# **Oral presentations**

## Deciphering cacao pod defense responses to Phytophthora palmivora and P. megakarya using comparative RNA-seq analyses

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#### Abstract

Black pod rot, caused by *Phytophthora palmivora* and *P. megakarya*, significantly impacts cacao production worldwide, posing a major threat to this economically important crop. Given the limitations of current agronomic control methods, breeding resistant clones is critical for managing this disease. While black pod rot resistance is mainly described as a quantitative trait, bioassays using zoospores on unwounded pods suggested qualitative resistance in some clones. Our study aims to elucidate the molecular basis underlying resistance or susceptibility of cacao clones by characterizing their transcriptional responses to each *Phytophthora* spp. We selected ten cacao clones with varying degrees of resistance to each pathogen and used RNA-seq to profile gene expression on pods at 12h and 48h post-inoculation. Differential gene expression was similarly high across genotypes at 12 hpi regardless of the *Phytophthora* spp., but increased at 48 hpi, reflecting the genotype-specific susceptibility to the pathogens. Consistent with the absence of complete resistance to P. megakarya in all genotypes, a common set of 417 genes was upregulated at 48 hpi in response to P. megakarya, including genes involved in defense response and abscisic acid signaling. These and further findings will advance our understanding of host-pathogen interactions in cacao and may identify critical candidate genes for breeding resistant cultivars, ultimately contributing to improved crop protection strategies.

Keywords: Host, Pathogen Interaction, Disease Resistance, Transcriptomics

### Spatiotemporal dynamics of organelles during resistosome-mediated hypersensitive cell death triggered by an oomycete effector

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#### Abstract

Plant nucleotide-binding domain leucine-rich repeat (NLR) proteins act as immune receptors that detect pathogens and trigger resistance responses. Upon activation, many NLRs assemble into membrane-associated resistosomes that initiate immune signaling and lead to hypersensitive cell death. However, the sequential subcellular events linking resistosome activation to cell death remain poorly understood. In this study, we investigated the spatiotemporal dynamics of organelles during resistosome-mediated cell death triggered by the oomycete effector AVRblb2. Using a copper-inducible system to express AVRblb2 alongside constitutively expressed Rpi-blb2, we performed time-lapse imaging of NRC4 resistosome activation. Tracking various subcellular markers, we found that cytoplasmic streaming and organelle movement were disrupted upon the appearance of visible resistosome puncta. Coinciding with puncta formation, actin filaments and microtubules depolymerized, followed by plasma membrane (PM) integrity loss, endoplasmic reticulum fragmentation into vesicle-like structures, and nuclear envelope breakdown. Eventually, cells collapsed, with the PM and tonoplast retracting toward the cell center. Our findings define the temporal sequence of subcellular events during resistosome-mediated hypersensitive cell death triggered by oomycete and provide a framework for future studies on the molecular mechanisms underlying immuneinduced cellular reprogramming.

**Keywords:** nucleotide binding domain leucine rich repeat (NLR), NLR required for cell death (NRC), hypersensitive cell death, organelle dynamics, time lapse imaging

### A Phytophthora Effector Targets the Host Chloroplast Movement Mechanism

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#### Abstract

Pathogens secrete a variety of effector proteins to act as virulence factors in the host apoplast and cytoplasm. Effectors promote pathogen infection by various mechanisms including suppression of host immune response, alteration of organelle function and promotion of nutrient acquisition from the host. Chloroplasts, besides being a central hub in plant metabolism, play a key role in plant immunity by synthesising defence hormone precursors and generating reactive oxygen species, and hence tend to be a prime target of effectors. Recent evidence indicates that chloroplasts alter morphology and accumulate at the plant/pathogen interface called haustorium during infection. We identified an RXLR effector from *Phytophthora palmivora* that promotes the pathogen infection when transiently expressed in Nicotiana benthamiana leaves. It is conserved in various Phytophthora species and appears to have a unique structural fold. The effector targets a specific kinesin involved in regulating chloroplast-actin filaments and cytosolic actin filament density is reduced in plant cells expressing the effector. Additionally, we observe an impact on nuclear movement. Ongoing experiments clarify whether the altered nuclear dynamics are an indirect effect due to altered chloroplast-nucleus linkages. Our work points towards Phytophthora species using effectors to target host chloroplast movement towards the haustorium to possibly mitigate host immunity.

Keywords: Phytophthora palmivora, RXLR effector, chloroplast, nucleus, actin

### Characterizing Phytophthora palmivora Virulence Gene Variation Using Targeted Capture Sequencing and RNA-seq

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#### Abstract

*Phytophthora palmivora* is a globally distributed pathogen affecting economically important tropical crops, including cacao, durian, and papaya. Understanding its genetic diversity and transcriptional activity during infection is crucial for improving disease management and identifying resistance mechanisms. Our study integrates targeted capture sequencing for virulence genes and dual RNA-seq for gene expression analysis to characterize virulence genes variation in a diverse P. palmivora collection. We analyzed 87 P. palmivora isolates from multiple hosts and regions, using probe sets targeting 2,198 virulence-related genes. Conserved effectors, including RxLR and CRN proteins, were identified, and sequence variation was assessed to infer pathogen adaptability. Dual RNA-seq of Theobroma cacao pods at 12 and 48 hours post-inoculation identified differentially expressed genes in 10 clones selected for their broad susceptibility-resistance profiles. Pathogen transcript recovery was highest at 48 hours, highlighting key virulence genes active during infection. These expression data guided the selection of priority genes for the analysis. By integrating targeted sequencing with expression profiles, we identified genes essential for host colonization and assess their variation across P. palmivora populations. This approach refines effector characterization and advances strategies for disease mitigation in cacao.

Keywords: Phytophthora. Cacao. RNA, seq. Capture, seq. Effectors

<sup>\*</sup>Speaker

### How does Phytophthora make the cut?

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#### Abstract

Phytophthora pathogens breach plant surfaces using a specialized infection structure called the appressorium. The appressoria is able to breach the plant surface by reorganizing the actin cytoskeleton. However, the molecular mechanisms that translate host perception into the development of an appressoria remains poorly understood. Our work aims to decipher the successive steps in the molecular cascade that perceives mechanical stimuli leading to the reorganization of the actin cytoskeleton and appressoria formation.

We hypothesise that the appressoria formation requires two steps: (i) perceiving the host surface by the use of mechanosensitive channels and (ii) actin cytoskeleton reorganization. In Phytophthora, we have identified several homologs of mechanosensitive channels including PIEZO and MCA calcium-permeable channels with PIEZO being absent in fungi. The opening of these channels facilitates the influx of calcium molecules, acting as a downstream signalling molecule. Calcium likely binds to the actin-binding proteins. We have identified actin-binding proteins such as alpha-actinin and fimbrin in Phytopththora that contain a calcium-binding domain. This underscores that calcium is likely the signal molecule that links perception to actin cytoskeleton remodelling and appressoria formation. To illuminate the role of calcium, we have generated a calcium indicator based on GCaMP to study the role of calcium.

This work aims to decipher the molecular pathway of the appressoria formation by using several techniques. These techniques include chemical treatments and CRISPR-Cas to interfere with the mechanosensitive channels. Additionally, tools like the calcium indicator based on GCaMP and proximity labelling will help us explore the players of the signalling pathway. Our ultimate goal is to discover the key molecular players enabling Phytophthora to convert mechanical input from the plant surface into a precise developmental response, paving the way for the development of anti-penetrant oomycides.

Keywords: Phytophthora, mechanosensitive channels, appressoria

### Investigating the Molecular Architects of Phytophthora Zoospores

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#### Abstract

Polarity is essential for the development, cellular organization, and signaling of all living organisms. In animals and plants, this process involves DIX domain-containing proteins (DDPs). These proteins all share a ubiquitin fold-like domain that oligomerizes, called the DIX domain. We identified a set of four DDPs in the SAR supergroup, named Musketeer proteins, that exhibit unique domain compositions compared to known DDPs. Musketeer proteins combine a DIX domain with either an ELMO, DnaJ, or kinase domain, suggesting potential roles in cytoskeleton dynamics, protein folding, and signaling that can lead to distinct biological functions. These proteins are predominantly present in micro-swimmers and species with a motile stage in their life cycle. Interestingly, at least two Musketeer proteins localize near the ventral groove of *Phytophthora* zoospores, where flagella are inserted, and they physically interact *in vivo*. Whether these proteins function exclusively in zoospores or have broader roles across other life stages remains to be determined. Ongoing research will clarify whether Musketeer proteins are linked specifically to motility or more generally to polarity regulation. Uncovering their function could enable the development of novel, evolution-informed strategies against oomycete pathogens and related parasites within the SAR supergroup.

Keywords: DIX domains, motility, polarity, zoospores, SAR

<sup>\*</sup>Speaker

### A Plant GTPase-Activating Protein Inhibits Autophagy and Immune Trafficking Against P. infestans

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#### Abstract

Plants rely on autophagy and membrane trafficking to manage stress, counter infections, and maintain cellular homeostasis. Yet, the molecular interplay between these processes remains poorly understood. Using an AI-guided discovery approach, we identified Rab3GAPL as a key membrane trafficking regulator that suppresses autophagy in plants. Rab3GAPL inhibits autophagy by interacting with ATG8, a core autophagy adaptor, and by inactivating Rab8a, a small GTPase essential for autophagosome biogenesis and defense-associated secretion. This inhibitory function is conserved across three model plant species, and its loss in *Marchantia polymorpha* enhances recovery following heat stress. Importantly, Rab3GAPL also restricts immune secretion specifically targeting the oomycete pathogen *Phytophthora infestans*, thereby modulating focal immunity. Together, these findings position Rab3GAPL as a molecular switch that fine-tunes autophagic flux and pathogen-directed immune secretion by controlling Rab8a-mediated trafficking. This study reveals a previously unrecognized regulatory axis involving a RabGAP-Rab pair and ATG8, offering new insights into the membrane trafficking mechanisms that orchestrate plant autophagy and immunity against *P. infestans*.

Keywords: Autophagy, Vesicle Trafficking, Phytophthora infestans

### Systematic Optimization of LAMP Assays for Kauri Dieback Diagnostics

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#### Abstract

Kauri dieback, a serious disease threatening New Zealand's native forests, is primarily caused by the oomycete pathogens *Phytophthora agathidicida* and *Phytophthora cinnamomi*. Due to the lack of a cure for kauri dieback, rapid and field-deployable diagnostic tools are essential for effective management and containment strategies. Loop-mediated isothermal amplification (LAMP) presents a promising molecular technology for the detection of oomycete pathogens, offering advantages in speed, sensitivity, and the ability to run without complex laboratory equipment.

This study presents a systematic evaluation of the interactions among detection dyes, reaction additives, and primer designs to optimize LAMP protocols for *P. agathidicida* and *P. cinnamomi*. Nine detection dyes and multiple additive conditions were screened to assess their impact on reaction sensitivity, specificity, and visual signal clarity. The results reveal key parameters influencing LAMP performance for oomycete detection, with optimized conditions significantly improving assay robustness and reliability.

These optimized protocols are designed to serve as a foundation for future integration into capillary microfluidic platforms, with the aim of creating portable, easy-to-use diagnostic tools for detecting kauri dieback pathogens in the field.

Keywords: Kauri dieback, Loop mediated isothermal amplification (LAMP), Phytophthora species

### Transcriptomic and functional analysis of effector-mediated immune suppression in red needle cast disease caused by Phytophthora pluvialis

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#### Abstract

*Phytophthora pluvialis*, the causative agent of Red Needle Cast (RNC), is a destructive foliar pathogen of pine. Like other oomycetes, it deploys host-translocated effector proteins to facilitate infection. To elucidate the differential expression of effector genes associated with *P. pluvialis* infection, we conducted quantitative RNA sequencing of mycelium and infected pine needles from both susceptible and resistant pine lines at 3- and 5-days post-infection (dpi).

A total of 311 effector genes were identified as differentially expressed, comprising 136 Crinklers, 115 RxLRs, and 60 Elicitins. Downstream analyses focused on RxLR effectors with significant expression differences between susceptible and resistant pine lines at 3 dpi. RxLR effectors are known to translocate into host cells and play key roles in infection establishment and suppression of host immune responses.

Protein sequence and structural conservation of early-expressed RxLR effectors was assessed using sequence similarity networks and phylogenetic trees. Over 90% contained at least one WY domain. Eight candidates were transiently expressed in *Nicotiana benthamiana*, showing diverse subcellular localizations. Expression was confirmed by Western blotting, and all candidates were tested for cell death induction or suppression. To identify host targets, Yeast Two-Hybrid (Y2H) screens were performed using *P. infestans*-derived potato and *P. pluvialis* pine cDNA libraries. Identified targets were further validated through computational predictions and co-localization in N. benthamiana.

Here, we will focus in more detail on the effector PPR04, which demonstrated a robust cell death suppression phenotype in INF1-triggered cell death. In the Y2H screen, PPR04 showed a strong and consistent interaction with Probable serine/threonine-protein kinase With No Lysine (WNK) 4. The interaction site was identified as the kinase domain, which is conserved within angiosperms and gymnosperms, including the original host radiata pine.

<sup>\*</sup>Speaker

Subsequently, WNK4 and PPR04 were co-expressed in *N. benthamiana* and confirmed cytosolic co-localisation of the two proteins, further supporting a functionally relevant interaction during infection. These findings highlight PPR04 as a candidate RxLR effector with a putative role in modulating host kinase-mediated signaling pathways to suppress immunity. This interaction and its mechanistic consequences will be further elucidated in future studies.

Keywords: Phytophthora pluvialis, Red Needle Cast, Pinus radiata, RXLR effectors

### Phytophthora cinnamomi populations affecting avocado in the U.S.A. exhibit large phenotypic variability and potential introductions from Mexico

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#### Abstract

Phytophthora root rot (PRR), caused by *Phytophthora cinnamomi* (Pc), is the most devastating avocado disease worldwide. A global Pc population study identified two A2 panglobal clonal lineages, PcG1-A2 and PcG2-A2. Avocado Pc populations from California (CA) belong to two A2 clonal lineages (clade I and II) with A2 clade II from southern CA containing more virulent and potassium phosphite (PP)-resistant isolates. We extended the Pc phenotypic and genetic characterization to other U.S.A. avocado producing states and determined their genetic relationships with Mexican populations. Significant variability was found among isolates in growth rate, PP sensitivity, and virulence to avocado seedlings and pear fruits. Isolates from Hawaii (HI), Florida (FL), and southern CA grew faster at 28°C, suggesting adaptation to warmer climates. PP-resistant isolates were found in CA

<sup>\*</sup>Speaker

and FL, likely reflecting the continued overuse of this chemical. Principal component and phylogenetic analyses clustered the U.S.A. and Mexican populations into the previously identified A2 clades. By including isolates of the two panglobal A2 lineages, we assigned the A2 clades I and II to the PcG1-A2 and PcG2-A2 lineages, respectively. The PcG1-A2 lineage contained the majority of U.S.A. isolates and a few isolates from Mexico. The PcG2-A2 lineage contained isolates from southern CA, Hawaii, and most of the Mexican isolates, providing support for the Mexican origin of this clade in CA and movement of PcG2-A2 isolates within California and between Mexico and California. Our study highlights the adaptative capacity of clonal Pc populations to local environments and control measures and provides evidence for pathogen movement between avocado growing regions which could impact the sustainability of the avocado industries.

Keywords: Population genomics

### Exploring the Evolution of Saprolegniales: Were ancestral Oomycetes Parasites?

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#### Abstract

Saprolegniales are a group of Oomycetes that include both plant and animal parasites, renowned for their devastating effects on agriculture and wildlife. Despite numerous studies focusing on plant-parasitic oomycetes, the evolution of this group and it's contribution to the diverse lifestyles within the order remains poorly understood. Investigating the evolutionary processes behind these lifestyles is essential to unravel the ecological roles and adaptive strategies of Saprolegniales in diverse environments. To study the evolutionary patterns and rates of the 'lifestyle' trait, we applied comparative phylogenetic methods (Bayesian inference and Maximum likelihood) to a large nuclear internal transcribed spacer (ITS) database. Our results revealed a strong phylogenetic signal for lifestyle traits within Saprolegniales, suggesting that ancestral oomycetes were primarily parasites, most likely parasitizing plants, while the saprotrophic lifestyle appears to be a derived character. This transition to saprotrophy occurred early in the evolution of Saprolegniales, while plant parasitism is a more recent innovation, particularly evident during the diversification of the genus Aphanomyces. Understanding the mechanisms driving this shift is crucial, as it highlights evolutionary trends in ecological adaptability, providing insight into the broader evolutionary history of protists and their environmental interactions.

Keywords: evolutionary history, phylogenetic, ancestral state reconstruction, pathogens, parasites

<sup>\*</sup>Speaker

### High throughput image analysis and quantification of aggregation of an aquatic fungus on its algal host: Paraphysoderma sedebokerense almost never attacks Haematococcus spp. alone

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#### Abstract

Haematococcus spp. is a freshwater green microalga cultivated worldwide to produce astaxanthin, a high value antioxidant and pigment used in nutraceutical and feed industries. Large production of Haematococcus spp. is threatened by the aquatic fungal pathogen Paraphysoderma sedebokerense (Blastocladiomycota). Depending on favourable or unfavourable growth conditions, P. sedebokerense the life cycle of which is complex and incompletely known, switches between vegetative thin-walled cysts and resting thick-walled cysts. Both types of cysts are known to release different types of propagules, such as flagellated zoospores, amoeboid swarmers and coatless spores. In an attempt to develop a quantitative and reproducible fungal inoculation protocol and bioassay, we observed a non-random distribution of P. sedebokerense cells on its algal host. Accordingly, we developed and validated a high throughput cell classification method based on a digital slide scan, that enables us to quantify the parasite distribution on its host cells, and thus the aggregation of P. sedebokerense. This method allows the screening of thousands of algal cells as well as automatised calculation of infection prevalence even at low infection rates. This method could be of interest for other host-pathogens systems to quantify the pathogen distribution.

Keywords: aquatic fungus, image analysis, parasite aggregation

<sup>\*</sup>Speaker

### Optimizing dsRNA-Based Strategies for Controlling Downy Mildew in Pea

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#### Abstract

Downy mildew, caused by *Peronospora viciae* f. sp. *pisi* (*Pvp*), poses a significant threat to pea (Pisum sativum) production, leading to severe yield losses. Traditional fungicidebased control raises concerns about sustainability and resistance. Exogenous double-stranded RNA (dsRNA) application offers a promising alternative via RNA interference (RNAi). This study aimed to optimize dsRNA-based approaches for controlling Pvp by selecting and validating key gene targets. Using insights from Hyaloperonospora arabidopsidis (Hpa)-Arabidopsis interactions, we identified 28 candidate Pvp genes and tested their roles in pathogen development using short synthesized dsRNAs (SS-dsRNAs). Genes like *Cellulose* Synthase 3 (CesA3) were further analysed using longer in vitro-produced dsRNAs, which significantly reduced Pvp spore germination and disease progression both in vitro and in planta. Furthermore, small RNA (sRNA) sequencing revealed bidirectional cross-kingdom RNA interactions between pea plant and Pvp. We identified three Pvp genes targeted by pea-derived sRNAs and two Pvp genes encoding sRNAs that regulate pea gene expression. Targeting these five genes with SS-dsRNAs completely inhibited *Pvp* infection in pea plants. These findings highlight the Spray-Induced Gene Silencing (SIGS) as a scalable, eco-friendly strategy for downy mildew control in pea and provide a framework for optimizing dsRNA applications by refining target selection and delivery methods for enhanced efficacy.

**Keywords:** Downy mildew, Peronospora viciae f. sp. pisi (Pvp), double, stranded RNA (dsRNA), Spray, Induced Gene Silencing (SIGS), CesA3, cross, kingdom RNA interactions, plant disease management.

# Sugar metabolism regulates Phytophthora root and stem rot

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#### Abstract

Successful infection by pathogenic microbes requires effective acquisition of nutrients from their hosts. Root and stem rot (RSR) caused by *Phytophthora sojae* is one of the most important diseases of soybean (*Glycine max*). However, the specific form and regulatory mechanisms of carbon acquired by *P. sojae* during infection remain unknown. Here, we show that *P. sojae* boosts trehalose biosynthesis in soybean through the virulence activity of an effector PsAvh413. PsAvh413 interacts with soybean trehalose-6-phosphate synthase 6 (GmTPS6) and increases its enzymatic activity to promote trehalose accumulation. *P. sojae* directly acquires trehalose from the host and exploits it as a carbon source to support primary infection and development in plant tissue. Importantly, *GmTPS6* overexpression promoted *P. sojae* infection, whereas its knockdown inhibited the disease, suggesting that trehalose biosynthesis is a susceptibility factor that can be engineered to manage RSR in soybean.

Keywords: Phytophthora sojae, soybean, effector, metabolism

### A Phytophthora apoplastic trypsin-like serine protease targets the receptor-like kinase BAK1 to dampen plant immunity

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#### Abstract

Perception of microbial pathogens by plant cell-surface pattern recognition receptors (PRRs) activates pattern-triggered immunity in plants. BAK1, a receptor-like kinase serving as a critical co-receptor for multiple PRRs, operates as a central signaling hub coordinating immune responses. Nevertheless, the molecular strategies employed by phytopathogens to subvert BAK1-mediated surveillance in the apoplast remain poorly characterized. Through a functional genomics screen of *Phytophthora* effector arsenals, we identified an apoplasitc protease named PsAP1 from *Phytophthora sojae* capable of suppressing *Phytophthora* elicitin INF1-induced cell death in plants. PsAP1 associates with BAK1 in soybean, and widely suppress immune responses triggered by different pathogen-associated molecular patterns. Proteolytic cleavage assays revealed PsAP1 specifically targets the extracellular leucine-rich repeat domain of GmBAK1, with enzymatic activity being indispensable for its immunosuppressive function. Evolutionary analysis uncovered broad conservation of PsAP1 across Phytophthora species, with functional orthologs exhibiting convergent capacities to inactivate BAK1 through proteolytic processing. This work illuminates a previously unrecognized proteolytic disarming mechanism whereby oomycete pathogens achieve broad-spectrum immunosuppression through targeted cleavage of a central immune kinase.

Keywords: Phytophthora effectors, pattern triggered plant immunity, BAK1, cleavage

<sup>\*</sup>Speaker

### A Phytophthora infestans RXLR effector S04373 subverts plant ETI by blocking nuclear-cytoplasmic shuttling in plant cells

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#### Abstract

Potato late blight, caused by the oomycete pathogen *Phytophthora infestans*, poses a significant threat to potato production. Previously, we cloned an immune receptor R04373 from Solanum americanum, which specifically recognizes the P. infestans effector PITG\_04373 but failed to confer effective resistance against P. infestans. To investigate how R04373-mediated immunity is compromised, we screened an RXLR effector library of *P. infestans* and identified another RXLR effector S04373, which specifically suppresses the hypersensitive response (HR) triggered by R04373/PITG\_04373. S04373 inhibits ETI by targeting key components of host nuclear transport, importin  $\alpha$  and  $\beta$ . Notably, S04373 also suppresses the HR mediated by *Rpi-chc1* and *R2*. Nucleocytoplasmic trafficking is a critical bidirectional process for the transport of RNA and proteins between the nucleus and cytoplasm, ensuring normal cellular functions. This study reveals that the nuclear localization of R04373 is essential for its activation. Furthermore, we observed that R04373 forms oligomers upon activation, though whether these oligomers localize to the nucleus remains to be explored. These findings demonstrate that nuclear trafficking is indispensable for ETI activation and serves as a key target for pathogen effectors. Understanding the ETS mechanism will contribute to designing the plant immune system and restoring the impaired R genes.

Keywords: Oomycete, Effectorome, ETI suppression, Importin, NLR nuclear localization

<sup>\*</sup>Speaker

### Apoplastic small RNAs contribute to plant immunity against Phytophthora pathogen

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#### Abstract

Plant small RNAs (sRNAs) not only function intracellularly in gene regulation but are also exported to the apoplastic compartment. These extracellular sRNAs can undergo crosskingdom transfer into pathogenic microbes, where they induce trans-kingdom gene silencing to suppress infection. However, alternative mechanisms by which apoplastic sRNAs modulate plant-pathogen interactions remain unexplored, and the molecular basis for their selective extracellular sorting is poorly understood. Using the tomato-Phytophthora capsici pathosystem, we systematically identified apoplastic sRNAs responsive to P. capsici infection. Our findings demonstrate that pathogen invasion triggers selective secretion of specific tomato miRNAs and 22-24 nt siRNAs into the apoplast, while 24 nt sRNAs dominate the crosskingdom transfer into Phytophthora hyphae. Notably, one miRNA orchestrates systemic immunity through apoplastic trafficking to neighboring cells, while two specific siRNAs directly target and cleave virulence-related mRNAs in Phytophthora. This work uncovers the dual functionality of apoplastic sRNAs in simultaneously enhancing plant immunity and attenuating pathogen virulence. These insights advance our understanding of plant defense mechanisms and provide novel molecular targets for designing RNA-based biocontrol agent.

Keywords: tomato, apoplast, trans, kingdom RNAi, systemic immunity

<sup>\*</sup>Speaker

### Analysis of a lettuce cell surface immune receptor mediating nlp-triggered immunity

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#### Abstract

Necrosis- and ethylene-inducing peptide 1-like proteins (NLPs) are present in microbes from three different kingdoms of life (bacteria, fungi, and oomycetes). A short, conserved region of 24 residues from NLP3 (nlp24) of the Arabidopsis downy mildew Hyaloperonospora arabidopsidis acts as an immunity-triggering pattern (PAMP) in multiple plant species, including lettuce. No receptors for nlp24 (or other PAMPs) have yet been identified or characterized in lettuce or any other Asteraceae species. The lettuce reference genome does not have an ortholog of the Arabidopsis nlp24 receptor RLP23, which is a cell surface-localized receptor-like protein. Furthermore, the recognition specificity of the two plant species is different suggesting NLP recognition arose by convergent evolution. We performed an extensive characterization of early and late nlp24 recognition signaling outputs in lettuce, confirming it as a potent activator of immunity. Our PAMP time-course RNAseq experiment with nlp24 in lettuce identified candidate transcriptional regulators of PAMP-triggered immunity. By combining association mapping, comparative genomics, and genetic complementation assays, we identified a cell surface immune receptor, different from RLP23, to be responsible for nlp24 recognition in lettuce. Analysis of the nlp24 variants (also of the lettuce downy mildew Bremia lactucae) revealed the NLP regions required for the timely and robust activation of lettuce immunity. We will report on specific features of this novel receptor gene and its potential role in activating plant immune responses and disease resistance.

Keywords: PAMP, immunity, lettuce, downy mildew

<sup>\*</sup>Speaker

## Posters

### Strategic application of essential plant nutrients to manage Citrus gummosis by inducing activation of defense-related enzymes

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#### Abstract

The nutritional status of host plants plays a rational role in defining their resistance or susceptibility against invading pathogens. In light of growing concerns over chemical use in agriculture, nutrient-based products are emerging as promising alternatives to synthetic fungicides. However, their role in managing destructive pathogens like Phytophthora nicotianae particularly in citrus and their influence on plant antioxidant defense mechanisms remains underexplored. This study evaluated the efficacy of two nutritional products, Krystafeed and Micro Plus, applied at three concentrations via soil drenching, against citrus gummosis. Additionally, we investigated their impact on the antioxidant defense components in citrus plants. Correlation analyses were performed to determine the relationship between various enzymes of the antioxidant defense system. Results indicated that the combination of Krystafeed and Micro Plus was found the most effective with (27.01, 26.59%) disease incidence, followed by Micro Plus (29.56, 32.35%) and Krystafeed (38.21, 41.15%), both in greenhouse and field conditions, respectively. Moreover, the combination of Krystafeed and Micro Plus significantly increased the concentration of SOD (27.53, 108.96)%, POD (37.29, 45.65)%, CAT (19.33, 95.33)%, H2O2 (22.13, 118.98)%, TPC (27.39, 17.37)%, chlorophyll a (21.80, 35.74)%, chlorophyll b (57.57, 18.25)%, total chlorophyll (30.21, 19.83)%, Tocopherol (13.08, 33.66)%, TrxR (5.03, 36.56)%, MDA (13.84, 54.79)%, ascorbate (4.72, 17.28)%, Proline (5.94, 59.31)%, and phytoalexin (11.33, 55.08)% in the treated plants of resistant and susceptible citrus varieties, respectively, as compared to the untreated plants. Pearson's correlation heat-map analysis showed that all the enzymes of antioxidant defense system were found positively correlated with each other. It is concluded that the improvement of plant resistance by the application of plant nutrient related products may be viable alternatives to synthetic chemicals for managing citrus gummosis and potentially other pathogens.

**Keywords:** Oomycetes, Phytophthora nicotianae, Nutrition, Citrus Gummosis, Defense related Enzymes.

### A Phytophthora infestans Effector Restructures the Host-Pathogen Interface by Targeting Defense-Associated Vesicle Trafficking

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#### Abstract

Pathogens have evolved sophisticated mechanisms to manipulate host membrane dynamics, a critical adaptation that allows them to thrive in the face of innate immune defenses. In plants, specialized membrane compartments that surround the invasive hyphae of fungal and oomycete pathogens are key sites that shape the outcome of infection. However, how pathogens exploit these interfaces to their advantage remains poorly understood. In this study, we uncovered a conserved oomycete effector, PiE354, which targets a host Rab GTPase-activating protein (RabGAP) named TOPGAP to remodel the host-pathogen interface. PiE354 co-opts TOPGAP as a susceptibility factor by exploiting its GAP activity to inactivate Rab8a, a Rab GTPase essential for immune-related secretion. Through this interaction, PiE354 drives the displacement of Rab8a from the plasma membrane, thereby rerouting Rab8a-dependent immune trafficking away from the pathogen interface. This strategy highlights a remarkable evolutionary innovation, whereby a pathogen effector subverts a host trafficking regulator to suppress defense-related secretion, offering novel mechanistic insight into how pathogens reprogram host membrane dynamics to facilitate infection.

Keywords: Effector, Vesicle Trafficking, Phytophthora infestans

# Cancelled

## The first secretome landscape of Phytophthora capsici: Discovery of a key effector in Piper nigrum pathogenesis

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#### Abstract

*Phytophthora capsici*, a hemi-biotrophic oomycete, causes quick wilt (foot rot) disease in *Piper nigrum*, the most used spice in the world also known as the 'king of all spices'. The crop is indigenous and originated in the Western Ghats of India, which contributes to 95% of the India's area of production. Root rot is responsible for up to 30% of vine mortality and crop losses of up to 40% in Kerala state, a major producer of black pepper in India. Phytophthora species causes infection in the host plant by modulating the plant innate immune system through various secreted effector proteins. However, the underlying mechanisms by which *Phytophthora capsici* effectors manipulate host plant defense remain largely unknown. To date, there are no published reports of secretome analysis of *P. capsici*. We have done the first complete characterization of the secreted proteins of *P. capsici* by LC-MS/MS. More than 200 secreted proteins of P. capsici were identified after 12 days of incubation under two conditions. The mycelia were either grown in PDB media, or were first allowed to infect *Piper nigrum* for 6 hours before incubation in PDB. We selected one effector protein AB hydrolase-1 domain-containing protein (UniProt ID: A0A081AI76) for functional studies. The effector gene (1029 bp) was cloned into pCAMBIA 1302 binary vector which was transformed into Agrobacterium tumefaciens GV3103 strain. Transient foliar overexpression in Nicotiana benthamiana provided evidence for the critical role of this effector in the pathogenicity of Phytophthora capsici in the host P.nigrum

Keywords: Agroinfiltration Piper nigrum Secretome Phytophthora capsici Virulence

<sup>\*</sup>Speaker

### Phytophthora infestans suppresses host immune responses by targeting two unrelated helper NLRs

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#### Abstract

Pathogens deploy effector proteins to manipulate host processes and suppress immunity. We previously showed that the *Phytophthora infestans* RxLR-(L)WY effector Pi82 suppresses cell death mediated by helper NLRs NRC1 and NRC4. To explore the structural basis of this suppression, we generated Pi82 truncation mutants. Our findings suggest that Pi82 employs distinct (L)WY motifs to selectively suppress NRC1 and NRC4, highlighting a modular strategy that disrupts specific branches of the NRC immune signaling network. To identify the host target of Pi82, we performed immunoprecipitation-mass spectrometry and discovered a protein family involved in vesicle trafficking, mycorrhization, and lignin biosynthesis, which we named Target of *Phytophthora* effector (TOPe). Overexpression of TOPe enhanced NRC1-, but not NRC4-mediated cell death. We propose that Pi82 exploits the diversity of TOPe proteins to suppress specific branches of the NRC network. These findings offer insights into how *Phytophthora infestans* subverts plant immune networks and implicate TOPe as modulators of NRC immunity. Ongoing work aims to validate the functional significance of the Pi82-TOPe-NRC interactions *in planta*.

Keywords: Phytophthora infestans, effector suppression, NRC network

<sup>\*</sup>Speaker

### Understanding resistance to Aphanomyces cochlioides in sugar beet using Genome-wide association studies

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#### Abstract

Aphanomyces cochlioides, the causal agent of seedling damping-off and root rot of sugar beet can cause significant yield reduction. These diseases are primarily managed by use of fungicide seed treatments, incorporation of factory waste-lime, and selecting moderately resistant varieties. Breeding efforts have focused on improving adult plant resistance although the mechanisms of disease resistance are unclear. Ninety-six USDA-ARS pre-breeding lines were screened for seedling resistance using a zoospore inoculation method and scored using a standardized 0-3 ARR rating scale. Individual plants within an accession were selected and pooled into equally sized (n=106) highly resistant or highly susceptible pools. Genomic sequencing was conducted on the pools for an extreme phenotype genome-wide association study (XP-GWAS) to identify potential genomic variants associated with ARR resistance which identified 29 statistically significant SNPs. Allele frequency analysis was also used to identify additional novel genomic regions with a high proportion of divergent SNPs.

Keywords: oomycetes, GWAS, seedling disease

<sup>\*</sup>Speaker

### Boosting the performance of Pythium oligandrum for biocontrol and biostimulation in potato

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#### Abstract

Potato (Solanum tuberosum) ranks as the world's third most important food crop, following rice and wheat, with an annual production of 330 million tons, making it crucial for food security globally. However, pathogens like *Phytophthora infestans*, the causal agent of late blight, pose significant threats to potato cultivation globally. The situation is exacerbated by climate change, which is expected to further reduce yields. The oomycete Pythium oligandrum is a mycoparasite with potential as a biocontrol agent and biostimulator in potato. Although commercial preparations of *P. oligandrum* are approved for use in potato and other crops within the EU, adoption of *P. oligandrum* remains limited due to challenges such as inconsistent standardization and limited shelf life, which deter farmers from opting for it over chemical alternatives. Potato crops are typically cultivated in sandy soils; however, much of the research on *P. oligandrum* has been conducted either *in vitro*, in compost or in nutrient-rich soils, leading to uncertainties when applied to field conditions. We evaluate the growth-promoting properties of *P. oligandrum* across 96 potato cultivars on sandy soils and investigate correlations with specific genetic markers, as a prelude to breeding for potato cultivars that better host with biocontrol agent. Furthermore, we will investigate how inoculation with P. oligandrum alters root exudate release to attract specific microbes, using metabolomic and metagenomic analyses under field conditions. Our findings aim to improve our understanding of *P. oligandrum* efficacy under crop production systems, with the goal being to enhance its effectiveness as a biocontrol and growth-promoting agent.

Keywords: Potato cultivation, Pythium oligandrum, Biocontrol agent, Metagenomics, Metabolomics

<sup>\*</sup>Speaker

### Specific selection on XEG1 and XLP1 genes correlates with host range and adaptability in Phytophthora

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#### Abstract

In diverse Phytophthora-plant pathosystems, Phytophthora secretes XLP1 (PsXEG1-Like Protein), a non-enzymatic paralog that functions as a decoy to protect XEG1 (Xyloglucan-specific Endoglucanase) from host inhibitors. Here, we show that the genus-specific selection pressures on the XEG1/XLP1 gene pair are crucial for host adaptation and are closely linked to Phytophthora host range. Our findings reveal that the XEG1/XLP1 gene pair originated within Phytophthora and subsequently evolved into genus-specific genes, undergoing functional divergence driven by preferential selection. Positive selection sites within the XEG1/XLP1 gene pair in Phytophthora contribute to this functional divergence and are associated with the host range variability of Phytophthora as evidenced by multivariate statistical analyses. Furthermore, mutations at key selection sites in Phytophthora sojae and Phytophthora capsici significantly impair their pathogenicity, with P. capsici exhibiting almost no colonization expansion on tobacco and pea. Notably, natural Phytophthora populations harbor mutations at the positive selection sites, indicating ongoing evolutionary pressures on the XEG1/XLP1 gene pair.

Keywords: Phytophthora, host range

<sup>\*</sup>Speaker

### A molecule switch orchestrates potato receptor signaling against oomycete and bacterial pathogens by coupling to immune complexes

Jie Li , Aifang Ma , Jiahan Ying , Xiuli Qin , Wenjie Liu , Zhengyu Chen , Zhiyuan Yin , Maofeng Jing , Guangyuan Xu , Ingo Hein , Xiangxiu Liang , Paul Birch , Daolong Dou , and Xiaodan Wang<sup>\*1</sup>

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#### Abstract

Plants utilize plasma-membrane-localized pattern recognition receptors (PRRs) to sense and respond to microbial infections. The downstream regulatory components have been studied extensively, but the mechanisms ensuring appropriate immune responses to diverse pathogens remain enigmatic. We report that a core regulatory component named StBPA1 (BINDING PARTNER OF ACD11-1) is a molecular switch that modulates both antioomycete and anti-bacterial immunity. *StBPA1*-knockout displays dwarfed growth, enhanced pattern-triggered immunity (PTI), and broad-spectrum resistance to potato late blight and bacterial wilt diseases. StBPA1 negatively regulates the StSOBIR1-StBAK1/StFLS2-StBAK1 immune complex formation and inhibits StFLS2 kinase activity to prevent constitutive immune responses. In turn, StBAK1 specifically phosphorylates StBPA1, this modification is enhanced by oomycete PAMP INF10r bacterial PAMP flg22 perception and impairs the negative regulatory role of StBPA1, thereby ensuring proper immune signaling. These findings reveal an StBPA1-PRR complex regulatory module and highlight inhibitions by StBPA1 as key mechanisms to ensure efficient yet strictly regulated immune responses against oomycete and bacterial pathogens.

**Keywords:** potato late blight, potato bacterial wilt, immune regulatory components, pattern recognition receptors (PRRs), PAMP, triggered immunity (PTI), phosphorylation

### High-efficiency green management of potato late blight by a self-assembled multicomponent nano-bioprotectant

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#### Abstract

Potato late blight, caused by *Phytophthora infestans*, is a highly destructive disease worldwide. Unlike other plant pathogens, double-stranded RNA (dsRNA) is poorly taken up by P. infestans, which is a key obstacle in using dsRNA for disease control. Here, a self-assembled multicomponent nano-bioprotectant for potato late blight management is designed based on dsRNA and a plant elicitor. Nanotechnology overcomes the dsRNA delivery bottleneck for P. infestans and extends the RNAi protective window. The protective effect of nano-enabled dsRNA against infection arises from a synergistic mechanism that bolsters the stability of dsRNA and optimizes its effective intracellular delivery. Additionally, the nano-enabled elicitor enhances endocytosis and amplifies the systemic defense response of the plants. Co-delivery of dsRNA and an elicitor provides a protective effect via the two aspects of pathogen inhibition and elevated plant defense mechanisms. And dsRNA could be effectively delivered into both oomycetes and plants with the help of SPc, leading to higher RNAi efficiency; dsRNAs homologous to two selected target genes; Interestingly, the particle size of cellobiose/SPc/dseGFP complex increased to 88nm due to the adhesion of dseGFPto the surface of the cellobiose/SPc complex. The multicomponent nano-bioprotectant could still protect dsRNA from degradation, and deliver dsRNA to P. infestans and plant cells. The multicomponent nano-bioprotectant exhibited the best protective effect with the lowest disease index, and its protective effect of 68% was significantly higher than the 53% protective effect of the widely-used mancozeb fungicide at 29 dpi in the field. The multicomponent nano-bioprotectant exhibits superior control efficacy compared to a commercial synthetic pesticide in field conditions. This work introduces a sustainable strategy for managing severe plant diseases and pest infestations.

Keywords: the multicomponent nano, bioprotectant, RNAi, dsRNA, SPc

## Cancelled

### Detecting the undetectable: Metagenomic analysis of uncultured Phytophthora

Tage Rosenqvist\*1 and Michelle Cleary1

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#### Abstract

Oomycetes of the genus *Phytophthora* are causing damage to economically and ecologically valuable plants all over the world. Current methods for detection and quantification of *Phytophthora* rely on isolation and/or amplification of specific genes (metabarcoding, qPCR). Metabarcoding studies have revealed the existence of uncultured lineages of *Phytophthora*. However, these studies provide limited information regarding the functional potential of uncultured *Phytophthora*. Additionally, as metabarcoding studies have been developed and validated on cultured species, currently applied primer sets may not necessarily amplify all lineages of uncultivated *Phytophthora*.

This project aims to identify and characterize the genomes of uncultured *Phytophthora* using a cultivation independent and amplification-free metagenomics approach. The chemotaxis of *Phytophthora* towards compounds associated with plant damage will be used to enrich cells on "synthetic baits", from which PCR-free metagenomes will be sequenced using a combination of long-read and short-read technologies. Resulting metagenome-assembled genomes will be compared to previously sequenced *Phytophthora* genomes to assess their phylogeny. Additionally, short-read sequences from the baited soils will be mapped to the assembled genomes, to estimate the relative abundances of the uncultured *Phytophthora* in soil.

With this project, we hope to develop a method for discovering and characterizing threats to agriculture, forestry, and natural environments posed by unculturable, possibly undetectable *Phytophthora*. The approach could potentially be extended to reveal hidden diversity in other oomycete genera.

Keywords: Phytophthora, metagenomics, methods

<sup>\*</sup>Speaker

### New self-updating automated COX2 database for Oomycete identification

Thomas Heinrichs<sup>\*1</sup>, Emma Zimmermann<sup>1</sup>, Melanie Sapp<sup>1</sup>, and Laura E. Rose<sup>1</sup>

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#### Abstract

For the identification of Oomycetes, the ITS1 locus is commonly used, but Choi et al. (2015) found that this marker provides less taxonomic precision compared to the mitochondrial Cytochrome C Oxidase Subunit II (COX2) locus. However, the COX2-primers designed by Choi et al. (2015) are not suitable for Illumina sequencing. Therefore, Sapp et al. (2019) designed a new reverse primer for Illumina-based metabarcoding and demonstrated its performance on the microbiome of oak trees. To perform taxonomy assignment of Oomycetes, Sapp et al. (2019) created a custom COX2 sequence database based on COX2 sequences downloaded from NCBI's GenBank database.

In this study, we updated the COX2 database from Sapp et al. (2019) for our current microbiome metabarcoding research. The updated COX2 database covers approximately 200 more species and drastically reduces the number of sequences per species, resulting in higher taxonomic precision. Revisiting Sapp et al. (2019) and unpublished Arabidopsis metabarcoding data from Sapp et al. (2018), the updated database detected up to 10% more species compared to the original database.

To ensure the database stays up to date, the process of updating the database is automated using a custom SnakeMake workflow and Python scripts, allowing the database to be easily integrated with existing metabarcoding pipelines.

To further validate the database, I am looking to partner with other research groups studying microbiomes containing Oomycetes or working with Oomycete cultures that are currently unidentified at the species level.

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Keywords: COX2, COII, Database, Illumina

## Cancelled

## Late blight effector PiAvr3a suppresses plant immunity via mediating modification of negative and positive immune regulators

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#### Abstract

Oomycetes are fungus-like eukaryotic microorganisms and represent major threats to global food security and ecosystem stability. A notable example is the potato late blight pathogen Phytophthora infestans that caused the Irish Potato Famine in the 1840s, and remains the major constraint to global potato production, the world's third-largest staple crop. Effectors are pathogen-encoded, small, secreted proteins, playing crucial roles in mediating pathogen interactions with host plants and have received extensive attention. However, the molecular and biochemical mechanisms underlying effector-mediated suppression of host immunity are poorly understood. Here, we report that the P. infestans effector PiAvr3a mediates modification of three known host target proteins: the E3 ubiquitin ligase CMPG1, the Dynamin-Related protein DRP2, and the cinnamyl alcohol dehydrogenase subfamily protein CAD7. Mutational analysis reveals that the conserved modified residues in CAD7 and CMPG1 are indispensable for their immune functions. Functional mimicry via site-specific amidation showed that PiAvr3a-induced modification stabilizes CAD7, enhancing its negative regulatory role of immunity, while abolishing the positive immune function of CMPG1 and DRP2. These findings establish the biochemical basis of PiAvr3a-mediated virulence, uncovering a previously unrecognized strategy by which eukaryotic pathogens employ target protein modification to subvert host defense systems.

Keywords: Phytophthora infestans, PiAvr3a, CAD7, CMPG1, DRP2, plant susceptibility

<sup>\*</sup>Speaker

### The Oomycete and Fungal Culture Collection of the Real Jardín Botánico (RJB-CSIC) as a key resource for Oomycete studies

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<sup>1</sup>Real Jardín Botánico (RJB-CSIC) – Spain

#### Abstract

The Oomycete and Fungal Culture Collection of the Real Jardín Botánico (RJB-CSIC) is a globally unique microbial repository comprising over 5,000 cultures from about 200 species, including Oomycetes mainly of the order Saprolegniales and fungi. The collection houses type specimens and singular isolates not preserved elsewhere, making it a strategic resource for biodiversity conservation and systematic, and genomic research. Actively expanding through research projects, many entries are accompanied by molecular data (e.g., DNA sequences), enhancing their scientific value. Subcollections include taxa of high ecological and conservation relevance, such as pathogens of agriculture and aquaculture economic importance, or those implicated in biodiversity decline (e.g., Aphanomyces astaci, Aphanomyces euteiches, Aphanomyces cochlioides, Saprolegnia parasitica, Saprolegnia diclina, Fusarium falciforme, Fusarium keratoplasticum). Recent outputs include over 60 peer-reviewed publications across disciplines such as mycology, protistology, conservation biology, and microbial ecology. The collection supports both academic and applied sectors, with potential services in axenic culturing and technical consultancy for public and private stakeholders. This collection positions the RJB-CSIC as a key hub for taxonomic, evolutionary, genomic and biodiversity research, with strong alignment to strategic European programs such as H2020, LIFE, SYNTHESYS+, and it's currently a resource being used in the project Pathogens of Algae for Biocontrol and Biosecurity (PHABB) of the PEOPLE" MARIE CURIE ACTION "Initial Training Networks" (ITN). The collection's integration into a unified database, developed with the Technical Unit for Biodiversity Informatics (UTIB), will support open access, standardization, and international collaboration.

Keywords: Culture collection, Oomycete, Saprolegnia, Aphanomyces

### Plant Cell Wall Integrity Mechanisms: Growth Control and Oomycete Susceptibility

Xiaoxuan Zhu<sup>1</sup>, Mathieu Brisson<sup>1</sup>, Sulyvann Chereau<sup>1</sup>, Celso Gaspar Litholdo Junior<sup>1</sup>, Naïma Minet<sup>1</sup>, Enora Panek<sup>1</sup>, and Aurélien Boisson-Dernier<sup>\*1</sup>

<sup>1</sup>Institut Sophia Agrobiotech – INRAE, Université Côte d'Azur (UCA) – France

#### Abstract

Plant cells have developed complex signaling pathways to coordinate their intracellular growth machinery with their extracellular cell wall, that shields them from the environment. These pathways, or cell wall integrity mechanisms, are governed in higher plants by receptor-like kinases, including FERONIA (FER). Interestingly, such mechanisms discovered in the flowering model plant Arabidopsis thaliana are, at least in part, conserved in the bryophyte Marchantia polymorpha. Our recent multi-omic approach on a knockdown mutant for Marchantia Mp*FER*, has revealed that FER regulates plant defence responses, in particular against the parasitic oomycete *Phytophthora palmivora*. Considering that parasitic pathogens can manipulate plant defence to infect and establish disease, and cell wall mechanisms are central to plant development, we hypothesized that the FER-dependent pathways could regulate the outcome of plant-Phytophthora interactions. Here, we will present our AI-based macroscopic symptoms phenotyping pipeline as well as the microscopic and molecular characterization of the responses of cell wall integrity mutants in both *M. polymorpha* and A. thaliana. Interestingly, in both Arabidopsis and Marchantia, FER and some of its signaling partners, clearly contribute positively to plant immunity, suggesting a conserved function for FER in promoting plant defense mechanisms during land plant evolution.

Keywords: Arabidopsis, Marchantia, Phytophthora palmivora, FERONIA

<sup>\*</sup>Speaker

### Identification and Functional Characterisation of Extracellular Targets in Phytophthora Infestans

Callum Scott<sup>\*1</sup>, Thomas Hainaux<sup>2</sup>, Carol Munro<sup>1</sup>, Mike Csukai<sup>2</sup>, and Pieter Van West<sup>1</sup>

<sup>1</sup>University of Aberdeen – United Kingdom <sup>2</sup>Biotalys – Belgium

#### Abstract

Phytophthora infestans is the infamous plant pathogen causing late blight on potato. The devastating effects of *P. infestans* are widespread, with huge costs associated with food loss and control measures. P. infestans infection is facilitated by the appressorium, a specific infection structure which forcibly punctures the leaf cuticle to facilitate hyphal growth within. Therefore, the appressorial stage, or earlier, are ideal points in the infection to target P. infestans, but genes associated with these stages are poorly understood and require functional characterisation. This project will use transient RNAi silencing and stable CRISPR/Cas12a knockouts to identify extracellular targets on *P. infestans*, with a focus on genes highly expressed during appressorium development. Recently, CRISPR/Cas12a has been introduced/developed in *P. infestans*, allowing for greater opportunity to elucidate gene function and identify targets for novel control methods. To induce transient silencing, double stranded RNA was synthesised to target a 150-300bp region of a target gene. Highest silencing efficiency peaks between day 12 and 15 post-transfection. Interesting gene candidates will be stably knocked out using the CRISPR/Cas12a vector (pSTUC-1), in which a specific guide RNA is inserted into the vector to create insert/deletion mutations in the target gene. Validating the essential role of appressorial stage genes in infection, will provide important candidate targets against which variable heavy domain of heavy chain antibodies (VHHs) can be synthesised as a novel control method in the field. VHH's are highly specific and environmentally friendly, which can make them good alternatives for pathogen disease control in agriculture.

Keywords: Phytophthora, Appressorium, infection, CRISPR, Antibody

<sup>\*</sup>Speaker

### The transcription factor MpGRAS7 is a novel susceptibility factor with a role in reproductive development in Marchantia

David Hoey<sup>1</sup>, Darius Kosmützky<sup>\*1</sup>, Philip Carella<sup>2</sup>, and Sebastian Schornack<sup>1</sup>

<sup>1</sup>Sainsbury Laboratory Cambridge University – United Kingdom <sup>2</sup>John Innes Centre [Norwich] – United Kingdom

#### Abstract

While it is known that vascular plants integrate biotic and abiotic signals for their development, knowledge of this in non-vascular plants is lacking. The transcription factor Mp*GRAS7* from the model bryophyte *Marchantia polymorpha* has recently been found to respond to abiotic stresses such as drought and far-red light. Furthermore, Mp*GRAS7* shifts the balance of reproductive strategy of *M. polymorpha* towards the sexual rather than asexual mode. Here we show that in addition to the responsiveness to abiotic signals, infection of *Marchantia* with the pathogen *Phytophthora palmivora* leads to increased systemic expression of Mp*GRAS7*. Treatment of *Marchantia* with *P. palmivora* cell-free supernatant was sufficient to elicit a similar increase in expression, confirming that the causal agent is a molecule rather than infection structures. Knock-out mutants of Mp*GRAS7* in *M. polymorpha* and *M. paleacea* showed increased resistance to infection by *P. palmivora*, implicating Mp*GRAS7* as a susceptibility gene. Mp*GRAS7* therefore functions as a positive regulator of infection and is nevertheless maintained in *Marchantia* due to its role in coordinating reproductive development. Future work will determine the integration of these functions.

Keywords: Marchantia, transcription factor, Phytophthora, infection, abiotic stress, susceptibility

<sup>\*</sup>Speaker

# Cancelled

## Characterization, Pathogenicity and Fungicide Sensitivity of Phytophthora Palmivora, A New Pathogen Causing Citrus Gummosis in Punjab Pakistan

Nasir Ahmed Rajput<sup>\*1</sup>, Hadeed Ahmad<sup>\*1</sup>, Muhammad Atiq<sup>1</sup>, and Muhammad Wahab<sup>1</sup>

<sup>1</sup>University of Agriculture Faisalabad – Pakistan

#### Abstract

In 2023, Kinnow (*Citrus reticulata*) trees in Sargodha, Punjab, Pakistan exhibited symptoms including leaf chlorosis, twig dieback, gum exudation on stems, poorly colored or discolored fruits, tip wilting, and leaf withering. Based on morphological characteristics and multilocus sequence analysis of the ITS-1, Cox-I,  $\beta$ -tubulin, and EF-1 $\alpha$  gene regions, the pathogen associated with citrus gummosis was identified as *Phytophthora palmivora*. Pathogenicity assays using mycelial plugs and zoospore suspension showed that *P. palmivora* induces disease symptoms on various parts of citrus trees, including both aerial and basal regions. This is the first report of *P. Palmivora* as the causative agent of citrus gummosis on various trees. Further, *P. Palmivora* was sensitive to all tested fungicides, including Folio Gold, Ridomil Gold, Aliette, Dynasty CST and Topsin-M. These findings have significant implications for developing effective management strategies against citrus gummosis in citrus orchards.

Keywords: Twig dieback, gum exudation, zoospore suspension, Folio Gold, Ridomil Gold

### Hunting crop resistance genes from the wild

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#### Abstract

Crop diseases cause severe yields and economic losses. Deploying plant resistance genes is a sustainable way to protect crops from different diseases. However, the limited gene pools of many crops often lack sufficient resistance genes, making the wild relatives of crop plants valuable sources for these genes. Previously, we used the wild Solanaceae species *Solanum americanum* and the potato late blight pathogen *Phytophthora infestans* as a model to develop a high-throughput pipeline for resistance gene mining. This approach successfully cloned several Rpi (resistance genes against *Phytophthora infestans*) genes, including Rpi-amr1, Rpi-amr3, Rpi-amr4, R04373, and R02860, along with their corresponding Avr effectors Avramr1, Avramr3,  $PITG_04373$ , and  $PITG_02860$  from P. *infestans*. To extend this methodology to combat other oomycete diseases, such as pepper *Phytophthora* blight (P. capsici) and tobacco black shank (P. parasitica), we have collected and assessed a large collection of wild Solanaceae species and are pursuing novel resistance genes from these wild plants. This work will accelerate R gene cloning from wild plants and contribute to breeding broad-spectrum and durable oomycete-resistant crops.

**Keywords:** R gene, Plant immunity, Phytophthora disease, Potato late blight, Wild plants, Effecotomics

### SCAR Wars: The dual role of barley SCAR/WAVE proteins in root interactions with pathogens and symbionts

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#### Abstract

Plant-microbe interactions are shaped by host molecular processes, including cytoskeletal reorganization. Susceptibility (S) genes, often linked to broader metabolic functions, play key roles in pathogen infection and are promising targets for improving crop resistance. SCAR/WAVE proteins are part of the SCAR/WAVE complex which regulates ARP2/3mediated actin filament nucleation. In Medicago truncatula, the SCAR protein API is a susceptibility factor to the oomycete pathogen Phytophthora palmivora by controlling cell wall properties near the root tip. However, a conserved role for SCARs as S genes in monocots has not yet been demonstrated. To explore this, we generated barley lines where individual SCAR/WAVE proteins are inactivated and assessed their impact on plant development and microbial interactions. We find that HvSCAR-B and HvSCAR-C contribute to root susceptibility to P. palmivora, with stronger resistance in double mutants. HvSCAR-B/HvSCAR-C doubled mutants did not show any pleiotropic defects in growth or fertility. Colonization by Arbuscular Mycorrhiza (AM) was unaffected or enhanced, suggesting a differential role for SCARs in different microbial interactions. These findings establish SCAR/WAVE proteins as susceptibility factors in monocots but reveal distinct functional roles among barley SCAR paralogs. Future research should explore their biochemical impact on root-microbe interactions, particularly in localised cell wall remodelling.

Keywords: Susceptibility, Crop, Symbiosis, Monocot, Actin, Phytophthora palmivora

<sup>\*</sup>Speaker

### Plant cell wall: a dynamic frontier in plant-microbe interactions

Zhenchuan Ma<sup>1</sup>, Yeqiang Xia<sup>1</sup>, Guangzheng Sun<sup>1</sup>, Yan Wang<sup>1</sup>, and Yuanchao Wang<sup>\*1</sup>

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#### Abstract

Plant cell walls act as the first line of defense against pathogen invasion. Microbial pathogens secrete cell wall-degrading enzymes to breach plant cells, while plants deploy diverse strategies to deactivate these enzymes. For instance, the soybean root rot pathogen *Phytophthora sojae* secretes the glycoside hydrolase XEG1, which degrades xyloglucan and facilitates infection. In response, soybean produces the inhibitor GmGIP1 to block XEG1 activity, the aspartic protease GmAP5 to degrade XEG1, and the receptor-like protein RXEG1 to recognize XEG1 to mount defenses. *P. sojae* also secretes pectin methylesterase PsPME1, which reduces pectin methylesterification and synergizes with polygalacturonase PsPG1 to weaken cell walls. However, soybean produces the pectin methylesterase inhibitor GmPMI1, preserving pectin integrity by targeting both soybean and *P. sojae* pectin methylesterases. Leveraging AlphaFold, GmPMI1 was precisely engineered to confer broad-spectrum disease resistance without compromising soybean growth. Together, these findings highlight the importance of maintaining cell wall integrity in plant adaptation and engineering resistance.

**Keywords:** cell wall, cell wall degrading enzymes, molecular decoy, arms race, AI assisted resistant gene engineering

### Effector gene silencing coordinated by histone methylation and small RNAs enhances host adaptation in Phytophthora sojae

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<sup>3</sup>London Research and Development Centre, Agriculture and Agri-Food Canada – Canada

#### Abstract

Pathogen adaptability driven by epigenetic processes remain poorly understood and poses a significant challenge to sustainable disease management. Histone H3 lysine 27 trimethylation (H3K27me3) and small RNA (sRNA) mediated gene silencing are two classical mechanisms employed by pathogens to evade host recognition and enhance adaptability. However, the co-regulatory relationships between these two mechanisms in modulating effector gene expression remains poorly explored. In this study, we investigated the coordinated roles of H3K27me3 and sRNAs in silencing avirulence effector genes in *Phytophthora sojae*. Gene editing of PsSu(z)12, a core subunit of H3K27me3 methyltransferase complex, disrupted both H3K27me3 and sRNA silencing marks on multiple RxLR effector genes, including Avr1b and Avr3a. Differential complementation effects at these loci, combined with analyses of H3K27me3-enriched ChIP-seq, RNA-seq and sRNA-seq data, revealed a strong locusspecific association between H3K27me3 and sRNAs in regulating gene expression. Notably, 11 out of 12 H3K27me3 regulated RxLR effectors were co-regulated by sRNAs. These data reveal the role of PsSu(z)12 in coordinating H3K27me3- and sRNA-mediated gene silencing in *P. sojae*. In summary, our study suggests that an oomycete plant pathogen simultaneously exploits histone modifications and sRNA-mediated gene silencing to enhance the diversity of gene expression plasticity, enabling adaptability in response to host immunity.

Keywords: Epigenetic regulation, Effector gene silencing, Immune escaping, H3K27me3, small RNA

### Dissecting the pathogenicity mechanisms of the oomycete Phytophthora palmivora using genetic scissors

Miaoying Tian<sup>\*1,2</sup>, Goutom Goswami<sup>1</sup>, Zhiying Cai<sup>2</sup>, Ana Caroline Conrado<sup>1</sup>, and Mehak Ghai<sup>1</sup>

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<sup>2</sup>Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa – United

States

#### Abstract

Phytophthora palmivora is one of the most destructive opmycete pathogens with a wide host range. Its genome encodes a large number of apoplastic and cytoplastic effectors, including over 700 RxLR effectors. P. palmivora is diploid with a whole genome duplication, leading to multi-copy genes. Due to this feature and low germination rate of oospores, its functional genomics study was impossible prior to the advent of CRISPR technology. By developing an efficient Agrobacterium-mediated transformation (AMT) and AMT-compatible CRISPR/Cas9 gene editing system, we have generated mutants of multiple effector proteins and determined their roles in pathogenicity, including the identification of a plant nuclearlocalized RxLR effector as a key pathogenicity factor for infection of cacao. Identification and characterization of the DNA-binding activity and host targets of this effector, and the host cellular processes it modulates are underway. In addition, we have been optimizing the functional genomics tools for *P. palmivora*. We have recently developed a significantly simplified AMT protocol, which allows to transform *P. palmivora* with ease and higher level of reproducibility. As it is challenging to use the currently available CRISPR/Cas9 system for multiplex gene editing to study the functions of gene families or multi-copy genes with sequence variations, we have been establishing an AMT-compatible CRISPR/Cas12a gene editing system. Details will be presented in the meeting.

**Keywords:** Effector biology: Functional genomics: CRISPR genome editing: Pathogenicity mechanisms

<sup>\*</sup>Speaker

### Molecular Characterization of a Natural Resistance Gene Stack in Lettuce

Pelin Yuksel<sup>\*1</sup>, Kelsey Wood<sup>2</sup>, Alex Kozik<sup>1</sup>, Maria Truco<sup>1</sup>, Lorena Parra<sup>1</sup>, Beth Rowan<sup>3</sup>, Megan Reeves<sup>1</sup>, and Richard Michelmore<sup>1</sup>

> <sup>1</sup>University of California , Davis – United States <sup>2</sup>University of Washington – United States <sup>3</sup>Oregon State University – United States

#### Abstract

Lettuce downy mildew (LDM), caused by Bremia lactucae, is a major threat to lettuce production worldwide. Resistance genes provide high levels of resistance but may only be effective for a limited time because B. lactucae rapidly evolves new virulence phenotypes. Stacking multiple resistance genes in a cultivar maximizes evolutionary hurdles for the pathogen, potentially providing more durable resistance. ViAE is a Lactuca sativa breeding line with resistance introgressed from L. virosa. In ViAE, recognition and cell death occur in response to seven B. lactucae effectors which is genetically linked to resistance on Chromosome 8. This suggests ViAE has a natural gene stack that could provide durable resistance to LDM. A T2T genome assembly of ViAE was generated using PacBio HiFi and Oxford Nanopore reads. Comparison to the susceptible line Salinas revealed a highly variable 35.2 Mb region on Chromosome 8, within the 65 Mb introgression interval mapped using RILs. This region contains 27 NLRs, 5 RLKs, and 2 RLPs as candidate resistance genes. Highly expressed NLRs with all three domains (N-terminus, NBS, LRR) were prioritized for functional validation. Over half of these candidate NLRs have been cloned and co-agrotransformed with the seven effectors. Two NLRs recognized at least two effectors, with both recognizing an effector in common. Different combinations of CRISPR-mediated knockouts are being generated to test the number of effective NLRs in the resistance gene stack.

Keywords: Bremia lactucae, lettuce resistance, comparative genomics

### Deciphering Mechanosensing in Plants During Pathogen Invasion

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<sup>1</sup>Translational Plant Biology, Utrecht University – Netherlands <sup>2</sup>Plant Stress Resilience, Utrecht University – Netherlands

#### Abstract

Plants are constantly exposed to mechanical forces in their environment. Pathogen infection structures, such as hyphae and haustoria, can exert physical pressure on host cells, potentially serving as mechanical cues perceived by the plant immune system. However, the way these cues activate immune responses is still poorly understood. In our research, that is part of the Dutch Green Tissue Engineering (GreenTE) consortium, we investigate the emerging concept of Mechanically-Triggered Immunity (MeTI): a layer of defence where mechanical stimuli activate immune signalling pathways. We are using a multifaceted approach to characterize the molecular basis of MeTI. First, previously generated phosphoproteomic datasets have identified candidate proteins rapidly phosphorylated upon artificial mechanical stimulation. Arabidopsis mutants, perturbed in the corresponding proteins, will be tested for altered mechanical responses and altered susceptibility to two oomycete pathogens, Phytophthora palmivora and Hyaloperonospora arabidopsidis(Hpa). To complement this, we are performing a time course microscopy screen of Hpa and P. palmivora infection of different Arabidopsis tissues to visualise infection structures and their progression over time. In parallel, we are developing cell culture infection assays with the aim to generate new phosphoproteomic data to profile early signalling dynamics in response to pathogen attack. Given that the plant cell wall serves as the first line of defence against invading pathogens, we are investigating how its integrity influences mechanical susceptibility by screening cell wall receptor mutants using Hpa and P. palmivora disease assays. Together, these approaches aim to define the molecular underpinnings of MeTI and its intersection with immune pathways, shedding light on how plants sense and respond to mechanical stimuli in their environment.

**Keywords:** Mechanically, Triggered Immunity (MeTI), Plant mechanosensing, Phytophthora palmivora, Hyaloperonospora arabidopsidis (Hpa), Cell wall integrity

### Hypersensitive-like cell death and autophagy are two successive and broadly conserved lines of defence of brown algae against their oomycete pathogens

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#### Abstract

Brown algae (Phaeophyta) encompass key primary producers and ecosystem engineers of temperate and cold coastal seas, such as kelps, the cultivation of which is rapidly expanding worldwide. Using the biotrophic, intracellular oomycete Eurychasma dicksonii, we interrogated the cellular mechanisms resulting in innate or induced disease resistance in across brown algae species. Having identified clonal brown algal strains exhibiting contrasting degree of innate resistance against either pathogen, we find that across ten brown algal species, innate resistance against the intracellular oomycete pathogen Eurychasma dicksonii is mediated by local cell death and accompanied by cell-wide deposition of  $\beta$ 1-3 glucans and fluorescent metabolites, the accumulation of reactive oxygen species, and the expression of programmed cell death markers such as metacaspase and DNA degradation. This cell death-mediated response also occurs in compatible strains, though only for a fraction of the infected algal cells, which makes it a quantitative trait. The onset of this innate, cell-death mediated resistance is followed by the induction of autophagy both in the host (xenophagy) and the pathogen (abortive autophagy). This leads to the restriction of pathogen propagation upon new infection, thus providing the first known cellular mechanism for inducible and systemic acquired resistance in brown algae. A screen of 43 strains, spanning 27 algal species across 10 orders, shows that the HR and autophagy markers identified above strongly correlate with resistance against the two oomycetes Eurychasma dicksonii and Anisolpidium ectocarpii, as well as the phytomixid Maullinia ectocarpii. They are broadly conserved across brown algae, and include: i) cell wall reinforcements, namely local papilla formation beneath the infection site, sometimes accompanied of systemic cell wall thickening across the entire

individual; ii) the death of host cells challenged by a pathogen spore; iii) the upregulation of phlorotannin metabolism, which suggests a defensive role for these anti-herbivore secondary metabolites; iv) an inducible autophagic response of the host and the pathogen; v) hydrogen peroxide production, confirming the participation of oxidative stress in these algal-pathogen interactions. Our screen leads us to conclude that these responses are widely conserved across Phaeophyceae, and altogether account for innate and acquired resistance against the investigated oomycete and phytomixid pathogens. However, we also observed several resistant algal strains where these responses were not strong, suggesting that more undescribed mechanisms are contributing to brown algal immunity.

Keywords: innate immunity, induced immunity, defence markers

### Phylogenomics and Physiology of Oomycete Pathogens Infecting Red Algae, Brown Algae, and Diatoms Towards Biocontrol and Biosecurity

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#### Abstract

In 2022, red algal cultivation accounted for approximately 80% of the global seaweed production value according to the FAO Fishstat database (1). This multibillion-dollar industry (1) is also affected by various pathogens (2,3). This research project focuses on the oomycete pathogens which are understudied (4). This project will increase our understanding of the physiology and phylogenetic relationships of Olpidiopsis-like oomycetes of red algae to enable the mitigation of risks to cultivated and wild populations (3). We aim to characterize and increase an existing collection of Olpidiopsis-like oomycetes of red algae by increasing our understanding of the diversity and phylogenetic relationships between these pathogenic oomycetes of brown, red, and green macroalgae as well as diatoms. We will also analyze the virulence strategies of different oomycete pathogens of red algae. The acquired knowledge will be applied to improve diagnostic tools in red algal pathology and to increase biosecurity in the algal cultivation sector.

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Keywords: algal pathogens, phylogenetics, biosecurity, biocontrol

### Kānuka and mānuka oils have significant inhibitory effects on the growth of oomycete species

Ayelen Tayagui<sup>\*1</sup>, Yiling Sun<sup>1</sup>, Wendy Maddocks<sup>1</sup>, Christoph Goebl<sup>2</sup>, Breanna Smith<sup>1</sup>, Viji Sarojini<sup>3</sup>, Ishita Yellapurkar<sup>3</sup>, Volker Nock<sup>1</sup>, and Ashley Garrill<sup>1</sup>

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#### Abstract

Oomycetes can have profound effects on biodiversity and ecosystems, primary industries and on food biosecurity through the diseases that they cause. They are currently typically controlled using anti-fungal agrochemicals, such as phosphites, that may have undesirable environmental effects. It is thus important that less harmful treatments are found. Kānuka (Kunzea ericoides) and mānuka (Leptospermum scoparium) are members of the myrtle plant family that appear far less susceptible to plant pathogens than other members of the myrtaceae. We have tested the effects of oils extracted from kānuka and mānuka on the radial growth of four oomycete species: Phytophthora nicotianae, Phytophthora cinnamomi, Phytophthora agathidicida and Achlya bisexualis. Oils were obtained from various commercial producers in New Zealand. All of the oils tested had significant inhibitory effects on growth with IC50 values ranging from 0.01 to 0.23% (v/v), with the manuka oils more effective than kānuka. P. nicotianae was most sensitive to the oils with an average IC50 value of 0.05%(v/v) and Phytophthora cinnamomi and Phytophthora agathidicida the least sensitive, both with an average IC50 value of 0.14% (v/v). GS – MS and NMR analysis of the oil components suggest that the presence of naphthalene - containing compounds in the oils correlate with their inhibitory effects on growth. The application of these oils and/or the strategic planting of mānuka and kānuka around susceptible plants may present a new approach to tackling diseases caused by oomycetes.

Keywords: Phytophthora, kānuka oil, mānuka oil, growth inhibition, naphthalene

<sup>\*</sup>Speaker

### Characterization of a biological control agent of oomycetes in aquatic ecosystems

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#### Abstract

Pythium pathogens have emerged as a major economic threat to the hydroponic production of arugula, lettuce, spinach, and other leafy greens. However, once Pythium pathogens get introduced into this artificial environment, the hyphae produce zoospores that enable rapid dispersal throughout the system. Surface biofilms then provide a continuing inoculum source for new seedlings. Thus there is an urgent need to develop a biological control solution for *Pythium* management. We posited that there must exist in nature, oomycete specific pathogens and a strategic search using an appropriate selection system could identify these strains. Adoption of this strategy lead to the discovery of a Pseudomonas fluorescens strain now referred to as PythiumX. This strain is ac ontact dependent killer of of 50 isolates collected from GHs across the USA and Canada, the soybean pathogen Phytophthora sojae, and six species of Saprolegnia an oomycete fish pathogen. That this bacteria strain is effective against oomycete species separated by millions of years of evolutionary change suggests the development of resistance against this strain is not going to occur easily. The basis of the virulence of this particular strain of bacteria is the delivery of multiple effectors via a Type Six Secretion System. A common virulence strategy employed by several plant pathogens is the delivery of effectors that disrupt the actin cytoskeleton. Here we have used actin phalloidin staining to demonstrate that one or more of the effectors of the PythiiumX strain targets the actin hyphal skeleton of Pythium. @font-face {font-family:"Cambria Math"; panose-1:2453 5 4 6 3 2 4; mso-font-charset:0; mso-generic-font-family:roman; mso-font-pitch:variable; msofont-signature:-536870145 1107305727 0 0 415 0;}@font-face {font-family:Aptos; panose-1:2 11 0 4 2 2 2 2 2 4; mso-font-charset:0; mso-generic-font-family:swiss; mso-font-pitch:variable; mso-font-signature:536871559 3 0 0 415 0;}p.MsoNormal, li.MsoNormal, div.MsoNormal {mso-style-unhide:no; mso-style-qformat:yes; mso-style-parent:"; margin-top:0in; marginright:0in; margin-bottom:8.0pt; margin-left:0in; line-height:115%; mso-pagination:widoworphan; font-size:12.0pt; font-family:"Aptos", sans-serif; mso-ascii-font-family:Aptos; msoascii-theme-font:minor-latin; mso-fareast-font-family:Aptos; mso-fareast-theme-font:minorlatin: mso-hansi-font-family:Aptos; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi; mso-font-kerning:1.0pt; mso-ligatures:standardcontextual; }.MsoChp {mso-style-type:export-only; mso-default-props:yes; font-family:"Aptos", sans-serif; mso-asciifont-family: Aptos; mso-ascii-theme-font: minor-latin; mso-fareast-font-family: Aptos; mso-fareasttheme-font:minor-latin; mso-hansi-font-family:Aptos; mso-hansi-theme-font:minor-latin; msobidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi;}.MsoPapDefault {msostyle-type:export-only; margin-bottom:8.0pt; line-height:115%; div.WordSection1 {page:WordSection1;}

 $<sup>^*</sup>Speaker$ 

**Keywords:** Biological control, Pythiium, hydroponics.

### AGROBODYTM bioactives: novel biocontrol agents for oomycete plant disease control

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#### Abstract

Phytopathogenic oomycetes remain a significant threat to global food security, causing extensive crop losses and challenging current disease management strategies. While chemical crop protection remains the standard approach to controlling plant diseases, concerns over the environmental and health impacts of pesticide use, the emergence of resistant pathogen strains, and the recent ban of key fungicides are driving the need for innovative alternatives. Biotalys is committed to the development of an AGROBODY Foundry<sup>TM</sup>, to generate a new type of protein based crop protection solution aiming to replace or reduce conventional chemical pesticides and contribute to more sustainable and resilient agricultural systems.

In addition to standard antibodies, members of the Camelidae family also produce a distinctive type of antibody that does not contain light chains. The antibody fragments derived from these heavy-chain only IgG are dubbed VHHs. Their small size, high stability, strong binding affinity , simple and cost-effective production make them emerge as a promising tool in the development of therapeutics in different fields.

In the context of infectious diseases, VHHs have demonstrated the ability to effectively target bind and neutralize surface proteins and microbial toxins or blocking pathogen attachment and prevent infection. Additionally, VHH domains have shown great potential as enzyme inhibitors, expanding their utility in combating pathogen virulence mechanisms. EVOCATM, the first product candidate which targets fungal plant pathogens is undergoing registration in the USA and Europe, but the AGROBODYTM bioactives can also be applied to oomycete control and here we present promising results on the development of VHHs targeting *Phytophthora infestans* and *Plasmopara viticola*, highlighting their potential as effective biocontrol agents.

Keywords: phytophthora\_infestans, biocontrol, VHH, biotalys

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