Cacciola, University of Catania (Italy), Bruno Scanu, Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia (SPaVE), University of Sassari (Italy); Leonardo Schena, Dipartimento di Agraria, Università Mediterranea di Reggio Calabria (Italy); David E.L. Cooke, James Hutton Institute, Dundee (UK).



Speaker: Santa Olga Cacciola, (Italy) Professor of Plant Pathology, Department of Agriculture, Food and Environment, University of Catania, Italy, Via S. Sofia, 100 Catania +390957147371, olgacacciola(at)unict.it. Degree in Biological Science. PhD in Plant Pathology, University of Bari. Research lines: Phytophthora and fungal diseases of Mediterranean plant species; phylogeny and molecular diagnosis of Phytophthora and fungal pathogens. Senior Editor of Plant Disease Journal. Member of the Board of Italian Society of Plant Pathology. Coauthor of several Phytophthora species description. Representative of the Italian side in bilateral and trilateral projects with UK, Vietnam, Spain, Sweden, Iran. Member of Management Committee of COST FPS Actions (FP0801 and FP1406).

Abstract: The diversity of *Phytophthora* in Europe reflects the diversity of hosts, habitats and agricultural ecosystems in this continent as well as the adaptive ability of these oomycetes. More than 70 species have been reported from Europe, from *P. palmivora*, native to tropical rainforests, to the endemic *P. vulcanica*, with an optimum temperature of 15°C, found at an altitude of about 1800 m asl in Sicily. *P. nicotianae* and the emerging *P. niederhauserii* are the most frequent species in ornamental nurseries while *P. cinnamomi*, *P. cactorum*, *P. plurivora* and *P. xcambivora* prevail in forest nurseries. Natural ecosystems are a reservoir of *Phytophthora* diversity. *P. cinnamomi*, *P. plurivora*, *P. quercina*, *P. pseudosyringae* and *P. x cambivora* are common in forests of Fagaceae while *P. lacustris* and *P. gonapodyides* are typical of riparian ecosystems. Probably, *P. multivora* is widespread in southern Europe as it aestivates in the form of oospores. The propagation method of citrus may have played a major role in shaping citrus subpopulation of the polyphagous *P. nicotianae*, which is an example of the diversification process driven by selective pressure in agricultural ecosystems.

**10:45** - **11:00** am. Topic: "Phytophthora and other Oomycetes diversity in cultivated and natural environments in Japan". Koji Kageyama (Japan)



**Speaker: Koji Kageyama (Japan)** is Professor, River Basin Research Center, Gifu University, kageyama(at)green.gifu-u.ac.jp PhD in Plant Pathology, Hokkaido University. Member of Phytopathological Society of Japan, American Phytopathological Society, Mycological Society of Japan. Research fields: Taxonomy and ecology of Oomycetes. Editor of Journal General Plant Pathology and Mycoscience.

In Japan, Phytophthora, Phytopythium and Pythium species cause a lot of diseases in field crops, vegetables, ornamental plants, fruit trees and forest trees. Forty Phytophthora species causing 184 diseases in 157 plants has been so far reported in Japan. Since 2000, six and five species have been described as new species and re-identified as newly known species in Japan, respectively. Recently leaf blight and corm rot of taro caused by Ph. colocasiae is very serious in southern part of Japan. Although the survey had not been conducted in natural ecosystem, last year we surveyed Phytophthora species in natural forest soils and river water with Dr. Thomas. Jung, Dr. Marília Horta Jung, Dr. Clive Brasier and Dr. Joan Webber. The identification is in progress. Seven Phytopythium species whose four species, P. fagopyri, P. helicoides, P. oedochilum and P. vexans cause 11 plants have been discovered in Japan. Phytopythium fagopyri causing damping-off of buckwheat was transferred from Pythium helicoides, originally Phytophthora fagopyri in 2014. Phytopythium iriomotense and P. aichiense were isolated from river water and water sludge in 2015, respectively. Thirty nine Pythium species causing 184 diseases in 153 plants have been reported in Japan. And four species, Py. alternatum, Py. rishiriense, Py. senticosum and Py. takayamanum, were isolated from natural forest soils and in river water. In Phytopythium and Pythium, high temperature tolerant species, P. helicoides, Py. aphanidermatum and Py. myriotylum cause serious root rots in vegetables and ornamental plants especially in hydroponic culture systems.

**11:00 - 11:15 am. Topic:** "*Phytophthora* and other Oomycetes diversity in cultivated and natural environments in Latin America region" Grijalba P., Palmucci H., Greslebin A., Vélez M. L., Irribarren J. (Argentina); Gonçalves D., Costamilán L. (Brazil); Restrepo S. (Colombia); Ahumada R. (Chile); Aragón L. (Perú); Stewart S. (Uruguay).

**Speaker: Pablo Grijalba (Argentina)** is Professor of Plant Pathology, Department of Plant Production, Facultad de Agronomía, University of Buenos Aires, av. San Martín 4453 CABA. Argentina +541152870063, grijalbalga(at)agro.uba.ar. Degree in Agriculture Engineer Buenos Aires University, Msc. on Plant Pathology at Integrated Unit INTA-Balcarce (Argentina). PhD in Natural Sciences, finalized (exposition pending), La Plata University (Argentina). Member of the Argentinian Society of Plant Pathology. Research lines: Prevalent, emerging and quarantine diseases of agricultural crops. Etiology and Importance of Root and Stem Base Rot on Soybean. Morphological and Molecular diagnosis of oomycetes and fungi diseases and their management. Author and Co-author of several scientific and extension papers.

Latinoamérica shows a great geographical and biological diversity, also within Phytophthora, Pythium and Phytopythium. In the last fifteen years, various studies have been carried out in the different countries, in agriculture, natural ecosystems and hydroponics. In natural ecosystems P. austrocedri causes the main disease in native forests in Patagonia Argentina and P. pinifolia affects Pinus radiata in Chile. Phylogenetic analysis of sequences from Chile, Vietnam and Europe using different genes revealed that they belong to six new species of a new genus, Nothophytophthora gen. nov. In Brazil the description of Halophytophthora species was based on research done in a mangrove swamp. A new species, P. betacei, was identified from Solanum betaceum in Colombia. In agriculture, the main diseases are still Ph. infestans-Solanum tuberosum, Ph sojae-Glicine max and Ph. capsici- Capsicum annuum. The genetic diversity in P. capsici populations has been widely studied in Peru and in Argentina. P. infestans diversity is still being characterized with phenotypic and genotypic markers. Distribution and races of P. sojae have been studied due to the great importance of the crop in Argentina, Brazil and Uruguay.

**11:15 - 11:30 am. Topic: "Phytophthora, Downy Mildews and other Oomycetes diversity in cultivated and natural environments in North America region" Guillaume J. Bilodeau1, Gloria Abad2, Sylvia Fernandez3, John Bienapfl2, Herve Van der Heyden4, and Yazmin Rivera2. 1. Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, ON, Canada. 2.USDA-APHIS, USA, 3. Universidad Michoacana de San Nicolás de Hidalgo, Michoacán, México, 4. Cie de Recherche Phytodata, 291 rue de la cooperative, Sherrington Qc. Canada** 

**Speaker: Guillaume J. Bilodeau**, **(Canada)** Research Scientist, Canadian Food Inspection Agency (CFIA), 3851 Fallowfield Rd, Ottawa, Canada, K2H 8P9, (343)212-0283, Guillaume.Bilodeau(at)inspection.gc.ca Dr. Bilodeau is Research Scientist at CFIA and Adjunct Professor Universite Laval, Quebec, Canada. His research are on R&D in technologies of detection and identification of plant pests (fungi-oomycetes) of regulatory significance. Expertise: Fungal detection and genotyping, Phytophthora, Phytophthora ramorum, Verticillium, Real-Time PCR, Molecular biology, genomic, metagenomic.

In cultivated, forest and natural environment, Oomycetes such as Phytophthora, Pythium, downy mildews are causing plant diseases. An overview of the diversity of those organisms in North-America will be presented in outlining the report finding in Canada, United-States and Mexico. Some example of emerging issue will be briefly discussed and indication related to regulated one identified. A total of 69, 22 and 18 Phytophthora species have been reported respectively in United-States, Canada and Mexico. Other oomycetes diversity will be also presented.





11:30 - 11:45 am. Topic: "Phytophthora and other Oomycetes diversity in cultivated and natural environments in Oceania region". Treena Burgess (Australia), Giles Hardy Centre for Phytophthora Science and Management at Murdoch University, Australia.



**Speaker: Treena Burgess (Australia)** is an Associate Professor in Plant Sciences and the Director of the Centre for Phytophthora Science and Management at Murdoch University, Australia.

**Abstract:** Metabarcoding has opened Pandora's box; diversity studies are much easier to undertake and a lot of data can be generated, but what does it all mean? We are detecting a large number of species across a whole range of environments with what appears to be limited ecological filtering. Interpreting this data is difficult and moving forward we are looking at much more fine scale studies asking specific questions. In this presentation I will give a very brief overview of Phytophthora diversity in Oceania and some of the new projects that are underway.

**11:45 - 12:00 am.** The global spread of obligate biotrophic oomycetes – past epidemics and future challenges Marco Thines (Germany)



**Speaker: Marco Thines (Germany)** Professor, Biodiversity and Climate Research Centre (BiK-F) and the Goethe University Frankfurt am Main, Department of Biological Sciences. His research is centered on the evolutionary ecology of plant pathogens, in particular obligate biotrophic oomycetes. In my group we are studying a broad range of subjects related to this, including functional genetics, genomics, molecular plant-pathogen interactions, trait evolution, taxonomy, evolutionary ecology, and ecological modelling.

Downy mildews feature an endophytic stage, during which host plants may appear entirely healthy. Even if later no disease symptoms develop, biotrophic oomycetes have the capacity to enter seeds of the hosts to be transmitted vertically to the next generation. With increasing trade with seeds and plants, worldwide, the risk of introduction of new species has increased. This is also evident by the increased rates of newly occurring downy mildew diseases in crops and ornamentals. Plasmopara viticola was one of the first downy mildew species to be introduced from North America to Europe, from where it spread globally, already about 150 years ago. Later, also Plasmopara halstedii from sunflower made its way to Europe and other continents. In recent years, predominantly herbs and ornamentals are affected by new downy mildew diseases, e.g. Peronospora belbahrii from Africa, affecting sweet basil, and Plasmopara destructor and Plasmopara velutina from East Asia, affecting several ornamental Impatiens species, as well as Peronospora aquilegicola, the realisation that the species are distinct from previously described ones came too late to hinder the spread of the pathogens by appropriate regulations. This points to the importance to treat newly occurring infections of previously unaffected hosts with caution, as most downy mildew species are highly host specific in their native range, so that these occurrences likely represent introductions of a new species.

12:00 – 1:00 pm Lunch Break with posters view.

• 1:00 – 1:45 pm Management and control: Advanced IPM-tools, improved biocontrol, enhancing resistance, sustainable production, agricultural systems for the future.

Moderator: Nick Grünwald (USA) USDA ARS

1:00 - 1:15 pm. Topic: Quantifying Adaptation to Host Resistance in Phytopthora nicotianae. David Shew (USA)

**Speaker: David Shew (USA)** is Professor of Plant Pathology in the Department of Entomology and Plant Pathology at NC State University. He is currently an Alumni Distinguished Undergraduate Professor and a Philip Morris Professor at NC State. His research has focused on important soilborne pathogens of tobacco, turfgrass, and most recently, stevia in which he has elucidated the etiology and management of multiple diseases of tobacco, including target spot and yellow stunt, characterized the abiotic suppression of pathogens in acid soils by aluminum, and provided new insights into the basic biology of the black root rot and black shank pathogens of tobacco. Shew has been very active in the integration of technology into the classroom and online and has received numerous teaching awards including the Excellence in Teaching Award from APS. He became a Fellow of APS in 2018.

Abstract: Host resistance is the most effective means of controlling plant diseases. Resistance is an integral part of sustainable disease management and can greatly reduce the use of fungicides and other management inputs. However, pathogen races often quickly overcome single-gene resistance and they can adapt to multi-gene partial resistance via an increase in infection efficiency and lesion expansion, and through a shorter latent period and longer infectious period, all gradually eroding the effectiveness of the resistance. Various mechanisms of overcoming complete resistance have been described, either through the loss of a functioning Avr gene, or through the addition of gene products that suppress host resistance. How plant pathogens overcome partial resistance remains largely unknown. Phytophthora nicotianae has a broad host range and is the causal agent of tobacco black shank. In this presentation we will present our approaches to quantifying and visualizing how P. nicotianae adapts to resistance in tobacco and determine if there is a fitness cost to P. nicotianae during adaptation. If so, can we take advantage of the fitness cost in the design of disease management systems?



1:15 - 1:30 pm. Topic: Tracking a cucurbit killer: developing bio-surveillance tools for improved management of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis* . Lina M. Quesada-Ocampo and Alamgir Rahman. Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616. Frank Martin. Crop Improvement and Protection Research Station, USDA-ARS, Salinas, CA, 93905. Tim Miles. Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI, USA.



**Speaker: Lina Quesada (USA)** On March 1st of 2013, Dr. Quesada joined the Department of Plant Pathology at North Carolina State University (NCSU) as an Assistant Professor for Vegetable Pathology. Her research program uses a broad range of tools from traditional field studies to genomics to improve management strategies of vegetable diseases. She received her Ph.D. in Plant Pathology from Michigan State University (MSU) and her doctoral work focused on the host range, host resistance and population structure of Phytophthora capsici, an important pathogen of cucurbits and solanaceous crops. She then was a postdoctoral researcher at MSU where she studied populations of cucurbit downy mildew and bacterial canker of tomato. Right before joining NCSU, Dr. Quesada was a NIFA postdoctoral fellow working on maize-Fusarium interactions. She was awarded the Hewitt early career award by the American Phytopathological Society in 2017.

**Abstract:** Cucurbit downy mildew remains a threat to cucurbit production in the United States since its re-emergence in 2004. The pathogen, *Pseudoperonospora cubensis*, infects a broad range of cucurbit crops, has overcome previously effective resistance in cucumber, and easily develops fungicide resistance. Due to its obligate nature, *P. cubensis* overwinters in non-frost areas and as temperatures increase and cucurbits are planted in the eastern United States, airborne sporangia are spread causing outbreaks. Controlling cucurbit downy mildew requires intensive and frequent fungicide applications that can be reduced with an effective alert system for the pathogen. Using comparative genomics approaches, species-specific, crop risk, and fungicide resistance diagnostic markers were identified and developed into qPCR assays. Assays were tested for specificity and sensitivity in the laboratory and validated with field samples collected using roto rod spore traps in North Carolina cucurbit fields. Our findings allow for molecular biosurveillance of *P. cubensis* by providing growers information regarding presence and amount of inoculum, crops at risk of infection depending on the host specificity profile of the inoculum, and effective fungicides to apply for precision disease management.

**1:30 - 1:45 pm. Topic: Pathotypes and races of** *Pseudoperonospora cubensis* – great challenge for science and cucurbit breeding. Aleš Lebeda, Eva Křístková, Božena Sedláková. Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 27, 783 71 Olomouc-Holice, Czech Republic; \* Author for correspondence (E-mail: ales.lebeda(at)upol.cz)



**Speaker: Aleš Lebeda (Czech Republic).** Professor of agricultural botany and plant pathology, with more than 40 year experience in research, plant breeding and teaching. His research is mostly focused on vegetable crops and their pathogens (mostly downy and powdery mildews), as well as plant germplasm diversity and their exploitation. He attended and managed more than 30 national and international projects; organized and/or co-organized many international scientific meetings (e.g. EUCARPIA). He has long-lasting and broad international experience; he realized 51 middle- and long-time research stays and expeditions in abroad, he visited and lectured (more than 200 lectures) in 39 countries of five continents. Until now he published more 1,100 papers, book chapters and books. From that number ca 300 original scientific papers (on WOS) with high citation index (cca 2500 citations, h index = 27) and nearly 40 books and proceedings. He was president (2007-2012) of Czech Plant Pathology Society. Recently (from 2006-untill now) he is an Editor-in-Chief of international scientific journal Plant Protection Science (on Web of Science from 2012, in 2016 IF=0.742).

**Abstract:** Host-parasite interactions between Cucurbitaceae and *P. cubensis* exhibit significant variation. This contribution reviews the current state of knowledge regarding characterization of P. cubensis virulence variation on the level of pathotypes (variation in host genera and species host-range) and races (variation in intraspecific level). However, our knowledge of the interactions between P. cubensis isolates and the most important genera and species of cultivated cucurbits is limited. An improved differential set of six cucurbit genera and 12 genotypes (Benincasa, Citrullus, Cucumis, Cucurbita, Lagenaria, and Luffa) was developed to characterize pathotypes among P. cubensis isolates (Lebeda and Widrlechner, 2003), and is broadly used. Recently we developed the differential set of 21 genotypes of Cucumis melo for determination of P. cubensis races. Research of pathogen population in the Czech Republic showed broad spectrum of virulence patterns demonstrating existence of huge number of races. Combination of both approaches is important for science as well as for practical application in cucurbit resistance breeding.

1:45 - 2:00 pm. Topic: Managing Aphanomyces on Sugar Beet. Mohamed F. R. Khan, Professor and Extension Sugarbeet Specialist, North Dakota State University and University of Minnesota, 227 Walster Hall, Fargo, North Dakota, 58108. Telephone: 701-231-8596, Mohamed.khan(at)ndsu.edu



**Speaker: Mohamed Khan (USA)** is Professor and Extension Sugarbeet Specialist for North Dakota State University and the University of Minnesota. He develops, conducts and evaluates educational programs to improve sugarbeet production practices in North Dakota and Minnesota. Dr. Khan conducts research to improve management practices of sugarbeet diseases including Cercospora leaf spot, Rhizoctonia damping-off and root rot, Rhizomania, Fusarium yellows and Aphanomyces. Dr. Khan is the secretary of the Sugarbeet Research and Education Board of Minnesota and North Dakota responsible for funding and promoting research and educational programs in sugarbeet production. Dr. Khan Chairs the International Sugarbeet Institute that organizes an annual two-day trade show. Dr. Khan received his BS from the University of Guyana, MS from the University of Bath, UK, and his Ph.D. from Clemson

**Abstract:** Aphanomyces cochlioides Drechsler causes Aphanomyces damping-off and root rot of sugar beet (Beta vulgaris L.) in many production areas worldwide. Growers rely on hymexazol as a seed treatment to prevent damping-off. No pesticide is available that controls Aphanomyces root rot that occurs later in the season. Sensitivity of A. cochlioides to pyraclostrobin (Qol), prothioconazole, and tetraconazole (DMIs) were compared to hymexazol and evaluated in vitro using radial mycelial growth assay and by evaluating disease severity on inoculated plants treated with fungicides in the greenhouse. The EC50 values were 0.52, 0.78, 2.39, and 3.48 µg ml-1 for hymexazol, pyraclostrobin, prothioconazole, and tetraconazole, respectively. None of the newer DMIs or Qol evaluated was effective at reducing Aphanomyces damping-off and root rot invivo. Growers use a combination of tolerant varieties, hymexazol (tachigaren) seed treatment, planting into cool soils, improved drainage, and application of 7 to 16 t ha-1 of precipitate calcium carbonate (PCC), (a by-product of the sugar purification process) to manage Aphanomyces. Research shows that PCC applied at 11 to 44 t ha-1 provides control against early season stand loss, and results in higher tonnage and recoverable sucrose for up to 12 years after initial application.

• 2:00 - 2:45 pm. Plant pathogenic Oomycetes of concern: Trade regulations, advanced monitoring and certification.

#### Moderator: Yazmín Rivera, USDA-APHIS-PPQ-Science and Technology Beltsville Laboratory, USA

2:00 -2:15 pm. Topic: Emerging downy mildews: The same old pathogens or new enemies? Jo Anne Crouch (USA), Nicholas Le Blanc (USA), Catalina Salgado-Salazar (USA).

> **Speaker: Dr. JoAnne Crouch (USA)**, Research Molecular Biologist, USDA –ARS Mycology and Nematology Genetic Diversity & Biology Laboratory, Beltsville, MD, USA. Dr. Crouch's research focuses on molecular investigations of emergent and quarantine-significant agricultural pathogens, including downy mildews and boxwood blight. She works extensively with oomycete and fungal populations, genomes and microbiomes to determine pathogen identities, origins and diversity, taxonomy and biology.

**Abstract:** Global agriculture is increasingly threatened by downy mildew disease outbreaks. Specialty crops have been particularly hard hit by downy mildew diseases, with growers of impatiens, basil, sunflower, rudbeckia, rose, curcurbits and other crops incurring millions of dollars in losses annually. This presentation will provide an overview of how downy mildew pathogen populations are changing over time, before and after new disease epidemics occur, with emphasis on the recent emergence of impatiens downy mildew disease.

**2:15 - 2:30 pm. Topic:** An Update on *Phytophthora ramorum , P. tentaculata* and other species of regulatory concern in the USA. Susan J. Frankel, USDA Forest Service, Pacific Southwest Research Station, Albany, CA (USA)

**Speaker: Susan J. Frankel, (USA),** Plant Pathologist, USDA Forest Service, Pacific Southwest Research Station, Albany, CA 94710, 510-883-8825, sfrankel(at)fs.fed.us. Susan is a plant pathologist and leads Sudden Oak Death Research, at the USDA-Forest Service, Pacific Southwest Research Station (PSW) in Albany, CA. Susan is a co-leader of the Phytophthoras in Native Habitats Work Group, www.calphytos.org, responding to issues related to Phytophthora introductions into restoration areas in California.

Abstract: Phytophthora ramorum has killed millions of oaks, (Quercus spp.) and tanoaks (Notholithocarpus densiflorus) in the USA since the discovery of sudden oak death (SOD) in the mid-1990s in Mill Valley (Marin Co., CA). Well over a hundred plant species have been found to be infected by the pathogen causing leaf blight, shoot blight, stem cankers or dieback on woody and herbaceous plants, as well as some ferns. The pathogen is a concern in ornamental nurseries in all 50 states and is known to be present in wildlands in fifteen California counties and in Curry Co. in southeastern Oregon where the NA1 clonal lineage was first detected in 2001 and the EU1 lineage detected killing tanoak in 2015. Since 2002, through ongoing cooperative surveys, APHIS-PPQ have been tracking the extent of the pathogen's distribution in the USA and limiting its artificial spread beyond infected areas through quarantine and a public education program. In 2012, another species of regulatory concern was detected in California, Phythophthora tentaculata on symptomatic Diplacus aurantiacus, sticky monkeyflower. The first detection in the USA was from a native plant nursery in 2012 (Monterey Co.) and was followed in 2014 by finds of infected nursery stock outplanted in restoration areas in several San Francisco Bay Area counties. Those interceptions prompted surveys for Phytophthoras in California native plant nurseries and restoration sites which found over 50 Phytophthora species on CA native plants (shrubs, perennials and others), including the first detection in the USA of Phytophthora guercina on valley oak (Quercus lobata), in a restoration planting in San Jose, (Santa Clara Co). Some of the native plants infected with Phytophthora species are rare, threatened or endangered plants that are regulated for biodiversity and wildlife habitat values, introducing a new set of concerns and regulators.





2:30 - 2:45 pm. Topic: *Phytophthora* in plant nursery settings, engaging with stakeholders and early initiatives on accreditation in UK. Sarah Green (FR), David Cooke(JHI), Mariella Marzano (FR), Peter Thorpe (JHI), Mike Dunn (FR), Debbie Frederickson-Matika (FR), Beatrix Clark (JHI), Mhairi Clark (FR), Tim Pettitt (UW), Eva Randall (JHI), Leighton Pritchard (JHI), Alexandra Schlenzig (SASA), Jane Barbrook (APHA). FR, Forest Research (as above); JHI, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK; UW, University of Worcestor, Jenny Lind Building, 1013 Henwick Grove, Worcester, WR2 6AJ, UK; SASA, Science and Advice for Scottish Agriculture,1 Roddinglaw Rd, Edinburgh, EH12 9FJ, UK; APHA, Animal and Plant Health Agency, Plant Health and Seeds Inspectorate, Sand Hutton, York, YO41 1LZ, UK. (UK).

> **Speaker: Sara Green (UK).** Forest Pathologist, Forest Research, Northern Research Station, Roslin, Midlothian, Scotland EH25 9SY, +44 (0) 300 067 5941, sarah.green(at)forestry.gsi.gov.uk Sarah is a forest pathologist leading the pathology group at Forest Research's Northern Research Station. She also manages Forest Research's Programme on Understanding Biotic Threats to Resilience and is the Principal Investigator on the interdisciplinary PHYTO-THREATS LWEC Phase 3 project which is working withstakeholders to understand and mitigate spread of Phytophthoras in trade. Her main research interests inlcude distribution, detection methods, biology and evolutionary genetics of forest pathogens, with a particular recent focus on emerging Phytophthora diseases.

**Abstract:** Five new species of pathogenic *Phytophthora* have emerged in the UK over the past 15 years, killing trees and having a serious ecological and economic impact on UK landscapes. For all five of these pathogens, importation of traded plant material has been either confirmed or strongly implicated as the most likely route of introduction. The PHYTO-THREATS project is an interdisciplinary collaboration of seven institutions aimed at addressing the risks to UK forest and related ecosystems from *Phytophthora*. This three year project, which started in April 2016, is examining the diversity of Phytophthoras across a broad range of UK plant nurseries operating different management practices. Metabarcoding is being used to identify Phytophthora species in root and water samples collected from each nursery. Evidence generated from the nursery sampling is being combined with social and economic analyses to help support the development of a UK-wide accreditation scheme to reduce the risk of further Phytophthora introduction and spread.

• 2:45 – 3:15 pm Plant pathogenic Oomycetes: The era of genomics and microbiomics.		
Moderator: David Cooke, The James Hutton Institute, Dundee, UK		
2:45 - 3:00 pm. Topic: Genom	nics and adaptation in Oomycetes	
	Speaker: Brett Tyler (USA) Department of Botany and Plant Pathology, and Center for	
	Genome Research and Biocomputing, Oregon State University. Tel: 541-737-3347; E- mail:brett.tyler(at)oregonstate.edu	
	Brett Tyler has studied oomycete molecular biology, genomics, bioinformatics and systems biology for 30 years.	
Abstract: Many oomycete pla	nt pathogens are highly adaptable, readily overcoming chemical and genetic control measures,	
and jumping to new host species. Genomic studies have implicated hundreds to thousands of genes as potentially		
contributing to their virulence. In the soybean pathogen, Phytophthora sojae, the genome contains nearly 400 genes that		
encode RxLR effectors that can enter plant cells to promote infection. P. sojae strains differ in their repertoires of effector		
genes, in the DNA sequences of their effector genes, and to a surprisingly large extent, by which effector genes are		
transcribed. Using gene silencing, and more recently CRISPR/Cas9-mediated gene knockouts, we have shown that the		
pathogen relies on a relatively small subset of "elite" effectors that seem essential for full virulence. We have successfully		
targeted these genes for find new soybean resistance genes. Genetic selection for strains that come overcome the loss of		

targeted these genes for find new soybean resistance genes. Genetic selection for strains that come overcome the loss o essential effector genes has however revealed that the pathogen is extraordinarily effective at recovering from losses of essential effectors through epigenetic changes that affect the expression of other genes in the genome.





#### 3:00 - 3:15 pm. Topic: Comparative Genomics of Downy Mildews. Richard Michelmore (UC Davis;

rwmichelmore(at)ucdavis.edu), Kyle Fletcher (UC Davis; kfletcher(at)ucdavis.edu), Frank Martin (USDA ARS, Salinas; frank.martin(at)ars.usda.gov), Juliana Gil (UC Davis; giljuliana(at)gmail.com), Lin Zhang (UC Davis; linzhang(at)ucdavis.edu), Kelsey Wood (UC Davis; klsywd(at)gmail.com), Aubrey Kenefick (UC Davis; awkenefick(at)ucdavis.edu), Fe Dela Cueva (U Philippines, Los Banos; fmdcueva(at)yahoo.com), Doug Luster (USDA ARS Ft. Detrick MD; doug.luster(at)ars.usda.gov), Yazmin Rivera (USDA, APHIS; yazmin.rivera(at)aphis.usda.gov), Clint Magill, (Texas A&M; c-magill(at)tamu.edu), Rajan Sharma (ICRISAT; r.sharma(at)cgiar.org), Steve Klosterman (USDA ARS, Salinas; steve.klosterman(at)ars.usda.gov), Yan Zhang (UC Davis; xxyzhang(at)ucdavis.edu), Gloria Abad (USDA, APHIS; gloria.abad(at)aphis.usda.gov), Sebastian Reyes-Chin-Wo (UC Davis; sreyeschinwo(at)ucdavis.edu).



Speaker: Richard Michelmore, (USA) Professor and Director, The Genome Center, University of California, Davis. 530-752-1729. rwmichelmore(at)ucdavis.edu His multidisciplinary research utilizes a synthesis of molecular, genetic and evolutionary approaches (http://michelmorelab.ucdavis.edu). His interests span basic research into the molecular basis of specificity in plant-pathogen interactions to translational plant genetics and crop improvement. His research is focused on comparative and functional genomics with an emphasis on plant disease resistance and pathogen variability. In addition, his program coordinates and hosts the bioinformatics component of the Compositae Genome Project. Richard's interests also include applications of next-generation DNA sequencing approaches to all areas of biology and its imminent impact on society in general. In particular, he aims to exploit such approaches for information-driven deployment of resistance genes in plants to provide more durable disease resistance. In addition, he is interested in fostering research to enhance global food security.

**Abstract:** We have sequenced the genomes of several temperate and tropical downy mildews including *Bremia lactucae*, *Peronospora effusa*, *P. schachtii*, *P. destructor*, and *P. tabacina* as well as *Sclerospora graminicola*, *Peronosclerospora sorghi*, *P. maydis*, *P. philippinensis*, and *P. sacchar* i. For some, it was possible to sequence multiple historical and contemporary isolates. These genomes could be assembled to varying degrees of completeness and contiguity. *Peronospora spp.* have small genomes (~50 Mb), whereas the tropical downy mildews have larger genomes (>200 Mb). Heterozygosity varies widely; some species are mainly homozygous and were relatively easy to assemble, while several are more heterozygous and remain challenging to assemble. Deep sequencing revealed that some isolates of *B. lactucae* are heterokaryotic. Extensive collinearity is detectable among downy mildews and *Phytophthora* spp. These assemblies combined with published downy mildew genomes are being used to elucidate taxonomic and evolutionary relationships, to study genome architectures, fluidity, and synteny, and to characterize expression and variation of effector repertoires. Phylogenetic analysis supported multiple origins for biotrophy in downy mildews.

3:15 - 4:00 pm Break with posters view.

• 4:00 – 5:00 pm New technologies for Oomycete research: looking to the future.

Moderator: Frank Martin (USA)

**4:00 - 4:15 pm. Topic: New tools for the diagnostics and identification of Oomycetes. Yazmin Rivera**, Gloria Abad, John Bienapfl, Marco Galvez, Jin-Hsing Huang (Taiwan).

**Speaker: Yazmin Rivera (USA)** Dr. Yazmín Rivera, Molecular Biologist, USDA APHIS PPQ S&T, Center for Plant Health Science and Technology, Beltsville MD, USA. Dr. Rivera's research interests are in population genetics of fungi and oomycetes as well as new technologies for molecular diagnostics. She earned a PhD in Forest Pathology and Mycology from SUNY College of Environmental Sciences and Forestry in Syracuse NY (USA). She then joined Dr. Jo Anne Crouch's research group at USDA ARS as a postdoctoral research associate studying populations of emerging downy mildew pathogens such as Plasmopara halstedii and P. obducens She now works at the USDA APHIS Center for Plant Health Science and Technology and her work focuses on developing and evaluating molecular diagnostic tools for plant pathogens of concern. In the past two years she has worked with the Select Agent pathogens that cause Philippine downy mildew (Peronosclerospora philippinensis) and Brown stripe downy mildew (Sclerophthora rayssiae).

New technologies for the diagnostics and identification of pathogens are being developed at a fast pace. In the last few years we have seen improvements in portability, faster time of detection and a focus on simpler sample preparations geared towards the untrained hands. Although these technological advances are largely driven by medical research, a lot can be learned and applied to the detection of plant pathogens. Early detection is particularly important for diseases caused by oomycetes, since signs and symptoms can indicate an already well-established population that may be harder to control as time progresses. In this talk, we will provide an overview of emerging technologies for diagnostics. We will also discuss how new technologies such as isothermal amplification and nanopore sequencing can aid in the diagnostics and identification of oomycetes pathogens and the challenges associated.



**4:15 - 4:30 pm. Topic: Nanotech+Molecular Biology+Electronics: Combining complicated technologies to simplify your life. Jimmy Botella**, M. G. Mason; Y.P. Zou; M. Trau; P.J. Blackall. University of Queensland, Brisbane Australia. M. G. Mason; University of Queensland; michael.mason(at)uq.edu.au; Y.P. Zou; University of Queensland; yiping.zou(at)uq.net.au; M. Trau; University of Queensland; m.trau(at)uq.edu.au; P.J. Blackall; University of Queensland; p.blackall(at)uq.edu.au



**Speaker: Dr. Jimmy Botella, (Australia)** Professor of Plant Biotechnology, School of Agriculture and Food Sciences, University of Queensland, Brisbane Australia. Phone +61-7-33651128. email j.botella(at)uq.edu.au. Dr Botella's research interests are in genetic engineering, molecular biology and signal transduction in plants. Dr. Jimmy Botella obtained a degree in Quantum Chemistry from the University of Madrid (Spain) and a PhD in Biochemistry from the University of Malaga (Spain). After postdoctoral positions at Michigan State University and Pennsylvania State University he joined the University of Queensland in 1995. At UQ he founded the Plant Genetic Engineering Laboratory (PGEL) specialising in the fields of tropical and subtropical agricultural biotechnology for almost 15 years. J. Botella has eleven international patents in the field of Plant Biotechnology and is a founding member of two biotechnology companies (Coridon Ltd. and Origo Biotech).

**Abstract:** Nucleic acid-based bioassays that can be performed on-site are in high demand, however a number of challenges need to be overcome before new technologies can be widely adopted. We need to create reliable, portable and simple methodologies that do not require a laboratory environment or sophisticated equipment. We have used a combination of nanotechnology and molecular biology to create a suite of rapid and easy-to-perform diagnostic tools suited to laboratory and point-of-need applications. Our platform technologies include: 1) nucleic acid purification dipsticks that allows extraction of DNA/RNA in less than 30 seconds without the use of specialized equipment, 2) an emulsion that enables visualization of amplification reactions in 10 seconds and 3) a low-cost, hand-held device that can perform 'quantitative' isothermal amplification, interpret results and report positive samples using mobile phone. The combination of these technologies allows us to reliably perform diagnostic assays in approximately 1 hour in rural and remote environments. These tools are suitable for a wide range of applications and can easily be adapted to identify biomarkers in plants, animals or humans.

**4:30 - 4:45 pm. Topic:** Real time DNA sequencing using Oxford Nanopore Technologies to enable portable sequencing for Disease Detection, Diagnosis and Discovery. **James Brayer** (USA)



**Speaker: James Brayer (USA),** Associate Director, Market Development, Oxford Nanopore Technologies Inc.

Oxford Nanopore Technologies has developed a disruptive platform to allow diagnostic field based approach for disease detection, diagnosis and discovery. Our platform leverages the direct, electronic analysis of single molecules. This approach enables the rapid sequencing of DNA and RNA molecules at a single base resolution. At the heart of our platform is a biological protein called a 'nanopore'. A single nanopore creates a hole in a membrane made from a synthetic polymer. When an electric potential is applied to our system the resulting current flow motivates individual DNA or RNA molecules to pass through the aperture of the nanopore. Single molecules that enter the nanopore cause characteristic disruptions in the current, by measuring these disruptions the DNA molecules can be read with single base resolution. Our device, 'The MinION' is small and can fit in the palm of your hand. It is designed for portability and simplicity of its workflow. The MinION plugs into a standard PC or laptop. Oxford Nanopore is integrating the data produced by the MinION into automated data analysis workflows to help researchers to track, trend and predict biological data resulting in real time actionable interpretation of their data. There is a growing list of publications on the many uses for our nanopore sensing platform that include field based applications, real time pathogen detection and surveillance, metagenomics analysis, anti-microbial resistance detection, education and many more including sequencing on the International Space Station. I look forward to sharing with you the unique opportunities enabled by our nanopore sensing approach.

**4:45 - 5:00 pm. Topic: Analysis of global populations of Phytophthora cinnamomi suggests presence of two dominant clonal lineages and evidence of sex in Southeast Asia.** Shankar K. Shakya, **Niklaus J. Grünwald**, Jerry Weiland, Valerie Fieland, Brian J. Knaus (USA), Marilia Horta Jung, Cristiana Maia (Czech Republic), André Drenth, David I. Guest, Edward C.Y. Liew, Coline Crane (Australia), Bruno Scanu (Italy), Thomas Jung (Czech



**Speaker: Niklaus J. Grünwald, (USA)** Research Plant Pathologist, USDA, ARS and courtesy Professor, Department of Botany & Plant Pathology & Center for Genome Biology and Biocomputing, Oregon State University as well Adjunct Professor, Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell. He works with epidemiology, genetics and evolution of exotic and reemerging Phytophthora species.

Abstract: *Phytophthora cinnamomi* is a heterothallic, broad host range plant pathogen causing dieback and root rots of more than 3000 plant species. Several independent studies have suggested the existence of clonal lineages, primarily of the A2 mating type, and rarely sexual reproduction. However, a rigorous study of population diversity at a global scale is currently lacking. We analyzed 197 isolates of *P. cinnamomi* sampled from 11 countries. Genotyping by sequencing was performed using two restriction enzymes (PstI and MspI) and sequenced on the Illumina HiSeq 3000 platform. Raw reads were mapped to the *P. cinnamomi* reference genome using bowtie2 and variants were called with the GATK HaplotypeCaller. We tested the hypothesis of clonal reproduction in *P. cinnamomi* populations. A neighbor-joining tree based on 1,027 variants indicated that populations of *P. cinnamomi* from Southeast Asia (Taiwan & Vietnam) consisting of A1 and A2 mating type isolates were highly diverse whereas most isolates from the remaining countries formed two distinct clonal lineages of the A2 mating type. A1 mating type isolates from Papua New Guinea, South Africa and Australia grouped within the diverse Taiwanese and Vietnamese populations which were inferred to be partially sexual based on the index of association. This suggests that Southeast Asia might be a candidate center of origin for *P. cinnamomi* as speculated previously. These results provide novel insights into the existence of both mating types in Southeast Asia and probably sexual reproduction that could potentially give rise to novel aggressive genotypes or lineages.

5:00 - 5:15 pm. Topic: IDphy: Molecular and Morphological Identification of *Phytophthora* Based on the Types. Official release of the Online Resource at the ICPP 2018. Gloria Abad (USA), Treena Burgess (Australia), John Bienapfl (USA), Amanda Redford (USA), Michael Coffey (USA) and Leandra Knight (USA).



**Speaker: Gloria Abad (USA)** is a Senior Plant Pathologist at the USDA APHIS PPQ S&T Beltsville Laboratory and Adjunct Professor at Pennsylvania State University. She is an expert in oomycetes with an emphasis in *Phytophthora* taxonomy and nomenclature. She is the chair of the International Society of Plant Pathology, Subject Matter Committee of Oomycetes established in August 2016.

At the present the Genus Phytophthora with 182 species (161 culturable) contains a great number of species causing important diseases around the world. In order to facilitate accurate and efficient identification to species for the genus, using the type specimens from the original descriptions wherever possible we have implemented the "IDphy: Molecular and Morphological Identification of Phytophthora based on the Types". IDphy emphasizes species of high economic impact and species of biosecurity concern. The tool includes DNA sequence-based identification support based on ITS rDNA and COI, an interactive Lucid key along with a tabular key for the 161 culturable species, images, diagrams, fact sheets, a glossary, and an image gallery. We are also including other additional four nuclear and mitochondrial genes. To further support molecular identification, we are providing SOPs and strategies to obtain a higher level of confidence in the determination of unknown species. This resource is presented to the international community working with the Genus Phytophthora with the occasion of the 11th International Congress of Plant Pathology (ICPP-2018) at the "6th International Oomycetes Workshop" presented by the ISPP-SMC of the Oomycetes. This valuable online resource, is the first website of this nature implemented for plant pathogens.

#### 5:15 – 6:00 pm Discussion, certificates, memories.

#### POSTERS

#### POSTER #1

SESSION: Diversity in cultivated and natural environments: The global spread of pathogens.

#### POPULATION GENETICS ANALYSIS ON Pythium myriotylum REVEALS THE HOST SPECIFICITY

Auliana Afandi1, Chasuna Borjigen2, Kayako Otsubo2, Shinichi Fuji3, Ayaka Hieno2, Haruhisa Suga4, Koji Kageyama2.

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*Pythium myriotylum* is a warm temperature soil and water borne pathogen which has a wide host range. The purpose of this study is to analyze the population genetics of *P. myriotylum* in Japan. Two hundred and sixty six isolates of *P. myriotylum* collected from 30 geographic locations were analyzed for their diversity using microsatellite markers which amplified 6 loci. Phylogenetic analysis on the isolates revealed that the isolates were grouped primarily according to host plant rather than the geographic origin. The phylogenetic tree were divided into ginger family group, tomato group, legume group, and -'others'- group. In the ginger family, the isolates of the isolates collected from tomato in Mie, Kagoshima, and Shizuoka. The legume group consisted of the isolates collected from kidney bean, peanut and soya from Hokkaido, Shizuoka, Hiroshima, and Fukui. The - 'others'- groups consisted of several clades from different host and geographic origin.

#### POSTER #2

SESSION: Management and control: Advanced IPM-tools, biocontrol, resistance, sustainable production.

#### PREDICTION AND SCREENING OF CANDIDATE RESISTANCE GENES OF OCIMUM BASILICUM IN RESPONSE TO THE BASIL DOWNY MILDEW PATHOGEN PERONOSPORA BELBAHRII

Greg DeIulio1,2, Kelly Allen2, Robert M. Pyne3, Rob Wick2,4, James E. Simon3, Li-Jun Ma1,2

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Basil downy mildew, caused by the biotrophic oomycete *Peronospora belbahrii*, is a destructive disease of sweet basil (*Ocimum basilicum*) that results in high yield losses worldwide. Chemical and cultural control options have offered insufficient control for growers, and breeding has been a slow process due to the lack of annotated genetic resources and the necessity for retaining plant morphological and chemical characteristics for marketability. Following phenotypic screening for disease resistance, RNA-seq was used to compare the host-pathogen interactions of a downy mildew resistant sweet basil cultivar 'Mirihani' to a susceptible sweet basil line Rutgers SB22 during the inoculation and infection process. Following a previously-reported *de novo*-assembly based data analysis pipeline, this data has been used to predict candidate resistant genes uniquely present in the resistant

cultivar 'Mirihani'. Both nucleotide -binding leucine rich repeat proteins and receptor-like kinases are represented in the candidate genes. These protein classes are known to act upstream or alongside defense triggering pathways, including plant hormone responses. An alysis of hormone pathways shows that unlike 'Mirihani', SB22 does not accumulate some mRNAs associated with salicylic acid signaling and biosynthesis while both the jasmonic acid and ethylene pathways are consistent between cultivars. The predicted resistance gene sequences were used for primer design and expression profiles observed in the data were validated using qRT-PCR. This research presents the analytical approach for prediction of these candidate resistance genes, as well as the validation of the analysis using gene expression data.

#### POSTER #3

SESSION: Identification and diagnostics: From the traditional tools to the state of the art NGS and TGS.

### EVALUATION OF HIGH RESOLUTION MELTING ANALYSIS TO DIFFERENTIATE BETWEEN PARENTAL AND HYBRID *PHYTOPHTHORA* SPECIES

M. Ratti, E. M. Goss. Plant Pathology Department, University of Florida, Gainesville, FL, U.S.A.

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*Phytophthora* hybridization events have been reported repeatedly, resulting in new species that conserve some of their parental phenotypic traits but differ in genotypes and host range. *P. andina* and *P. x pelgrandis* are pathogens of Solanaceae and ornamentals respectively, although the extent of their host ranges and distributions are unknown. *P. andina* emerged from hybridization of *P. infestans* and an unidentified related species, *P. x pelgrandis* from *P. nicotianae* and *P. cactorum*. Considering that hybrids and parental species can co-exist in the same areas and identification of hybrids usually involves sequence analysis, we aimed to develop a rapid tool to discern hybrids from parental species. We used High Resolution Melting (HRM) assays to differentiate genotypes based on their amplicon melting profiles. We designed primers for *P. x pelgrandis* based on available sequences of nuclear genes of *P. nicotianae* and *P. cactorum* containing one or more polymorphisms. For *P. andina*, heterozygous sites from Illumina short reads mapped against *P. infestans* were used for the same purpose. We evaluated the discriminative potential of amplicons using uMelt prediction software; then we ran HRM experiments to obtain actual melting profiles. Significant differences in melting curves were detected for each species and hybrid. These assays could be used to identify *P. andina* and *P. x pelgrandis* hybrids, and potentially as a versatile diagnostic tool when suspected infections arise.

#### POSTER #4

SESSION: Identification and diagnostics: From the traditional tools to the state of the art NGS and TGS.

#### PREVALENCE OF PINEAPPLE HEART ROT IN PUERTO RICO

Estévez de Jensen C.1. Intriago D. 1 and Abad G.2 1University of Puerto Rico, 2USDA-APHIS-PPQ CPHST Beltsville Laboratory, USA. Corresponding author: <u>consuelo.estevez(at)upr.edu</u>

Pineapples (*Ananas comosus* L. Merril) in fields in Santa Isabel, Puerto Rico showed discoloration of the basal leaf tissue, root necrosis and stem rot. In vegetative growth stage plants were stunted with the central leaves of pale green to yellow color and the base discolored with a brown margin. In 6 and 12 month old plants leaf tips become curled, brown and dried. The AGDIA ImmunoStrip for *Phytophthora* spp. resulted positive.

*Phytophthora* isolations from leaf basal tissue on potato dextrose agar (PDA) amended with streptomycin and PARP media were obtained. Coenocytic hyphae grew 3-4 days after incubation at 25 oC on PDA. Hyphal swellings and abundant terminal and intercalary clamydospores average of 23.2 to 34.5 µm were observed on PDA media. The isolates developed oval non-caduceous papillate sporangia of 32.5 to 66.0 µm. The 18-00210 isolate could also be differentiated based on the ITS sequence which showed 100% identity in the base pair rDNA fragment, as *P. nicotianae* (GenBank Accession No. MH537599). Implementing cultural practices and promoting the use of beneficial organisms may prevent losses due to the disease.

#### POSTER #5

SESSION: Identification and diagnostics: From the traditional tools to the state of the art NGS and TGS.

### Use of LAMP detection to identify potential contamination sources of *Pythium irregulare* in hydroponic culture system of eustoma

Feng W.1, Suga H. 2, and Kageyama K. 3.

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Hydroponic culture systems are subject to high risks of diseases caused by zoosporic plant pathogens. Control is generally difficult because of the rapid spread of zoospores in water. In Japan, the cut flower eustoma cultivated using the nutrient film techniques is susceptible to root rot caused by the *Pythium irregulare*. We used loop-mediated isothermal amplification to identify potential contamination sources of this pathogen by monitoring its presence in the water supply wells, seedling terraces, nutrient solutions, plants, and ground soils of a eustoma greenhouse complex. The results indicated that the infestation of *P. irregulare* had become established and more and more serious, and the pathogen may enter the culture system from the soils. Entry most likely occurs when seedlings are moved from the seedling terraces to the greenhouses, and sterilization of the system may not be sufficient. What's more, there is the possibility of the infestation of *P. irregulare* in the healthy plants before the disease symptoms are visible. Therefore, the periodic monitoring of pathogens in the culture system and ground soils is very important for the management and prevention of disease.

#### POSTER #6

#### GENETIC STRUCTURE OF POPULATIONS OF *Phytophthora sojae* FROM NORTH AND SOUTHEAST OF BUENOS AIRES PROVINCE (ARGENTINA)

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*Phytophthora* root and stem rot, caused by *Phytophthora sojae*, is an important disease of soybean in Argentina. Race 1 was prevalent in the North of Buenos Aires province (NBA) until 2001. In southeast Buenos Aires province (SEBA) soybean is a relatively new crop. This study aims at elucidating the genetic structure of *P. sojae*  isolates in this area. Ninety-three isolates obtained between 2011 and 2015 were utilized. Genetic diversity was evaluated using 8 polymorphic microsatellite markers, and pathotype diversity using 8 soybean differentials. Genetic variation was larger in NBA; non-significant differences were detected between SEBA and NBA. Significant differences were found among localities and samples within the same locality. Discriminant Analysis of Principal Components determined six genetic groups and Network analysis produced three that did not correlate with their geographic origins. Virulence was expressed as 22 different formulas. None of the *Rps*-gene differentials was resistant to all isolates, but no isolate deployed all the differential genes used. The soybean cultivation system and the anthropic intervention may have contributed to the present genetic structure.

#### POSTER #7

SESSION: Identification and diagnostics: From the traditional tools to the state of the art NGS and TGS.

### SPECIFIC DETECTION OF QUARANTINE SPECIES, *Phytophthora ramorum*, *P. kernoviae* and *P. lateralis* BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY.

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*P. ramorum* is known as a causal agent of sudden oak death, most commonly observed on *Camellia, Magnolia, Pieris* and *Quercus* spp.. *P. kernoviae* causes bleeding stem cankers on members of the *Fagaceae* and foliar blight and shoot dieback on other hosts in UK and New Zealand. *P. lateralis* is highly aggressive to *Chamaecyparis lawsoniana*, a native forest tree in California and Oregon. Spread of these pathogens were probably associated with import/export ornamental plants. Therefore, an effective quarantine control of these *Phytophthora* species is highly required. In this study, we developed a species specific detection method using LAMP and simple DNA extraction kit. LAMP primer sets were designed and the specificity were checked with extracted DNA from 27 species of *Phytophthora*, 13 species of close genus *Pythium* and *Phytophthium*, and 7 genus of other soil-borne pathogens. Selected LAMP primer sets, designed in *cox*1 gene for *P. ramorum* and *P. lateralis*, and in rDNA-ITS for *P. kernoviae* were able to detect each target from DNA samples extracted from inoculated leaves within 20 min. This method can be utilized for effective detection of the three species in quarantine.

#### POSTER #8

**SESSION:** Identification and diagnostics: From the traditional tools to the state of the art NGS and TGS.

#### **OOSPORE PRODUCTION OF** Pythiogeton romosum AND P. zizanaiae

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*Pythiogeton* is a little-studied genus of pythialean Oomycete. During a survey of paddy fields in Taiwan, *Pythiogeton* species were actually quite common, and a total of nine *Pythiogeton* species were identified. Among these 9 *Pythiogeton* species, only *P. ramosum* and *P. zizaniae*, which were isolated from rice seedlings and water bamboo, could produce sexual structures. The former is heterothallic and can produce aplerotic oospores; the latter is homothallic and can produce plerotic oospores. *Pythiogeton ramosum* could produce abundant oogonia and oospores on rye sucrose agar, while few on 10% clarified V8-juice agar (10% CVA). *Pythiogeton zizaniae* 

could produce abundant oogonia on 10% CVA, while only 0–45% of these oogonia could proceed to form oospores. However, abundant oospores could produce on 10% CVB culture. The range of temperature for the production of sexual structures was between 20 to 32°C (optimal 24°C) for *P. ramosum* and 16 to 24°C (optimal 20°C) for *P. zizaniae*. Light could inhibit the production of oospore.

#### POSTER #9

SESSION: Diversity in cultivated and natural environments: The global spread of pathogens.

#### CHARACTERIZATION OF APHANOMYCES EUTEICHES POPULATIONS

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Aphanomyces root rot (ARR), caused by *Aphanomyces euteiches*, is a devastating disease that nearly eliminated fresh pea production from Wisconsin, Iowa, and Minnesota. ARR was first identified in North Dakota in 2014 and survey results indicate that it was present in over 50% of the fields surveyed in 16 North Dakota counties in 2015-16. Although studies have reported that the *A. euteiches* populations present in the many European countries and parts of the U.S. display a high level of genetic diversity, the studies focused primarily on European *A. euteiches* populations using RFLP, AFLP and SSR methods. The objective of this study was to characterize *A. euteiches* populations from North America and Europe morphologically and using next generation sequencing technology. Preliminary results for genotypic diversity indicate that isolates collected from across North America tended to cluster by region, and that European isolates cluster separately from North American isolates. Morphological characterizations of the isolates correspond to genotypic characterizations. Understanding the diversity of *A. euteiches* is an important first step in deploying resistance to the devastating pathogen.

#### POSTER #10

SESSION: Identification and diagnostics: From the traditional tools to the state of the art NGS and TGS. INVESTIGATING INTRA-SAMPLE SEQUENCE VARIANTS FROM MITOCHONDRIAL AND NUCLEAR LOCI USED FOR DOWNY MILDEW IDENTIFICATION

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The potential for multiple individuals infecting a single plant and heterozygosity within nuclear loci constrain the use of DNA sequence data for proper identification of biotrophic downy mildews. The purpose of this work was to quantify sequence variation from the mitochondrial *cox2* and nuclear ITS loci within individual downy mildew samples to test for evidence of mixed infections and heterozygosity. DNA was extracted from individual downy mildew lesions on the ornamental plants *Agastache*, *Cleome*, and *Monarda*. Amplicon libraries generated from a portion of *cox2* and two regions of ITS were sequenced using the Illumina MiSeq platform. Unique sequence variants in each library were identified using DADA2 and mapped to reference sequences to remove contaminants. Data from the *cox2* library showed that single sequence variants represented 99% of sequence data in individual samples. In contrast, data from the ITS locus showed multiple samples were composed of two common variants, with each variant making up approx. 30% or 70% of the sample. Though the underlying reason

for the observed sequence variants in the ITS libraries is unknown, this work highlights the need to account for potential variation when using this locus to identify or describe new types of downy mildews.

#### POSTER #11

SESSION: Management and control, Advanced IPM tools, biocontrol, resistance, sustainable production.

Synergistic integration of rhizobacteria, resistance elicitors and fungicides for effective management of *Sclerospora graminicola* incited downy mildew in pearl millet Chandra Nayaka Siddaiah, Niranjan Raj Satyanarayana and Huntrike Shekar Shetty

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Pearl millet is the most important millet species grown for food worldwide and 1.98 million hectares are cultivated with pearl millet in India, with an annual production of 2.4 million tones. Major constraint to yield is downy mildew caused by the oomycete *Sclerospora graminicola*. Integrated management of downy mildew by combining rhizobacteria with fungicide (half dosage) was conducted in multilocational trails across different pearl millet growing areas in India. The most effective strains *Bacillus pumilus* and *Pseudmonas fluorescens* were used in combination with half dosage of the systemic fungicide Apron 35 SD. Treatments promoted pearl millet growth and significantly reduced the downy mildew, the reduction of downy mildew was higher when they were used in combination with half dosage of the fungicide. Mycelial extract and cell wall glucans elicitors of *Trichoderma hamatum* UOM 13 and Lipopolysaccharides (LPS) isolated from *P. fluorescens* UOM SAR 14 effectively induced systemic and durable resistance against downy mildew. LPS pre-treatment affects defense signaling through the central regulator NO which triggers the activities of POX, PAL, PR-1, PR-5 and HRGPs.

#### POSTER #12

SESSION: Diversity in cultivated and natural environments: The global spread of pathogens.

#### THE GENUS PHYTOPHTHORA IN ARGENTINA

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The genus *Phytophthora* includes pathogens, affecting a wide host range causing severe damage and economic losses. In order to achieve a more comprehensive vision of *Phytophthora* in Argentina, a review was carried out since the first reports at the beginning of the twentieth century till December 2017. Information was taken from printed and on line primary and secondary sources such as Proceedings of national and international Scientific Meetings, Bulletins from National Institutions and Universities, periodical Journals, books and data bases. It was analyzed and categorized to update the status or inventory of *Phytophthora* species recorded in the country, considering its geographical distribution, type of crops affected, host-pathogen relationships, symptoms and

nomenclature changes. A quick and comparative access to different aspects related to these pathologies were present. To date 20 species and 1 taxon have been cited affecting 224 hosts pathogen relationships in Argentina. The diversity of *Phytophthora* species in the world suggests that a wider variety of species, still not cited, could be present in Argentina.

#### POSTER #13

#### SESSION: Identification and diagnostics: From the traditional tools to the state of the art NGS and TGS. EVALUATION OF HIGH RESOLUTION MELTING ANALYSIS TO DIFFERENTIATE BETWEEN PARENTAL AND HYBRID PHYTOPHTHORA SPECIES

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*Phytophthora* hybridization events have been reported repeatedly, resulting in new species that conserve some of their parental phenotypic traits but differ in genotypes and host range. *P. andina* and *P. x pelgrandis* are pathogens of Solanaceae and ornamentals respectively, although the extent of their host ranges and distributions are unknown. *P. andina* emerged from hybridization of *P. infestans* and an unidentified related species, *P. x pelgrandis* from *P. nicotianae* and *P. cactorum*. Considering that hybrids and parental species can co-exist in the same areas and identification of hybrids usually involves sequence analysis, we aimed to develop a rapid tool to discern hybrids from parental species. We used High Resolution Melting (HRM) assays to differentiate genotypes based on their amplicon melting profiles. We designed primers for *P. x pelgrandis* based on available sequences of nuclear genes of *P. nicotianae* and *P. cactorum* containing one or more polymorphisms. For *P. andina*, heterozygous sites from Illumina short reads mapped against *P. infestans* were used for the same purpose. We evaluated the discriminative potential of amplicons using uMelt prediction software; then we ran HRM experiments to obtain actual melting profiles. Significant differences in melting curves were detected for each species and hybrid. These assays could be used to identify *P. andina* and *P. x pelgrandis* hybrids, and potentially as a versatile diagnostic tool when suspected infections arise.

#### POSTER #14

### Detection and quantification of *Peronospora destructor* inoculum in air and soil samples by Real-Time qPCR.

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After a truce of nearly 10 year, the incidence of Onion Downy Mildew (ODM), caused by *Peronospora destructor* [Berk.] Casp, increased annually in southwestern Québec since the early-2000s, reaching up to 33% of onion fields diseased. Information of ODM epidemiology is limited; wind-dispersed sporangia are known to

be the main secondary inoculum; however, sources of initial inoculum remains unknown. Knowledge on both types of inoculum is crucial for ODM management. Hence, this work aimed at developing a real-time TaqMan qPCR assay allowing the detection and quantification of *P. destructor* from soil and air samples. The assay specificity was tested against 28 isolates of *P. destructor*, 25 peronosporales and various onion pathogens. Validation against artificially inoculated soil and air samples suggested a sensitivity of 10 sporangia g-1 of dry soil and 1 sporangium m-3 of air while validation with environmental air samples shows a strong relationship between microscopic and qPCR counts (R2 = 0.96). Naturally infested soils ranged from 0 to 146 sporangia equivalent g-1 of dry soil. This assay will facilitate studies on the role of soil and air inoculum in the development of ODM epidemics.

#### POSTER #15

SESSION: Plant pathogenic Oomycetes of concern: Trade regulations, advanced monitoring and certification.

### RARE PLANTS AND PHYTOPHTHORAS DON'T MIX. THE PHYTOPHTHORAS IN NATIVE HABITATS WORK GROUP

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The Phytophthoras in Native Habitats Work Group (www.calphytos.org) was created to respond to *Phytophthora tentaculata* detections on California native plants outplanted into restoration areas and intercepted in native plant nurseries. The detections of *P. tentaculata* were followed by the discovery of *P. quercina* in a restoration planting as well as new, or new hybrid *Phytophthora* taxa (i.e. *Phytophthora* taxon *mugwort*, and *Phytophthora* taxon *juncus*). Preliminary surveys demonstrated that Phytophthoras are common in California native plant nurseries and restoration areas with over 40 species detected on numerous herbaceous and woody hosts. This voluntary coalition strives to protect native vegetation, especially sensitive, threatened and endangered species by minimizing the introduction and spread of Phytophthoras. A restoration nursery accreditation program is under development as are treatments for infested field sites, such as steam application, solarization or other non-chemical treatments.