QRW Abstracts: Plant–Microbe Interactions Meeting

PM1: A fungal pathogen interferes with the cell wall to colonize wheat

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The plant cell wall (PCW) is a complex and dynamic structure that plays a crucial role in plant defense against pathogens and is a source of oligosaccharides acting as elicitors of the immune responses. Some pathogenic fungi degrade the PCW through the action of a diverse set of secreted cell wall-degrading enzymes (CWDEs) that facilitate host colonization. Here, we demonstrate that ZtGH45, a conserved β-glucanase from *Zymoseptoria tritici*, is induced during the necrotrophic phase and hydrolyzes wheat cell wall polymers, releasing mixed-linked β-1,3/1,4-glucans (MLGs) and cello-oligosaccharides that activate wheat immune responses and hinder pathogen infection. Misexpression of *ZtGH45* leads to an earlier release of MLGs, premature induction of host immunity and impairment of fungal virulence. Altogether, these findings demonstrate that the balance between PCW degradation and the release of resistance inducers by fungal CWDEs might dictate the evolution of regulatory mechanisms governing fungal enzyme expression to promote plant colonization.

PM2: Catalysts of deception: unveiling fungal Nudix effector protein function in plant-fungal interactions

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Our research endeavours to understand the molecular mechanisms employed by pathogenic fungi to manipulate host plants and induce disease. Additionally, we aim to understand how plants recognise fungal effector proteins to activate defence pathways. Here I will present our latest findings focusing on two families of effector proteins utilised by diverse pathogenic fungi during plant infection. These effector families both belong to the ancient enzyme superfamily known as Nudix hydrolases. We show that fungal Nudix effectors can be categorised into two evolutionary and functionally distinct groups. The first group is exclusive to rust fungi from the *Melampsora* genus. Our results demonstrate that these enzymatic effectors specifically hydrolyze the protective 5′ cap structure on mRNA[1]. The second group of Nudix effectors is utilised by numerous economically significant pathogenic fungi, including *Magnaporthe oryzae*, the causative agent of rice blast disease. We demonstrate that this conserved class of effector proteins specifically hydrolyse inositol pyrophosphates. In plants these effectors activate the phosphate starvation response. By hijacking this pathway, we propose that fungal pathogens deceive the plant into suppressing its own defence mechanisms, thereby aiding in colonization and disease progression[2]. To conclude, I will discuss how we are currently leveraging this newfound knowledge to develop innovative approaches aimed at reducing fungal virulence and enhancing plant disease resistance.

1. McCombe, C.L.[†], A.M. Catanzariti[†], J.R. Greenwood, A.M. Desai, M.A. Outram, D. Yu, D.J. Ericsson, S.E. Brenner, P.N. Dodds, B. Kobe, D.A. Jones[§] and S.J. Williams[§] (2023). *A rust-fungus Nudix hydrolase effector decaps mRNA and suppresses plant immunity.* New Phytologist. 239: 222-239.

2. McCombe, C.L.[†], A. Wegner[†], C.S. Zamora, F. Casanova, S. Aditya, J.R. Greenwood, L. Wirtz, S. de Paula, E. England, S. Shang, D.J. Ericsson, E. Oliveira-Garcia*, S.J. Williams* and U. Schaffrath*. *Plant pathogenic fungi hijack phosphate starvation signaling with conserved enzymatic effectors*. BioRxiv https://doi.org/10.1101/2023.11.14.566975.

PM3: Decoding the unknown: unveiling the role of an effector protein during myrtle rust infection by *Austropuccinia psidii*

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Myrtle rust is caused by the invasive fungus *Austropuccinia psidii*, which is incredibly infectious and physically devastating to plants in the Myrtaceae family. The disease was first detected in New Zealand in 2017 and continues to spread rapidly across the country. *A. psidii* has already caused major declines in Myrtaceae populations worldwide. It threatens many Aotearoa-New Zealand natives, including taonga species such as pōhutukawa (*Metrosideros excelsa*), mānuka (*Leptospermum scoparium*) and rātā (*Metrosideros umbellata*). Localised extinctions of Myrtaceous plants are already occurring in Aotearoa.

Transcriptomic experiments have identified several proteins that are expressed during the first 24–48 hours of infection by *A. psidii* on mānuka. This expression pattern is a signature of their important role in the successful infection of plant cells. In other plant pathogens these 'effector proteins' are known to manipulate the host plant's cellular processes to boost pathogen fitness.

We aim to elucidate the role of the *A. psidii* effector protein, AP1260, during infection through bioinformatic and biophysical analysis. *A. psidii*'s dikaryotic nature has led to the presence of two haplotypes of AP1260, with both being present during infection. Biophysical studies have sought to determine the physical characteristics of both haplotypes in solution. These include analytical ultracentrifugation, circular dichroism, small-angle X-ray scattering, and nuclear magnetic resonance. Functional analysis of AP1260 uses *Agrobacterium*-mediated transformation of *Nicotiana benthamiana* and yeast-two-hybrid to determine its localisation and potential *in planta* interaction partners. This study represents the first investigation of an *A. psidii* effector protein. Characterisation of AP1260 improves the knowledge of the mechanisms of *A. psidii* infection and may lead to the development of a novel and effective method to treat and control myrtle rust.

PM4: Developing a disease-suppressive microbial community against a bacterial pathogen

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Foliar diseases managed by suppressive phyllosphere communities is the next frontier. We investigated whether suppressive phyllosphere communities could be developed through artificial passaging to suppress disease using the model pathosystem of *Pseudomonas syringae* pv. *tomato* (*Pto*) and tomato. Tomato plants were inoculated with a field phyllosphere metacommunity and then challenged with *Pto* or $MgCl_2$ buffer. Disease progression following inoculation was recorded for eight days. Microbial material was then collected from plants with the least disease (*Pto*-inoculated plants) or at random (control plants) to inoculate naïve plants for the next passage and for sequencing. Metabarcoding and metagenomics were used to access the microbiome composition at the end of each passage and assessed antagonist gene abundance at key passages based on disease. Disease severity increased from the initial passage (~15%), peaked at passages 4-5 (25-45%), followed by a sharp decline until passage 9 (~10%). Sterilising the passage 9 communities showed elevated disease over several subsequent passages. Sequencing identified two dominant bacterial genera, whereas hundreds of fungal genera were present at low relative abundance. Taxonomic richness and evenness were low within samples, with clustering occurring for suppressive or non-suppressive microbiomes. The relative abundance of genes associated with antagonism increased at passage 4, corresponding to the highest observed disease severity. Overall, we were able to develop disease-suppressive phyllosphere communities in which suppression is due to specific microbial composition and activity.

PM5: Dissection of the epoxyjanthitrem pathway in *Epichloë* sp. LpTG-3 strain AR37 by CRISPR gene editing

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Epichloë festucae var. *lolii* and *Epichloë* sp. *Lp*TG-3 are filamentous fungal endophytes of perennial ryegrass (*Lolium perenne*) that have a substantial impact on New Zealand's agricultural economy by conferring biotic advantages to the host grass. *Epichloë* endophyte strain AR37 provides ryegrass with improved agronomic performance, insect protection and plant persistence, with this endophyte alone estimated to contribute NZ$3.6 billion to the New Zealand economy over a 20-year period. This strain produces secondary metabolites, including epoxyjanthitrems, which are a class of indole diterpenes, associated with the observed effects of AR37 on livestock and insect pests. Until very recently, AR37 was intractable to genetic modification but this has changed with the application of CRISPR-Cas9-based gene editing techniques. We used gene inactivation by CRISPR-Cas9 to deconvolute the genetic basis for epoxyjanthitrem biosynthesis and manipulate this secondary metabolite pathway to reduce or remove endophyte-induced mammalian toxicity whilst retaining insect activity against some important agricultural pests. This provides a step change in the future use of animal-safe *Epichloë* strains in New Zealand pastures which will significantly reduce chemical inputs and increase animal welfare.

We also show that gene editing of *Epichloë* can be achieved without off-target events or introduction of foreign DNA through an AMA1-based plasmid that simultaneously expresses the CRISPR-Cas9 system and selectable marker. Furthermore, since a nucleic acid template was not used to guide Cas9 and the repair mechanism was through NHEJ, gene edits generated with this delivery system are classified as site-directed nuclease 1 (SDN-1), which are not regulated as genetically modified organisms (GMO) in selected jurisdictions, including Australia.

PM6: Interactions of the apple pathogen *Venturia inaequalis* with microorganisms from the apple phyllosphere

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Fungal pathogen *Venturia inaequalis* is the causal agent of apple scab disease, the most economically costly disease affecting apple crops worldwide, largely due to the heavy reliance on fungicides for disease control[1]. The microorganisms present in the phyllosphere may present a reservoir of potential biological control agents to replace this heavy reliance on fungicides, and thus were investigated for their potential as antagonists against *V. inaequalis*.

A microorganism collection, comprising twenty-seven yeasts and bacteria, was isolated from the apple phyllosphere, and identified to genus level via Sanger sequencing. These microorganisms were then used in interaction assays where they were co-inoculated onto potato dextrose agar plates with *V. inaequalis* (ICMP13248) conidiospores. The plates were observed at 24 hours post inoculation for *V. inaequalis* germ-tube length and germination rate. They were further observed at four weeks to check for total growth of macroscopically visible *V. inaequalis* growth.

For co-inoculation assays the initial germ tube lengths or germination rates of the conidiospores did not predict the final growth area covered by the *V. inaequalis* mycelium. However, most of the microorganisms were found to inhibit *V. inaequalis* growth to some extent. More surprisingly, two isolates of the *Rathayibacter* genus were found to have increased growth when in direct or close contact to *V. inaequalis* growth.

Investigating the antagonistic relationships of these microorganisms with *V. inaequalis* could be a step in discovering novel biological controls or antimicrobial compounds to augment or replace fungicide control. Interrogating the benefit that *V. inaequalis* is providing to the *Rathayibacter* isolates could enable greater understanding of the complex relationships that take place in the apple phyllosphere.

1. Carisse, O. and J. Bernier (2002). *Effect of environmental factors on growth, pycnidial production and spore germination of* Microsphaeropsis *isolates with biocontrol potential against apple scab.* Mycological Research. 106: 1455-1462.

PM7: The role of the transcription factor TRSYMB1 in secondary metabolism and symbiosis in *Trichoderma*

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Endophytic microorganisms protect plants against biotic and abiotic stresses, enhancing growth, productivity, biodiversity, and ecosystem function. Surprisingly, fungi that can be devastating and widespread plant pathogens, including *Fusarium* spp., have also been identified as asymptomatic endophytes in healthy host and non-host plants. Moreover, strains of *Trichoderma*, a facultative fungal symbiont and the fungal species applied most successfully in agriculture, have shown positive, neutral, and sometimes detrimental effects in plants. In this presentation, we will describe the role of a membrane-bound transcription factor called TRSYMB1, which regulates *Trichoderma*-plant root symbiosis. A deletion mutant lacking the *trsymb1* gene (Δ*trsymb1*) causes inhibition of seedling emergence and produces severe maceration in tomato and *Arabidopsis* plants. Interestingly, *trsymb1* is conserved in some fungal pathogens. The lack of the *trsymb1* gene affects nitrate assimilation by regulating the nitrate gene cluster in *T. virens*. Remarkably, different secondary metabolites are differentially expressed by ammonium or nitrate through TRSYMB1. This finding creates a unique opportunity to understand the molecular mechanisms that control the endophyte-pathogen equilibrium in host and non-host plants, not only with *Trichoderma* but most likely with pathogens such *as Fusarium oxysporum.*

PM8: Immune signalling by cell surface-localised receptor-like proteins (RLPs) reveals its secrets

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The interaction between the apoplastic pathogenic fungus *Fulvia fulva* (formerly known as *Cladosporium fulvum*) and tomato, has been subject of study for various decades. Next to the *Cf* resistance genes, most of which were identified by the research team of Jonathan Jones (The Sainsbury Laboratory, Norwich, UK), our team has identified the matching effectors that are secreted by *F. fulva*. Cf proteins are receptor-like proteins (RLPs), which are cell-surface receptors that stand at the basis of the first layer of the plant innate immune system. During gene-for-gene co-evolution, *F. fulva* has evaded recognition by Cf proteins through mutation of its matching effectors. We found that the receptor-like kinases SOBIR1 and BAK1 are the regulatory co-receptors of RLPs and tomato Cf-4, which detects the Avr4 effector secreted by *F. fulva*, also requires these co-receptors to mediate resistance against this fungus. We have established (i) reactive oxygen species (ROS) burst, (ii) MAPK activation and (iii) cell death activation as indicators of the strength of the immune output and have transformed the *Cf-4* gene to the model plant *Nicotiana benthamiana*, in which the encoded protein is functional. Receptor-like cytoplasmic kinases (RLCKs) are well-recognized to act as the initial cytoplasmic transducers, bridging cell-surface receptor complexes with their downstream signalling partners. The family of RLCKs is extremely large, with more than 100 members in tomato and in *N. benthamiana*. We have knocked-out multiple genes belonging to different *RLCK class VII* subfamilies in *N. benthamiana:Cf-4* and observed that members encoded by RLCK-VII-6, -7, and -8, differentially regulate the Avr4/Cf-4-triggered biphasic ROS burst. In addition, members of RLCK-VII-7 were found to play an essential role in the Avr4/Cf-4-triggered hypersensitive response (HR) resulting in cell death, and in resistance against the oomycete pathogen *Phytophthora palmivora*, which in *N. benthamiana* is mediated by the RLP RESPONSIVE TO ELICITINS (REL).

PM9: Engineering NLR immune receptors for disease resistance in cereal crops

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Natural plant populations maintain high diversities of resistance (*R*) genes that provide effective resistance to disparate pathogens; however, such diversity has been significantly reduced by the genetic bottlenecks associated with plant domestication and breeding. Resistance based on the reduced diversity is often short-lived, as pathogens evolve rapidly to overcome *R* genes by varying their recognised components. The *mildew resistance locus A* (*MLA*) *R* gene family in barley and wheat represents an extraordinary source of natural genetic variation that is ideal for mining disease resistance specificities. The *MLA*-*R* genes encode nucleotide-binding leucine-rich repeat (NLR) immune receptor proteins that confer disease resistance against multiple unrelated plant pathogens through recognising and binding to pathogen-specific proteins, termed effectors. The barley NLRs MLA13 and MLA7 confer resistance against different strains of the barley powdery mildew pathogen, through interaction with the pathogen effectors AVR_{A13} and AVR_{A7} respectively. Using DNA shuffling, we generated a variant library recombining the *MLA7* and *MLA13* genes *in vitro*. The diversity library was cloned into yeast generating circa 40,000 independent clones and was screened for interaction with effectors AVR_{A13} and AVR_{A7} using a high throughput yeast-two-hybrid (Y2H) assay. Y2H screening of the variant library yielded a number of clones that interact with AVR_{A13}. Protein sequence and structural analysis showed that the interacting clones can be sub-grouped into three clusters and all of the corresponding recombined genes contain a critical site from *MLA13*. Mutation of this single site in MLA7 to that from MLA13 enabled the mutated MLA7 to interact with AVR_{A13} in yeast and trigger AVR_{A13}-dependent immune response *in planta*. Our study has given insights into the fundamental mechanisms of pathogen effector recognition by plant NLRs. We established a NLR engineering pipeline that evolves MLA-NLRs to recognise distinct pathogen effectors, which will help to develop made-to-order resistant crops in the future.

PM10: Activation and translocation of plant membrane-associated NAC transcription factors: key in stress response and target of pathogen effectors

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Membrane-associated transcription factors are pivotal for eukaryotic cell adaptation and stress response signalling in plant cells. Land plants encode over 300 variants of these transcription factors, highlighting their significance in environmental sensing. Tethered to cellular membranes, these transcription factors need to undergo activation and cleavage as initial functional steps. This is often induced by environmental cues, involving post-translational modifications and protein interactions. The subsequent step is the translocation of the cleaved transcription factor domain to the nucleus, where they regulate gene expression.

Here we investigate activation and translocation mechanisms of membrane-associated NACs (NAM, ATAF1/2, and CUC2). We focus on the Arabidopsis ANAC013 and ANAC017 cluster within the membrane-associated NACs, which are key regulators of cellular reactive oxygen species levels and organelle signalling. The inactive forms of these proteins are tethered to the endoplasmic reticulum via a C-terminal transmembrane domain. Proteolytic cleavage, mediated by rhomboid proteases, releases the transcription factor domain. However, the activation processes leading to cleavage and translocation are not well understood. Our data suggest that the translocation of these NACs, induced by certain pathogen-associated molecular patterns and drought stress, depends on phosphorylation at a conserved site and interaction with 14-3-3 proteins. We hypothesize that this phosphorylation is essential for rhomboid protease cleavage and subsequent nuclear translocation. Secondly, we confirmed the inhibition of translocation by pathogen-derived RXLR effector proteins by interacting with critical domains, thereby potentially preventing phosphorylation or cleavage. These identified RXLR effectors co-localize with the NACs at the endoplasmic reticulum upon transient expression in *Nicotiana benthamiana*. Interestingly, sequence analysis of NAC-interacting effectors revealed variations at the protein sequence level, with only one distinct conserved motif present in the transmembrane domain. This motif is potentially involved in both NAC-effector and effector-effector interactions. We will further discuss our results within the context of plant immunity and signalling crosstalk.

PM11: Are we closer to understanding the function of the PR1 protein in plant immunity?

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In recent years it has emerged that some necrotrophic fungi facilitate disease through a strict gene-for-gene mechanism as observed in biotrophic pathogens. For the wheat pathogen *Parastagonospora nodorum*, the basis of this host-specific interaction is small cysteine-rich effector proteins secreted during infection (ToxA, Tox1 and Tox3). These effectors interact with specific dominant susceptibility genes in the host leading to a programmed cell death response and disease. However, whilst we now understand the requirement of these effector proteins for disease, their modes of action remain poorly understood.

I will present our latest findings on dissecting the dual functionality of the Tox3 effector protein. Together with its function in causing cell death through its interaction with Snn3, we demonstrate that Tox3 has an important role in mediating PR-1 defence signalling and is required for disease development. Furthermore, I will discuss new and exciting data on how PR1 proteins function and contribute to disease resistance through peptide release and signalling. Collectively, these data have not only significantly advanced our understanding of necrotrophic diseases, but also provided a rare insight into the function and mechanism of the enigmatic plant PR-1 proteins.

PM12: Delivery of RXLR effectors from haustoria into plant cells

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The oomycete pathogen *Phytophthora infestans* causes late blight, the number one disease threatening production of potatoes and tomatoes globally. *P. infestans* develops haustoria – finger-like infection structures that form an intimate interaction with the host plasma membrane during the early stages of disease. Haustoria are major sites for secretion of effector proteins and cell wall-degrading enzymes that facilitate disease development. Amongst the former are a class called RXLR effectors, so-named for the conserved RXLR motif that is required for these virulence proteins to be translocated inside living host cells. There are hundreds of RXLR effector genes predicted in the genome of *P. infestans*, demonstrating a remarkable potential for manipulation of host processes. RXLR effectors interact directly with a range of regulatory proteins in the host cell to suppress or otherwise manipulate plant defences. In contrast, they are also targets for host resistance proteins which activate immune responses that prevent further colonization by the pathogen.

In this talk I will focus on the secretion of RXLR effectors and their uptake into host cells. We have found that many RXLR effectors are associated with extracellular vesicles (EVs), whereas apoplastic effectors and cell wall-degrading enzymes mostly are not. We have discovered novel EV markers that are co-secreted with RXLR effectors. Evidence is presented that RXLR effectors can be taken into host cells by clathrin-mediated endocytosis[1], raising the question of how they are released from endosomes to reach their targets in a range of subcellular destinations.

1. Wang, H., S. Wang, W. Wang, L. Xu, L. Welsh, M. Gierlinski, S.C. Whisson, P.A. Hemsley, P.C. Boevink and P.R.J. Birch (2023). *Uptake of RXLR effectors into host cells by clathrin-mediated endocytosis*. The Plant Cell doi: 10.1093/plcell/koad069.

PM13: The role of *Phytophthora pluvialis* RxLR effectors during early infection of *Pinus radiata*

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Phytophthora pluvialis is the causative agent of Red Needle Cast and is a destructive foliar disease of pine. Like other oomycetes, it utilises host-translocated effector proteins to promote successful infection. An RNA sequencing approach was chosen to elucidate differential expression of effector genes in mycelium and pine needles of susceptible and resistant pine lines infected with *P. pluvialis* at two different time points (3- and 5-days post infection). Overall, 311 effector genes were identified to be differentially expressed (136 were Crinklers, 115 RxLRs, and 60 Elicitins). Further down-stream analysis focused on RxLR effectors expressed during the early stage of infection. First, we employed a computational approach to acquire understanding on protein sequence and structural conservation. As expected, conservation on sequence level was limited. Interestingly, structural modelling showed that more than 90% of the early expressed RxLR effectors contained at least one WY-motif. Transient expression in *N. benthamiana* for eight selected candidates showed diverse localisation *in planta*. Successful protein expression and stability was confirmed by Western blotting. Three WY-motif containing effectors candidates, PpR03, PpR06, PpR07 were expressed *in E. coli,* purified, and characterised in detail. PpR04 and PpR08 each displayed a suppression phenotype in INF1 cell death assays. Work is ongoing to identify the host targets of *P. pluvialis* RxLRs via yeast-2-hybrid screening against both a potato-based and pine-based cDNA library, with co-immunoprecipitation and virulence assays to support results.

PM14: Disruption of the infection process of Sclerotiniaceous fungi *Botrytis cinerea* and *Ciborinia camelliae* using common plant secondary metabolites

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Fungi play important roles in plant survival in both beneficial and pathogenic ways. The Sclerotiniaceae fungal family includes a large number of plant pathogens with similar life cycles. This family includes the generalist *Botrytis cinerea*, the causal agent of grey mould, which affects many crops worldwide with high financial impact as well as *Ciborinia camelliae*, a specialist, that causes *Camellia* Petal Blight uniquely on *Camellia* blooms. From our previous studies it is known that *Camellia lutchuensis* is inherently resistant to *C. camelliae* and this is associated with the early activation of the phenylpropanoid pathway upon infection[1].

We tested if these secondary metabolites could inhibit the infection of susceptible *Camellia* hybrid 'Nicky Crisp' with *C. camelliae*, and *Arabidopsis thaliana* with *B. cinerea*. We applied nine phenylpropanoids by means of droplet incubation, whole-petal/leaf sprays and by feeding through the vascular system. The alterations in the infection process were visualized using non-destructive and destructive methods. Five phenylpropanoids effectively inhibited mycelial growth and lesion progression. Spores of both fungi were unable to germinate in the presence of Cinnamic and Coumaric acids. Mycelium development and the capability to penetrate the upper epidermis decreased using Conifer aldehyde and with Ferulic acid and Coniferyl alcohol uniquely affected spore germination of *C. camelliae.* We hypothesise that spore cell wall integrity is being compromised when exposed for extended periods of time and we are currently testing this hypothesis.

1. Kondratev, N. *Identification of mechanisms defining resistance and susceptibility of* Camellia *plants to necrotrophic petal blight disease*. PhD Thesis in Plant Biology. 2019, Massey University: Manawatū. Chapter 4.

PM15: Effector proteins required for virulence of the fungal pathogen *Neonectria ditissima*

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European canker, caused by the necrotrophic fungal pathogen *Neonectria ditissima*, is one of the most damaging apple diseases in New Zealand and north-western Europe. During plant colonisation, it is hypothesised that *N. ditissima* secretes virulence factors, also known as effectors, a subset of which can be recognised by plant susceptibility proteins to activate a hypersensitive response (HR). The HR-generated dead tissue then serves as an ideal nutrition source for this necrotrophic pathogen to allow extensive plant colonisation. Understanding the molecular basis of *N. ditissima* virulence may ultimately lead to the formulation of novel control strategies. Following transcriptome profiling of *N. ditissima* during colonisation of apple fruit and twigs, the most upregulated fungal genes *in planta* were selected for their effector features using bioinformatics tools (SignalP 5.0, SecretomeP 2.0 and EffectorP – fungi 3.0). The tertiary structures of the candidate effectors (CEs) were predicted using AlphaFold2 and then investigated for similar protein structures using the Dali server. Unique predicted functions were identified for three CEs (encoded by *g5185*, *g2092* and *g11625*) with similarity to virulence proteins in other plant necrotrophic fungi. These CE genes were knocked out in *N. ditissima*, through CRISPR-Cas9 gene editing, and a reduction of the virulence phenotype in apple fruit and twigs was observed for the *g5185* knock-out (KO) and the *g2092* KO. Only the *g11625* KO showed a reduction of virulence in apple twigs, which aligns with the *g11625* transcriptomic profile with high upregulation of expression only during twig infection, not fruit infection. The CE encoded by *g5185* is likely associated with the export of plant phytoalexins as the *g5185* KO grew significantly less than wild-type in media augmented with the apple phytoalexin phloretin. Our study reveals novel effectors in the necrotrophic pathogen *N. ditissima*, their predicted function and the potential interplay of their virulence roles in different host tissues.

PM16: Dissecting the mechanism of translocation of *Magnaporthe oryzae* effectors into plant cells

Oliveira-Garcia, E.[1], Vu, A.H.[1], Hamilton, A.J.[1], Martin-Urdiroz, M.[2], Rodriguez-Herrero, C.[2], Dalby, M.[3], Talbot, N.J.[2], Park, J.[4], Park, S.[4], Valent, B.[3]

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Fungal and oomycete pathogens deliver effector proteins directly into plant cells to suppress the plant immune system and reprogram cellular processes to facilitate pathogen biotrophic invasion of plant tissue. Cytoplasmic effectors of the rice blast fungus, *Magnaporthe oryzae*, are secreted via the conventional Golgi-dependent, Brefeldin A (BFA)-sensitive pathway. By contrast, cytoplasmic effectors are secreted by a non-conventional, BFA-insensitive secretion pathway involving the exocyst and SNARE protein Sso1. To date, little is known about the mechanism by which these pathogens translocate effector proteins across the plasma membrane into the plant cytoplasm. We have shown that cytoplasmic effectors within BICs are packaged into dynamic, vesicle-like membranous effector compartments (MECs) that are occasionally observed in the host cell cytoplasm. Live-cell imaging with fluorescently labelled proteins in rice revealed that these MECs colocalize with the plant plasma membrane and with CLATHRIN LIGHT CHAIN 1, a component of clathrin-mediated endocytosis (CME). Inhibition of CME using virus-induced gene silencing and chemical treatments resulted in cytoplasmic effectors in swollen BICs lacking MECs. Furthermore, we identified the first plant plasma membrane-associated effector, Bas83, that appears to play a role in recruiting plant membrane for the endocytic machinery at the BIC. Silencing of the *BAS83* gene suggests a potential role of this effector in fungal virulence. We also identified a BIC-localized lipase-like effector that may be associated with effector release from MECs into the rice cytoplasm. Our study provides evidence that cytoplasmic effector translocation is mediated by CME in BICs and suggests a role for *M. oryzae* effectors in coopting plant endocytosis.

1. Oliveira-Garcia, E., et al. (2023). *Clathrin-mediated endocytosis facilitates the internalization of* Magnaporthe oryzae *effectors into rice cells*. The Plant Cell. 35:2527–2551.

PM17: Sequential breakdown of the *Cf-9* leaf mould resistance locus in tomato by *Fulvia fulva*

de la Rosa, S.[1], Schol, C.R.[2,3], Ramos Peregrina, A.[2,3], Winter, D.J.[4], Hilgers, A.M.[2,3], Maeda, K.[5], Iida, Y.[5], Tarallo, M.[4,6], Jia, R.[3], Beenen, H.G.[3], Rocafort, M.[1], de Wit, P.J.G.M.[3], Bowen, J.K.[7], Bradshaw, R.E.[4,6], Joosten, M.H.A.J.[3], Bai, Y.[2], Mesarich, C.H.[1,6]

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Leaf mould, caused by *Fulvia fulva*, is a devastating disease of tomato plants. During infection, *F. fulva* resides in the leaf apoplast, where it secretes virulence factors, termed effectors, to promote host colonization and disease. In resistant tomato cultivars, however, one or more of these effectors can be recognised by cognate cell surface-localised receptor-like proteins (RLPs) to trigger defence responses that halt *F. fulva* growth. In most commercial tomato cultivars, leaf mould resistance is governed by the *Cf-9* locus, which encodes the RLPs Cf-9C and Cf-9B. Of these, Cf-9C recognizes the previously identified Avr9 effector and provides resistance during all stages of plant growth, whereas Cf-9B recognises the yet-unidentified Avr9B effector and provides resistance in mature (flowering/fruiting) plants only. Over the last decade, *F. fulva* strains have emerged worldwide that can overcome the *Cf-9* locus, with *Cf-9C* circumvented through *Avr9* deletion. To understand how *Cf-9B* is circumvented, we set out to identify *Avr9B*[1]. Comparative genomics, transient expression assays and gene complementation experiments were used to identify *Avr9B*, with *Cf-9B* circumvention shown to be through gene deletion/mutation. Gene sequencing was used to assess *Avr9B* allelic variation across a worldwide strain collection. A strict correlation between *Avr9* deletion and resistance-breaking mutations in *Avr9B* was observed in strains recently collected from *Cf-9* cultivars, whereas *Avr9* deletion but no mutations in *Avr9B* were observed in older strains. This suggests that *F. fulva* has evolved to sequentially break down the *Cf-9* locus, with *Cf-9C* circumvented prior to *Cf-9B*.

1. de la Rosa, S., C.R. Schol, A. Ramos Peregrina, D.J. Winter, A.M. Hilgers, K. Maeda, Y. Iida, M. Tarallo, R. Jia, H.G. Beenen, M. Rocafort, P.J.G.M. de Wit, J.K. Bowen, R.E. Bradshaw, M.H.A.J. Joosten, Y. Bai and C.H. Mesarich (2024). *Sequential breakdown of the* Cf-9 *leaf mould resistance locus in tomato by* Fulvia fulva. New Phytologist. https://doi.org/10.1111/nph.19925.

PM18: *Phytophthora* cytoplasmic effectors undergo two independent cleavage events

Xu, L.[1], Wang, S.[1], Wang, W.[1], Wang, H.[1,2], Welsh, L.R.J.[3], Whisson, S.C.[3], Boevink, P.C.[3], Birch, P.R.J.[1,3]

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Phytophthora species continue to present a serious threat to agriculture and the natural environment. Secreted effectors are critical to the pathogens' infection success and the translocated RXLR class appear to be the most important. We have shown that RXLR effectors are secreted from *Phytophthora* non-conventionally, despite having signal peptides. We are investigating what this non-conventional secretory pathway involves. It was first shown 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 for western blotting and proteomic analyses we show consistent cleavage at the RXLR, including at some degenerated motifs. We reveal that the EER motif represents a second cleavage site and define the exact sites of cleavage at the two motifs. Analysis of the secreted protein fractions indicates that cleavage of the effector influences the secretion pathway. We propose a model for how the RXLR motif may function in the secretory pathway selection.

1. Xu et al. (2024). *Proteolytic processing of both RXLR and EER motifs in oomycete effectors*. bioRxiv https://doi.org/10.1101/2024.04.16.589758.

PM19: RXLR40, a broad cell death suppressor of the kauri dieback pathogen *Phytophthora agathidicida*, targets a plant BTB domain-containing protein

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Phytophthora agathidicida is a soil-borne pathogen that causes root and collar dieback of New Zealand kauri (*Agathis australis*), which is one of the world's largest ancient Araucariaceae conifer species and has immense cultural significance for Māori. *Phytophthora* pathogens produce both intracellular and extracellular virulence factors, termed effector proteins, that they use to infect their hosts and to suppress the host's immune system. The most well studied of these are the intracellular RXLR effectors. Many *Phytophthora* RXLR effectors have a role in virulence by targeting different host molecules to suppress host immunity. A recent study identified 147 RXLR-encoding genes from the *P. agathidicida* genome. Among those, we identified a gene that encodes an RXLR effector, PaRXLR40, which localizes to the nucleus and suppresses plant immunity triggered by a range of other intracellular and extracellular *P. agathidicida* effector proteins. Using a 'yeast-two-hybrid' method that enables the identification of proteins that interact with each other, and a model host plant, we identified the putative plant target of PaRXLR40: a BTB domain-containing 'ARIA' protein that also localizes to the nucleus. The target ARIA protein also suppresses cell death elicited by intracellular and extracellular cell death-eliciting proteins of *P. agathidicida*. This suppression activity was lost when a hairpin RNA-mediated silencing construct was used to transiently knockdown the genes encoding the ARIA target in the alternative host *N. benthamiana*. Moreover, both the RXLR40 effector and the ARIA host target protein appear to have important roles in enhancing colonization by *P. agathidicida*. This study provides an important foundation for studying the molecular basis of plant–oomycete interactions in gymnosperm forest trees. Moreover, the identification of host targets might ultimately provide molecular markers for selection or breeding for disease resistance in forest trees. Alternatively, RNAi-based silencing of key pathogen virulence factors might be an effective pathogen control strategy.

PM20: Saving your smashed avocado toast – Biochemical analysis reveals the multi-modal function of the oomyceticide phosphite in an oomycete pathosystem

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Phytopathogenic oomycetes constitute some of the most devastating plant pathogens and cause significant natural biodiversity and economic losses. The phytopathogen *Phytophthora cinnamomi* causes dieback disease in native vegetation and major horticultural crops including avocadoes, nuts and stone fruits. The oomyceticide phosphite is a commonly used chemical to control dieback caused by *P. cinnamomi* infection. Despite its widespread use, the mode of action of phosphite is not well understood. It is unclear whether it targets the pathogen, the host, or both. Resistance to phosphite is emerging in *P. cinnamomi* isolates and other oomycete phytopathogens. The mode of action of phosphite on phosphite-sensitive and -resistant pathogen isolates and through a model host was investigated using label-free quantitative proteomics. *In vitro* treatment of sensitive *P. cinnamomi* isolates with phosphite hinders growth by interfering with metabolism, signalling and regulation of gene expression; traits that are not observed in the resistant isolate. When the model host *Lupinus angustifolius* was treated with phosphite, an increased abundance of defence-related proteins was also observed. Surprisingly, we also observed that host proteins associated with photosynthesis, carbon fixation and lipid metabolism were enriched in abundance. This suggests that phosphite may function as a biostimulant in the plant which we are validating with crop growers. We hypothesise the multi-modal action of phosphite and present several models constructed using comparative proteomics that demonstrate mechanisms of pathogen and host responses to phosphite.

PM21: Effector knockout competition reveals individually redundant effectors are collectively required for successful virulence

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The 2010 incursion of Pseudomonas syringae pv. actinidiae (Psa) in New Zealand devastated susceptible kiwifruit orchards. The kiwifruit industry has since recovered following the deployment of more tolerant cultivars. However, little is known about the extent to which the Psa population is evolving in orchards and further adapting to its kiwifruit hosts. Effector proteins help pathogens invade their host, extract nutrients, and suppress immunity. Psa is an emergent kiwifruit pathogen with over thirty functional effectors, providing a unique opportunity to understand how host selection shapes pathogen evolution. In particular, we sought to understand whether Psa requires all its effectors to cause disease. Recent research suggests that only a few Psa effectors are required for virulence. Further still, resistant kiwifruit hosts such as Actinidia arguta can recognise several Psa effectors. Why, then, does Psa retain so many effectors in its repertoire? Using complementary approaches of orchard-based genome biosurveillance and effector knockout strain competition, we sought to examine effector requirements, redundancies, and repertoire refinement across different kiwifruit hosts. The selective pressure exerted by serially passaging a competitive pool of effector knockout strains in planta allowed us to detect subtle contributions of effectors to virulence. While the majority of Psa's effectors previously appeared to be non-essential, competition results suggested they may be collectively required for successful virulence. Competition has also revealed new host-specific effector requirements. This research provides important insights into the evolution of emergent pathogens and will ensure future resistance breeding efforts are robust and sustainable.

PM22: Unlocking the secrets of tandem kinase proteins: direct binding of a fungal effector sparks plant defence

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Plant immunology focuses on two well-characterized receptor classes: surface localized Pattern Recognition Receptors (PRRs) and intracellular Nucleotide-binding Leucine-Rich Repeat (NLR) proteins. These receptors have been described in exquisite molecular detail. Recently, tandem kinase proteins (TKPs) have emerged as new immune regulators conferring robust pathogen resistance. Here, we focus on the wheat RWT4 TKP, that confers resistance to the devastating fungal pathogen *Magnaporthe oryzae.* We established a rice protoplast system, revealing RWT4 specifically recognizes the *Magnaporthe* AvrPWT4 effector, leading to the transcription of defense genes and inducing cell death. RWT4 possesses both kinase and pseudokinase domains, with its kinase activity essential for defense. RWT4 directly interacts with and transphosphorylates AvrPWT4. Sequence similarity and structural modeling revealed an integrated partial kinase duplication in RWT4's kinase region as critical for effector interaction and defense activation. Investigation of TKPs throughout the plant kingdom revealed diverse integrated domains are common. Collectively, these experiments provide some of the first mechanistic insights into TKP activation.

PM23: Smut fungal conserved *N*-glycosylated effector manipulates plant cell wall dynamics

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Pectin is dynamically regulated through methyl-esterification by pectin methylesterase (PME) and its inhibitor, PMEI, influencing plant resilience against pathogens. De-methylesterified pectin by PMEs renders the cell wall susceptible to polygalacturonase degradation, facilitating pathogen invasion, while PMEI inhibits PME activity. However, how fungal pathogens overcome the defensive role of PMEI remains unclear. Here, we report that the smut fungus *Ustilago maydis* effector Nge1 regulates host cell wall dynamics by blocking PMEIs in an *N*-glycosylation-dependent manner. Through the combination of coimmunoprecipitation-LC-MS/MS and AlphaFold pairwise structure alignment, we discover that Nge1 targets at least two PMEIs in a glycosylation-dependent manner. Protein-protein interaction analysis further reveals that Nge1 rescues PME19 from the inhibition of PMEI45 and PME20 from the inhibition of a yet-to-be-identified PMEI. The overexpression of Nge1 increases the level of low-methylated pectin and promotes fungal virulence, suggesting that rescuing PMEs from inhibition by specific PMEIs to demethylate pectin, presumably loosening cell walls, is a critical virulence function of Nge1. The successful complementation of Δ*nge1* with Nge1 orthologs harboring the glycosylation site highlights a novel and widely conserved *N*-glycosylation-dependent virulence function in smut fungi. This is conferred by its ability to counteract PMEIs to manipulate host cell wall dynamics, thereby favoring fungal virulence.

PM24: Comparative genomics identifies *Ecp5* as the *Avr6* avirulence effector gene of *Fulvia fulva* corresponding to the recently deployed *Cf-6* leaf mold resistance locus of tomato

Schol, C.R.[1,2], Hu, A.[1], Jia, R.[1], Veenendaal, L.[2], de la Rosa, S.[3], Mesarich, C.H.[3,4], Fokkens, L.[1], Bai, Y.[2], Joosten, M.H.A.J.[1]

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Fulvia fulva (previously known as *Cladosporium fulvum*) is a fungal pathogen causing tomato leaf mold. Genetic resistance, mediated by cell-surface anchored, receptor-like Cf proteins that recognize their corresponding *F. fulva*-secreted Avr effectors, is an important defense strategy against the disease. For example, the gene products of two genes at the widely deployed *Cf-9* locus, *Cf-9B* and *Cf-9C,* mediate resistance by recognition of the *F. fulva* effectors Avr9B and Avr9, respectively. In recent years, however, the *Cf-9* resistance locus has been overcome by *F. fulva* strains harbouring mutated and/or deleted *Avr9B* and *Avr9* genes, prompting breeders to deploy an alternative resistance locus, *Cf-6*, of which the putative gene product recognizes the so-far unidentified effector Avr6. In this study, we set out to identify *Avr6* by means of a comparative genomics strategy. Whole genome sequencing of a panel of *F. fulva* isolates, some of which break *Cf-6*-mediated resistance, revealed that a previously characterized *F. fulva* effector, *Ecp5*, is either deleted or mutated. We then validated that Ecp5 is Avr6 by means of potato virus X (PVX)-mediated expression of Ecp5 in *Cf-6* tomato plants, gene knockout of *Ecp5* in *F. fulva* and complementation of *Cf-6*-breaking strains with wild-type *Ecp5*, after which *Ecp5* was renamed to *Avr6*. Additionally, we studied naturally occurring allelic variation in *Avr6* and found that several Argentinean strains harbouring polymorphisms in *Avr6* allow these to overcome *Cf-6*-mediated resistance. Finally, we applied AlphaFold to generate a high confidence structural prediction to show that Avr6-like proteins are present in multiple plant-pathogenic fungi, which could potentially advance resistance breeding in other crops. The identification of *Avr6* and strains that can overcome *Cf-6* is valuable information for breeders, as it demonstrates that a recently deployed resistance gene is already broken and more durable resistance breeding strategies should be considered.

PM25: Discovering early infection strategies by plant-pathogenic fungi of the Ascomycota phylum

Shrestha, D.K.[1], Dijkwel, P.D.[1], Sehrish, T.[1,*], Pilkington, K.M.[1,*], Dassanayaka, M.P.[2,**], Fan, B.[3], Hallett, I.C.[4,***], Sutherland, P.W.[4,***], David, C.C.[4], Frampton, R.A.[4,***], Angelin-Bonnet, O.[4], Butler, R.C.[5,***], van Toor, R.F.[5,***]

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The phylum Ascomycota includes important plant-pathogenic fungi that cause a significant reduction in crop production and threaten food security. Application of chemical pesticides against these pathogens adversely affect ecosystems and human health, and more sustainable strategies are required. In this project, we aim to understand the conserved pathways that regulate the early infection phase of pathogenic fungi in three representative model pathogen-host combinations of the Sclerotiniaceae family of Ascomycota. *Botrytis cinerea* and *Sclerotinia sclerotiorum* are broad-range fungi that cause grey and white mould, respectively. In contrast, *Ciborinia camelliae* is host-specific, exclusively infecting camellia blooms. For the early infection-phase transcriptomics study, RNA samples from pathogen spore-inoculated substrates were obtained at 0, 3, 6, 12 and 24 hours post inoculation (hpi). Leaves of susceptible Arabidopsis were inoculated with *B. cinerea* and *S. sclerotiorum*, while camellia petals were inoculated with *C. camelliae* and *B. cinerea*. Comparative transcriptomics from the spore-inoculated samples showed that the change in pattern of pathogen gene expression in all the tested pathosystems occurred after 12 hpi. Therefore, we focused on 12 hpi gene expression and identified 10 common pathogen genes that were induced at that time. The identified genes are related to pathogen development, host-cell wall degradation, protein degradation and intercellular transportation. *B. cinerea* knock-out mutants are being constructed to validate the functionality of these genes, and the efficacy of RNA interference constructs that target these genes in the three pathogens will be evaluated in pathogenicity experiments.

PM26: The role of mini-chromosomes in adaptive evolution of the blast fungus

Barragan, A.C.[1], Latorre, S.M.[2], Malmgren, A.[1], Harant, A.[1], Win, J.[1], Sugihara, Y.[1,4], Burbano, H.A.[2], Kamoun, S.[1], Langner, T.[3]

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Genome structure and maintenance determine the evolvability of organisms. The genomes of fungal plant pathogens are often structured heterogeneously, harboring highly variable compartments and compartments of relative stability. Often, rapidly evolving, virulence-related genes are associated with dynamic regions that are rich in repetitive, transposable elements and accessory genomic regions. An extreme case of such genomic structural variation are supernumerary mini-chromosomes (mChr), that are present in only some individuals of a species. We identified diverse mChr in the wheat- and rice-infecting blast fungus, *Magnaporthe* (syn. *Pyricularia*) oryzae. These chromosomes are associated with intra- and inter-chromosomal rearrangements, copy number variation and horizontal transfer of genetic material and ultimately increase the genetic diversity and the adaptive potential of the blast fungus. Here, I will discuss recent progress we made towards understanding mChr biology and their contribution to gene flow and adaptive evolution in the blast fungus.

PM27: Applications of pan-genomics for phenotype-based effector discovery and crop-disease surveillance

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Crop diseases caused by fungal pathogens are coordinated by interactions between effectors and host receptors. Accurate effector prediction relies on combining multiple methods[1], but efficiently bridging the gap to functional validation is challenging due to mutation and sequence diversity. To improve existing approaches by incorporating statistical tests for association with disease phenotype scores, we developed a method called “EffectorFisher”, which accurately predicted known (cultivar-specific) effector loci from pan-genome data of the necrotroph *Parastagonospora nodorum*. EffectorFisher indicated the relative aggression of effector isoforms versus a panel of wheat cultivars. The hemibiotroph *Zymoseptoria tritici* produced similar results, indicating EffectorFisher can predict both gene-for-gene and inverse interactions. Simulation of low-quantity pan-genome data and non-quantitative phenotyping indicated that EffectorFisher is broadly applicable to datasets of varying quality, and could become a useful tool for monitoring crops for emerging effector-susceptibility. Towards this goal, and to enable reproducibility across multiple pathosystems, we incorporated EffectorFisher and other workflows into the “MycoProcessor” toolkit. We use MycoProcessor for these steps: 1) QC, contamination filtering and assembly; 2) gene annotation; 3) repeat analysis; 3) functional annotation; 4) effector and fungicide-resistance profiling (Predector[1], EffectorFisher, FRAST[2]); 5) variant calling and phylogeography; and 6) comparative genomics.

1. Jones, D.A.B. et al. (2021). *An automated and combinative method for the predictive ranking of candidate effector proteins of fungal plant pathogens.* Scientific Reports. 11: 19731.
2. Oliver, R.P. et al. (2024). *The 2023 update of target site mutations associated with resistance to fungicides and a web-tool to assist label designations.* Journal of Plant Diseases and Protection. 1-6.

PM28: Dikaryotic organisation and regulation of the complex wheat stripe rust fungus genome revealed by a telomere-to-telomere haplotype-phased assembly

Tam, R.[1], Möller, M.[1], Luo, R.[1], Luo, Z.[1], Jones, A.[1], Periyannan, S.[2], Rathjen, J.[1], Schwessinger, B[1].

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The stripe rust fungus (*Puccinia striiformis* f. sp. *tritici*) is a pathogen that threatens global wheat production. Its predominant asexual life stage that causes pandemics on wheat is associated with dikaryotic (two haploid nuclei) genome organisation. How this contributes to its extraordinary capacity for rapid host adaptation remains poorly understood because of the lack of accurately haplotype-resolved genomes for this species. We used Oxford Nanopore (ONT) Q30+ duplex sequencing combined with Hi-C proximity data to generate a telomere-to-telomere, fully nuclear-phased chromosome assembly for a long-term asexual lineage of this pathogen. We defined centromeres using Hi-C and ONT-derived CpG methylation data and demonstrated their lack of sequence conservation and enrichment for retrotransposons. A single methylation dip region appeared consistently across all centromeres, suggestive of sequence-independent kinetochore recognition sites. Each nucleus carries a unique array of ribosomal DNA (rDNA) units with over 200 copies, which harbour nucleus-specific variations within the intergenic spacers. We speculate that nuclear-specific rDNA homogeneity might be maintained by compartmentalised concerted evolution via the physically separated inheritance of each nucleus. We annotated the genome comprehensively using long-read ONT cDNA datasets representing six conditions including spore dormancy and host infections. We captured allele-specific expression (ASE) bias for up to 15% of the biallelic heterozygous genes across the sampling conditions. Secretome genes, which include virulence effectors, were enriched in ASE when compared to evolutionary conserved genes (BUSCOs). The secretome ASE genes exhibit significant methylation differences between differentially expressed alleles at their transcription start sites, whereas BUSCOs showed no methylation differences. This novel insight implies distinct mechanisms regulating core and accessory functions. Our study sheds new light into the genome biology and adaptive evolutionary potential of rust fungi, with important implications for managing their agricultural impacts.

PM29: Identification of candidate avirulence effector genes in the wheat stripe rust fungus

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The wheat stripe rust fungus *Puccinia striiformis* f. sp*. tritici* (*Pst*) secretes effectors to facilitate infection of its plant host. Some effectors, known as avirulence (Avr) proteins, are recognized by wheat resistance (R) proteins, triggering defence responses. Identifying and implementing suitable R genes is crucial for breeding disease-resistant wheat varieties. This study aims to identify Avr candidates in *Pst* and investigate their interactions with known R proteins using a novel wheat protoplast screening assay.

Combing genomic and transcriptomic analyses, we identified effector candidates that might be recognised by the products of extensively described wheat R genes. First, we identified hemizygous effectors and shortlisted the most highly expressed of these using a high-resolution Nanopore long-read cDNA infection time series. Based on this analysis, we synthesized 66 candidates Avr genes which we screened against specific R genes that had previously been overcome in Australia. Here, we will present several of these new *Pst* Avr candidates.

Identification of smartly chosen Avr effectors demonstrates the efficacy of protoplast screening in uncovering key components of pathogen-host interactions. These findings provide valuable insights into the molecular mechanisms underlying wheat resistance to stripe rust. Future research will focus on the functional characterization of these Avr-R gene interactions.

Summary of Abstracts for the Poster Session -
Plant-Microbe-Interactions

No.	Title	Presenter	Institutions
PM30	AvrRvi4 is a ToxA-like effector of the apple scab fungus, *Venturia inaequalis*, and is recognized by the Rvi4 NLR resistance protein of apple	C.H. Mesarich	School of Agriculture and Environment, Massey University, Palmerston North, NZ. Bioprotection Aotearoa, Massey University, Palmerston North, NZ
PM31	Conserved candidate effector proteins from fungal and oomycete foliar pine pathogens	R.E. Bradshaw	Bioprotection Aotearoa, School of Food Technology and Natural Sciences, Massey University, Palmerston North, NZ
PM32	CRISPR-Cas9-mediated analysis of virulence factors in the pathogenic fungus, *Neonectria ditissima*	R.S. Panting	School of Biological Sciences, University of Auckland, Auckland, NZ. The New Zealand Institute for Plant and Food Research Limited, Mt Albert, Auckland, NZ
PM33	Generation and characterisation of nanobodies targeting a *Magnaporthe oryzae* virulence effector	B. Silke	Research School of Biology, Australian National University, Canberra, ACT, Australia
PM34	Insights from a dark environment: osmotic stress and PAMP-triggered immunity modulate ROS and Ca2+ signaling in *Arabidopsis* roots	C.N. Meisrimler	Department of Biology, University of Canterbury, Christchurch, NZ. Bioprotection Aotearoa, Lincoln University, Christchurch, NZ. Biomolecular Interaction Centre, University of Canterbury, Christchurch, NZ, 4AgResearch, Lincoln, NZ

PM35	The contribution of neighbouring plants on native Maire Tawake (*Syzygium maire*) through the phyllosphere microbiome and mātauranga Māori	H. Ehau-Taumaunu	The New Zealand Institute for Plant and Food Research Limited, Private Bag 92169, Auckland Mail Centre, Auckland 1142, NZ. Bioprotection Aotearoa, PO Box 85084, Lincoln University, Lincoln 7647, NZ
PM36	The interactions of *Bremia lactucae* RxLR effector proteins BLR05 and BLR09 with NAC transcription factors	C. Mercer	School of Biological Science, University of Canterbury, Christchurch, NZ
PM37	The mess of stress: exploring 14-3-3 protein interaction with ANAC013 and ANAC017	T. Curtis	Department of Biology, University of Canterbury, Christchurch, NZ
PM38	The molecular basis of FOLD effector recognition by a resistance protein in the *Fol*-tomato pathosystem	H.N. Trantino	Research School of Biology, Australian National University, Canberra, Australia
PM39	The potential function of dormancy-associated (DRM) disordered proteins in plant-pathogen interactions	M. Wood	The New Zealand Institute for Plant & Food Research Limited, Mt Albert Research Centre, Auckland, NZ
PM40	Towards biochemical characterisation of wall-associated kinases (WAKs) from wheat and canola	N. Tasneem	Research School of Biology, College of Science, Australian National University, Australia

PM30: AvrRvi4 is a ToxA-like effector of the apple scab fungus, *Venturia inaequalis*, and is recognized by the Rvi4 NLR resistance protein of apple

de la Rosa, S.[1], Winter, D.J.[2], Arshed, S.[3], Bus, V.G.M.[4], Bowen, J.K.[3], Baudin, M.[5], Sannier, M.[5], Dagg, T.J.[1,6], Tarallo, M.[2,6], Mosen, A.M.[1,6], Rocafort, M.[1], Plummer, K.M.[7], Le Cam, B.[5], Schouten, H.J.[8], Bradshaw, R.E.[2,6], Mesarich, C.H.[1,6]

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Scab or black spot, caused by the biotrophic fungus *Venturia inaequalis*, is the most economically important disease of apples worldwide. During infection, *V. inaequalis* resides in the subcuticular environment of apple leaves and fruit, where it secretes an arsenal of virulence factors, termed effector proteins, to promote host colonization and disease. In resistant apple cultivars, however, one or more of these effector proteins can be recognised by cognate resistance proteins to trigger defence responses that ultimately halt *V. inaequalis* growth. In New Zealand, a strain of *V. inaequalis*, NZ203.1, was identified that could overcome the *Rvi4* resistance gene of apple, which encodes a nucleotide-binding/leucine-rich repeat (NLR) resistance protein. To understand how *Rvi4*-mediated resistance is overcome, we set out to identify the corresponding effector gene, *AvrRvi4*, using a bulked segregant analysis based on progeny from a sexual cross between NZ203.1 and a *V. inaequalis* strain that carries a functional copy of *AvrRvi4*, J222. Using this approach, we identified a single gene of interest that encodes a small, secreted, cysteine-rich protein with predicted structural homology to ToxA effectors from other plant-pathogenic fungi. In strain NZ203.1, this gene has been disrupted through the insertion of a transposable element. Notably, the candidate AvrRvi4 protein triggers a weak resistance response upon co-expression with Rvi4 in cells of the model non-host plant, *Nicotiana benthamiana*, indicating that it is AvrRvi4. In support of this, the gene that encodes this protein was found to be independently mutated in several Rvi4-breaking strains of *V. inaequalis* collected from France. Interestingly, *AvrRvi4* belongs to a seven-gene family in *V. inaequalis*, but also has homologs in other plant-pathogenic species of *Venturia*. We are currently investigating whether the proteins encoded by these homologs can be recognized by Rvi4, as this may have implications for cross-species resistance.

PM31: Conserved candidate effector proteins from fungal and oomycete foliar pine pathogens

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Dothistroma needle blight, Cyclaneusma needle blight and Red Needle Cast are devastating diseases of *Pinus radiata* in New Zealand, caused by the fungi *Dothistroma septosporum*, *Cyclaneusma minus* and oomycete *Phytophthora pluvialis*, respectively. These pathogens colonize the apoplastic host environment, secreting effector proteins to promote disease. If these effectors are recognized by corresponding host resistance proteins, they activate the plant immune system to stop pathogen growth. Two *D. septosporum* candidate effectors, Ds69335 and Ds131885, were identified with homologues in both *C. minus* and *P. pluvialis*. Their protein structures were analysed using AlphaFold2 and the corresponding genes disrupted through CRISPR/Cas9 to study their function during pine infection. Ds69335 is structurally similar to proteins with known roles in fungal virulence and *Ds69335*-disrupted strains showed decreased fungal biomass *in planta* compared to wild-type (WT), suggesting a possible role in virulence. Ds131885 elicited cell death in *Nicotiana* species and *P. radiata*, showed structural similarity to a cross-kingdom PAMP and was recognized by a *Nicotiana benthamiana* immune receptor, triggering defence responses. Disruption of *Ds131885* did not convincingly alter fungal biomass. Unexpectedly, none of the complementation strains generated for either Ds69335 or Ds131885 restored WT fungal biomass. Despite the ambiguous complementation results, these candidate cross-kingdom effectors deserve further exploration as they might ultimately hold the key to selection for broad spectrum resistance in pines.

PM32: CRISPR-Cas9-mediated analysis of virulence factors in the pathogenic fungus, *Neonectria ditissima*

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Neonectria ditissima is an ascomycete pathogen of apples and pears and is the causal agent of European Canker. This Sordariomycete can manifest symptoms at any point during the growing season, as a cankerous growth that slowly encircles and kills branches. This disease is responsible for tens of millions of dollars' worth of crop losses annually in New Zealand alone. It is imperative that we develop alternative control strategies for this disease, a goal which can only be accomplished by building our knowledge of the molecular interactions between pathogen and host plant.

Like many plant pathogens, *N. ditissima* uses effectors to enable infection in the host plant: small, genetically encoded molecules that can perform a variety of functions in the process of causing disease. A recently identified candidate effector, encoded by *g9941*, is thought to be important to the pathogenicity of this organism, as it is upregulated during infection and shares structural homology to a known ToxA-like effector in *Fusarium oxysporum* (Florez, 2024). By designing gRNA primers specific to the *g9941* gene, we were able to generate a plasmid construct with the goal of knocking out this gene in *N. ditissima* using CRISPR-Cas9 selective editing tools. We hypothesise that by utilising this CRISPR-Cas9-based methodology to selectively knock out *g9941* function, we can build our understanding of the necessity of this gene to the pathogen's ability to cause disease.

1. Florez, L. (2024). *Unravelling the virulence mechanisms of* Neonectria ditissima*, a fungal pathogen of apple*. [Unpublished PhD thesis]. The University of Auckland.

PM33: Generation and characterisation of nanobodies targeting a *Magnaporthe oryzae* virulence effector

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Rice blast disease, caused by the hemibiotrophic fungus *Magnaporthe oryzae,* is responsible for significant crop yield losses. We have previously identified an effector, MoNUDIX, which is required for full pathogen virulence[1]. MoNUDIX functions as an enzyme which hydrolyses inositol pyrophosphates. Indeed, this enzymatic activity is critical for full virulence. Therefore, targeting MoNUDIX has the potential to reduce the severity of rice blast disease. To inhibit MoNUDIX, we sought to develop MoNUDIX-binding nanobodies. We identified a set of nanobodies which bind to MoNUDIX. We optimised expression of these nanobodies in prokaryotic expression systems to enable characterisation of binding and inhibition. We also characterised nanobody-dependent inhibition of MoNUDIX in transient expression systems. These data characterise binding and inhibition for a repertoire of nanobodies which bind to MoNUDIX. This repertoire will aid understanding of the basis of MoNUDIX-dependent virulence and by extension allow design and development of specific immune receptors to protect rice against *M. oryzae.*

1. C. L. McCombe et al. (2023). *Plant pathogenic fungi hijack phosphate starvation signaling with conserved enzymatic effectors*. bioRxiv. 2023.2011.2014.566975.

PM34: Insights from a dark environment: osmotic stress and PAMP-triggered immunity modulate ROS and Ca^{2+} signaling in *Arabidopsis* roots

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Signal transduction enables plants to detect and respond to environmental stress. Calcium (Ca^{2+}) and reactive oxygen species (ROS) are key signalling molecules in plants, regulating plant immunity and programmed cell death. However, the specificity of Ca^{2+} and ROS signalling responses remains unclear. We established a bidirectional dual-flow RootChip microfluidic device to expose G-CaMP3 and HyPerGFP *A. thaliana* primary roots to asymmetric solute gradients. We utilized linear one-sided concentration gradients for PEG, NaCl, and pathogen-associated molecular patterns (PAMPs) flg22 and PEP13. Additionally, we infected *A. thaliana* roots with *Phytophthora* spores and mycelium. Fluorescent live-cell imaging was used to detect and quantify cytosolic Ca^{2+} and ROS in parallel to root growth measurements and spore localization. Importantly, the plants' Ca^{2+} and ROS spatiotemporal signal patterns in response to treatments are significantly different and indicate cell-type-specific response mechanisms. To gain further insight into the molecular regulations during osmotic stress and PAMP-triggered immunity, we used a label-free quantitative proteomics approach to compare changes in protein abundance and phosphorylation in roots 5 minutes post-PEG and post-flg22 treatment in comparison to control. A computational approach was chosen for network analysis, 3D structural modelling, and protein conservation studies for proteins of interest. We will further discuss specific proteins and their potential role in plant infection and increased pathogenicity during drought and osmotic stress conditions.

PM35: The contribution of neighbouring plants on native Maire Tawake (*Syzygium maire*) through the phyllosphere microbiome and mātauranga Māori

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Maire Tawake (*Syzygium maire*) is an Indigenous and endemic tree species of the Myrtaceae family found in swamp and wetlands in Aotearoa New Zealand. Within the country, this species is the sole representative of the genus *Syzygium* and is regarded as uncommon due to land clearing and swamp draining. Maire Tawake is now critically endangered due to its extreme susceptibility to myrtle rust (*Austropuccinia psidii*) and requires constant protection for survival. Research is currently underway to understand the microbiome composition and microbial antagonistic potential of the Myrtaceae family members. However, since this tree is rare, Maire Tawake research has been limited. The purpose of this research is to explore the contributions of neighbouring phyllosphere microbiomes to Maire Tawake and how the associated microorganisms are connected through Indigenous knowledge and whakapapa (genealogy). Leaf samples from the bottom canopy of Maire Tawake were collected from three geographical locations across the North Island of Aotearoa, with neighbouring plants (including trees, ferns, and vines) selected within five metres of the focal Maire Tawake at least one metre above the ground floor. Amplicon regions were targeted for metabarcoding analyses to cover bacterial, fungal, oomycete and protist diversity. We aim to investigate (1) the associated phyllosphere microbial composition and structure, (2) explore differences and similarities across geographical regions, and (3) propose unique whakapapa of the Maire Tawake microbiome using Indigenous knowledge.

PM36: The interactions of *Bremia lactucae* RxLR effector proteins BLR05 and BLR09 with NAC transcription factors

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The obligate biotrophic oomycete *Bremia lactucae* is a lettuce-infecting downy mildew with significant economic impact worldwide. Membrane-bound NAC transcription factors (TF) are conserved across many plant species (e.g., lettuce, potato and *Arabidopsis*) and translocate from the endoplasmic reticulum (ER) to the nucleus during pathogenic stress. Translocation of NAC TFs is hypothesised to regulate cellular reactive oxygen species and host cell death, hindering pathogen colonisation. *B. lactucae* produces host-translocated RxLR effector proteins, BLR05 and BLR09, which inhibit NAC translocation and cell death. This research aims to further characterise the relationship between BLR05, BLR09 and NAC TFs.

Preliminary data suggest dimerisation of BLR05 and BLR09. Co-Immunoprecipitation and yeast two-hybrid have confirmed the dimerisation pattern of BLR05 and BLR09. Transient expression of BLR05, BLR09, ANAC13 and ANAC17 in *Nicotiana benthamiana* epidermis cells have been imaged by confocal microscopy, confirming localisation at the ER membrane. The localisation patterns of independent replicates have additionally been quantified to evidence localisation differences in the ER. Previous studies of BLR05 and BLR09 identified the C-terminal transmembrane domain as being necessary for effector interaction with NAC TFs. Bioinformatic analysis and sequence alignment results have identified conserved amino acid sequences within oomycete effectors potentially necessary for effector-NAC interaction.

The characterisation of BLR05 and BLR09 and their interactions with conserved NAC TFs will reveal protein characteristics necessary for infection. Knowing these characteristics are beneficial for future development of *B. lactucae* and other oomycete-preventative treatments, which would reduce the effect of downy mildew disease in cultivated lettuce.

PM37: The mess of stress: exploring 14-3-3 protein interaction with ANAC013 and ANAC017

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Plant growth, development, and function are significantly impacted by biotic and abiotic factors. Plants rely on cellular signalling networks to allow for efficient adaptation and survival. 14-3-3 proteins are heavily involved in plant signalling pathways as they bind to phosphorylated targets. Phosphorylation is a protein post-translational modification frequently used in plant signal transduction. The presented research aims to elucidate the interaction of *Arabidopsis thaliana* 14-3-3 proteins and membrane-bound transcription factors, ANAC013 and ANAC017. Initially, the interaction of 14-3-3 with ANAC013 and ANAC017 was identified by co-immunoprecipitation (coIP) followed by mass spectrometry. Computational analysis confirmed a conserved phospho-hub in ANAC013/017 as potential targets of 14-3-3. Protein interaction modelling for ANAC017 with 14-3-3 identified an amphipathic binding groove that interacts with the NAC phospho-hub. Strengthening the likelihood of an interaction, the model identified the bond distance to be 3.3 Å, a strong non-covalent interaction. Transient expression in *Nicotiana benthamiana* was used for localisation and co-localisation assays, followed by Western blotting to confirm protein presence, stability and interaction. During single expression, 14-3-3 was observed to localise in the cytosol. In contrast, during co-expression of 14-3-3 with the NACs, localisation of both proteins was observed to be in the endoplasmic reticulum (ER), indicating an interaction. Currently, work is focusing on further confirming the hypothesised interaction through coIP and yeast-2-hybrid assays. Quantitative RT-PCR will be used to determine the effect of stressors on 14-3-3 gene expression levels. By elucidating NAC pathways, their interactions, and mechanisms, we gain insights on promising targets for crop enhancement associated to plant immunity and plant abiotic stress adaptation.

PM38: The molecular basis of FOLD effector recognition by a resistance protein in the *Fol*-tomato pathosystem

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Fungal plant pathogens secrete proteins, known as effectors, that function in the apoplast and inside plant cells to promote virulence. Despite their importance, our understanding of effector function and detection by immunity receptors remains poor. Within the secreted in xylem (SIX) effectors of *Fusarium oxysporum f. sp. lycopersici* (*Fol*), we recently identified a new structural class of effectors using X-ray crystallography[1,2]. We show that the recognised effectors Avr1 (SIX4) and Avr3 (Six1) represent founding members of the ***Fol* d**ual-domain (FOLD) effector class. Using the structure of Avr1 and modelling the interaction with the I receptor, we identify residues in both the effector and receptor that are crucial for recognition. We show that a single amino acid mutation is capable of rescuing recognition in an allelic variant of I that cannot recognise Avr1. Collectively, these insights will aid future studies to understand the molecular mechanisms governing effector function and recognition in *F. oxysporum*, potentially enabling the development of engineered immunity receptors with novel recognition specificities to combat *Fusarium* wilt disease more effectively.

1. Yu, D.S., M.A. Outram, E. Crean, A. Smith, Y. Sung, R. Darma, X. Sun, L. Ma, D.A. Jones, P.S. Solomon and S.J. Williams (2022). *Optimized production of disulfide-bonded fungal effectors in* Escherichia coli *using CyDisCo and FunCyDisCo coexpression approaches*. Molecular Plant-Microbe Interactions. 35:2, 109-118.

2. Yu, D.S., M.A. Outram, A. Smith, C.L. McCombe, P.B. Khambalkar, S.A. Rima, X. Sun, L. Ma, D.J. Ericsson, D.A. Jones, and S.J. Williams (2024). *The structural repertoire of* Fusarium oxysporum *f. sp.* lycopersici *effectors revealed by experimental and computational studies*. eLife 12:RP89280.

PM39: The potential function of dormancy-associated (DRM) disordered proteins in plant-pathogen interactions

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Intrinsically disordered proteins (IDPs) are prevalent within biological systems, constituting approximately 25% to 30% of eukaryotic proteomes. Further studies indicate that more than 50% of eukaryotic proteins and 70% of signalling-associated proteins feature extended disordered regions. Because of their flexible, unordered nature, IDPs readily undergo structural changes upon binding to biological partners. This inherent flexibility contributes to the characteristic versatility of IDPs, often playing pivotal roles as hub proteins in signalling pathways. Analysis of the amino acid composition of the plant-specific DoRMancy-associated (DRM) protein family reveals a notable enrichment in residues that promote disorder, contrasting with a depletion of residues that typically stabilize ordered structures. This pattern aligns with known characteristics of structurally defined IDPs. Focusing on specific members of the DRM protein family, AtDRM1 and AtDRM2, we present evidence suggesting their potential significance in a plant's response to various stresses, both biotic and abiotic. Interestingly, individual members may exhibit antagonistic functions, including the regulation of reactive oxygen species (ROS) levels following exposure to flg22, indicating their involvement in intricate regulatory mechanisms within plants.

PM40: Towards biochemical characterisation of wall-associated kinases (WAKs) from wheat and canola

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Plant cell surface receptors are crucial for initial perception of pathogen infection and coordination of downstream immune responses.[1–3] Several of these cell surface immune receptors have been identified as resistance proteins against fungal pathogens that colonise the apoplast. To date, extracellular receptors involved in resistance against plant disease have been broadly categorised into one of the following structural classes – LRR-RLP (Leucine-rich repeat–receptor-like protein), NB-LRR (Nucleotide-binding–Leucine-rich repeat), S-RLK (S-domain receptor-like kinase) and WAK (Wall-associated kinase).[1] Of these, WAKs represent a relatively understudied class of receptor kinases that are unique to the plant kingdom and have been implicated in conferring either broad-spectrum (cf. quantitave) or isolate-specific (cf. qualitative) resistance to various apoplastic pathogens.[1,4]

In this poster, I will present our work towards recombinant production, and biochemical characterisation of extracellular domains from WAKs that are known to be involved in conferring gene-for-gene type resistance against Septoria Tritici Blotch (STB)[5] disease in wheat and Black-leg[6] in canola.

1. Kanyuka, K. and J.J. Rudd (2019). *Cell surface immune receptors: the guardians of the plant's extracellular spaces.* Current Opinion in Plant Biology. 50, 1-8.
2. Zipfel, C. (2008). *Pattern-recognition receptors in plant innate immunity.* Current Opinion in Immunology. 20, 10-16.
3. Steinbrenner, A.D. (2020). *The evolving landscape of cell surface pattern recognition across plant immune networks*. Current Opinion in Plant Biology. 56, 135-146.
4. Verica, J.A. and Z.-H. He (2002). *The cell wall-associated kinase (WAK) and WAK-like kinase gene family.* Plant Physiology. 129, 455-459.
5. Saintenac C. et al. (2018). *Wheat receptor-kinase-like protein Stb6 controls gene-for-gene resistance to fungal pathogen* Zymoseptoria tritici. Nature Genetics. 50, 368-374.
6. Larkan, N.J. et al. (2020). *The Brassica napus wall-associated kinase-like (WAKL) gene* Rlm9 *provides race-specific blackleg resistance*. The Plant Journal. 104, 892-900.