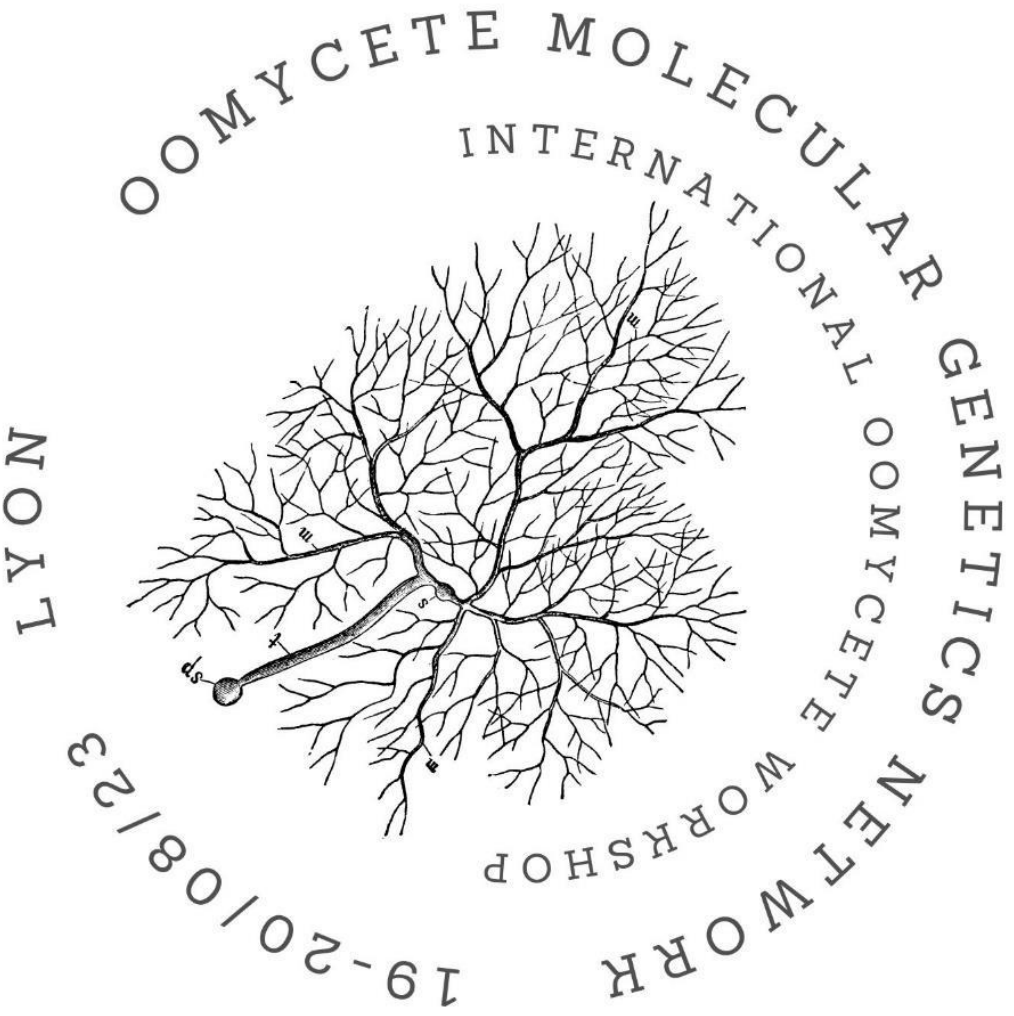
ABSTRACT BOOK

Joint ICPP Satellite Meetings

22nd Meeting of the Oomycete Molecular and Genomics Network

and 7th International Oomycete Workshop



OMGN-IOW – LYON 2023

Supported by The French Society of Plant Pathology

PROGRAM

8:00-9:15	**Registration**	
09:15-9:30		Welcome - Opening (OMGN-IOW), **Room 'Rhone 1'**

Oomycete Molecular Genetics Network

	SESSION 1	**Cell Biology, Signaling and Metabolism**
	Moderator :	*Steve Whisson*
09:30	Evangelisti Edouard	*Phytophthora* dix domain-containing proteins: an energy distribution platform?
09:45	Tayagui Ayelen	A lab-on-a-chip device to study the growth of oomycetes in O2 gradients
10:00	Breen Susan	Discovery of protein markers of oomycete ev's
10:15	Attard Agnès	Understanding the early events of plant infections by oomycetes, at new spatio-temporal scales: from attraction and aggregation of zoospores to host penetration
10:30-10:55		*Coffee break*
	SESSION 2	**Evolution, Diversity and Population Genomics**
	Moderator :	*François Delmotte*
11:00	La Spada Federico	DNA metabarcoding as a support tool of traditional isolation methods to describe the complexity of *Phytophthora communities*
11:15	Tandy Peter	Genomic investigations reveal atypical dynamic mitotic variation can rapidly drive diversity in spinach downy mildew
11:30	Goss Erica	Population structure of cacao pathogen *Phytophthora megakarya*
11:45	Kälin Carol	Comparative genomics of european *Aphanomyces euteiches* strains
12:00	Li Yufei	Evolution of LWY effector repertoire in *Phytophthora*
12:15	Murray Kevin	The coevolutionary race between *Hyaloperonospora arabidopsidis* and *Arabidopsis thaliana* at a transcontinental scale
12:30-1:55 pm		*Lunch*
2:00	Michelmore Richard	The telomere-to-telomere revolution : unveiling oomycete genomes
	SESSION 3	**Molecular Mechanisms of Pathogenicity**
	Moderator :	*Francine Govers*

02:30	Boevink Petra	Two independent cleavage events are involved in RxLR-EER effector processing
02:45	Delmotte François	Genome-wide association studies identify the oomycete mating-type locus sequence and avirulence candidate genes in grapevine downy mildew (*Plasmopara viticola*)
03:00	McLellan Hazel	Investigation of the role in virulence of *Phytophthora infestans* effector Pi06099
03:15	Dvorak Etienne	A QTL mapping approach leads to the identification of candidate avirulence genes of grapevine downy mildew
03:30	Whisson Steve	A *Phytophthora infestans* Myb transcription factor involved in sporulation and host penetration
03:45	Camborde Laurent	Targeted crispr-cas9-based gene knockouts in *Aphanomyces euteiches*
4:00-4:30 pm		*Coffee break*
4:30-5:30 pm		**POSTER SESSION**
5:30 pm		Free activity

Sunday, August 20th 2023

09:00	Kharel Aayushree	New approaches to expand our understanding of cryptic oomycete elicitin proteins
	SESSION 4	**Host Resistance Mechanisms**
	Moderator :	*Agnès Attard*
09:15	Schornack Sebastian	A Marchantia transcription regulator confers susceptibility to *Phytophthora palmivora* infection
09:30	Wang Yuanchao	xeg1: a case study of microbial attack and plant immunity in the apoplast
	SESSION 5	**Emerging Pathogens in the era of Globalization**
	Moderator :	*Magnus Karlsson*
09:45	Khan Mohamed	Serendipitous observation led to practice of using precipitated calcium carbonate in controlling *Aphanomyces cochlioides* in sugar beet
10:00	Cooke David	Re-emergence of the potato late blight threat in europe driven by an evolving population of *Phytophthora infestans*
	SESSION 6	**Innovations in Management and Control**
	Moderator :	*Paul Birch*
10:15	Zouaoui Mohamed	A culturomics approach identifies rhizospheric bacterial strains involved in legumes protection against the root rot agent *Aphanomyces euteiches*
10:30-10:55		*Coffee break*

11:00	Van den Ackerveken Guido	Innovations in optical plant disease imaging for improved resistance breeding
11:15	Krajaejun Theerapong	Potential anti-*Pythium insidiosum* therapeutics identified through screening of agricultural fungicides
11:30	Schena Leonardo	Kiwifruit vine decline syndrome: are we closing the circle?
11:45	Morris Paul	Control of *Pythium* pathogens in hydroponic greenhouses
12:00-1:00 pm		*OMGN-IOW Group photo + Lunch*
1:00-1:40 pm		**POSTER SESSION**

7th International Oomycete WorkShop (IOW)

	SESSION 7	**Taxonomy, Nomenclature, New Taxa**
	Moderator :	*To be defined*
01:45	Jung Thomas	Worldwide forest surveys reveal forty new phytophthora clade 2 species with fundamental implications for *Phytophthora* biodiversity, biogeography and evolution and global forest biosecurity
02:00	Nguyen Hai	Whole genome sequencing and phylogenomic analysis show support for the splitting of genus *Pythium*
02:15	Ristaino Jean	An open-access t-bas phylogeny for emerging *Phytophthora* species
02:30	Abad Gloria Z	*Phytophthora*: taxonomy and phylogeny and aspects of morphological and molecular characterization based on the types
02:45	Martin Frank	Mitochondrial genomics – a systematic approach for phylogenetics, taxonomy and development of diagnostic markers
	SESSION 8	**Identification and Diagnostics : From the traditional tools to HTS**
	Moderator :	*To be defined*
03:00	Huang Jin-Hsing	Heavy rainfall has given rise to severe crop diseases caused by *Phytophthora* spp. in Taiwan
03:15	Chanda Ashok Kumar	Detection and management of *Aphanomyces* root rot of sugar beet
03:30	Hamelin Richard	Genomic biosurveillance of sudden oak death pathogen *Phytophthora ramorum* reveals variants, hybrids and super-spreader events
03:45	Guaschino Micol	The role of the oomycete *Phytopythium vexans* as a biotic stress component of kiwifruit vine decline syndrome in Italy
4:00-4:30 pm		*Coffee break*
04:30-04:45 pm		**Concluding Remarks + Awards** (Poster/Talk)
6:00 pm		*ICPP Welcome Reception*

ABSTRACT ORAL PRESENTATIONS

SESSION 1 Cell Biology, Signaling and Metabolism

PHYTOPHTHORA DIX DOMAIN-CONTAINING PROTEINS: AN ENERGY DISTRIBUTION PLATFORM?

KOSTARELI M. (1), WESTERINK T. (1), WEIJERS D. (2), **EVANGELISTI E. (1)**

(1) Wageningen University, Laboratory of Phytopathology, Wageningen, NETHERLANDS; (2) Wageningen University, Laboratory of Biochemistry, Wageningen, NETHERLANDS

In animals, the DIshevelled and aXin (DIX) domain favours the aggregation of essential regulators of the Wnt-beta-catenin signalling cascade. DIX domains also occur in SOSEKI proteins that localize to specific corners of plant cells. Interestingly, oomycete genomes encode DIX-containing proteins with unique combinations of functional domains, suggesting their role differs from those identified in animals and plants. Using the cacao killer *Phytophthora palmivora* as a model system, we investigate the contribution of DIX domain-containing proteins in oomycete plant pathogens. *In vivo* imaging and interaction studies suggest these proteins contribute to energy distribution in hyphae. Our work aims to exploit oomycete genetic specificities to provide inroads for crop protection against this class of plant pathogens.

A LAB-ON-A-CHIP DEVICE TO STUDY THE GROWTH OF OOMYCETES IN O2 GRADIENTS

TAYAGUI A. (1), SUN Y. (1), NOCK V. (1), GARRILL A. (1)

(1) University of Canterbury, Christchuch, NEW ZEALAND

Diseases caused by oomycetes impact ecosystems with loss of biodiversity, and in the agricultural, horticultural, forestry and aquaculture sectors cause huge economic losses. It is thus important to understand how they grow and infect their hosts. The energy required for growth and infection is presumed to come from oxidative respiration, despite the fact that the infection structures and hyphae may be exposed to oxygen concentrations as low as 1 – 2% in and around the host. Pathogenic fungi, which utilize similar infective strategies, have been shown to sense and adapt to these hypoxic conditions, significantly altering their gene expression. As far as we are aware, there are as yet no reports of how oomycetes respond to differing oxygen concentrations. With a view to investigating this, we are developing oxygen sensor Lab-on-a-Chip (LOC) devices that expose oomycetes to oxygen gradients. Made from gas-permeable polydimethylsiloxan (PDMS), the devices comprise a central channel along which hyphae can grow and two side channels, one on each side of the central channel. These are filled with oxygen or nitrogen that diffuses through the PDMS. This creates an oxygen gradient in the central channel that can be measured with the hypoxia-sensing dye Pt(II) meso-tetrakis(pentafluorophenyl)porphine (PtTFPP) which is embedded in PDMS. I will describe experiments using these devices and the tropic responses of several oomycete species, including Phytophthora and Achlya.

DISCOVERY OF PROTEIN MARKERS OF OOMYCETE EV'S

BREEN S. (1), MCLELLAN H. (1), WANG W. (1), CHAPMAN S. (2), WHISSON S. (2), BOEVINK P. (2), BIRCH P. (1)

(1) University of Dundee, DUNDEE, UNITED KINGDOM; (2) The James Hutton Institute, DUNDEE, UNITED KINGDOM

The movement of effector proteins and RNAs from pathogen into host cells during infection is known to occur. However, the exact mechanisms facilitating this movement are still being widely studied. One possible delivery route involves the secretion and uptake of extracellular vesicles (EVs) between organisms.

In this study we isolated EVs from the oomycete Phytophthora infestans, cause of potato late blight, with the aim of identifying oomycete-associated EV markers and investigating the cargo of these bodies. This is being achieved by a proteomics approach to identify both secreted and vesicular proteins during in vitro growth. We have identified some known EV proteins found widely in EV proteomes, supporting our methodology and approach. Additionally, we have identified some oomycete-specific proteins that have as yet unknown functions but appear to be transmembrane proteins, including TMP1 (Trans-Membrane Protein 1). TMP1 accumulates in the same density fraction in sucrose gradients as the RXLR effector protein, PITG_04314, during EV isolation implying they could be associated with the same EV. The overall aim of this work is to find markers of EVs that we can use to determine how these EVs are secreted and taken up into the plant cell and whether this is a mode of transport for pathogenicity factors such as RXLR effectors.

UNDERSTANDING THE EARLY EVENTS OF PLANT INFECTIONS BY OOMYCETES, AT NEW SPATIO-TEMPORAL SCALES: FROM ATTRACTION AND AGGREGATION OF ZOOSPORES TO HOST PENETRATION

LE BERRE J. (1), MINET N. (1), KUHN M. (1), RANCUREL C. (1), LUPATELLI C. (1), THOMEN P. (2), COHEN C. (2), NOBLIN X. (2), FRÖSCHEL C. (3), GALIANA E. (1), DROEGE-LASER W. (3), **ATTARD A. (1)**

(1) INRAE-Université Côte d'Azur-CNRS, Sophia-Antipolis, FRANCE; (2) CNRS-Université Côte d'Azur, Nice, FRANCE; (3) Julius-Maximilians-Universität Würzburg, Würzburg, GERMANY

Plant pathogens have evolved a wide range of strategies enabling surface colonization and invasion of host despite the plant defense mechanisms. Current knowledge of small spatio-temporal scales on mechanisms allowing attraction toward hosts and progression across each first plant cell layer remains sparse. To characterize which host signals and plant cell functions regulate zoospore attraction and penetration, we developed a multidisciplinary study of the rhizospheric dialogue between the telluric oomycete, *Phytophthora parasitica* and Arabidopsis. On the one hand, we generated new phenotyping tools dedicated to the short time-scale quantification of both zoospores behavior swimming in the presence of ionic signals and aggregation on the root surface. On the other hand, we defined the transcriptome of roots and zoospores during attraction of *P. parasitica* and the translatome of each root cell layer during the penetration of zoospores. Thus, we showed that (1) the zoospores aggregated on root in the first minute after inoculation, (2) both roots and zoospores stimulated transcriptomic changes during attraction, and (3) when *P. parasitica* penetrated the rhizodermis, the translatomes were also modulated beyond in the cortex, the endodermis and the stele while these cell layers are not yet colonized. The implication of these results in understanding the early stages of infection, at short spatio-temporal scales, and their use for disease control will be discussed

SESSION 2 Evolution, Diversity and Population Genomics

DNA METABARCODING AS A SUPPORT TOOL OF TRADITIONAL ISOLATION METHODS TO DESCRIBE THE COMPLEXITY OF PHYTOPHTHORA COMMUNITIES

LA SPADA F. (1), ALOI F. (1), RIOLO M. (1,2), PANE A. (1), COCK P. (3), RANDALL E. (3), COOKE D. (3), CACCIOLA S. (1)

(1) UNIVERSITY OF CATANIA, DEPARTMENT OF AGRICULTURE, FOOD AND ENVIRONMENT, Catania, ITALY; (2) UNIVERSITY OF VALENCIA, FACULTY OF PHARMACY, LABORATORY OF FOOD CHEMISTRY AND TOXICOLOGY, Valencia, SPAIN; (3) THE JAMES HUTTON INSTITUTE, Dundee, UNITED KINGDOM

With the advent of the new millennium, traditional PCR has become the most reliable tool for the identification of cultured microorganisms. Recently, advancements in molecular technologies paved the way to 'omic sciences', new disciplines leading to the description and interpretation of communities of microorganisms in complex biological samples. Among these sciences, the DNA metabarcoding is emerging as the best support tool for the surveillance of *Phytophthora* communities within environmental samples carried out by traditional isolation. In this study, leaf baiting isolation and DNA metabarcoding were used to describe *Phytophthora* communities from soils of a nature reserve, a botanical garden and a citrus orchard. Overall, 155 baited isolates and the 32 metabarcoding-ASVs leaded to the identification of 21 *Phytophthora* taxa, including species exclusively recorded by baiting (*P. bilorbang*, *P. cryptogea*, *P. gonapodyides*, *P. parvispora* and *P. pseudocryptogea*), species exclusively detected by metabarcoding (*P. asparagi*, *P. occultans*, *P. psycrophila*, *P. syringae*, *P. aleatoria*/*P. cactorum*, *P. castanetorum*/*P. quercina*, *P. iranica*-like, and 5 unknown *Phytophthora* taxa) and species in common with both techniques (*P. citrophthora*, *P. multivora*, *P. nicotianae* and *P. plurivora*). Results suggested that the combination of leaf baiting and metabarcoding is the best approach to gain the most comprehensive diversity of *Phytophthora* communities in soil samples from different environments.

GENOMIC INVESTIGATIONS REVEAL ATYPICAL DYNAMIC MITOTIC VARIATION CAN RAPIDLY DRIVE DIVERSITY IN SPINACH DOWNY MILDEW

TANDY P. (1), LAMOUR K. (1), CORRELL J. (2), KLOSTERMAN S. (3), CLARK K. (3), FENG C. (2), ZIMA H. (2), VILLARROEL-ZEBALLOS M. (2), LIU B. (2)

(1) Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, Tn, UNITED STATES; (2) Department of Plant Pathology, University of Arkansas, Fayetteville, Ar, UNITED STATES; (3) United States Department of Agriculture, Agricultural Research Service, Salinas, Ca, UNITED STATES

Spinach (*Spinacia oleraceae*) downy mildew (SDM), caused by the oomycete pathogen *Peronospora effusa*, is the most important disease of spinach worldwide. Breeding resistant spinach cultivars is a critical management strategy. Race typing of SDM has been an important approach for tracking the evolution of the pathogen. Our objective was to investigate plasticity of the whole genome under controlled and field conditions for multiple race types. Genomic DNA was extracted from putative single lesions and PCR-free libraries were sequenced using Illumina HiSeqX in a 2x150bp configuration. Data were processed and heterozygous allele frequencies estimated using BWA and GATK as well as CLC Genomics Workbench. Heterozygous allele frequencies were visualized at the chromosome level. Most heterozygous allele frequencies were inconsistent with the expectations for a diploid organism and proved unstable between isolates as well as locations in the genome. Race type did not correlate with genotype and the SDM pathosystem appears to be dynamic. The plasticity of the genome has broad implications.

POPULATION STRUCTURE OF CACAO PATHOGEN PHYTOPHTHORA MEGAKARYA

GITTO A. (1), KOLAWOLE O. (1,4), TEN HOOPEN M. (3), BAILEY B. (2), BRAWNER J. (1), **GOSS E. (1)**

(1) University of Florida, Gainesville, UNITED STATES; (2) USDA ARS, Beltsville, UNITED STATES; (3) CIRAD, Montpellier, FRANCE; (4) University of Ibadan, Ibadan, NIGERIA

Phytophthora megakarya is an aggressive and extremely destructive pathogen that causes black pod disease of cacao, significantly limiting yield in the world's leading cacao producing region in West and Central Africa. To effectively use genetic breeding to improve cacao resistance to black pod disease, the genetic diversity of both host and pathogen populations must be considered. We examined genetic diversity and population structure of *P. megakarya* using genomic data from 166 isolates collected from Cameroon, Nigeria, and Ghana. We used reads from genotyping by sequencing of 150 isolates and from published whole genome sequences of 15 isolates to call 2,644 high quality SNPs relative to the reference genome Pm1/GH34 from Ghana. Isolates could be assigned to one of two major clades. One clade contained isolates from Nigeria and Ghana and the other contained isolates collected in all three countries. The two major clades showed differing degrees of genetic variation among isolates and heterozygosity of SNPs. Genomic data will be integrated with isolate phenotypes determined using experimental inoculations of cacao pods to evaluate variation in genetic determinants of virulence in *P. megakarya*.

COMPARATIVE GENOMICS OF EUROPEAN APHANOMYCES EUTEICHES STRAINS

KÄLIN C. (1), PIOMBO E. (1), KOLODINSKA BRANTESTAM A. (2), DUBEY M. (1), ELFSTRAND M. (1), KARLSSON M. (1)

(1) Swedish University of Agricultural Sciences, Uppsala, SWEDEN; (2) Nomad Foods, Findus Sverige AB, Bjuv, SWEDEN

The plant pathogen Aphanomyces euteiches causes root rot in its broad host range of various legume species. The homothallic oomycete is known for its low genetic diversity due to predominant clonal reproduction. In a recent study using short sequence repeat markers, three genetically distinct groups were identified in pea-infecting A. euteiches strains from Europe. A central European population differed significantly from genetically distinct groups comprising strains from the most northern (eastern Sweden and Finland) and most southern (Italy) sampling regions. From this strain collection, 69 strains representing the three groups were genome sequenced and the genomes assembled and annotated. Initially, a genealogical concordance phylogenetic species recognition analysis will be performed to establish the species status of the northern and southern groups. We will further perform a comparative genomics analysis of A. euteiches with focus on gene content and gene family evolution. Effectors, CAZymes and proteases have been shown to play important roles in oomycete virulence, and these gene families will be characterised in detail. Further, the data set offers the possibility for scanning the genomes for signatures of positive selection using selective sweep analysis. This project will improve our understanding of the genetics underlying diversity and virulence in European A. euteiches populations. The annotated genomes will be made available through AphanoDB

EVOLUTION OF LWY EFFECTOR REPERTOIRE IN PHYTOPHTHORA

LI Y. (1), SHU H. (2), ZHANG Z. (2), ZHANG F. (2), LI H. (1), TANG B. (1), FENG L. (1), YE W. (2), DONG S. (2), WANG Y. (2), KAMOUN S. (1), MA W. (1)

(1) The Sainsbury Laboratory, Norwich, UNITED KINGDOM; (2) Nanjing Agricultural University, Nanjing, CHINA

Effectors are deployed to manipulate host cellular processes and promote infection by pathogens. Many *Phytophthora* effectors consist of tandem repeats of (L)WY motifs, each containing a conserved 3-5 α-helical bundle. Some of these (L)WY effectors have the WY1-(LWY)n arrangement in which neighboring (L)WY-LWY units are concatenated by a conserved linkage, resulting in an overall non-globular shape. Despite the structural conservation, the (L)WY units show divergence in surface-exposed residues, leading to the hypothesis that the shuffling of (L)WY units may contribute to the diversification of *Phytophthora* effector repertoire. To investigate potential (L)WY shuffling, we developed a bioinformatic pipeline, which identified 74-155 LWY sequences from five *Phytophthora* genomes. Many of these genes are arranged in multi-LWY gene clusters in the genome, which may serve as hotspots for effector evolution. Interestingly, up to ~64% of the LWY genes encode putative proteins without the canonical N-terminal secretion Signal Peptide, indicating the presence of a dynamic sequence reservoir that could promote effector evolution. Comparison of sister *Phytophthora* species revealed LWY effectors as recombined products, lending support to a recombination-based mechanism that could contribute to the birth of novel virulence activities. This study offers important insight into the functional diversification of an effector repertoire driven by modular protein architecture.

THE COEVOLUTIONARY RACE BETWEEN HYALOPERONOSPORA ARABIDOPSIDIS AND ARABIDOPSIS THALIANA AT A TRANSCONTINENTAL SCALE

MURRAY K. (1), SHIRSEKAR G. (1), PAUL F. (1), ALBA G. (1), SCHWAAB R. (1), TAHTSIDOU C. (1), DEUSCH O. (1), KAI DURR H. (1), KONTOS I. (1), MAROTZ A. (1), KARAWOUNOPOULOS S. (1), VELTHOVEN R. (1), LUCKE M. (1), LANZ C. (1), COLLENBERG M. (1), TEAM P. (1), WEIGEL D. (1)

(1) Max Planck Institut für Biologie Tübingen, Tübingen, GERMANY

Plants and their pathogens are locked in a perpetual coevolutionary battle for survival. We present a transcontinental investigation of coevolution in the Hyaloperonospora arabidopsidis – Arabidopsis thaliana pathosystem. We generate whole genome sequences of over 400 host-pathogen pairs from natural infections collected throughout both the native eurasian range and the human-commensal colonisation of North America, as well as new near-complete long-read genome assemblies with evidence-based annotation. We investigate the demographic history of both host and pathogen, examine coevolution both generally and of individual gene pairs, and describe variation in the genetic networks of interacting host and pathogen genes. Our results show that the negative-frequency dependent selection on both the pathogen and host genomes leads to the presence of balanced polymorphisms in the wild pathosystem, in contrast to the directional selection generally experienced by pathogens of crop pathosystems.

THE TELOMERE-TO-TELOMERE REVOLUTION: UNVEILING OOMYCETE GENOMES

FLETCHER K. (1), **MICHELMORE R. (1)**

(1) University of California, Davis, Davis, UNITED STATES

Oomycetes include diverse pathogens that include obligate biotrophs, hemibiotrophs, and necrotrophs that result in foliar and root diseases. Technological advances in genome sequencing have enabled the generation of near complete genome assemblies of diverse species; these are foundational resources that can be used to study how the pathogen rapidly adapts to evade control. We have generated telomere-to-telomere assemblies from several oomycete genera, focusing on the obligately-biotrophic oomycetes that cause downy mildew diseases as well as Phytophthora and Pythium spp. Comparative genomics determined that downy mildew causing oomycetes are polyphyletic and share a 17-chromosome ancestral state along with many Phytophthora clades. Annotation of these assemblies has identified horizontal gene acquisition events from phytopathogenic fungi and defined high-identity gene clusters that encode putative effectors. Sequencing of multiple isolates revealed hallmarks of allo- and auto-heterokaryosis. Alloheterokaryons are believed to arise by somatic fusions of distinct genotypes, while autoheterokaryons are believed to arise via somatic mutations in a single founding genotype; both result in distinct genotypes sharing a common cytoplasm. Heterokaryosis increases the adaptability of the pathogen and has consequences for determining virulence pathotypes and population genomic inferences.

SESSION 3 Molecular Mechanisms of Pathogenicity

TWO INDEPENDENT CLEAVAGE EVENTS ARE INVOLVED IN RXLR-EER EFFECTOR PROCESSING

XU L. (2), WANG S. (3), PHAM J. (2), WANG W. (2), WANG H. (2), WELSH L. (1), WHISSON S. (1), **BOEVINK P. (1)**, BIRCH P. (2)

(1) The James Hutton Institute, Dundee, UNITED KINGDOM; (2) University of Dundee, Dundee, UNITED KINGDOM; (3) University of California, Riverside, UNITED STATES

Phytophthora plant pathogens are a serious and continuing threat to agriculture and the natural environment. Secreted effectors are critical to their infection success and the translocated RXLR class are a key set of these. We have shown that RXLR effectors are secreted from *Phytophthora* in a non-conventional fashion, despite having typical signal peptides. We have been investigating what this secretory pathway involves. It was shown, first with Avr3a, and subsequently for other RXLRs, that there is cleavage at the RXLR motif during secretion from the pathogen. Using a range of RXLR effector variants and mutations we show consistent cleavage at the RXLR, including at some degenerated motifs. Furthermore, we reveal that the EER motif represents a second cleavage site. We propose a model for how the RXLR motif may function in the secretory pathway selection

GENOME-WIDE ASSOCIATION STUDIES IDENTIFY THE OOMYCETE MATING-TYPE LOCUS SEQUENCE AND AVIRULENCE CANDIDATE GENES IN GRAPEVINE DOWNY MILDEW (PLASMOPARA VITICOLA)

PAINEAU M. (2), DUSSERT Y. (3), MINIO A. (2), FABRE F. (1), COUTURE C. (1), DEMEAUX I. (1), CANTU D. (2), MESTRE P. (4), **DELMOTTE F. (1)**

(1) INRAE, Bordeaux, FRANCE; (2) Department of Viticulture and Enology, Univ California, Davis , UNITED STATES; (3) CNRS, EBI, Poitiers, FRANCE; (4) INRAE, Colmar, FRANCE

The availability of high-quality reference assemblies for oomycetes enables genome-wide association studies (GWAS) to link genotype and phenotype variation, identifying the genomic architecture of pathogens' life-history traits. In P. viticola, mating can occur only between individuals of different mating types (heterothallism). Using GWAS, we identified a genomic region of 570 kb associated with the mating-type phenotype (Dussert et al. 2020). P2 individuals were homozygous for the MAT-a allele at the mating-type locus, whereas P1 individuals were heterozygous, carrying the MAT-a and MAT-b alleles. The mating-type region features a gene that encodes a transmembrane protein that might act as a hormone receptor; this is noteworthy since hormones have previously been identified as mating-type factors in Phytophthora spp. Our subsequent research delved into the genomic factors that drive the breakdown of grapevine's partial resistance to downy mildew, specifically focusing on Rpv3. Using GWAS, we discovered a distinct structural variation exclusively present in the genomes of strains that are virulent on grapevines carrying the Rpv3 locus. The structural variation consisted of in a deletion of 30 kb encompassing two closely-related genes that encode proteins of 800-900 amino acids with a signal peptide. The predicted structures of both proteins contain repeats that form structural elements typical of the LWY-fold, a conserved structural module in oomycete effectors.

INVESTIGATION OF THE ROLE IN VIRULENCE OF PHYTOPHTHORA INFESTANS EFFECTOR PI06099

MCLELLAN H. (1), SUNNY S. (1), LIU X. (1), WELSH L. (2), GILROY E. (2), BIRCH P. (1,2)

(1) University of Dundee, Dundee, UNITED KINGDOM; (2) James Hutton Institute, Dundee, UNITED KINGDOM

Plant pathogens secrete many effector proteins which are translocated inside plant cells and act to suppress host defences and promote pathogen colonisation. The RxLR effector Pi06099 from potato late blight pathogen *Phytophthora infestans* interacts with the plant red light receptor Phytochrome B (PhyB). Red light promotes plant immunity by accelerating cell death in response to the *P. infestans* MAMP INF1. Silencing the *Pi06099* effector using Host Induced Gene Silencing (HIGS) and stable RNAi transgenic lines demonstrated that it contributes to the virulence of *P. infestans* on Potato and *Nicotiana benthamiana*. Furthermore, domain swapping and mutagenesis of Pi06099 has been used to investigate the disruption of the interaction with PhyB and phenotypes associated with effector virulence.

A QTL MAPPING APPROACH LEADS TO THE IDENTIFICATION OF CANDIDATE AVIRULENCE GENES OF GRAPEVINE DOWNY MILDEW

DVORAK E. (1), FOULONGNE-ORIOL M. (2), MAZET I. (1), COUTURE C. (1), DELMOTTE F. (1)

(1) INRAE, Université de Bordeaux, Bordeaux Sciences Agro, SAVE, ISVV, Villenave D'ornon, FRANCE; (2) INRAE, MYCSA, Villenave D'ornon, FRANCE

Plasmopara viticola is a biotrophic oomycete responsible for grapevine downy mildew, one of the most destructive diseases in viticulture. Breeding efforts for new resistant cultivars are based on the introgression of *Resistance to Plasmopara viticola* (*Rpv*) factors from wild grape species. However, in recent years, a number of isolates able to overcome the resistance conferred by different *Rpv* genes have been reported. The risk of rapid breakdown of resistance makes it urgent to understand the genetic factors underlying the virulence of the pathogen.

We carried out a QTL mapping study focused on *P. viticola* adaptation to three major resistance genes: *Rpv3*, *Rpv10* and *Rpv12*. Sexually compatible strains were crossed to generate two F1 progenies, on which targeted genotyping-by-sequencing was performed. We built a set of unprecedented linkage maps of the *P. viticola* genome, with a consistent number of seventeen linkage groups that likely correspond to chromosomes.
Each offspring was cross-inoculated on a panel of grapevine cultivars carrying one of the aforementioned *Rpv* genes or none. Linkage analysis shows that resistance breakdown is under the control of one major QTL for each *Rpv* gene. These QTLs map to regions enriched in predicted secreted effectors, in which large deletions are observed for strains virulent on *Rpv3* and *Rpv12*. These results pave the way for the functional validation of the interaction between *Rpv* genes and candidate *P. viticola* avirulence genes.

A PHYTOPHTHORA INFESTANS MYB TRANSCRIPTION FACTOR INVOLVED IN SPORULATION AND HOST PENETRATION

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Completion of the *Phytophthora* lifecycle involves the precise expression of thousands of genes, typically regulated by the action of transcription factors. We aimed to identify transcription factors that bound to conserved sequence motifs in the promotors of infection-regulated genes from the potato late blight pathogen, *Phytophthora infestans*. Using a motif found in the promotors of many effector coding genes, we conducted a yeast-1-hybrid screen, which identified a single *P. infestans* candidate MYB transcription factor. Silencing of this transcription factor revealed that it regulated sporulation and pathogenicity phenotypes. In particular, silenced lines were unable to penetrate intact leaves, but could colonise wounded leaves. Transcriptome analysis of silenced lines, compared to wild type, identified over 1000 differentially expressed genes, including 42 RXLR effectors and 79 carbohydrate active (CAZy) proteins. Additional transcription factors and kinases were also discovered and, when silenced, also led to loss of pathogenicity.

TARGETED CRISPR-CAS9-BASED GENE KNOCKOUTS IN APHANOMYCES EUTEICHES

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Oomycete genome editing using CRISPR/Cas9 system represents the most promising and powerful technique to study genes involved in pathogenicity such as effectors. However, most of the reported successful studies have been obtained on *Phytophthora sp*. using polyethylene glycol-mediated protoplast transformation to insert Cas9 DNA plasmids for gene disruption. This method presents some disadvantages. Firstly, PEG-mediated transformation protocol needs strain adaptation and includes difficulty in obtaining high concentrations of viable protoplasts, high transformation efficiency or stable transformants. Secondly, the random integration of foreign DNA can lead to undesired gene disruption or reduced transcription rates. Furthermore, the transgene overexpression of Cas9 can result in off-target cleavage or toxicity. Here, we propose to circumvent these problems by adapting a protocol previously described on brown algae. By combining microprojectile bombardment for delivery of ribonucleoprotein complexes, we successfully transformed for the first time *Aphanomyces euteiches*, the root rot pathogen of legumes. We report that mutations at specific target sites are generated following the introduction of CRISPR-Cas9 ribonucleoproteins into *A. euteiches* cells. By transposing the positive selection system described for algae, we next propose a double mutation approach on a selected effector gene. Potentially, this method should be readily transferable to other oomycete species.

NEW APPROACHES TO EXPAND OUR UNDERSTANDING OF CRYPTIC OOMYCETE ELICITIN PROTEINS

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Phytophthora cinnamomi, one of the most devastating plant pathogens, poses a serious threat to the biodiversity and has a host range of almost 5000 plant species. Belonging to the class Oomycetes, *P. cinnamomi* is a sterol auxotroph and relies on external sterol sources for completion of its life cycle. Our recent review paper on elicitins of *Phytophthora* outlined the importance of these highly conserved proteins in recruiting sterols and providing it to the pathogen, however, the process is largely unknown. Research on the dependency of *P. cinnamomi* on plants for sterols, the process following sterol recruitment and thereafter, sterol signalling, will help us better understand the importance of sterols for oomycete survival. Thus, through a targeted metabolomics technique, we explored the dynamics of sterol acquisition by *P. cinnamomi*. We have also begun to explore gene editing which, in a limited *Phytophthora* species has assisted in gene functionalisation. Thus, to expand the possibilities of gene editing in *P. cinnamomi*, we have optimized a protoplast isolation process from the multinucleated hyphae of this pathogen. To understand the cell biology of the protoplasts, we utilized various vital stains to visualize internal structures, along with fluorescent dye to quantify viable protoplasts. These methodologies provide an opportunity to establish CRISPR/Cas-mediated gene editing of elicitins in *P. cinnamomi* to expand our fundamental knowledge of these proteins.

SESSION 4 Host Resistance Mechanisms

A MARCHANTIA TRANSCRIPTION REGULATOR CONFERS SUSCEPTIBILITY TO *PHYTOPHTHORA PALMIVORA* INFECTION

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Phytophthora palmivora is a broad range pathogen infecting plants as divergent as angiosperms and non-vascular bryophytes allowing us to study conserved and plant lineage specific responses to its infection. In previous work, we have established infections of *P. palmivora* with the liverwort *Marchantia polymorpha* and have identified 30 differentially expressed transcription regulator genes. We study a GRAS gene which is conserved across the green lineage, but absent from Arabidopsis and thus not characterised previously. Orthologous GRAS genes in different host plants seem to respond to *Phytophthora* infection. The Marchantia GRAS gene is induced by *P. palmivora* infection as well as by cell-free *Phytophthora* culture supernatant. To understand its contribution to the infection process we generated CRISPR/Cas9 mutants and found that GRAS mutants are more resistant to infection. Our data suggests that the GRAS gene is part of a biotic stress response which is exploited by *Phytophthora* to achieve full infection in *Marchantia*. Future work should address whether it fulfils similar functions in angiosperm host plants and would allow engineering quantitative resistance to *Phytophthora* infection.

XEG1: A CASE STUDY OF MICROBIAL ATTACK AND PLANT IMMUNITY IN THE APOPLAST

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The apoplast constitutes a major interaction niche in plant-microbe interactions. During infection, microbial pathogens secrete a large repertoire of effectors that act in the apoplast to modulate host conditions for infection. Plants respond to microbial attack via perception of conserved molecular patterns or apoplastic effectors using cell surface immune receptors to mount defense. The apoplastic effector XEG1 is a glycoside hydrolase 12 protein secreted by the soybean root rot pathogen Phytophthora sojae. XEG1 displays hydrolase activity toward xyloglucans and essential for Phytophthora infection. As a countermeasure, soybean secretes the inhibitor GmGIP1, which binds directly to XEG1 and inhibits its hydrolase activity, to increase soybean resistance. P. sojae secretes a paralogous XEG1-like protein, XLP1, with no enzyme activity. XLP1 binds GmGIP1 more tightly than XEG1, and acts as a decoy protecting XEG1 from the inhibitor GmGIP1. XEG1 is degraded by host aspartic protease GmAP5 in the apoplast. However, XEG1 undergoes N-glycosylation, which protects XEG1 from GmAP5 degradation. In addition, XEG1 can be recognized by a plant membrane-localized receptor-like protein RXEG1 to mount defense. Structural analyses revealed that RXEG1 inhibits the hydrolase activity of XEG1 and plays a dual immunogenic role in plant defense. Together, these studies revealed that co-evolutionary arms race tailored the multi-layered defense and counter-defense in plant-microbe interactions.

SESSION 5 Emerging Pathogens in the era of Globalization

SERENDIPITOUS OBSERVATION LED TO PRACTICE OF USING PRECIPITATED CALCIUM CARBONATE IN CONTROLLING APHANOMYCES COCHLIOIDES IN SUGAR BEET

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Sugar beet (*Beta vulgaris* L.) major crop worldwide for sugar production. Minnesota and North Dakota, adjoining states in the US produce 57% of the US sugar beet production that results in $5 billion in total economic activity. One of the major limiting factors for sugar beet production is *Aphanomyes cochlioides* that causes seedling damping off and Aphanomyces root rot. Tachigaren/hymexazol as a seed treatment is the only fungicide labeled for and widely used on sugar beet for protecting seedlings from *A. cochlioides*. In the 2003, research conducted in Minnesota to determine whether precipitated calcium carbonate (CaCo3) could help to reduce the time that sugar beet could be grown in fields with carryover herbicide showed that the CaCo3 reduced the impact of root with symptoms of Aphanomyces root rot. Further research using 11 to 44 tonnes per hectare of precipitated CaCo3 significantly reduced damage caused by *A. cochlioides.* As a result, sugar beet growers in the USA with fields identified with A. cochliodes have used the practice of applying 7 to 16 tonnes per hectare of CaCo3 before planting sugar beet for a high quality crop. Research further showed that the CaCo3 provided protection from *A. cochlioides* for over 12 years.

RE-EMERGENCE OF THE POTATO LATE BLIGHT THREAT IN EUROPE DRIVEN BY AN EVOLVING POPULATION OF PHYTOPHTHORA INFESTANS

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Successful integrated pest management (IPM) is dependent on knowledge of the pathogen, its host and the environment and how each influences management practices. Late blight, caused by Phytophthora infestans, continues to threaten potato and tomato crops on a global scale. An evolving pathogen population and increasingly rigorous regulations on chemical use are driving a transition from prophylactic fungicide use to more integrated approaches combining host resistance, knowledge of pathogen spread and infection risk with smarter use of fungicides and biological products. The evolving population of P. infestans prompted the EuroBlight consortium to collect data on the diversity of P. infestans, analysed with simple sequence repeat genetic markers. Surveys of late blight infected crops by many collaborators from 2013–2022 has resulted in over 16 thousand genotyped samples from across Europe held in an isolate database with associated analysis tools and a mapping interface. The population is dominated by relatively few clonal lineages that we have tracked over time and space (www.euroblight.net). We have identified traits such as fungicide resistance that drive the emergence, evolution and spread of some clones and share the knowledge with the industry to tailor IPM practices. In contrast to the clones, 20-30% of the European population comprises genetically diverse strains consistent with oospore-derived sexual populations and their evolving traits are challenging to predict.

SESSION 6 Innovations in Management and Control

A CULTUROMICS APPROACH IDENTIFIES RHIZOSPHERIC BACTERIAL STRAINS INVOLVED IN LEGUMES PROTECTION AGAINST THE ROOT ROT AGENT APHANOMYCES EUTEICHES

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In recent years, using a consortium of selected bacteria from the root microbiota, also known as a "synthetic community" (SynCOM), to promote plant health has become increasingly common. The objective of this study is to elaborate a model SynCOM from the rhizosphere of *Medicago truncatula* (Mt) to unravel the role of root microbiota in mediating plant-microbe interactions in the con of biotic stress caused by *Aphanomyces euteiches*, a devastating oomycete that causes root rot in legume plants. A high throughput culturomics protocol was used to obtain 1364 isolates from the rhizosphere of Mt. The collection of isolated bacteria was genotyped using Illumina 16S metabarcoding sequencing. The UCLAST algorithm was employed with a 97% identity to select 812 pure isolates with 79 unique OTUs. The relative abundance analysis of the collection showed that the uppermost taxa were similar to those observed in molecular identification obtained from soil DNA. Among the selected 812 pure isolate 12 were found to inhibit *A. euteiches* growth in a dual culture assay. In planta testing, only one strain of Pseudomonas sp. showed significant difference from the untreated control. This collection of 79 unique OTUs was used to constitute a synthetic community as a model of the root microbiota of *Medicago* plants. A multi-omics approach will be used to analyze the behavior of this SynCOM in a gnotobiotic system to study its role in plant microbe interactions.

INNOVATIONS IN OPTICAL PLANT DISEASE IMAGING FOR IMPROVED RESISTANCE BREEDING

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Quantitative disease resistance (QDR) is gaining importance in breeding of resilient crops. This is caused by a scarcity of strong monogenic resistance traits for some crops or the short lifespan these have because of the rapid evolution of resistance-breaking pathogen isolates. However, QDR faces the challenge of reproducible and sensitive phenotyping to determine the underlying genetics and utilize it in breeding. Precise plant phenotyping is required so that differences in susceptibility or resistance between genotypes can be detected and quantified. Optical sensors allow to complement scoring of visual features with the human eye. They can increase accuracy as well as throughput, e.g. by measuring microscopic changes, using wavelengths outside the visible spectrum, or automating the phenotyping.We will present our advances in optical imaging of lettuce downy mildew caused by the obligate biotrophic oomycete *Bremia lactucae*. Resistance to downy mildew is an essential trait in lettuce cultivars and of high priority for both lettuce breeders and growers. We have developed two methods to image disease progression before visible symptoms can be observed: (1) optical coherence tomography for real-time 3D imaging of downy mildew hypha in living tissue, and (2) UV fluorescence imaging for quantitative visualization of early plant responses to infection. We will report on these methodologies and their potential use in exploring QDR to aid the development of resilient crops.

POTENTIAL ANTI-PYTHIUM INSIDIOSUM THERAPEUTICS IDENTIFIED THROUGH SCREENING OF AGRICULTURAL FUNGICIDES

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Pythiosis is a life-threatening infectious disease of humans and animals caused by the oomycete microorganism *Pythium insidiosum*. Clinical manifestations of pythiosis include blood vessel, eye, skin, or gastrointestinal tract infections. According to geographical distribution analysis, pythiosis has been increasingly reported in 23 countries across the world, with an overall mortality rate of 28%. Pythiosis is treated with a combination of surgery, immunotherapy, and antimicrobial drugs. Radical surgery, which usually results in a handicap, is often required to save patients' lives due to the limited efficacy of conventional antimicrobial drugs. Immunotherapy could reduce the need for surgeries and improve recovery rates in a few cases. However, new and effective medical treatments are urgently needed for pythiosis. This study aims to find potential anti-*P. insidiosum* agents by screening 17 agricultural fungicides that inhibit plant-pathogenic oomycetes and validating their efficacy and safety. We found that cyazofamid, fenamidone, and fluopicolide could effectively inhibit *P. insidiosum* and showed relatively low toxicity to human cells. Cyazofamid was the most promising chemical with the highest calculated therapeutic ratio, and its mode of action may involve binding cytochrome c reductase of *P. insidiosum*. In conclusion, pythiosis still lacks effective treatment. This study demonstrates some agricultural fungicides that could be repurposed for treating pythiosis.

KIWIFRUIT VINE DECLINE SYNDROME: ARE WE CLOSING THE CIRCLE?

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The Kiwifruit Vine Decline Syndrome (KVDS) is a disease that severely affects the root system of kiwifruit plants, causing their sudden collapse. While the causes behind KVDS are still being debated, previous work shows that this syndrome has a biotic origin, and it is potentially induced by oomycetes. In this con, our work focused on clarifying causes and mechanisms behind KVDS. Our field surveys identified three major sites where we isolated several oomycetes identified as Phytophtora spp., Pythium spp., and Phytopythium spp. Amplicon metagenomics focused on bacterial, fungal, and oomycete communities show marginal differences in the diversity and structure of microbial communities between symptomatic and asymptomatic plants. However, more detailed analyses showed a clear presence of Phytopythium sp. only in presence of KVDS symptoms. Furthermore, Phytopythium vexans was the most frequently isolated pathogen using a baiting approach. Then, we performed the high-throughput isolation of potential biocontrol agents, which yielded a pool of bacterial isolates with a strong antagonistic activity against representative isolates of Phytophtora sp., Pythium sp. and Phytopythium sp.. Collectively, our results strongly support the biotic origin of KVDS, potentially caused by Phytopythium spp. through a complex interaction with the environment, the plant, and the plant microbiome, building up the base for sustainable control strategies.

CONTROL OF PYTHIUM PATHOGENS IN HYDROPONIC GREENHOUSES

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Hydroponic greenhouses are more efficient in their use of water than conventional soil-based systems. With the use of energy efficient light, and elevated CO2 conditions, these production systems enable the year-round production of leafy greens in northern climate zones. However, this novel environment is also ideally suited for the spread of pythium pathogens in the recirculating water. These pathogens can be introduced via air dust, contaminated water, insects, foot traffic, contaminated soil etc. In the US, Pythium pathogens have emerged as particularly economically impactful pathogens of spinach, and other leafy greens. Our research has identified a group of Pseudomonas fluorescens strains capable of contact-dependent killing of isolates obtained from greenhouse operations from California, Indiana, Ohio, and New Jersey. To identify species-specific virulence factors, we have implemented a sequential strategy of bioinformatic analysis; DNA synthesis of boundary regions containing in-frame deletions of target genes, electroporation of the vector containing gene KOs; selection of vector and assays of virulence following operon disruption by integration of vector; counter-selection for removal of vector; and finally, verification of gene deletion events and changes in gene virulence.

SESSION 7 Taxonomy, Nomenclature, New Taxa

WORLDWIDE FOREST SURVEYS REVEAL FORTY NEW PHYTOPHTHORA CLADE 2 SPECIES WITH FUNDAMENTAL IMPLICATIONS FOR PHYTOPHTHORA BIODIVERSITY, BIOGEOGRAPHY AND EVOLUTION AND GLOBAL FOREST BIOSECURITY

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During surveys of global *Phytophthora* diversity 40 new species were detected mainly in natural forests and streams in Europe, Asia and the Americas and assigned to the five subclades of *Phytophthora* Clade 2 based on a multigene phylogeny of nine nuclear and three mitochondrial gene regions. The evolutionary history of the Clade appears to have involved the pre-Gondwanan divergence of three extant subclades, 2c, 2d and 2e, all having disjunct natural distributions on separate continents and comprising species with a soilborne and aquatic lifestyle and a few partially aerial species in Clade 2c; and the post-Gondwanan evolution of subclades 2a and 2b in South-/East Asia and South America, respectively. Clade 2b comprises soil inhabiting and aerial species whereas Clade 2a has evolved further towards an aerial lifestyle comprising only species which are predominantly or partially airborne. The 74 described Clade 2 species result from both allopatric non-adaptive and sympatric adaptive radiations. They represent most morphological and physiological characters, breeding systems, lifestyles and forms of host specialism found in the genus *Phytophthora* demonstrating the strong biological cohesiveness of the genus. The finding of 40 previously unknown species from a single *Phytophthora* clade highlights a critical lack of information on the scale of the unknown pathogen threats to forests demonstrating the anachronism of plant biosecurity protocols based on lists of named organisms.

WHOLE GENOME SEQUENCING AND PHYLOGENOMIC ANALYSIS SHOW SUPPORT FOR THE SPLITTING OF GENUS PYTHIUM

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The genus *Pythium* (nom. cons.) sensu lato (s.l.) is composed of many important species of plant pathogens. Early molecular phylogenetic studies suggested paraphyly of *Pythium*, which led to a formal proposal by Uzuhashi and colleagues in 2010 to split the genus into *Pythium* sensu stricto (s.s.), *Elongisporangium*, *Globisporangium*, *Ovatisporangium* (=*Phytopythium*), and *Pilasporangium* using morphological characters and phylogenies of cox2 and 28S rDNA. Although the split was fairly justified by the delineating morphological characters, there were weaknesses in the molecular analyses, which created reluctance in the scientific community to adopt these new genera for the description of new species. In this study, this issue was addressed using phylogenomics. Whole genomes of 109 strains of *Pythium* and close relatives were sequenced, assembled, and annotated. Phylogenomic analyses were performed with 148 single-copy genes represented in at least 90% of the taxa in the data set. The results showed support for the division of *Pythium* s.l. The status of alternative generic names that have been used for species of *Pythium* in the past (e.g., *Artotrogus*, *Cystosiphon*, *Eupythium*, *Nematosporangium*, *Rheosporangium*, *Sphaerosporangium*) was investigated. Based on our molecular analyses and review of the *Pythium* generic concepts, we urge the scientific community to adopt the concepts proposed by Uzuhashi and colleagues in 2010 in their work going forward.

AN OPEN-ACCESS T-BAS PHYLOGENY FOR EMERGING PHYTOPHTHORA SPECIES

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Phytophthora species cause severe diseases on food, forest, and ornamental crops. We developed an open access phylogenetic tool using the Tree-Based Alignment Selector Toolkit (T-BAS) for 192 formally described species of Phytophthora and 33 informal taxa in the genus *Phytophthora*. The phylogenetic tree uses sequences of eight nuclear genes and was inferred using the RAxML maximum likelihood program. A search engine was developed to identify microsatellite genotypes of *P. infestans* based on genetic distance to known lineages. The T-BAS tool provides a visualization framework allowing users to place unknown isolates on a curated phylogeny of all *Phytophthora* species. The tree can be updated in real-time as new species are described. The tool contains metadata including clade, host species, substrate, sexual characteristics, distribution, and reference literature, which can be visualized on the tree and downloaded for other uses. This phylogenetic resource will allow data sharing among the global *Phytophthora* community and the database will enable users to upload sequences and determine the phylogenetic placement of an isolate within the larger phylogeny and download sequence data and metadata. The database will be curated by *Phytophthora* researchers and is housed on the T-BAS web portal in the Center for Integrated Fungal Research at NC State. The T-BAS web tool can be leveraged to create similar metadata enhanced phylogenies for other Oomycete, bacterial or fungal pathogens.

PHYTOPHTHORA: TAXONOMY AND PHYLOGENY AND ASPECTS OF MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION BASED ON THE TYPES

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Phytophthora with 212 species contains many members that cause diseases of economic and environmental impact in nurseries, horticulture, agricultural and natural ecosystems; many species are of regulatory concern. This status requires the implementation of robust tools for morphological and molecular identification based on the reference specimen (type), which has been designated to represent the name for each described species. Since 2014, members of the USDA S&T Plant Pathogen Confirmatory Diagnostics Laboratory and Pest Identification Technology Laboratory have been collaborating with national and international experts to implement robust systems for species identification. In September 2019, the "*IDphy*: morphological and molecular identification based on the types" international resource with Lucid and Tabular Keys was launched online to cover the 161 species described until 2018 and in 2021 the *IDphy* app with Lucid key for morphological characterization. In 2023, we are working on *IDphy* version 2 to cover the 212 species described, and the innovative molecular toolbox with seven genes, and databases for Sanger sequencing and metabarcoding high-throughput sequencing technologies. In addition, a manuscript for the Revision of the Taxonomy and Phylogeny of *Phytophthora* based on the types is in progress and implemented to operate in conjunction with *IDphy* V2. We expect that these resources will be of great benefit for the international community working with *Phytophthora*.

MITOCHONDRIAL GENOMICS – A SYSTEMATIC APPROACH FOR PHYLOGENETICS, TAXONOMY AND DEVELOPMENT OF DIAGNOSTIC MARKERS

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A database of approximately 800 oomycete mitochondrial genomes representing 260 species in 19 genera has been assembled and used in a systematic approach for phylogenetic studies, design of a new barcode locus for species identification and development of diagnostic assays. Extracting the same gene from a broad range of taxa helps identify the most useful loci for phylogenetic analysis; the ability to examine flanking regions from a range of taxa for genes of interest also helps in the design of conserved amplification primers. Comparison of gene order differences facilitated development of a new barcode locus for species identification. In oomycetes the *rps10* gene is flanked by a unique order of tRNAs that enabled design of conserved amplification primers. Since this gene order is not observed in plants or Eumycotan fungi there are low levels of nonspecific amplification from environmental samples. Comparison of *rps10* with *cox1* sequences from the same isolates indicate an equal level of discrimination among taxa. The Grunwald lab developed a metabarcoding approach for oomycetes using this locus (Phytobiomes 6:214-226). Unique gene order differences among genera have also proved useful for design of diagnostic assays for *Phytophthora, Plasmopara, Aphanomyces* and current work with *Pythium* spp. For some taxa the identification of unique putative open reading frames by BLAST analysis of the entire database were useful for development of diagnostic assays.

SESSION 8 Identification and Diagnostics: From the traditional tools to HTS

HEAVY RAINFALL HAS GIVEN RISE TO SEVERE CROP DISEASES CAUSED BY PHYTOPHTHORA SPP. IN TAIWAN

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In Taiwan, heavy rainfall has been the most typical severe weather event in summer, causing various crop diseases. Most diseases were caused by *Phytophthora* spp., including Phytophthora blight of Welsh onion, Phytophthora blight of cucurbits, Phytophthora heart rot of pineapple, Phytophthora blight of orchids, Phytophthora blight of passion fruit, Phytophthora leaf blight, and fruit and root rot of papaya, and so on. Since 2009, continuous heavy rainfall has been a big problem in the summer in Taiwan, often causing flooding in the fields. It usually led to poor growth, the death of large areas of Welsh onion, as well as severe fruit rot of melon. The crop yield loss was initially thought to be the poor water tolerance of the cultivars in the rainy season. Later studies proved it was a severe disease caused by *Phytophthora nicotianae*, *P. melonis*, and other *Phytophthora* spp. induced by continuous heavy rainfall. Because Phytophthora blight develops fast and can cause severe infections in several days, it will be too late to apply pesticides after the rainy season. Field trial results show that the regular application of phosphite weeks before the rainy season or/and the precise application of fungicides before and during the rainy season could significantly reduce the disease severity. In addition, healthy seedlings and weather forecasting are also crucial for disease prevention.

DETECTION AND MANAGEMENT OF APHANOMYCES ROOT ROT OF SUGAR BEET

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Aphanomyces cochlioides is the causal agent of seedling damping-off and Aphanomyces root rot (ARR) of sugar beet. The pathogen can persist in the soil as dormant oospores. It is essential for the growers to know the risk of Aphanomyces prior to planting so that they can develop a suitable disease management approach to mitigate yield losses. Aphanomyces root rot can look very similar to root rots and seedling diseases caused by other fungi and oomycetes, so developing a diagnostic assay would be of great value to the sugar beet industry. A specific quantitative PCR (qPCR) assay targeting mitochondrial DNA was developed to detect and quantify *A. cochlioides* DNA in infested field soils and infected sugar beet samples. The assay has a detention limit of 0.1 pg of pathogen DNA and was able to detect A. cochlioides in naturally infected sugar beet root samples. Currently seed treatments offer protection during the first few weeks after planting. However, if the soil moisture remains high later in the season, significant yield losses can occur due to chronic root rot. Precipitated calcium carbonate (PCC), a byproduct of the sugar beet factories is very effective in protecting plant stands and reducing ARR severity up to 12 years after a single application. Use of PCC is widely adopted by the growers in Minnesota and North Dakota. A strong positive correlation between soil extractable calcium (SEC) and root yield suggesting that calcium is playing a vital role in reducing ARR.

GENOMIC BIOSURVEILLANCE OF SUDDEN OAK DEATH PATHOGEN PHYTOPHTHORA RAMORUM REVEALS VARIANTS, HYBRIDS AND SUPER-SPREADER EVENTS

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Invasive alien tree pathogens often have complex invasion histories. Understanding the source and pathways of invasion is crucial to improve prevention. Genomic biosurveillance can help untangle the invasion history. The BioSAFE project sequenced and analysed more than 500 genomes of a global collection of the pathogen responsible for the sudden oak death, the sudden larch death and Ramorum blight (*Phytophthora ramorum*). Variants within clonal lineages of P. ramorum were often geographically and/or chronologically restricted. We detected a shift in variants of the EU1 and NA2 lineages of *P. ramorum* in nurseries in British Columbia, Canada. One of the EU1 variants replaced all previous variants and spread to many nurseries, a signature of a potential super-spreader event. We also identified interlineage hybrids that are F1 progenies. They produce viable sporangia and chlamydospores and are infectious to rhododendron, a common host. Comparison of variant composition in nurseries, following treatment, revealed instances of eradication success and failure. For rapid and high-throughput biosurveillance, we have developed SODseq, a tool that generates high-throughput sequence data for 355 informative amplicons that recapitulate the patterns obtained with whole genome sequencing. This tool can be used with DNA extracted from cultures or directly from environmental samples or infected host tissues and provide useful genomic data that can inform mitigation approaches.

THE ROLE OF THE OOMYCETE PHYTOPYTHIUM VEXANS AS A BIOTIC STRESS COMPONENT OF KIWIFRUIT VINE DECLINE SYNDROME IN ITALY

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Several soilborne oomycetes belonging to the genus Phytopythium have been associated to kiwifruit vine decline syndrome (KVDS) which is defined as a multifactorial syndrome where both abiotic and biotic stressors are involved. Phytopythium vexans is one of the main species isolated in affected orchards also revealing several interactions with other taxonomic groups composing the microbiome associated to the syndrome. Microbiome network analysis between taxa revealed strain-specific interactions, requiring further studies on the genomics of involved oomycetes. Furthermore, plant response to the presence Phytopythium vexans in Actinidia deliciosa roots, characterized through gene expression analysis approach, revealed an upregulation of ROS scavenging pathways and hormonal stress signaling in response to the pathogen presence and flooding at specific time points. Further transcriptomic studies on Actinidia roots will reveal a major understanding of all the involved pathways in oomycete pathogenesis on fruit trees. The model of KVDS as a multifactorial syndrome, where climate change plays an important role in defining the onset of the syndrome, requires the application and combination of different omics techniques for reaching a wider comprehension of oomycete pathogenesis in complex systems.

POSTERS

REVEALING PRINCIPLES OF PHYTOPHTHORA ZOOSPORES SENSING AND MOTION PROPERTIES THROUGH A BIO-PHYSICAL APPROACH

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In the soil, early infection events of *Phytophthora* species are mediated by sensory and propulsion capabilities of biflagellate unicellular zoospores, orchestrated by rhizospheric guidance factors. Lack of detailed information on zoospores plasma membrane proteins prevents a comprehensive understanding of how they contribute to the perception of rhizospheric environment, particularly during migration toward host plant. A bio-physical approach was developed to identify the molecular key-players mediating host-driven taxis. At first, the membrane protein repertoire of *Phytophthora parasitica* zoospores was investigated through LC-MS/MS approach, resulting in a distinct peptide signature between zoospores cell body and flagella plasma membranes. Then, using a microfluidic set-up, functional biomechanics analyses were developed to quantify both zoospore motion (velocity, trajectory and cell rotation) and flagella beating (frequencies and oscillation amplitude) in response to distinct rhizospheric stimuli. The set-up further enabled to discriminate zoospores specific stimuli response among other rhizospheric microbial species. Altogether, the obtained results contribute to elucidate the mechanism of protein-mediated sensing and motion response of *Phytophthora* zoospores and improve the understanding of the complex rhizospheric interaction network driving oomycete dispersal.

INSIGHTS INTO THE GENOME OF PHYTOPHTHORA AGATHIDICIDA, THE KAURI DIEBACK PATHOGEN

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Phytophthora agathidicida is the main causal agent of a devastating dieback disease that kills iconic and ancient kauri (*Agathis australis*) in New Zealand. Like for so many other *Phytophthora* pathogens, there is no simple way to control or eradicate it. Studying the genomes and genes of species such as *P. agathidicida* will provide a much deeper understanding of how *Phytophthora* pathogens work and, ultimately, how we might combat them. We recently assembled the genome sequence of *P. agathidicida* to chromosome level - one of the first for any *Phytophthora* species (Cox et al. 2022 Frontiers in Microbiology 13: 1038444). The complete genome sequence shows the extent of duplication and diversification of genes such as effectors that are predicted to have roles in virulence. Here we describe recent progress in analysing the *P. agathidicida* genome and assessing effector gene expression *in planta*, as well as functional studies of both cytoplasmic and apoplastic effectors. The *P. agathidicida* genome sequence shows many similar genomic and gene features to those of other sequenced *Phytophthora* species. Because of this, it is anticipated that this genome sequence will be a useful resource for the broader *Phytophthora* research community as we make a collective global effort to control these plant killers.

PHASE-SPECIFIC TRANSCRIPTIONAL PATTERNS OF THE OOMYCETE PATHOGEN PHYTOPHTHORA SOJAE UNRAVEL GENES ESSENTIAL FOR ASEXUAL DEVELOPMENT AND PATHOGENIC PROCESSES

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Oomycetes are filamentous microorganisms easily mistaken as fungi but vastly differ in physiology, biochemistry, and genetics. This commonly-held misconception lead to a reduced effectiveness by using conventional fungicides to control oomycetes, thus it demands the identification of novel functional genes as target for precisely design oomycetes-specific microbicide. The present study initially analyzed the available transcriptome data of Phytophthora sojae and constructed an expression matrix of 10,953 genes across the stages of asexual development and host infection. Hierarchical clustering, specificity, and diversity analyses revealed a more pronounced transcriptional plasticity during the stages of asexual development than that in host infection, which drew our attention by particularly focusing on transcripts in asexual development stage to eventually clustered them into 6 phase-specific expression modules. Three of which respectively possessing a serine/threonine phosphatase expressed during the mycelial and sporangium stages, a histidine kinase expressed during the zoospore and cyst stages, and a bZIP transcription factor exclusive to the cyst germination stage were selected for down-stream functional validation. In this way, we demonstrated that PP2C, HK, and bZIP32 play significant roles in P. sojae asexual development and virulence. Thus, these findings provide a foundation for further gene functional annotation in oomycetes and crop disease management.

ABPP-MS METHOD IDENTIFIES ORIGINAL MODULAR EXTRACELLULAR PROTEASES FROM A. EUTEICHES IN PEA APOPLAST DURING INFECTION

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In the field *A. euteiches* causes the root rot disease of legume crops as pea and alfalfa and interacts in the lab with the legume model *Medicago truncatula.* During infection, *A. euteiches* secrete myriad of proteins to both combat the host plant defense mechanisms and to survive in adverse environmental conditions [1]. Microbial proteases are predicted to be crucial components of these systems. Here, we identified an overrepresentation of tandemly repeated proteases within *A. euteiches* genome, which are upregulated during host infection. We developed an Activity Based Protein Profiling and mass spectrometry (ABPP-MS) approach [2,3] on apoplastic fluids isolated from pea roots infected by the pathogen. We characterized 35 active extracellular microbial proteases, which represents around 30% of the genes expressed encoding serine and cysteine proteases during infection. Notably, eight of the detected active secreted proteases carry an additional C-terminal domain [4]. This work demonstrates ABPP-MS as an efficient tool to quickly substantiate genomics prediction of oomycete pathogenicity factors. This system can be easily translated to other pathosystems and will facilitate the selection of microbial candidate genes for functional analysis.

[1] Camborde et al., New Phytol 2022.
[2] Morimoto and van der Hoorn, Plant Cell Physiol. 2016.
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AN AGO PROTEIN IS REQUIRED FOR AVIRULENCE GENE SILENCING IN AN OOMYCETE PLANT PATHOGEN

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Successful pathogens can rapidly overcome host resistance through epigenetic silencing, but the underlying mechanisms of epigenetic variation are largely elusive. Based on genome-wide association study, we identified a natural allele of an Argonaute protein in Phytophthora sojae that confers adaptability to resistance soybean cultivar. Knockout of PsAGO2 impaired avirulence gene Avr1b silencing and the psago2 mutants were recognized by soybean cultivar carrying Rps1b. Further data revealed that PsAGO2 can bind 24-26 nt sRNAs and recruit the histone methyltransferase complex PRC2 to establish H3K27me3 at Avr1b loci. Our finding supports a model in which H3K27me3 formation is mediated by sRNA in oomycete, highlighting the role of a new function of AGO protein in epigenetic gene silencing in a plant pathogen.

IDENTIFYING PROTEIN-PROTEIN INTERACTIONS WITH TURBOID IN PHYTOPHTHORA INFESTANS

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Determining how proteins function and interact with one another is a key step in understanding how cellular systems function. There are a variety of methods for determining protein-protein interactions, such as co-immunoprecipitation, yeast-2-hybrid, and proximity labelling methods. One of the most recent developments is TurboID, a proximity labelling method that uses a high efficiency biotin ligase to tag interacting or nearby proteins with biotin, followed by streptavidin purification and mass spectrometry. We have adapted TurboID for use in the potato late blight pathogen, Phytophthora infestans, with the aim of identifying proteins interacting with effectors as they are secreted. We have generated transgenic lines of P. infestans expressing effector-TurboID fusions. These have been assessed for correct protein localisation, conditions for biotin labelling, and protein purification. Progress towards identifying proteins involved in secretory pathways will be presented.

SCREENING OF ALFALFA VARIETIES RESISTANT TO PHYTOPHTHORA CACTORUM AND RELATED RESISTANCE MECHANISM

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Alfalfa is one of the most important legume forages in the world. Root rot caused by soil-borne pathogens severely restricts the production of alfalfa. The knowledge of the interaction between alfalfa and root rot-pathogens is still lacking in China. Phytophthora cactorum was isolated from symptomatic seedlings of an alfalfa field in Nanjing with high levels of damping-off. We observed the different infection stages of P. cactorum on alfalfa, and found that the purified P. cactorum strain was aggressive in causing alfalfa seed and root rot. By evaluating the resistance of 37 alfalfa cultivars from different countries to P. cactorum, we found Weston is a resistant variety, while Longdong is a susceptible variety. We further compared the activities of various enzymes in the plant antioxidant enzyme system between Weston and Longdong during P. cactorum infection, as well as gene expression associated with plant hormone biosynthesis and response pathways. The results showed that the disease-resistant variety Weston has stronger antioxidant enzyme activity and high levels of SA-responsive PR genes, when compared to the susceptible variety Longdong. These findings highlighted the process of interaction between P. cactorum and alfalfa, as well as the mechanism of alfalfa resistance to P. cactorum, which provides an important foundation for breeding resistant alfalfa varieties, as well as managing Phytophthora-caused alfalfa root rot.

PECTIN METHYLESTERASES INHIBITOR MODULATE PLANT HOMOGALACTURONAN STATUS IN DEFENSES AGAINST THE PHYTOPHTHORA SOJAE

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Pectin Methylesterases Inhibitor Modulate Plant Homogalacturonan Status in Defenses against the *Phytophthora sojae*

Hosts and pathogens are engaged in a continuous struggle for physiological dominance that drives the evolution and specialization of key defense and virulence proteins. A major site on the struggle is the plant cell wall. Here, we show the involvement of the dynamic remodeling pectin methylesterification of cell wall in the co-evolutionary struggle between host and microbe. Pathogen-secreted apoplastic pectin methylesterases, PsPME1, that loosening the plant cell wall and synergizing the activity of pathogen secreted endo-polygalacturonases by decreased the degree of pectin methylesterification. However, GmPMEI, a soybean produced pectin methylesterases inhibitor protein, expression controlled by PME-related damage-associated molecular patterns that binds to both soybean and P. sojae pectin methylesterases and inhibits them enzyme activity to remodeling the pectin to high methylesterification status for protecting themselves from enzymatic degradation. Totally, our work highlights that plants exploit induced defense mechanisms based on biochemical modification on the cell wall in shaping the balance of the arms race in the co-evolutionary conflict between host and microbe.

THE SOYBEAN (GLYCINE MAX) LYSM RECEPTOR KINASES GMNFR5A AND GMCERK1 MEDIATE CHITOOLIGOSACCHARIDES-TRIGGERED IMMUNITY

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Chitin is a major component of fungal cell walls and serves as a molecular pattern for the recognition of potential pathogens in the innate immune systems of plants. Previous research suggested that chitin has different immune signaling pathways in Arabidopsis and rice, including extracellular receptor recognition and intracellular signal transduction. The mechanism of induced resistance of chitin oligosaccharide (COSNAC) and its deacetylated product chitosan oligosaccharide (COS), collectively referred to as chitooligosaccharides, is not clear in soybean. Herein, we report that chitooligosaccharides trigger immune responses and plant disease resistance in soybean. GmNRF5a and GmCERK1 are required for chitooligosaccharides recognition in soybean. Unexpectedly, COSNAC is directly recognized by GmNFR5a and GmCERK1, whereas COS only binds GmNFR5a. In addition, we confirmed that GmCERK1 and GmRLCK5 transduce intracellular signals of chitooligosaccharides through proteins interaction and phosphorylation. Taken together, our results suggest GmNFR5a and GmCERK1 play a key role in the perception of chitooligosaccharides elicitors, and the existence of a complete phospho-signaling transduction pathway from GmNFR5a and GmCERK1 mediated chitooligosaccharides recognition to GmRLCK5 activation in soybean.

PERFORMANCE OF COMMERCIAL VARIETIES AGAINST PHYTOPHTHORA SOJAE

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Phytophthora stem and root rot of soybean, caused by Phytophthora sojae, is mainly managed with single or stacked qualitative disease resistance genes. As pathotype complexity of the population increases, management through the tolerance of the commercial varieties would be advisable. The goal of this study was to evaluate the response of commercial genotypes against the pathogen. We used the hypocotyl inoculation and infected rice techniques. Six commercial genotypes were used together with “Sloan” variety as susceptible control, and 3 pathotypes of P. sojae (which differed in virulence on 1 to 6 Rps genes and are the most representative on the pampeana region). In the first technique, the response of the genotypes was identified as susceptible (70 % or more seedlings killed) or resistant (30% or less seedlings killed). The infected rice technique was carried out in a Randomized Design with 3 repetitions per treatment. The total length of the roots of the surviving plants in the pots was evaluated 21 days after sowing. The data obtained were subjected to ANAVA and Fisher's LSD test ($p<0.05$). The variety identified as commercial 4 is the only one that presented significant differences compared to the control and the rest of the varieties. It was classified as resistant and reached 316% more growth in length. We conclude that it could be possible to use this variety as a tolerant control for future field resistance trials against Phytophthora sojae in Argentina.

CONSERVATION AND DIVERGENCE OF RAR1-MEDIATED NONHOST RESISTANCE DURING LAND PLANT EVOLUTION

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Over 450 million years of co-evolution, plants and pathogens have developed sophisticated strategies to manipulate one another. In plants, nonhost resistance (NHR) describes a collection of molecular and cellular mechanisms that neutralize non-adapted pathogens. Although NHR has been extensively studied in angiosperms, the origin and evolution of NHR in land plants is largely unknown. Here, we demonstrate the conservation of a NHR mechanism mediated by the RAR1-SGT1-HSP90 chaperone complex and identify lineage-specific divergence in terrestrial ferns. The RAR1-SGT1 interaction is highly conserved among lineages and even occurs between distantly related ortholog pairs. Intriguingly, we identified a single exception in the C-fern, whose homologs were incapable of interactions outside of its lineage. We hypothesize that lineage-specific differences in the SGT1-interacting CHORD2 domain determines this specificity, which is supported by protein-modeling studies. To determine a role for RAR1-mediated NHR in divergent lineages, we generated a liverwort (*Marchantia polymorpha*) *Mprar1* mutant and screened it against 28 diverse *Phytophthora* isolates. Excitingly, the *Mprar1* mutant exhibited significant defects in NHR to candidate pathogens that we are now examining in more detail. Overall, our findings suggest that the core mechanism of RAR1-mediated NHR is conserved in land plants, while plant lineages have fine-tuned the system during their evolutionary histories.

BIOCONTROL OF POTATO LATE BLIGHT BY NATURAL COMPOUNDS FROM TRAMETES VERSICOLOR WITH POTENTIAL ANTIMICROBIAL AND BIOSTIMULANT EFFECTS

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Potato late blight is a devastating disease infecting cultivated potato (*Solanum tuberosum*), caused by the oomycete *Phytophthora infestans*. The use of biocontrol agents and plant resistance inducers are sustainable and alternative ways to fungicides to contrast the disease in field. These can include microorganisms capable to contain the pathogen or compounds derived from them that act as plant defense biostimulants. In this study we tested the effect of natural compounds derived from the liquid culture of the fungus *Trametes versicolor*, known for antioxidant and antimicrobial properties, against potato late blight. For this purpose we conduced both *in vitro* and *in planta* assays in which we also tested chitosan and ß-aminobutyric acid (BABA), known respectively as antimicrobial against *P. infestans* and biostimulant on potato. In the first assay, the oomycete growth was analyzed via spectrophotometer and microscopy, whereas the *in planta* effect was investigated via gene expression and mass spectrometry. We showed that the cultural filtrate (CF) of *T. versicolor* inhibits the growth of *P. infestans* with an effect comparable to chitosan. Moreover, when sprayed on potato leaves, both CF and BABA are capable to enhance plant defense hormones. Further studies will focus on the mode of action of CF based on its chemical components (mainly polysaccharides and peptides) and the use of natural compounds are promising for small-scale field trials in order to control potato late blight.

SYNCHROSPORA GEN. NOV., A NEW PERONOSPORACEAE GENUS WITH AERIAL LIFESTYLE FROM A NATURAL CLOUD FOREST IN PANAMA

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During a survey of *Phytophthora* diversity in Panama fast growing oomycete isolates were obtained from naturally fallen leaves of an unidentified tree species in a tropical cloud forest. Phylogenetic analyses of ITS, LSU, beta–tubulin, cox1 and cox2 sequences revealed they belong to a new species of a new genus, officially described here as *Synchrospora* gen. nov., which resided as basal genus within the *Peronosporaceae*. The type species *S. medusiformis* has unique morphological characters. The sporangiophores show de-terminate growth, multifurcating at the end forming a stunted, candelabra-like apex from which multiple (8 to >100) long, curved pedicels are growing simultaneously in a medusa-like way. The caducous papillate sporangia mature and are shed synchronously. The breeding system is homothallic, hence more inbreeding than outcrossing, with smooth-walled oogonia, plerotic oospores and paragynous antheridia. Optimum and maximum temperatures for growth are 22.5 and 25–27.5 °C, consistent with its natural cloud forest habitat. It is concluded that *S. medusiformis* is adapted to a lifestyle as canopy-dwelling leaf pathogen in tropical cloud forests. More oomycete explorations in the canopies of tropical rainforests and cloud forests are needed to elucidate the diversity, host associations and ecological roles of oomycetes and, in particular, *S. medusiformis* and possibly other *Synchrospora* taxa in this as yet under-explored habitat.

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