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QMB Plant-Microbe Interactions Abstracts

P1: The global movement of plant pests and pathogens: implications for food security

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Over the past centuries, crop diseases have led to the starvation of the people, the ruination of economies and the downfall of governments. Of the various challenges, the threat to plants of fungal infection outstrips that posed by bacterial and viral diseases combined. Indeed, fungal diseases have been increasing in severity and scale since the mid 20th Century and now pose a serious threat to global food security and ecosystem health.

We face a future blighted by known adversaries, by new variants of old foes and by new diseases. Modern agricultural intensification practices have heightened the challenge - the planting of vast swathes of genetically uniform crops, guarded by one or two inbred resistance genes, and use of single target site antifungals has hastened emergence of new virulent and fungicide-resistant strains. Climate change compounds the saga as we see altered disease demographics - pathogens are on the move poleward in a warming world.

This presentation will highlight some current notable and persistent fungal diseases. It will consider the evolutionary drivers underpinning emergence of new diseases and allude to the accelerators of spread. I will set these points in the context of recent disease modelling, which shows the global distributions of crop pathogens and their predicted movement and will discuss the concept of crop disease saturation. I shall conclude with some thoughts on future threats and challenges, on fungal disease mitigation and of ways of enhancing global food security in Australasia.

P2: Modulation of cysteine proteases in the maize – *Ustilago maydis* interaction

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The outcome of plant-microbe interactions is determined by the interplay of the attackers' virulence repertoire with the plant immune system. Central hubs for the coordination of plant defence are papain-like cysteine proteases (PLCPs). Despite their crucial role in plant immunity, it remains unknown, how PLCPs are activated, and which downstream signals they induce to trigger defence responses. Previously, we identified a set of five apoplastic PLCPs to be crucial for orchestration of salicylic acid (SA)-dependent defense signaling in maize. During the interaction of the biotrophic corn smut fungus *Ustilago maydis,* activity of the PLCPs is inhibited by the maize cystatin CC9, as well as the fungal effector protein Pit2.

Using a mass spectrometry approach, we discovered a novel immune signalling peptide, *Zea mays* immune signalling peptide 1 (Zip1). The peptide is released from its precursor PROZIP1 by PLCPs, which in turn are activated by the plant hormone SA. Strikingly, Zip1 itself activates *de-novo* synthesis of SA and induces transcriptional changes that resemble the SA-mediated defence response.

The *U. maydis* effector Pit2 efficiently blocks those maize PLCPs, which are responsible for Zip1 release. This modulation of host immune activation depends on a 14aa sequence motif within the Pit2 effector. Biochemical analyses revealed that Pit2 acts as substrate for the maize PLCPs, to release the inhibitory domain, which in turn blocks the plant PLCPs. We therefore propose that the Pit2 effector evolved as a molecular decoy to overcome PLCP-mediated plant defenses.

P3: The broad host range fungal pathogen *Rhizoctonia solani* adjusts its infection strategy depending on the host but can be kept at bay by ethylene and phytoalexin mediated defences

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Rhizoctonia solani is a fungal pathogen causing substantial damage to many of the worlds' largest food and fibre crops. The broad host range AG8 isolate inflicts losses on cereals, canola and legumes and we have exploited the interaction with different hosts to identify common and unique mechanisms of host resistance and fungal pathogenesis. For example, in legumes, transcriptomic analyses revealed ethylene signalling, reactive oxygen species metabolism and isoflavonoid biosynthesis pathways were specifically associated with resistance. Metabolite profiling revealed accumulation of isoflavonoids and related intermediates in the resistant line, and over-expression of a key biosynthetic enzyme confirmed an important role for isoflavonoids in resistance. On the pathogen side of the interaction, *in-silico* secretome analysis revealed Rhizoctonia employs largely unique secretomes and effectors and the infection strategy is adapted to the host being encountered. Among many characteristics tested, diversifying selection and up-regulation during infection were key characteristics for identification of effectors causing plant cell death. Inhibitor I-9 domain containing proteins inducing plant cell death were conserved among Rhizoctonia isolates and the family was found to be expanded in pathogens with a necrotising life stage. Together these findings provide a complementary understanding of how losses to Rhizoctonia diseases may be tackled by addressing host resistance and avoiding fungal pathogenicity in agriculture.

P4: Taming a root rot pathogen: Integration of geospatial technologies for disease management

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Geospatial technologies greatly enhance evaluation of large production areas to track pathogen movement, and when combined with on ground monitoring, a complete picture of disease progression can be elucidated. A common problem growing alfalfa in the southwest U.S.A. is the presence of a prevalent root rot pathogen, *Phymatotrichopsis omnivora*. Alfalfa fields infested with *P. omnivora* have reduced stand life and are taken out of production prematurely. To understand the spatio-temporal dynamics of the disease, we are monitoring disease progression in an alfalfa production field utilizing multiple techniques. Aerial imagery, taken with manned or unmanned aircrafts, provide a bird's eye view of pathogen spread and can be combined with a handheld GPS tracker to demarcate active diseased areas. During a single growing season (June to October) the disease front can move up to 4.5 m and in a heavily infested field, over 20% of the stand can be lost within a single year. The pathogen can be readily detected in the roots and soil by PCR using species-specific primers but is shown to be more concentrated around the disease front. Plants that have survived the disease often have an altered root morphology, where the main taproot has disappeared and the plant has compensated with development of crown roots. A spray regime with the fungicide Topguard™ (active ingredient flutriafol), is being developed for alfalfa with some success in disease suppression. Combining multiple technologies has provided insight into the *P. omnivora*-alfalfa disease complex, which will be integrated with decision-making tools associated with precision agriculture to reduce the severity of this root rot disease.

P5: *Pseudomonas syringae* pathogenesis of plants: Effectors and immunity

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The bacterial pathogen *Pseudomonas syringae* uses a type III secretion system to inject type III effector proteins into plant cells to favor pathogenicity. When plants are infected by pathogens, two types of plant immunity can be triggered. Conserved molecules known as pathogen-associated molecular patterns (PAMPs) can be recognized by surface-localized receptors known as pattern recognition receptors (PRRs) inducing pattern-triggered immunity (PTI). Pathogen effectors can be recognized by specific NOD-like receptors (NLRs) leading to effector-triggered immunity (ETI). The majority of type III effectors in *P. syringae* pv. tomato DC3000 can suppress PTI and/or ETI. I will give an update on the type III effectors that we are currently working on in the lab. One of these is HopE1, which recently was published to use the calcium sensor calmodulin as a co-factor and to target MAP65-1, a member of the microtubule-associated protein 65 family, a protein that functions in the Arabidopsis microtubule network. I also will talk about a newer project in the lab focused on Arabidopsis orosomucoid (ORM) proteins and their involvement in plant immunity. ORMs are known regulators of sphingolipid biosynthesis. Arabidopsis plants over-expressing ORM1 or ORM2 lack signalling from the FLS2 PRR and are more susceptible to *P. syringae*. Moreover, plants over-expressing ORM1/2 are greatly reduced in their FLS2 levels; ORM1/2 mutants have enhanced levels of FLS2. This portion of the talk will be focused on the molecular explanation behind these phenotypes.

P6: Peptide hormones: positive and negative regulators of root and nodule development in response to nitrogen availability

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Symbiotic nitrogen fixation requires legumes to provide energy to the rhizobia in root nodules. Thus, legumes must coordinate nodule formation and number according to their demand for nitrogen and the availability of carbohydrate. We have identified and characterised two classes of small-secreted peptides that regulate nodulation. In the model legume *Medicago truncatula*, we have shown that nodule specific CLE (CLAVATA 3/ENDOSPERM SURROUNDING REGION related) peptides inhibit nodulation in a receptor dependent manner mediated through long distance signalling. On the other hand, we also show that another class of peptides called CEPs promote nodulation and requires a different shoot-localised receptor. The CEP peptide mediated enhancement of nodulation is partially tolerant to nitrate levels that suppress nodulation and the CEP peptide-dependent increase in nodulation requires the symbiotic signalling pathway and ethylene signalling pathways. We also show that peptide processing and post-translational modifications are required for peptide activities. Overall, our work demonstrate that signalling peptides are important positive and negative regulators of root development and nodulation, and provide alternative avenues for modulating plant growth and symbiosis.

P7: Symbiotic phenotypes of exopolysaccharide mutants of *Mesorhizobium loti* indicate that the host plant differentially perceives both wild-type and truncated EPS

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Mesorhizobium loti strain R7A synthesizes and secretes an O-acetylated acidic exopolysaccharide (EPS) composed of octameric subunits comprising a backbone of one galactose (Gal) and three glucose (Glc) residues, with a branch of two Glc, one glucuronic acid (GlcA) and a terminal riburonic acid (RibA)[1]. In a previous study[2], it was shown that *exoU* mutants are defective in infection thread (IT) initiation and elongation, while *exoB* mutants form nitrogen-fixing nodules. It was proposed that full-length EPS produced by wild-type *M. loti* R7A acts as a signal to allow efficient IT development and bacterial release. R7A EPS mutant strains that produce no EPS, due to mutations that affect initial steps of EPS biosynthesis, avoid or suppress the plant surveillance system and can form nodules, while mutants that are disrupted midway through EPS biosynthesis are impaired in IT development due to secretion of truncated forms of EPS. In this work, we have further tested these hypotheses. Symbiotically-proficient suppressor mutants of R7AΔ*exoU* were all found to have second-site mutations in genes involved in EPS backbone synthesis, strongly suggesting secretion of the truncated EPS caused the symbiotic defects observed for R7AΔ*exoU*. Characterization of other EPS backbone mutants revealed that they were delayed in nodulation on *L. japonicus* cv. Gifu compared to wild-type R7A. Examination of one of these new mutants, R7AΔ*exoYF*, showed that it formed about half the number of ITs formed by R7A at 14 days post-inoculation. A delay in infection of nodule primordia was also observed. A mutation within a gene responsible for EPS transport, *exoT*, could be isolated in the R7AΔ*exoYF* background but not in wild-type or R7AΔ*exoU* backgrounds, suggesting that failure to secrete wild-type or truncated EPS was lethal. Taken together, the results support the findings that the *Lotus japonicus* receptor EPR3 binds exopolysaccharide directly and distinguishes compatible and incompatible exopolysaccharides[3].

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P8: *Pseudomonas syringae* pv. *actinidiae* effector HopZ5 is an acetyltransferase triggering accession specific HR, dependent on *SOBER1*, in Arabidopsis

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Actinidia deliciosa and *A. chinensis*, the two most economically important species of kiwifruit in the world, are both susceptible to the bacterial pathogen *Pseudomonas syringae* pv. *actinidiae* (*Psa*), the causal agent of canker of kiwifruit. Through our research, we sought to identify the resistance mechanisms against *Psa* used by the non-host cruciferous plant, *Arabidopsis thaliana*.

Bacterial virulence proteins that target plant defence when delivered through a bacterial type III secretion system, thus termed type III secreted effectors (T3Es), are critical in disease establishment. Plant recognition of certain T3Es can trigger a secondary line of defence that are mediated by disease resistance (*R)* genes that act in a gene-for-gene manner, turning the pathogen avirulent. This avirulence, when the pathogen is delivered in high numbers, is often accompanied by a macroscopic *R*-mediated programmed cell death response, termed the hypersensitive response (HR). Our study of *Psa-Arabidopsis* system has identified a putative acetyltransferase T3E, HopZ5 that triggers disease resistance on a few *Arabidopsis* accessions. HopZ5 is an acetyltransferase from the YopJ/AvrA superfamily of acetyltransferases and cysteine proteases. Mutation analysis within HopZ5 indicates that it is a *bona fide* plasma membrane-localized acetyltransferase with autoacetylating activity[1].

We found that while HopZ5 acts as a classical avirulence T3E triggering HR in *Arabidopsis* accession Catania-1 (Ct-1), accessions Wassilewskija-2 (Ws-2) and Columbia-0 (Col-0) are unable to produce an HR in response to *Pseudomonas*-delivered HopZ5. However, like Ct-1, Col-0 is resistant to *Pseudomonas* carrying HopZ5 despite the lack of an HR while Ws-2 is not, indicating a separation of immunity and HR. We mapped the HR-linked locus in recombinant inbred lines (RILs) between Col-0 and Ct-1 and found that a previously identified suppressor (*SOBER1*) of another YopJ/AvrA superfamily T3E, AvrBsT from *Xanthomonas*[2], was responsible for suppressing the HR in Ct-1 plants. We used transgenic lines and heterologous expression systems to demonstrate that this suppression is specific to HopZ5-triggered HR but not immunity, and that HR triggered by a subset of effectors from the YopJ/AvrA superfamily are suppressed by SOBER1.

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P9: The Delivery and Activity of Effector Proteins from *Phytophthora infestans* to Suppress Host Immunity

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The oomycete *Phytophthora infestans* causes late blight, globally the most serious disease of potato. *P. infestans*, during its interaction with potato and other hosts, such as the model solanaceous plant *Nicotiana benthamiana*, produces finger-like protrusions called haustoria, which form intimate interactions with host plant cells. We show that haustoria are the sites of secretion of both apoplastic effectors, which act outside of host cells, and cytoplasmic effectors, which are delivered inside living plant cells to suppress defences and manipulate other host processes. We show that these effectors are delivered by different secretion pathways. Much effort has been focussed on the roles of cytoplasmic RXLR effectors: identifying their target proteins in the host and characterising their roles in manipulating those host proteins to facilitate disease development. This presentation will reveal the latest progress we have made in studying host target proteins of RXLR effectors, in particular focussing on so-called susceptibility (S) factors that are used by the pathogen to negatively regulate immunity.

P10: Effector discovery and functional analysis in the kauri dieback pathogen.

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Kauri dieback is a devastating disease of iconic and culturally significant native trees, caused by a root-infecting pathogen*, Phytophthora agathidicida*. Efforts to manage the disease include measures to prevent movement of the pathogen to new sites, and phosphite treatment of individual trees to delay disease progression. But to preserve the species in the long term, it will be necessary to identify individuals showing resistance to this disease, to determine the genetic basis of this resistance, and to harness this knowledge for breeding resistant cultivars of kauri. We are taking a molecular genomic approach to elucidate interactions between *P. agathidicida* and its host, by mining the pathogen genome for RxLR effectors and performing functional screening assays. A set of 78 RxLR effector genes was identified using a conservative approach involving three methods. Sequence comparisons revealed that 32 of these are unique to *P. agathidicida* and about 26 of them are conserved in many other *Phytophthora* species. An extensive phylogenetic study and analysis of domain structures suggests common ancestry amongst *P. agathidicida* RxLR effectors. Functional screens carried out using a transient *Agrobacterium* transformation assay with non-host *Nicotiana* spp. revealed that eight of the RxLR effectors triggered an HR-like necrosis response, and three of them could suppress defence responses initiated by the *P. agathidicida* PAMP-like elicitin, Inf1-1. Amongst these are putative orthologues of Avh147 and Avh238 from the well-studied soybean pathogen *Phytophthora sojae*. In future we will develop methods to screen for responses in kauri tissue using purified RxLR proteins and aim to identify host targets that interact with these proteins. Ultimately we hope to determine the genetic basis of resistance in kauri and to help prevent decimation of the species by the plant destroyer *P. agathidicida*.

P11: The responses of WRKY transcription factors and pathogenesis-related proteins to *Venturia inaequalis* challenge in apple

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Apple scab, caused by the fungus *Venturia inaequalis*, is one of the most serious diseases affecting the apple industry. However, the molecular mechanisms of the apple response to *V. inaequalis* are not well understood. RNA-seq analysis was used to determine the host differential expression (DE) profiles associated with *V. inaequalis* challenge in two resistant accessions, *Rvi5* (accession A248R04T010, derived from host (5), carrying the resistance gene *Rvi5*) characterised by a hypersensitive response, and *Rvi8* (accession GMAL3631-W193B, derived from host (8), carrying the resistance gene *Rvi8*) characterised by a delayed stellate necrosis response, and a susceptible accession 'Royal Gala' (RG). WRKY proteins are a superfamily of transcription factors (WRKYs) and key regulators of many processes in plants, including germination, senescence and responses to biotic and abiotic stresses. Indeed, WRKYs play roles in regulating plant responses to pathogens. In particular, they often act as repressors, as well as activators, of the two partly interconnected branches of plant innate immunity, pathogen-associated molecular pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). Pathogenesis-related (PR) proteins, with diverse functionality including chitinase and glucanase activity, are associated with resistance responses in many plant-pathogen interactions. There were more up-regulated DE WRKY and PR protein genes in *Rvi5* than in *Rvi8* and RG, and there were strong positive correlations between the expression of some WRKY and PR protein genes in *Rvi5*, which possibly indicates that the expressions of these two gene families may be related to the resistant phenotype in *Rvi5*. Transient dual luciferase assays on *Nicotiana benthamiana* leaves confirmed the interactions between WRKYs and the promoters of PR protein genes. The correlation analysis and the transient assay of interactions between WRKYs and PR protein promoters may imply that some WRKYs act upstream of some PR proteins in the apple molecular defense mechanisms in response to *V. inaequalis* challenge.

P12: Host Induced Gene Silencing and Exogenous RNA application for the control of *Puccinia* species on wheat cultivars.

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Puccinia species are a global threat to agriculture due to the rapid evolution of these pathogenic fungi which can quickly overcome resistance in common wheat varieties. Much research is focused on identifying new resistance genes against these pathogens, from both crop plants and non-host species, to control these pathogen. However given the propensity of rust fungi to overcome resistance, developing alternative methods of control is an important goal. Recent studies have explored the use of RNAi methods to control the spread of fungal pathogens. Yin *et al*. (2015) used a Barley stripe mosaic virus (BSMV) mediated host induced gene silencing (HIGS) system to target *Puccinia graminis* f. sp. *tritici transcripts and identified 10 genes whose silencing* resulted in reduced infection of susceptible cultivars (Yin *et al*, 2015). Alternative RNAi approaches involve the use of constructs targeting pathogen genes applied externally to plant tissue in order to inhibit fungal infection (Wang *et al*, 2016). The application of small RNAs (sRNAs) and double stranded (dsRNAs) that target Botrytis DCL1 and DCL2 genes significantly inhibited grey mould disease (Wang *et al*, 2016).

In this project we are exploring RNAi methods to downregulate key fungal genes in order to control infection of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) and stripe rust (*P*. *striiformis* f. sp. *tritici*). Transgenic wheat lines have been generated which express a hairpin construct targeting key factors involved in the growth and regulation of *P*. *striiformis* f. sp. *tritici*. These plants have undergone screening to determine what effect these hairpin constructs have on the fungal transcripts during infection. An alternative method for control of wheat stem rust is to exogenously apply a dsRNA construct to the leaf surface. The project aims to determine if *Puccinia species can take up and process this dsRNA into small interfering RNAs (siRNAs) resulting in downregulation of fungal transcripts and an altered infection profile.*

P13: Genome guided interrogation of a cereal invader

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Recent disease outbreaks caused by (re-)emerging plant pathogens have been associated with expansions in pathogen geographic distribution and increased virulence. For example, in the past two decades' wheat yellow (stripe) rust, *Puccinia striiformis* f. sp. *tritici*, has seen the emergence of new races that are adapted to warmer temperatures, have expanded virulence profiles, and are more aggressive than previous races, leading to wide-scale epidemics. Here, we integrated next-generation sequencing technologies into the surveillance of such emerging filamentous plant pathogens. Accordingly, we are leading the genome and transcriptome sequencing of hundreds of isolates of the wheat yellow rust pathogen from across the globe. Our rapid "field pathogenomics" strategy, that uses gene sequencing of PST-infected wheat leaves taken directly from the field, has enabled us to gain insight into the population structure of PST over successive seasons. For instance, our analysis uncovered a dramatic shift in the PST population in the UK and supports the hypothesis that a recent introduction of a diverse set of exotic PST lineages may have displaced the previous populations. In addition, we uncovered potential seasonal and varietal specificity for specific genotypes of PST. This knowledge regarding which wheat varieties are susceptible to specific PST isolates, and when those isolates are prevalent throughout the year, represents a powerful tool for disease management.

P14: Using machine learning to decipher how plant pathogens cause disease

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Plant pathogens cause disease through secreted virulence proteins called effectors that alter host cell structure and function. Effector discovery is crucial for enhancing host-plant resistance, but user-driven effector prediction methods based on small protein size or high cysteine content return hundreds of candidates and suffer from high false positive rates. This leads to low validation success rates and incomplete understanding of pathogen biology. A contrasting data-driven approach is machine learning that encompasses a family of statistical learning methods with the ability to learn to identify patterns in data. In particular, machine learning can exploit the cryptic signatures that effectors carry in regards to their *in planta* localization. For example, plant pathogens deliver effectors into the apoplast as well as the host cell cytoplasm, but in the absence of conserved host translocation sequence motifs, no computational method is available to discriminate between these localizations.

We present ApoplastP, the first machine learning method for predicting if an effector or plant protein localizes to the apoplast. ApoplastP predicts apoplastic effector localization with sensitivity of 75% and false positive rate of 5%, improving accuracy of cysteine-rich classifiers by over 16%, and correctly predicts the localization of unconventionally secreted plant and effector proteins. The secretomes of fungal saprophytes, necrotrophic pathogens and extracellular pathogens are enriched for predicted apoplastic proteins and whilst rust pathogens show the lowest percentage of apoplastic proteins in their secretomes, they are highly enriched for predicted effectors. ApoplastP is the first program for predicting apoplastic localization and will facilitate functional studies. In particular, it will be valuable for predicting if an effector localizes to the apoplast or if it enters plant cells. ApoplastP is available at http://apoplastp.csiro.au.

P15: 'Ome Truths: bioinformatics approaches for the study of adaptation mechanisms in pathogen genomes and their pathogenicity effector proteins.

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Fungal genomes exhibit a handful of characteristics either unique to, or enhanced within certain taxa, many of which contain high numbers of important plant-pathogenic species. These include 1) repeat-induced point mutation (RIP), associated with the Pezizomycotina (and possibly Agaricomycotina); 2) high rates of intra-chromosomal structural rearrangements (mesosynteny), associated with the Pezizomycotina; 3) high rates of lateral gene transfer (LGT), enriched in Pezizomycotina, and; 4) numerous reports of accessory chromosomes (ACs) or regions (often Pezizomycotina). These features contribute to genome plasticity in fungal genomes, which can play significant roles in the co-evolution of pathogens with their hosts. Detection of these genome features can be also used to predict genes or regions of the genome that are likely to be important in host-pathogen interactions (i.e. effectors).

We present findings from recent bioinformatic studies that leverage comparative genomics to focus on the prediction of genes/regions of fungal pathogen genomes that are relevant to pathogenicity. While previous tools such as RIPCAL have been able to quantify the prevalence and type of RIP mutations in repeat families, a recently developed method OcculterCut measures the locations and proportion of AT-rich genome regions affected by RIP or RIP-like processes. This has allowed for both comparison of AT-rich content across the fungal kingdom, and the prediction of AT-rich-associated loci that may be 'hyper-mutated' by leakage of RIP-mutations. Using orthology comparisons across 200+ species, we have also developed a comprehensive database of lateral-transfer candidates for species with published genomes, focussing on those with effector-like properties. Our investigations of the protein structures of known effectors and the role of disordered amino acids have also led to development of new methods for effector prediction from secretome datasets. We also report investigations of chromosome structure variations in fungal pathogens and prediction of accessory genome content, which have been reported in several species to contain host-specific pathogenicity genes or clusters.

P16: A finished *Epichloë festucae* genome demonstrates links between genome structure and function.

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Epichloë species comprise a genus of ascomycete fungi that form symbiotic relationships with particular cool season grass species. This symbiosis can have profound effects on the grass, providing resistance to pathogens and herbivores, and tolerance of drought conditions. *Epichloë festucae* is a common symbiont of a number of agriculturally important pasture grasses, and has become a model species for *Epichloë* research. At present, the only reference genomes available for *E. festucae* are highly fragmentary; a situation which limits the ability of *Epichloë* researchers to study gene structure, organization and regulation at the chromosome level. Here we combine modern sequencing techniques, including long read sequencing and high throughput chromosome conformation capture (HiC), to generate a gapless telomere-to-telomere assembly of *E. festucae* strain Fl1 genome.

Our results reveal a "patchwork" genome, in which long gene-rich segments are interspersed by AT-rich regions made almost entirely of inactivated repetitive elements. Using HiC data, we show that these repeat-rich regions have a considerable impact on the three-dimensional structure of the genome. AT-rich regions have relatively condensed chromatin states and frequently act to isolate neighbouring gene-rich regions from each other within the nucleus. Using RNAseq data, we show the three-dimensional structure of the genome contributes to the regulation of gene expression. In particular, genes that are highly upregulated *in planta* co-locate within the nucleus of cells grown in culture more frequently than would be expected by chance and are frequently found in genomic regions with relatively condensed chromatin states. In addition to providing the first finished genome for an *Epichloë* species, these results suggest genome structure and repetitive elements may make important contributions to the maintenance of a symbiotic relationship between fungal and grass species.

P17: Plant malectin kinase targets of the bacterial effector AvrPtoB

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Host detection of plant pathogens occurs directly or indirectly through a system of receptors that operate either at the cell surface or within the plant cell. Surface receptors are typically membrane-embedded receptor proteins or receptor kinases (RK), whereas intracellular recognition is mediated by receptor complexes that generally contain at least one nucleotide binding-leucine rich repeats protein (NB-LRR). Pathogens of all classes secrete virulence effector proteins to modulate host signalling pathways and modify the local environment to their benefit. We are interested in the role of the RK FERONIA in host-pathogen interactions. With others, we showed that necrotrophic fungi encode RALF peptides[1] which are known ligands for FER, which has multiple roles in plant development, pathogen responses, and hormone and sugar signalling. In particular, plants lacking FER accumulate the phytohormone abscisic acid (ABA) and are hypersensitive to ABA treatments[2]. We found that one effector from the bacterium Pseudomonas syringae, AvrPtoB, interacts with FER and is able to suppress RALF responses. This is striking because AvrPtoB has previously been shown to induce ABA levels[3]. We found that FER is necessary for early responses triggered by bacterial PAMPs, and that treatment of plants with ABA dampened PAMP responses. We are now testing whether FER is able to influence sugar signalling within the plant. Lastly, because the kinase domain of FER is strongly homologous to Pto kinase, a receptor that recognises AvrPtoB together with the NB-LRR protein Prf, we are testing if these pathways interact. Overall, we are learning more about pathogenicity targets within the plant cell and how the host protects them.

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[2]Chen, J. et al. FERONIA interacts with ABI2-type phosphatases to facilitate signaling cross-talk between abscisic acid and RALF peptide in Arabidopsis. Proceedings of the National Academy of Sciences, 201608449 (2016).
[3]de Torres-Zabala, M. et al. Pseudomonas syringae pv. tomato hijacks the Arabidopsis abscisic acid signalling pathway to cause disease. The EMBO journal 26, 1434-1443 (2007).

P18: Elucidating the structural basis of effector-induced susceptibility in the *Parastagonospora nodorum* – wheat interaction

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Parastagonospora nodorum is an important fungal pathogen of wheat, and a key model system to understand necrotrophic fungal-plant interactions. *P. nodorum* secretes small cysteine-rich effector proteins (ToxA, Tox1 and Tox3) which interact with corresponding dominant host sensitivity gene products, leading to programmed cell death and disease in a process known as effector-induced susceptibility. However, whilst the importance of these effector proteins in causing disease is well known, their modes of action remain poorly understood.

Recent studies though suggest these effectors have roles independent of inducing cell death. It has been recently demonstrated that Tox1 plays a dual-role during infection by binding chitin to protect the fungus from wheat chitinases, while also promoting cell-death via the direct-interaction with its corresponding sensitivity protein, Snn1 (1, 2). Similarly our laboratory has now shown that Tox3 directly interacts with host pathogenesis-related 1 (PR-1) proteins whilst inducing necrosis in the persence of the *Snn3* susceptibility gene (3). In planta assays have confirmed that the interaction of Tox3 with PR-1 does mediate disease progression. Despite PR-1 proteins being amongst the most abundantly produced proteins in plants upon pathogen attack, their role in plant defence remains poorly understood.

We are utilising a protein structure-function approach to investigate the interactions between necrotrophic effectors and their host targets. We have established protein production systems for most of the key players to facilitate these studies (4) and are using these proteins to generate crystal structures to complement ongoing functional analyses. Here we will discuss the current state of these structures and provide insights into how the dual roles of these effector proteins contribute to disease.

1. Z. Liu *et al.*, SnTox1, a Parastagonospora nodorum necrotrophic effector, is a dual-function protein that facilitates infection while protecting from wheat-produced chitinases. *The New phytologist* **211**, 1052--1064 (2016).
2. G. Shi *et al.*, The hijacking of a receptor kinase-driven pathway by a wheat fungal pathogen leads to disease. *Science advances* **2**, e1600822 (2016).
3. S. Breen, S. J. Williams, B. Winterberg, B. Kobe, P. S. Solomon, Wheat PR-1 proteins are targeted by necrotrophic pathogen effector proteins. *Plant Journal* **88**, 13-25 (2016).
4. X. Zhang *et al.*, Production of small cysteine-rich effector proteins in Escherichia coli for structural and functional studies. *Molecular Plant Pathology* **18**, 141-151 (2017).

P19: Recognition of the *Magnaporthe oryzae* effector AVR-Pia by the decoy domain of the rice NLR immune receptor RGA5

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Nucleotide binding domain and leucine-rich repeat proteins (NLRs) are important receptors in plant immunity that allow recognition of pathogen effectors. The rice (*Oryza sativa*) NLR RGA5 recognizes the *Magnaporthe oryzae* effector AVR-Pia through direct interaction. Here, we gained detailed insights into the molecular and structural bases of AVR-Pia-RGA5 interaction and the role of the RATX1 decoy domain of RGA5. NMR titration combined with in vitro and in vivo protein-protein interaction analyses identified the AVR-Pia interaction surface that binds to the RATX1 domain. Structure-informed AVR-Pia mutants showed that, although AVR-Pia associates with additional sites in RGA5, binding to the RATX1 domain is necessary for pathogen recognition but can be of moderate affinity. Therefore, RGA5-mediated resistance is highly resilient to mutations in the effector. We propose a model that explains such robust effector recognition as a consequence, and an advantage, of the combination of integrated decoy domains with additional independent effector-NLR interactions.

P20: Towards understanding TIR-domain function during plant NLR immunity receptor signaling

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NLRs (nucleotide binding, leucine-rich repeat receptors) are used by the plant immune system to detect effector proteins secreted into the plant cell by potential pathogens, as part of effector-triggered immunity. To signal downstream, NLRs contain either a TIR (Toll/interleukin-1 receptor) domain or a CC (coiled-coil) domain at their N-termini. TIR domains are found in animals, plants and bacteria, e.g. in TLRs (Toll-like receptors) and TLR adaptors in animals. While it is known that signaling depends on self-association and homotypic association of TIR domains, crystal structures have revealed few common association modes [1,2]. We have targeted TIR domains from mammals, plants and bacteria (determining crystal structures for human TLR adaptor proteins MAL [3] and SARM (unpublished), the bacterial protein TcpB from *Brucella melitensis* [4] and the plant immune proteins L6 from flax [5], RPS4, RRS1 [6], SNC1 and RPP1 from Arabidopsis [7] and MrRPV1 from grape [8]). These crystal structures have started to reveal common trends in association modes, in particular for bacterial and plant TIR domains. Furthermore, for the TLR adaptors MAL and MyD88, we have been able to reconstitute large assemblies and determine the structure of the filamentous assembly of MAL by cryo-electron microscopy (9). As an unexpected twist, we (unpublished) and other (10) have shown that the TIR domain of the TLR adaptor SARM possesses self-association-dependent NAD+ cleavage activity. Jointly, these studies suggest a general mechanism of function of TIR domains, which involves "signaling by cooperative assembly formation (SCAF)" with prion-like features that is consistent with signaling in other innate immunity pathways.

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2. Nimma et al (2017) Curr Opin Struct Biol 43, 122
3. Valkov et al (2011) Proc Natl Acad Sci USA 108, 14879
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8. Williams et al (2016) Frontiers Plant Sci 7, 1850
9. Ve et al (2017) Nat Struct Mol Biol
10. Essuman et al (2017) Neuron 93, 1334

P21: Membrane dynamics during arbuscule formation in rice

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'Arbuscules' are immensely fascinating fungal feeding structures, produced inside root cortex cells by members of the fungal phylum Glomeromycota. Arbuscules are built by consecutive dichotomous hyphal branching, ultimately adopting a complex tree-like shape at microscopic scale. As the arbuscule develops, the hosting plant cell undergoes fundamental architectural adaptations to accommodate the intracellularly expanding fungus. For instance, the plant cell dramatically increases membrane biogenesis to envelope the growing hyphal structure in the so-called peri-arbuscular membrane (PAM). The hugely enlarged membrane surface area between the two organisms appears ideal for the exchange of signals and nutrients. Remarkably, despite what seems a considerable metabolic investment, arbuscules collapse after a few days, and host cell architecture is restored to that of a non-colonized cell. Therefore, the life of an arbuscule is marked by the highly dynamic continuum of development and collapse without static intermediate stages. To capture arbuscule formation and turnover in 4D, and at ultrastructural resolution, we combined advanced multiphoton confocal imaging of living mycorrhizal rice roots with high resolution electron microscopy. I will introduce our observations which have led us to propose fundamentally new communication mechanisms operating during the intimate plant-fungal engagement in the generation of the peri-arbuscular interface.

P22: Plant immunity impacting beneficial interactions

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The extent to which plant immunity functions and defense homeostasis shape and are themselves shaped by microbes remains an open question. New sequencing technologies combined with pathology, cell biology and physiology have enabled a radical re-evaluation of the phylogenetic relationships between saprotrophs, mutualists and pathogens. They also now begin to reveal genomic and transcriptomic signatures of endophytic lifestyles and colonization strategies. As found for well-characterized pathogenic strains, mutualistic fungi have panels of effector-like genes and secreted proteins with the potential to modulate the responsiveness of the host and other microbes. There is also evidence that root-associated microbes enhance plant disease resistance. It is therefore likely that the immune system is a host-specificity determinant of beneficial endophytes. We present here recent advance on molecular communication in root-sebacinoid fungal endophyte interactions.

P23: A new origin for *ToxA* in three Dothideomycete wheat pathogens

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During analysis of the promoter region of the human *MEST* gene, we noted apparent non-*Bipolaris sorokiniana* is the causal agent of multiple diseases on wheat and barley and is the primary constraint to cereal production throughout South Asia. Despite its significance, the molecular basis of disease is poorly understood. To address this, the genomes of three Australian isolates of *B. sorokiniana* were sequenced and screened for known pathogenicity genes. Sequence analysis revealed that the isolate BRIP10943 harboured the *ToxA* gene which was previously associated with disease in the wheat pathogens *Parastagonospora nodorum* and *Pyrenophora tritici-repentis*. The proteinaceous necrotrophic effector, ToxA, was first identified in culture filtrates of the fungal wheat pathogen *Pyrenophora tritici-repentis* where the culture filtrate alone caused necrotic lesions on susceptible wheat cultivars, in a genotype-specific manner. Later, a near identical 11kb region containing *ToxA* was found in the genome sequence of the wheat pathogen *Parastagonospora nodorum.* Susceptibility of wheat to ToxA was eventually linked to the presence of a host immune-like gene, *Tsn1.*

Analysis of the regions flanking *ToxA* within *B. sorokiniana* revealed that it was embedded within a 12kb genomic element nearly identical to the corresponding regions in *P. nodorum* and *P. tritici-repentis*. A screen of 35 Australian *B. sorokiniana* isolates confirmed that *ToxA* was present in 12 isolates. We completely assembled the genomes of *B. sorokiniana* BRIP10943 and *P. nodorum* SN15 using single molecule real-time (SMRT) sequencing and annotated the repeat families in each genome with REPET. We've also assembled several additional genomes with the Oxford Nanopore MinIon, to look at structural genome re-arrangements near *ToxA.* Syntenic alignments between the three genomes reveal shared repeat elements between *P. tritici-repentis* and *P. nodorum* outside of the 12kb region of high homology nearing 68kb in length. Further examination of these repeat elements throughout the genomes of all three species will be presented. We propose that *ToxA* and its surrounding 12kb is a highly promiscuous genomic element that has the ability to cross species barriers to facilitate disease on wheat. Acquisition of this element dramatically increases the pathogenicity of these *B. sorokiniana* strains on wheat in a *Tsn1* susceptible background, which in an agricultural setting can have devastating economic and social impacts.

P24: The origin of an emergent plant disease

McCann, H.C.[1], Hui, P.[2], Kim, G.H.[3], Colombi, E.[1], Straub, C.[1], Kok, D.[1], Li, L.[2], Liu, Y.[4], Sohn, K.H.[5], Koh, Y.J.[3], Huang, H.[4], Takikawa, Y.[6], Rainey, P.B.[1,7,8]

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Recurring epidemics of kiwifruit (*Actinidia chinensis*) bleeding canker disease are caused by separate lineages of *Pseudomonas syringae* pv. *actinidiae* (*Psa*), whose emergence has coincided with domestication of its host. Kiwifruit is among the most recently domesticated crops, common cultivars are but a few generations removed from their wild ancestors. The geographical origin of most *Actinidia* spp. is in China, though some species are broadly distributed across East Asia. Earlier outbreaks of *Psa* were limited to Japan, Korea and China, however in 2008 a pandemic affected nearly all kiwifruit-growing regions in the world. We first investigated the phylogeography of *Psa* and identified recombination between lineages of *Psa* responsible for separate outbreaks. We hypothesized contemporary and past outbreaks in cultivated crops were initiated by the transmission of *Psa* from a diverse, recombining source population associated with wild kiwifruit. We therefore sampled *Pseudomonas* from both cultivated and wild *Actinidia* spp. across China, South Korea and Japan. Although wild *A. chinensis* has a habitat range that overlaps with areas of kiwifruit cultivation, *Psa* was not identified among any wild *Actinidia* spp. sampled in China. Moreover, all *Psa* isolated from cultivated kiwifruit in China were members of the same lineage responsible for the global pandemic (*Psa*-3), while multiple lineages of *Psa* are present in both Korea and Japan. *Psa* was isolated from wild *A. arguta* in both Korea and Japan, indicating the ancestral pathogen population may not have evolved on wild kiwifruit but rather in association with a more distantly related and broadly distributed host, forming a large metapopulation of *Psa* across East Asia. The identification of a wild reservoir of *Psa* presents a rare opportunity to understand the relationship between wild populations of both plants and microbes and the ecological and evolutionary factors driving the origins of disease.

P25: Evolution of copper resistance in the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae* through acquisition of integrative conjugative elements and plasmids

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Horizontal gene transfer can precipitate rapid evolutionary change. In 2010 the global pandemic of kiwifruit canker disease caused by *Pseudomonas syringae* pv. *actinidiae* (*Psa*) reached New Zealand. At the time of introduction, the single clone responsible for the outbreak was sensitive to copper, however, analysis of a sample of isolates taken in 2015 and2016 showed that a quarter were copper resistant. Genome sequences of seven strains showed that copper resistance – comprising czc/cusABC and copABCD systems – along with resistance to arsenic and cadmium, was acquired via uptake of integrative conjugative elements (ICEs), but also plasmids. The ICEs acquired by *Psa* belong to a diverse family of ICEs present exclusively in plant-associated *Pseudomonas* spp[1]. Comparative analysis showed ICEs to have a mosaic structure, with one being a tripartite arrangement of two different ICEs and a plasmid that were isolated in 1921 (USA), 1968 (NZ) and 1988 (Japan), from *P. syringae* pathogens of millet, wheat and kiwifruit respectively. Two of the *Psa* ICEs were nearly identical to two ICEs isolated from kiwifruit leaf colonists prior to the introduction of *Psa* into NZ. Additionally, we show ICE transfer *in vitro* and *in planta*, analyze fitness consequences of ICE carriage, capture the *de novo* formation of novel recombinant ICEs, and explore ICE host-range.

1. Colombi, E., *The role of integrative conjugative elements in evolution of the kiwifruit pathogen Pseudomonas syringae pv. actinidiae. PhD Thesis* in *Genetics*. 2017, Massey University: Auckland. Chapter 3.

P26: A trojan horse in the kiwifruit phyllosphere?

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Commensal bacteria colonizing eukaryotes play an important role in growth, development and disease protection. However, the role of commensals in the evolution of plant pathogens has been little explored. The transition from a commensal to a pathogenic lifestyle (or vice versa) can occur via pathoadaptive mutations or acquisition (loss) of virulence genes through horizontal gene transfer events. *Pseudomonas syringae* is a ubiquitous plant pathogen of often agricultural importance with an extensive phylogenetic diversity. Recently, a new clade (Phylogroup 3a) of nonpathogenic commensal *P. syringae* was discovered. Clade 3a appeared to be solely associated with kiwifruit and demonstrated antagonistic effects against the kiwifruit canker pathogen *Pseudomonas syringae* pv. *actinidiae* (*Psa*). A total of 23 commensal kiwifruit *P. syringae* strains were sequenced. A comparative genomics approach was used to assess their pathogenicity attributes and traits involved in host range determination. In particular focus was the Type III Secretion System (T3SS), a major virulence characteristic of *P. syringae*. Isolates in clade 3a carried an extremely reduced set of Type 3 Secretion effectors, but possessed genes involved in syringomycin/syringopeptin production. Other PG3 commensal strains retained a larger effector repertoire, but were lacking the syringomycin gene cluster. The presence of a typical tripartite pathogenicity island (T-PAI) with a *hrp/hrc* cluster was confirmed for all commensal strains, although the functionality of the T3SS remains to be elucidated. It appears as if the kiwifruit resident strains in clade 3a have adapted to their more general lifestyle by acquiring additional phytotoxin pathways, paired with a reduced effector set. Phylogroup 2c showed a similar trade-off, suggesting this is not a unique occurrence and might be a common approach for *P. syringae* not specialized on infecting particular hosts.

P27: Live cell imaging of pathogenic fungi reveals novel fungicide mode of action

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Live cell imaging of fluorescent proteins has become a core technique in molecular cell biology. Our group pioneered this approach in plant pathogenic fungi and provided insight into fundamental principles of fungal cell organisation and pathogenicity. The recent development of a live cell imaging toolset for the wheat pathogen *Zymoseptoria tritici* has enabled us to take a more applied approach, using cell biological methods to understand the way by which anti-fungal chemistries affect the pathogen (fungicide mode of action). In this talk, I shall provide our unpublished results on such investigations, using well-established fungicides. I shall demonstrate the power of this novel experimental strategy and provide insight into how live cell imaging can help to develop new fungicides, needed to secure our future food supplies

P28: Intimate bacterial-fungal interactions mediated by interkingdom chemical communication

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The polymicrobial consortium, within and around plant tissues, communicate by chemical signalling that ultimately impacts survival in symbioses through shifts in morphology, development, and metabolism. The recent expansion of microbial genomic information indicates that bacteria and fungi harbour a diversity of unique biosynthetic genes involved in creating tailored messages which can often impact their interactions with one another and other members of their local community. By coupling *in vitro* coculture methodologies, imaging mass spectrometry, microscopy, and genetics of bacteria and fungi, we explore the chemical and biological drivers of intermicrobial communications. Specifically, we have found a number of metabolic signals which mediate interactions between endosymbiotic bacteria and their fungal hosts, as well as how these interactions change metabolic output. With this ongoing research we aim to elucidate the dynamics of these novel microbial symbiosis and their potential impacts on plant ecology and health.

P29: Cell wall composition and biosynthetic machinery of the fungal pathogen Fusarium graminearum

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The fungus *Fusarium graminearum* is responsible for the head blight disease in cereals. It is a threat to our food supply due to its negative impact on crop production and produce mycotoxins in infected plant tissues, making them unsuitable for food or feed. The fungal cell wall biosynthetic enzymes are ideal targets for the development of fungicides, however identifying the key genes is challenging without fundamental knowledge of the *F. graminearum* cell wall composition. Here we present a detailed carbohydrate analysis of the *F. graminearum* cell wall and a comprehensive expression profile of genes involved in cell wall metabolism and host pathogenesis. Key members of the sugar interconversion pathway have been identified, supporting the presence of sugars found at low levels within the fungal cell wall. Genes that are stably expressed *in vitro* and *in vivo* samples provide an overview of the cell wall synthesis machinery, whilst genes that are upregulated across an in vivo time course highlight genes required for pathogenesis and nutrient acquisition. Each gene set contains novel targets for the targeted development of fungicides that either prevent the growth of the fungus by preventing the synthesis of new cell walls or the degradation of the host.

P30: Remodeling of cell wall chitin in endophytic hyphae of *Epichloë festucae* in the symbiotic interaction with *Lolium perenne* and role of LysM proteins

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Epichloë festucae is a filamentous fungus, which forms symbiotic associations with aerial tissues of *Lolium* and *Festuca* grass species. Scanning confocal microscopy (SCM)-based analysis of leaf tissue infiltrated with the chitin-specific molecular probe, WGA-AF488, showed that just septa of endophytic hyphae bound this probe while the entire cell wall was labelled in epiphyllous hyphae. These results suggest that hyphal cell wall chitin is either masked or remodeled in endophytic hyphae. The aims of this project are (i) to test whether *E. festucae* LysM-containing proteins have a role in masking chitin and thereby preventing PAMP-triggered immunity as has been shown for the *Cladosporium fulvum*-tomato pathogen interaction and (ii) to analyze the composition of the cell wall of endophytic and epiphytic hyphae.

An analysis of the *E. festucae* genome identified seven genes encoding proteins with LysM domains. Expression of two of these genes, *lymA* and *lymB*, was down-regulated in the transcriptome of three different *E. festucae* symbiosis mutants. Interestingly, both are divergently transcribed from chitinase (*chi*)-encoding genes, which are also down-regulated in the symbiosis mutants. Single deletion mutants of *lymA, lymB, chiA* and *chiB* genes as well as the double Δ*lymA/B* mutant were generated and their plant interaction phenotype analysed. Plants infected with Δ*lymA*, Δ*lymB* or Δ*chiA* had the same plant-interaction phenotype as wild type whereas Δ*chiB* and Δ*lymA/B* mutants had defects in hyphal growth within the leaves. Analysis of hyphal cell wall structure using chitin (CBP) and chitosan (CAP and OGA-488)- specific eGFP-based biosensors suggest that cell wall chitin is converted to chitosan in endophytic hyphae. This structural change is consistent with a lack of a defence response when *E. festucae* forms a mutualistic symbiotic association with *L. perenne*. Functional analysis of *E. festucae* chitin deacetylase (*cda*) genes is currently in progress.

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
P31	Dissecting the genetics of multiple rust disease resistance in a wild relative of bread wheat using comparative genomics	N Athiyannan	Agriculture and Food, CSIRO, Australia
P32	Genome wide association mapping of the Vavilov wheat diversity panel to identify novel sources of genetic resistance (and susceptibility) to septoria nodorum blotch	Kar-Chun Tan	Curtin University, Australia
P33	Transcriptome profiling of the potato plant response to zebra chip disease	Margaret Carpenter	Plant & Food Research Limited, NZ
P34	Molecular dissection of resistance to aphids in the model legume *Medicago truncatula*	Jacques Silke	CSIRO Agriculture & Food, Australia
P35	Exploiting diverse landraces for novel broad spectrum powdery mildew resistance	Cynthia Ge	Curtin University, Australia
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P41	Dissecting the role of lysin motif receptor-like kinases (LYKs) in chitin-	Laura Davies	CSIRO, A&F, Adelaide, South Australia,

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P44	Conjugation dynamics of mobilisable and self-transmissible plasmids into *E. coli* O157:H7 on *Arabidopsis thaliana*	Mitja Remus-Emsermann	University of Canterbury, Christchurch, NZ
P45	Cell wall composition of the fungal pathogen *Blumeria graminis* f. sp *hordei*	T.A Pham	University of Adelaide, Waite Campus, South Australia
P46	An Echinocandin Treatment to Improve Transformable Protoplast Yield for Effector Characterisation of the Fungal Phytopathogen *Venturia inaequalis*	Linda Collard	Plant & Food Research Limited, Auckland, New Zealand
P47	Multiple functional interfaces in plant TIR domains	Xiaoxiao Zhang	CSIRO, Canberra, ACT 2601, Australia
P48	Metagenomics for an unexpected guest: *Candidatus* Liberibacter europaeus in broom psyllids and broom plants	Claudia Lange	Landcare Research, Lincoln, NZ
P49	Proteomic analysis of *Ciborinia camelliae* necrosis-inducing culture filtrate revealed potential pathogenicity factors	N Kondratev	Massey University, Palmerston North, NZ
P50	Identification of candidate genes encoding the avirulence effector *AvrRvi8* from the plant pathogenic fungus *Venturia inaequalis*	Brogan McGreal	Plant & Food Research, Auckland, NZ
P51	The role of novel effectors HopZ5 and HopH1 in the pathogenicity of *Pseudomonas syringae* pv. actinidiae (*Psa*)	O.P van der Linden	Wageningen University, The Netherlands and Plant & Food Research, Auckland, NZ
P52	A biosecurity project: The *Ceratocystis fimbriata* species complex, Rapid Ohi'a Death and what it means for New Zealand	C Smith	Plant & Food Research, Auckland, NZ
P53	Molecular mechanisms of	Artemio	Bio-Protection

	communication between endophytic *Trichoderma* and plant roots.	Mendoza-Mendoza	Research Centre, Lincoln University, NZ
P54	Identification and characterization of novel Kiwifruit proteins that interact with effector hopH1 from *Pseudomonas syringae* pv. *actinidiae* (*Psa*) biovar III	W. Cui	Plant & Food Research, Auckland, NZ
P55	Role of *nod* gene expression in competitive nodule formation by clover rhizobia	Shaun Ferguson	University of Otago, Dunedin, New Zealand
P56	Positively-charged amino acid disorder may correspond to functions of virulence or host-cell entry in fungal effector proteins	Darcy Jones	Curtin University, Perth, Australia
P57	The role of BoiA, a Boi1-like protein, in fungal-plant symbiosis	Carla Eaton	Massey University, Palmerston North, NZ
P58	Biomimetic materials for understanding plant-microbe interactions	A Little	University of Adelaide, Adelaide, Australia
P59	Regulation of *Epichloë festucae* secondary metabolite genes by CclA, KdmB and H3K4 trimethylation	Yonathan Lukito	Massey University & AgResearch, Palmerston North, NZ
P60	Molecular organization of long lasting wheat stem rust resistance gene *Sr26* introgressed from *Thinopyrum ponticum*	Jianping Zhang	CSIRO, Agriculture & Food, Canberra, Australia
P61	Metagenomics and culturing to investigate the diverse fungi associated with cabbage and their bio-control potential	M Kuchár	Bio-Protection Research centre, Lincoln University, Lincoln, NZ
P62	Identification and characterization of the *Bcvic1* and *Bcvic2* vegetative incompatibility genes in *Botrytis cinerea*	S Arshed	Plant & Food Research, Auckland, NZ & University of Auckland, NZ
P63	Analysis of secondary metabolite gene clusters in recently described endophytic and pathogenic Epicoccum (Ascomycota, Didymellaceae) species	M Fokin	University of Auckland

P31: Dissecting the genetics of multiple rust disease resistance in a wild relative of bread wheat using comparative genomics

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Global wheat production is under threat due to frequent evolution of highly virulent forms of the *Puccinia* fungus that cause rust diseases. Sustainable management of these diseases through the effective utilization of the host mediated genetic resistance requires constant supply of unexplored resistance. Wild relatives of cultivated wheat are an important resource for such resistance; in particular the diploid progenitors that share genomes of cultivated wheat. In this study an accession, CPI110672 of *Aegilops tauschii*, the D-genome progenitor of bread wheat, was deemed a highly valuable resource because of its ability to resist leaf, stem and stripe rust infecting wheat.

To determine whether the triple rust resistance is expressed by a single locus or by multiple loci, we conducted a genetic analysis using an F_2 mapping population derived from the cross between CPI110672 and susceptible accession CPI110717. Through rust infection screening and 90K SNP marker analysis, the chromosome position and closely linked markers were identified. High-resolution mapping of the target genomic regions using the D-genome reference sequences of Chinese Spring[1, 2] and *Ae. tauschii* accession AL8/78[3] further identified tightly linked markers and gene candidates for cloning the targeted rust resistance.

P32: Genome wide association mapping of the Vavilov wheat diversity panel to identify novel sources of genetic resistance (and susceptibility) to septoria nodorum blotch

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The necrotrophic fungus *Parastagonospora nodorum* is the causal agent of septoria nodorum blotch (SNB) of wheat. In Australia, yield loss is estimated up to AUD$108 million. The pathosystem is complex and mediated by interaction of multiple fungal proteinaceous necrotrophic effector-host sensitivity gene systems. Three effector-sensitivity gene systems are well characterised in this pathosystem; SnToxA-*Tsn1*, SnTox1-*Snn1* and SnTox3-*Snn3*. Despite this, we observed that *P. nodorum* that lacked *SnToxA*, *SnTox1* and *SnTox3* (*toxa13*) retained wildtype-like ability to infect certain wheat cultivars which suggests evidence of undiscovered effectors or novel dominant susceptibility genes in the host. Resistance to SNB is further complicated through minor SNB quantitative loci (QTL) and effector epistasis. Recently, we have acquired 253 wheat accessions from the N. I. Vavilov Institute of Plant Genetic Resources in Russia which is comprised of genetically diverse landraces and historic breeding lines genotyped with DArT-Seq. To further dissect the SNB pathosystem, we subjected the Vavilov wheat panel to effector bioassays, infection with *P. nodorum* wildtype and *toxa13*. Association mapping was then performed using the resulting phenotypes to identify QTL that are associated with necrosis caused by effectors or pathogen infection. QTL were detected on chromosomes 5BL (*Tsn1*), 1BS (*Snn1*) and 5BS (*Snn3*) when the panel was assayed with SnToxA, SnTox1 and SnTox3 proteins, respectively. Association mapping of phenotypes from SN15 infection resulted in the detection of significant QTL on 1BS (*Snn1*), 2AS, 2DL, 3AL, 4BS, 5AS, 5BS (*Snn3*), 5BL (*Tsn1*), 6BS and 7AS. Association mapping using phenotypes from *toxa13* infection detected most of the above QTL, with the exception of *Tsn1*, *Snn1* and *Snn3*. Haplotype analyses confirmed the 3A QTL contributed most significantly to disease response, where resistance was conferred by a rare haplotype variant. The opportunity to introgress the 3AL resistance allele into modern bread wheat will be discussed.

P33: Transcriptome profiling of the potato plant response to zebra chip disease

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Zebra chip is a relatively new disease having a devastating impact on commercial potato production in New Zealand and the USA. It inhibits the growth of plants and reduces tuber yield and quality. When the diseased tubers are fried they acquire an unappealing dark colouration and an unpleasant taste. The disease is caused by a phloem-limited bacterium *Candidatus* Liberibacter solanacearum (CLso) and spread by an insect vector, the tomato potato psyllid (TPP). Currently the disease is being controlled by increased insecticide use, as resistant germplasm is proving difficult to find, although cultivars with some tolerance are known.

To explore the response of potato plants to zebra chip disease at a molecular level, plants were infected with CLso using infected TPP. Control plants were exposed to uninfected TPP or untreated. Eight weeks after infection, leaf, stem and tuber samples were collected for transcriptome sequencing.

The presence of CLso caused many genes to be differentially expressed (DE) in all three tissues. The greatest changes were observed in the tuber where 6895 genes were down- or up-regulated at least two-fold ($p<0.01$). Similarly, the leaf and stem revealed 2463 and 1691 DE genes, respectively. In the tuber, the starch synthesis pathways were severely disrupted by the disease. SWEET transporters, which have crucial roles in cellular sugar efflux processes, and are induced by bacterial pathogens for nutritional gain, were up-regulated in all tissues. Genes in secondary metabolite pathways involved in plant defence, such as phenylalanine ammonia lyase and 4-coumarate-CoA ligase, were also up-regulated.

Overall, zebra chip disease caused a severe disruption of plant metabolism, particularly in the tuber.

P34: Molecular dissection of resistance to aphids in the model legume *Medicago truncatula*

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Aphids are ubiquitous sap-sucking insect pests with over 4,000 species causing substantial yield losses to agriculture worldwide. Damage is caused both by direct feeding, thereby draining plant nutrients from the phloem and as major vectors for disease transmission, transmitting over 50% of all plant viruses. Resistance to aphids has been identified in a number of plant species, yet the molecular mechanisms underlying aphid resistance remain largely unknown.

We have focused on one pair of near isogenic lines of the model legume *Medicago truncatula*; A17 (susceptible) and Jester (resistant) in which single dominant genes condition resistance to bluegreen aphid (BGA), pea aphid (PA) and spotted alfalfa aphid (SAA). All three aphid resistance genes have been fine-mapped and efforts are underway to clone specific genes under their endogenous promotors via a meticulously constructed cloning system, specifically designed to overcome challenges typically correlated with cloning large, repetitive NB-ARC-LRR type of resistance genes. Large scale transcriptomic studies enabled us to pinpoint the downstream signalling pathways, transcription factors and plant defence mechanisms in place against BGA and SAA infestation. Resistance to BGA has been examined in further detail by following the aphid feeding behaviour via electrical penetration graph technique and profiling the plants metabolic response to infestation. A range of reverse genetic approaches based on the principle of RNA interference was developed to enhance our understanding of plant-aphid interactions, including the molecular mechanisms underlying plant resistance and those employed by aphids to evade these defences.

P35: Exploiting diverse landraces for novel broad spectrum powdery mildew resistance

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Barley Powdery mildew is a significant and widespread threat to barley production with the most cost-effective long term management provided by genetic resistance. The pathogen has evolved to overcome most major race-specific resistance genes deployed in Western Australia and therefore we have screened diverse landraces to discover new broad-spectrum resistance genes. A subtle new broad-spectrum resistance variant based on the *mlo* resistance in landrace Eth295 has been characterised and other candidates are under investigation.

In Eth295, resistance was found to be recessive and genetic and complementation studies indicated the involvement of *mlo*. However, unlike known *mlo* alleles, the resistance was developmentally controlled and quantitative without spontaneous cell wall appositions or extensive necrosis. This resistance has two copies of the mlo-11 repeat units, compared to 12 copies in commonly grown cultivars and was designated mlo-11 (cnv2). *mlo*-11 repeat unit copy number dependent DNA methylation, SiRNA and histone modification corresponded with macroscopic phenotypic, cytological and transcriptional differences between copy number variants. Sequence data indicated *mlo*-11 (cnv2) formed via recombination between progenitor *mlo*-11 repeat units and the 3' end of an adjacent stowaway MITE containing region. *mlo*-11 (cnv2) is the only example of a moderated *mlo* variant discovered to date and may have arisen by natural selection against the deleterious effects of the progenitor mlo-11 repeat unit configuration.

In summary, Eth295 *mlo*-11(cnv2) has no deleterious pleiotropic effects as with 'normal' *mlo*'s, so does not need special 'compensating' genes bred in. Three additional promising lines are under development for which recent data will be presented.

P36: Integrative and Conjugative Elements (ICEs) found in *Pseudomonas syringae* define a new ICE family

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Horizontal gene transfer plays an important role in evolution. Conjugative transmissible elements, such as plasmids and integrative conjugative elements (ICEs), are able to move functional genetic units over broad phylogenetic distances. ICEs are plasmid-like entities with attributes of temperate phages that disseminate vertically as part of the bacterial chromosome and horizontally by virtue of endogenously encoded machinery for conjugative transfer[1].

I describe a novel ICE family, referred to as PsICEs. Members of this family are isolated exclusively in plant-associated *Pseudomonas* spp., have the same integration loci and backbone genes. Comparative analysis reveals that the evolution of PsICEs is characterized by extensive inter-ICE recombination events that produce chimeras with variable patterns of similarity to each other, yet maintaining a syntenic backbone. The diversity of PsICEs is also driven by sequence divergence and the presence of cargo genes located in conserved intergenic loci across the backbone. Although there are different classes of PsICE cargo genes, one set was frequently recovered: the set carried on the sub-element Tn*6212*[2]. This work demonstrates that the ICEs previously described in *P. syringae* [2,3,4,5,6] are not unrelated solo elements but that they are part of a more extensive family of ICEs that spread into the *P. syringae* species a long time ago, possibly distantly related to the PAPI-1 family of ICEs in *P. aeruginosa*[7].

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P37: Moving towards whole plant screening for red needle cast resistance in radiata pine

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Phytophthoras remain a concern for several of New Zealand's primary industries, and the ability to respond rapidly to incursions will benefit from the development of molecular screening tools. Red needle cast (RNC) in radiata pine is caused by *Phytophthora pluvialis*, producing masses of sporangia in infected needles which are then prematurely cast from the tree. The impact of this defoliation on photosynthetic capability can result in significant productivity losses, and the ability to select for resistant germplasm remains a target for the industry.

Genomic testing is being enabled through the Radiata Pine Breeding Company's Genomic Selection programme, through which a high-density SNP panel has been developed. The training of genomic predictive models requires meaningful, robust phenotypic information if they are to add value to breeding programmes. To date, our largest screening datasets for RNC consist of lesion measurements from detached needle assays, revealing a broad spectrum in the response of radiata to infection. As part of Scion's Healthy Trees, Healthy Future programme, we have been exploring alternative means of assessing resistance/tolerance, in particular in non-detached, living material. Using grafted industry-relevant material, we investigated the relationship between various disease-related symptoms at progressive time-points. These more natural infections with field inoculum are, however, complicated by co-infections with other pathogens, in addition to posing logistical challenges of transporting material to sites with extensive disease, and reliance on favourable infection conditions. The establishment of a fogging chamber for plant inoculations may mitigate some of these challenges, and allow for more controlled environments in which to perform on-plant infections, and collect robust phenotypes for the development of genomic prediction models. Indications from the field study are that the temporal host/pathogen dynamic is a more useful determinant of disease outcome than the single time-point, single phenotype observations of detached assays, and may yield improved predictive models.

P38: Dothistroma needle blight: Chasing effectors in a forest pathogen

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Small proteins secreted by pathogens to modulate host defence responses are called effectors. Effectors have been a major focus of plant pathologists in recent years, combining a steadily improving computational toolset and *in planta* experiments. However, effector biology in pathogens of non-crop plants and particularly gymnosperms has received little research attention. The filamentous fungus *Dothistroma septosporum* causes a serious foliar disease, dothistroma needle blight (DNB), on *Pinus radiata* in New Zealand and on many pine species worldwide. Potentially correlated to changes in climate, this disease has been on the rise for 20–30 years, and current countermeasures often struggle to contain the damage it causes. Here we present a molecular approach to combat DNB based on the identification and functional characterisation of effectors. Effector candidates were selected using a series of computational prediction tools, as well as RNAseq data, from a compatible *D. septosporum*-pine interaction. In total, 20 effector candidates were screened for induction of cell death in the model plants *Nicotiana benthamiana* and *N. tabacum* using Agro-infiltration. Four effector candidates induced cell death in at least one of these species, indicating recognition by the plant defence machinery, or toxicity. Of the four candidates, one is a homolog of an *Alternaria alternata* allergen and one is a Dothideomycete core effector (Ecp2). Effector screening methods are not available for pine, thus various approaches to achieve this have been trialled. Current research is focused on delivering extracted effector proteins into young clonal *P. radiata* shoots. These shoots may ultimately be used to screen for resistant pine genotypes. Selection of genotypes at this early stage could speed up DNB resistance screening for pine breeding. This research will also contribute to the molecular understanding of forest diseases and effectors that may be common among pathogens of distantly related hosts.

P39: Harnessing molecular determinants of virulence and adaptation in kauri dieback pathogens

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Phytophthora agathidicida is an oomycete pathogen responsible for the devastating kauri dieback disease. Since its formal identification in 2008, this pathogen has killed thousands of kauri trees in New Zealand. However, the genetic diversity of *P. agathidicida* or the mechanism by which this pathogen infects its host is poorly understood. Genomes of fourteen isolates of *P. agathidicida* were sequenced in order to assess the genetic diversity of its population in New Zealand. We show here that these isolates display very low levels of sequence diversity, which is compatible with a clonal population, suggesting conserved infection mechanisms and host targets. Oomycete plant pathogens, like their fungal counterparts, secrete effector proteins to facilitate disease. Contrary to fungal effectors, oomycete effectors contain highly conserved protein motifs that allow for their identification. These include the well-known RxLR and CRN (crinkler) motifs. Comprehensive bioinformatics analysis of the 14 *P. agathidicida* genomes identified 78 RxLR and 65 CRN candidates based on their domain compositions. Their domain structure, similarities to effector sequences from other oomycete species and potential evolutionary selection signatures in these effectors, were also studied. Some of the effectors are conserved in most *Phytophthora spp.* while others are unique to *P. agathidicida.* The RxLR effectors were also functionally assessed and potential virulence and avirulence factors were identified. A better understanding of *P. agathidicida* effectors could lead to the discovery of plant resistance genes that could be selected in kauri breeding programs in order to improve resistance to the kauri dieback disease.

P40: The genome of the broad host range plant pathogen Sclerotinia sclerotiorum exhibits polymorphic microregions containing genes associated with virulence and heterokaryon incompatiblity

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The fungus *Sclerotinia sclerotiorum* is a plant pathogen with a broad host range. Adaptive genomic processes in this fungus are elusive. In many narrow host range pathogenic fungi, genes encoding secreted virulence proteins and secondary metabolite biosynthesis genes are under enhanced selective pressure and frequently polymorphic. These types of genes are often contained in specific regions of the genome that favour mutation and instability due to an overabundance of transposable elements. There is another major group of genes known to be polymorphic in fungi that is involved in heterokaryon incompatibility. This is a process whereby two unerlated individuals prevent hyphal fusion, which may be important for preventing the spread of deleterious genetic material.

To determine what types of genes are polymorphic in *S. Sclerotiorum* and whether they exhibit a location bias, we compared genomic reads from 26 isolates with diverse geographical origins. We used both *de novo* assembly and mapping to the existing reference genome to infer positions of SNPs and InDels relative to reference gene annotations.

We found that secondary metabolite biosynthesis, secreted protein encoding and heterokaryon incompatibility associated genes frequently cluster within polymorphic microregions with an overabundance of transposable elements. Genes in these groups were overrepresented in gene subsets with presence/absence polymorphisms, SNP and InDel polymorphisms and evidence of positive selection. We conclude that selective pressures on heterokaryon incompatibility and adaptation to a changing host environment have possibly led to similar genomic traits of underlying gene sequences.

P41: Dissecting the role of lysin motif receptor-like kinases (LYKs) in chitin-triggered immunity in grapevine

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A key aspect of the plant innate immune system is the recognition of invading pathogens. This occurs through plasma membrane localised pattern recognition receptors (PRRs) detecting conserved pathogen signatures, termed pathogen-associated molecular patterns (PAMPs). In *Arabidopsis thaliana* CERK1 is a lysin motif receptor-like kinase (LYK), which is involved in the perception of chitin released from invading fungal pathogens. In comparison to the five members of the *LYK* gene family in Arabidopsis, we have identified ten members of the gene family in grapevine (*Vitis vinifera*), three of which (*VvLYK1-1*, *VvLYK1-2* & *VvLYK1-3*) are highly homologous to *CERK1*. VvLYK1-1:GFP was shown to localise to the plasma membrane. Expression of *VvLYK1-1* in the *Atcerk1* mutant background restored chitin-induced defense responses as demonstrated by MAPK activation and infection assays with the non-adapted grapevine powdery mildew pathogen, *Erysiphe necator*. This suggests that VvLYK1-1 plays a key role in PAMP-triggered immunity to powdery mildew in grapevine.

The kinase domain of *VvLYK1-1* was used as a bait in a yeast two-hybrid screen to search for interacting factors. The yeast two-hybrid screen identified a U-box E3 ubiquitin ligase, which shows high homology to the Arabidopsis PUB13 protein. PUB13 has been previously demonstrated to polyubiquitinate FLS2, the receptor of bacterial flagellin (flg22), and promote flagellin-induced FLS2 endocytosis and subsequent degradation.

P42: Dissecting pathogenesis in wheat by the fungal hemibiotroph, *Bipolaris sorokiniana*

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Bipolaris sorokiniana, a broad-range cereal pathogen, is a significant agronomic threat to wheat and barley production in the world's warmer cropping regions. The hemibiotrophic fungus infects all parts of its host, causing foliar Spot Blotch (SB) and Common Root Rot (CRR). In the warm and humid wheat-growing regions of South-East Asia, the Indian subcontinent and South America, these diseases are a major constraint on wheat production. Yield losses range around 20% with potential for 100% losses in environmental conditions favouring disease development. Impacts are exacerbated under the resource limited growing conditions common in these regions[1,2]. Despite the agronomic impact of *B. sorokiniana,* little is understood of the virulence mechanisms employed during pathogenesis. The horizontally-acquired effector ToxA, is the only host-specific virulence factor identified to date and provides a fitness advantage on *Tsn1* wheat lines[3]. Screening of *in vitro* secretomes of *ToxA*-positive isolates suggests the effector is strongly expressed in specific axenic conditions, alongside non-host specific phytotoxins.

To begin addressing this knowledge gap, we are generating a comprehensive annotation of a PacBio genome assembly of the isolate CS10 to allow mining for virulence-associated genes and an initial characterisation of the wheat-*B. sorokiniana* interaction. A non-redundant transcriptome was generated using Illumina RNAseq data from a range of *in vitro* culture conditions to direct gene structure annotation. Transcript-guided *ab initio* and sequence homology-based gene predictions are incorporated in our informatics pipeline to produce a set of annotations supported by multiple lines of evidence. The annotated genes will be subsequently analysed using EffectorP[4] to identify candidate effector-encoding genes. These will be functionally characterised through gene editing and heterologous protein expression approaches to assess their role in virulence. It is anticipated that this comprehensive approach will fundamentally advance our understanding on our *B. sorokiniana* causes disease on key cereal crops.

[1] Duveiller, E. and Sharma, R. C. (2012) Wheat resistance to spot blotch or foliar blight. In: *Disease Resistance in Wheat.* (Sharma, I., ed.). Oxfordshire, UK: CABI, pp. 120-135.

[2] Duveiller, E., Kandel, Y. R., Sharma, R. C. and Shrestha, S. M. (2005) Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. *Phytopathology,* **95,** 248-256.

[3] McDonald, M. C., Ahren, D., Simpfendorfer, S., Milgate, A. and Solomon, P. S. (2017) The discovery of the virulence gene ToxA in the wheat and barley pathogen Bipolaris sorokiniana. *Molecular Plant Pathology*.

[4] Sperschneider, J., Gardiner, D. M., Dodds, P. N., Tini, F., Covarelli, L., Singh, K. B., Manners, J. M. and Taylor, J. M. (2016) EffectorP: predicting fungal effector proteins from secretomes using machine learning. *New Phytologist,* 210, 743-761.

P43: Characterisation of pleiotropic leaf rust resistance gene *Lr13*

Hewitt, T.[1] [2], Zhang, J.[1] [2], Zhang, P.[2], Park, R.[2], Upadhyaya, N.[1], McIntosh, R.[2], Wulff, B.[3] [4], Steuernagel, B.[3] [4], Lagudah, E.[1]

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The group of fungal pathogens known as rusts continue to pressure wheat crops worldwide. Rust resistance (R) genes, which invariably encode proteins of the NBS-LRR (Nucleotide-binding site and leucine-rich repeat) family, have been a valuable resource for crop breeders to minimise yield losses due to infection. However, as R genes are usually bred one at a time into crop lines, the protection they provide is often overcome within a few seasons by pathogen evolution. Combining multiple R genes in a single line is considered a robust solution for providing durable resistance. With the advent of targeted gene enrichment and next-generation sequencing (NGS), the ability to rapidly clone specific R genes is helping this to become a reality (Steuernagel *et al*, 2016). Moreover, the ability to obtain the genetic sequences of individual NBS-LRRs facilitates research into their underlying mechanisms of resistance, much of which remains unknown. The many varieties of wheat and their relatives harbor a plethora of rust R genes that could be studied and cloned in order to engineer more sustainable disease control.

Several R genes appear to present altered phenotypes in certain genetic backgrounds, and cloning them would aid in uncovering the molecular interactions of the NBS-LRRs they encode. One such phenotype is that of hybrid necrosis, which has been observed in crosses of certain wheat genotypes involving R genes. One of which, *Lr13*, continues to provide resistance to leaf rust in Australia and Canada. Associated genes, *Ne1* and *Ne2,* occur in different allelic forms and when combined in the same genotype induce hybrid necrosis. Zhang *et al* (2016) concluded recently that *Lr13* and an allele of *Ne2* are in fact the same gene based on crosses and mutational studies. The capability of *Lr13* to incur both leaf rust resistance and hybrid necrosis cannot be answered without first cloning it. Current genetic markers for *Lr13* do not show close linkage due to reduced recombination in the near centromeric region where it resides. This does not make *Lr13* very amenable to traditional map-based cloning. The NGS-based pipeline known as MutRenSeq (mutagenesis and R-gene enrichment sequencing) was used on *Lr13* EMS (Ethyl methanesulfonate) induced, susceptible mutants along with support from comparative genomics to ascertain candidate gene sequences for *Lr13* which are currently being screened. Definite proof that a single gene is involved will only come with transformation studies when *Lr13* is successfully cloned and transformed into a susceptible line to see if it can confer both a resistance phenotype and a necrotic phenotype in the offspring of crosses between transgenic lines and lines possessing the corresponding *Ne1*gene.

Steuernagel, B., S. K. Periyannan, I. Hernandez-Pinzon, K. Witek, M. N. Rouse, G. Yu, A. Hatta, M. Ayliffe, H. Bariana, J. D. Jones, E. S. Lagudah, B. B. Wulff. 2016. "Rapid Cloning of Disease-Resistance Genes in Plants Using Mutagenesis and Sequence Capture." *Nat Biotechnol* 34(6): 652-5.

Zhang, P., C. W. Hiebert, R. A. McIntosh, B. D. McCallum, J. B. Thomas, S. Hoxha, D. Singh, and U. Bansal. 2016. "The Relationship of Leaf Rust Resistance Gene *Lr13* and Hybrid Necrosis Gene *Ne2m* on Wheat Chromosome 2bs." *Theor Appl Genet* 129(3): 485-93.

P44: Conjugation dynamics of mobilisable and self-transmissible plasmids into *E. coli* O157:H7 on *Arabidopsis thaliana*

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Less than a century since the discovery of antibiotics, bacterial diseases have yet again become a major threat to human welfare as infectious bacteria acquired antibiotic resistances that are able to overcome every antibiotic currently available. Most antibiotic resistance genes present in human pathogenic bacteria are believed to originate from environmental bacteria. This implies that, for an antibiotic resistance gene to reach a human pathogenic bacterium, there needs to be an exchange of genetic material from an environmental bacterium towards a pathogen. Plasmid conjugation is considered to be one of the major reasons for the increasing prevalence of antibiotic resistances. A hotspot for plasmid-based horizontal gene transfer is the so-called phyllosphere, i.e. the surfaces of aboveground organs of plants.

Bacteria that constitute the normal phyllosphere microbiota of plants are generally not considered harmful, but for antibiotic resistances conferring plasmids they might serve as intermediate hosts with transfer capability to human pathogenic bacteria. In this study, we evaluated the exchange of mobilisable and self-transmissible plasmids via conjugation. Me determined conjugation from the laboratory strain *E. coli* S17-1, the model phyllosphere coloniser *Pantoea eucalypti* 299R, and the model pathogen *E. coli* O157:H7 delta stx to the recipient strain *E. coli* O157:H7 delta stx in the phyllosphere of *Arabidopsis thaliana*. To do so, we employed a miniature plant system that enabled us follow plasmid conjugation dynamics to *E. coli* O157:H7 on plant leaves.

The here-presented rates of plasmid transfer will be important for future modelling approaches to estimate environmental antibiotic resistant spread in agricultural production environments

P45: Cell wall composition of the fungal pathogen *Blumeria graminis* f. sp *hordei*

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Blumeria graminis f. sp. *hordei* (*Bgh*) is an ectoparasitic obligate biotrophic fungal plant pathogen that exclusively infects barley and causes the disease powdery mildew. It is one of the most economically important diseases to barley as it can cause up to 20-40% losses in yield.

All fungi are encompassed by a complex network known as the cell wall. Cell wall synthesis is a fundamentally important process in the growth, survival, and morphogenesis in fungal cells. As the first point of contact between the environment and the fungi the cell wall holds a critical role in determining if the conditions of the environment are hostile or favourable. As the cell wall is important for survival and has many key functions it is an ideal target for cell wall fungicides.

While the overall cell wall structure and architecture is conserved across all fungal species, primarily consisting of an intertwined network of chitin, β-1-3-glucans and mannoproteins, it is not the same across all species. Deciphering the structure and composition of the *Blumeria* cell wall will give us a greater understanding of how it works and what aspects to target for inhibition. By examining the changes that occur in the cell wall composition during pathogenesis we may be able to find targets for antifungals.

P46: An Echinocandin Treatment to Improve Transformable Protoplast Yield for Effector Characterisation of the Fungal Phytopathogen *Venturia inaequalis*

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Venturia inaequalis is a host-specific fungal pathogen of Maloideae species and the causal organism of apple scab, a damaging disease of apples present in apple growing regions worldwide. The fungus parasitises its host in the sub-cuticular space where a specialised tissue, stroma, develops and forms an interface between pathogen and host. The mechanisms of nutrient acquisition and suppression of host defence are still to be discovered. Understanding the molecular mechanisms of virulence in this pathogen may inform resistance breeding strategies to secure the economic viability of valuable apple cultivars.

Pathogens employ small molecules, effectors, to establish infection by suppression and evasion of host defence mechanisms. However, no effectors required for virulence have yet been characterised in this organism, although many candidate effectors have been identified by bioinformatic analyses of the genomes of several isolates of *V. inaequalis*.

While *V. inaequalis* is transformable using *Agrobacterium* mediated transformation, characterisation of candidate effectors is hampered by the inability to create gene knock outs without off-target effects. Protoplast based transformation techniques are difficult because cell wall polysaccharides must be removed to generate protoplasts and available cell wall lysing enzymes are highly inefficient. Additionally, knocked out or silenced cell wall synthesis genes result in loss of pathogenicity. The echinocandin, caspofungin acetate, is a pharmaceutical grade (1→3) β-D-glucan synthesis inhibitor. We have trialled the use of caspofungin at sub-lethal concentrations to inhibit cell wall synthesis prior to enzymatic cell wall digestion in order to improve the yield of protoplasts. Recovery of cell wall synthesis and restoration of virulence following withdrawal have been evaluated. Transformation with a knock out vector of a cysteine knot containing protein, candidate effector atg7917, has been attempted.

P47: Multiple functional interfaces in plant TIR domains

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The Toll/interleukin-1 receptor (TIR) domain is widespread in animal and plant immune receptors. In plants, most immune receptors belong to the nucleotide-binding domain and leucine-rich repeat (NB-LRR) family. A major sub-family of NB-LRR proteins, such as flax L6 and *Arabidopsis* RPS4, contain a TIR domain at their N-terminus. Previous investigation of L6 and RPS4 TIR domains showed that isolated TIR domains can trigger pathogen effector-independent cell death and that TIR domain self-association is required for this signalling activity. L6TIR self-associates through an interface involving residues from the αD and αE helices (DE interface), while RPS4TIR self-associates through a different interface formed by the αA and αE helices (AE interface). Here we report the crystal structure of the TIR domain from the *Arabidopsis* TIR-NB-LRR protein SNC1. Analysis of the structure combined with site-directed mutagenesis reveal two distinct dimerization interfaces that resemble the AE and DE interfaces, respectively. Mutations in the AE interface abolish SNC1TIR self-association in solution. In addition, mutations in either AE or DE interface abolish SNC1TIR cell death signalling activity *in planta*. Although the AE interface was not identified in the L6TIR crystal structure and vice versa, mutations in the AE-interface equivalent regions of L6 disrupt L6TIR self-association in yeast and depress L6TIR signalling *in planta*. For RPS4, mutations in predicted DE interface abolish RPS4TIR self-association in yeast and affect RPS4TIR signalling. These data suggest that both interfaces are involved in plant TIR domain self-interaction and play distinct roles in TIR domain signalling function.

P48: Metagenomics for an unexpected guest: *Candidatus* Liberibacter europaeus in broom psyllids and broom plants

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The broom psyllid (*Arytainilla spartiophila*) was introduced to New Zealand (NZ) from the UK in 1993 as a biocontrol agent for the invasive Scotch broom (*Cytisus scoparius*). In 1994 a new genus of unculturable bacteria, *Candidatus* Liberibacter, was discovered that is obligately vectored by psyllids and often causes plant diseases (e.g. in tomatoes, potatoes and citrus), however some plants can harbour *Ca.* L. europaeus (leu) yet remain asymptomatic. In 2011-2013 leu was found widespread in Scotch broom and broom psyllids in NZ as well as in the UK. Their 16S sequences were identical, pointing to the UK as potential origin of leu in NZ. Even though a small number of kowhai trees and kowhai psyllids tested positive, the trees were healthy, and leu could not be found in other plants in NZ.

We investigated the pathway of leu introduction into NZ, the impact of leu on biocontrol efficiency of the psyllids and the potential that leu poses a biosecurity risk. A leu specific sequence of 16S was amplified by PCR and qPCR for detection and the bacterial community was determined by 16S metagenomics.

We found that leu-infected broom plants could not be “cured” by removing psyllids (insecticide treatment), indicating that the bacterium persists in the host. While we found that leu is only transmitted horizontally by broom psyllids, it can also be transmitted vertically by seeds. Plant symptoms did not reliably indicate leu-infection and we could not confirm a plant-damaging effect of leu in the absence of psyllids. Our results confirm that leu was likely introduced to NZ unwittingly via broom psyllids from the UK. The sum of these results and the fact that psyllids are highly host-specific indicate a relatively low biosecurity risk to NZ.

P49: Proteomic analysis of *Ciborinia camelliae* necrosis-inducing culture filtrate revealed potential pathogenicity factors

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The fungal pathogen, *Ciborinia camelliae* (Sclerotenіaceae), causes petal blight in plants of the *Camellia* genus which has important economic and cultural value. The fungus only infects floral organs causing browning and premature loss of blooms. The aim of our study is to identify fungal pathogenicity factors and determine their effects on *Camellia* plants. Since the secreted proteins are important components of the virulence machinery of pathogens, we are investigating proteins secreted by *C. camelliae.* We have collected fungal culture filtrate and showed that it causes necrosis when infiltrated into *Camellia* petals. Further experiments demonstrated that necrosis-inducing agents of the filtrate are of proteinacious nature. Using a trichloracetic acid precipitation method we extracted proteins from *C. camelliae* culture filtrate and sequenced them using MALDI/TOF mass spectrometry. In total, 94 proteins were detected in the fungal *in vitro* secretome. We annotated these proteins using their sequence simmilarity to proteins from public databases and classified them into five major groups based on their putative functions: proteases, carbohydrate-active enzymes, oxydoreductases, probable hypersensitive response elicitors and others. Overall, we observed a high similarity between the *in vitro* secretomes of host and organ-specific *C. camelliae* and closely related broad-host pathogen, *Botrytis cinerea*. We found *C. camelliae* homologs of endopolygalacturonases, cerato-platanin and NEP-like proteins which are known to contribute to *B. cinerea* virulence. In addition, the presence of enzymes involved in reactive oxygen species metabolism in the secretome (superoxide dismutase and thioredoxin) suggested that the fungus may use a redox balance shift in its infection strategy, similar to other Sclerotiniaceae necrotrophs. Currently, we are expanding our proteomic investigation into the *C. camelliae in planta* secretome by extracting and analyzing intercellular (apoplastic) fluids of infected *Camellia* petals. The study of the *C. camelliae* secretomes will help to understand pathogenicity and resistance mechanisms of the *C. camelliae–Camellia* interaction.

P50: Identification of candidate genes encoding the avirulence effector *AvrRvi8* from the plant pathogenic fungus *Venturia inaequalis*

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The fungus *Venturia inaequalis* causes scab disease of apple. The interaction between the host, *Malus*, and the pathogen follows the 'gene-for-gene' model, with 17 avirulence (*Avr*) and cognate resistance (*R*) gene pairings currently being recognised. Two *R* genes have been cloned from *Malus*, but no avirulence effector genes. Resistance responses in apple governed by 'gene-for-gene' interactions are phenotypically diverse and not restricted to a rapid hypersensitive response. It is hypothesised that the Rvi8 R protein recognises an effector (AvrRvi8), and initiates a stellate necrosis resistance response approximately 10-14 days post inoculation (dpi). Although this response restricts sporulation, rendering the host field-resistant, extensive sub-cuticular fungal growth, typical of a susceptible interaction, is also observed. A bulked segregrant/NGS approach was adopted to identify candidate *AvrRvi8* genes. Progeny (80) from an *in vitro* cross between isolates B04 (*AvrRvi8*) and 1639 (*avrRvi8)* have previously been phenotyped with respect to *AvrRvi8*[1]. DNA from two pools (*AvrRvi8* and *avrRvi8*), each comprising 12 progeny, were sequenced using Illumina technology, and 213 candidates for *AvrRvi8*, with SNPs in the orthologues between the pools, were identified by mapping the reads against the genomes of the parental isolates. Of these, only 64 candidates were situated between two novel markers for *AvrRvi8*, 587,867bp apart on a single scaffold in the B04 genome, of which nine encoded proteins which were predicted to be secreted and four had identical predicted protein sequence in two *AvrRvi8* isolates, and amino acid differences between the parental isolates. The primary candidate gene is predicted to encode a small, putatively secreted protein and had a 4 and 6 log2-fold increase in expression *in planta* compared with *in vitro* at 2 and 7 dpi, respectively, as measured by RNA-seq. The candidate gene belongs to a small gene family and is currently being functionally analysed by a complementation strategy.

1. Broggini, G. A. L., Bus, V. G. M., Parravicini, G., Kumar, S., Groenwold, R., and Gessler, C. 2011. *Genetic mapping of 14 avirulence genes in an EU-B04 × 1639 progeny of* Venturia inaequalis. Fungal Genet. Biol. 48:166-176

P51: The role of novel effectors HopZ5 and HopH1 in the pathogenicity of *Pseudomonas syringae* pv. actinidiae (*Psa*)

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Since 2008 the highly virulent and rapidly spreading biovar 3 of the kiwifruit pathogen *Psa* has been threatening the kiwifruit production worldwide. Comparative genomic analysis showed that the effector proteins HopZ5 and HopH1 are exclusively present in *Psa* biovar 3, and these effectors could potentially explain the virulence observed on cultivars of *Actinidia chinensis*.
In this study HopZ5 and HopH1 were both individually and simultaneously knocked out in the pandemic *Psa* isolate ICMP 18884. Highly susceptible golden kiwifruit variety *A. chinensis* 'Hort16A' and resistant kiwi berry *A. arguta* (07-03) were inoculated with *Psa* NZV-13 wt and the HopZ5/HopH1 KOs using the flood assay of tissue cultured plants. *A. arguta* shows a classical hypersentitive response to *Psa* infection suggesting the presence of a major resistance gene. Bacterial growth was monitored in 'Hort16A' and, after staining inoculated leafs, visual symptoms were monitored in both 'Hort16A' and *A. arguta*.
Here we show that the knockout of HopZ5 and/or HopH1 in *Psa* ICMP 18884 does not affect the ability to establish local infection in 'Hort16A' using this assay method. The high virulence of *Psa* strains within biovar 3 can thus not solely be explained by the acquisition of HopZ5 and HopH1. *Psa* ICMP 18884 wt elicits HR in *A. arguta* (07-03), and so do all KOs. HopZ5 and HopH1 are thus not exclusively responsible for the elicitation of HR.
Even though no effect of HopZ5 and HopH1 KO was found in the ability to establish and cause local infection, the role of these novel effectors in systemic infection using alternate assays has yet to be investigated.

P52: A biosecurity project: The *Ceratocystis fimbriata* species complex, Rapid Ohi'a Death and what it means for New Zealand

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First identified in 2015, Rapid Ohi'a Death (ROD) is the colloquial name for the disease caused by the ascomycete fungus *Ceratocystis fimbriata* that is currently spreading throughout Hawai'i. ROD is infecting and killing the Ohi'a tree (*Metrosideros polymorpha*), the dominant forest tree in Hawai'i. This plant pathogen is an important biosecurity concern for New Zealand as 12 of our native tree species are in the *Metrosideros* genus, including the iconic Pohutukawa (*Metrosideros excelsa*) and all of the Rata.

Throughout the world there are numerous strains of *C. fimbriata,* with the most common isolate infecting sweet potato (*Ipomoea batatus*) being found with a global distribution. It is believed this was spread clonally through distribution of sweet potato and it is this strain that is found in New Zealand. However, other host specific strains have been identified that infect a range of species, such as mango trees (*Mangifera indica*) and a variety of others. How these strains are related is poorly understood. Recent molecular research indicates that these different strains are closely related cryptic species, with at least two being described as separate species; *C. platani* and *C. cacaofunesta*. We have sequenced and assembled 11 isolates from Hawai'i and other countries to better understand the origin of ROD and how it is related to other strains of *C. fimbriata*. Phylogenetic analysis was undertaken through Bayesian inference of a set of ~3,300 core genes identified using BUSCO. Of the three isolates of ROD, two group together with *C. platani* and an isolate collected from a *Syngonium* sp at a Hawaiian nursery, whereas the third ROD isolate groups separately into a clade with *C. pirilliformis*. This indicates that what is being called ROD is actually two phylogenetically distinct groups. Further investigation may yield insight into the origin of ROD. Given the cryptic nature of the *C. fimbriata* species complex an accurate molecular identification test is necessary. As part of this goal we have developed a PCR-based assay that can positively identify ROD and can distinguish it from the strain of *C. fimbriata* that is already present in New Zealand.

P53: Molecular mechanisms of communication between endophytic *Trichoderma* and plant roots.

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Trichoderma fungi establish beneficial interactions with the roots of majority of higher plants and this interaction is predominantly a symptomless process. The fungi confer beneficial attributes to the host plant, for example, growth promotion, stress tolerance and disease resistance, while the fungi themselves obtain nutrients and derive protection from being inside the plant tissues. In this study, we provide insights into how *Trichoderma virens* behaves as an endophyte during the first stages of root colonisation, revealing several mechanisms that *T. virens* uses to communicate with roots of two model plant systems, *Arabidopsis thaliana* and *Zea mays*. We identified, by different strategies, important players in this interaction including potential effector proteins, phytohormones and volatiles. The final aim of this study is to elucidate the biological function of these molecules in root colonisation, immune response and hormonal regulation. *Trichoderma* colonisation is likely to be assisted by the secretion of cell wall degrading enzymes and potential suppressors of plant immunity. Volatiles emitted by *Trichoderma* had a significant impact in plant fitness and interestingly, this emission appears to be species/isolate dependent. In conclusion, *Trichoderma* communicates with its host plant by several mechanisms enabling mutualistic endophyte-plant interaction.

P54: Identification and characterization of novel Kiwifruit proteins that interact with effector hopH1 from *Pseudomonas syringae* pv. *actinidiae* (*Psa*) biovar III

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Pseudomonas syringae pv. *actinidiae* (*Psa*) belongs to Group I of *Pseudomonas syringae* and is responsible for bacterial canker in kiwifruit. *Psa* can be grouped into four biovars based on their genetic makeup and geographical origin. Previous genome sequencing has revealed the effector complement of these *Psa* biovars. *Psa* effector hopH1 is restricted to biovar III of *Psa*, but is also found in other *P. syringae* pathovars. Identification of the key plant proteins (R-genes) that recognise and/or interact with *Psa* effectors is a crucial step for understanding and management of the bacterial canker of kiwifruit. This project aims to identify the hopH1 host targets in *Actinidia chinensis*. We have generated an *Actinidia chinensis* yeast two-hybrid cDNA library from young leaves and shoots utilizing the highly potent homologous recombination machinery of *Saccharomyces cerevisiae* (Matchmaker Gold Yeast Two-Hybrid System). Yeast two-hybrid screening of the cDNA library using hopH1 as the bait has identified the interaction of hopH1 with a number of novel *Actinidia chinensis* proteins in yeast. The details of these proteins are further presented and discussed.

P55: Role of *nod* gene expression in competitive nodule formation by clover rhizobia

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Clovers are an important component of sustainable pastoral systems in New Zealand. Clovers enter a symbiotic relationship with rhizobium bacteria through a highly specific and complex signal exchange culminating in the formation of nitrogen-fixing root nodules. Plants exude flavonoids that are perceived by compatible rhizobia through a LysR-type regulator NodD. Activated NodD mediates expression of the nodulation *(nod)* genes, which produce a cocktail of signalling molecules known as Nod factors (NF). Recognition of NF by compatible legumes initiates nodule formation. Distinct strains of the clover-nodulating species, *Rhizobium leguminosarum* bv. *trifolii* (*Rlt*), vary in their ability to nodulate different clover species effectively, and to form nodules in competition with other strains. This is important agriculturally as indigenous strains often outcompete added inoculum strains that are superior at nitrogen fixation. To determine whether differences in *nod* gene expression contribute to host specificity or competitive ability, we investigated the induction of *nodA* and *nodF* promoters from four strains with different host specificities. We found significant variation in *nod* gene expression in response to flavonoid 7,4'-dihydroxyflavone, due to both the particular promoter and host strain background. Furthermore, we discovered most *Rlt* strains contain a previously unreported second copy of *nodD*, designated *nodD2*. Markerless deletion of *nodD1* from a strain containing *nodD2* revealed only slight reduction in nodulation on white clover. Deletion of both copies of *nodD* resulted in abolished nodulation, which was restored by complementation with either *nodD1* or *nodD2*. Together these results provide evidence of a functional NodD2 in some *Rlt* strains. Future work will determine whether differences in *nod* and other gene expression in the rhizosphere contribute to host-specificity and competitive ability, and the role *nodD2* plays. This will allow us to identify genetic factors contributing to successful symbiosis, to be used as selection criteria for high-quality clover inoculants in New Zealand.

P56: Positively-charged amino acid disorder may correspond to functions of virulence or host-cell entry in fungal effector proteins

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Sequence-based prediction of effectors using machine learning (ML) has become an integral step in effector discovery. EffectorP[1], the first ML predictor for fungal effectors, is a significant step towards prediction using empirically derived features (rather than arbitrary ranking scores from criteria e.g. cysteine count, no homology). However, the success of ML classification approaches depends on the choice of features offered to the model during training. Intrinsic disorder has been reported to be an important property of some oomycete and bacterial effector proteins. We hypothesise that disordered regions are likely to form protruding epitopes that may be important for membrane-interaction or similar functions in fungal effectors, present evidence of a relationship between disorder and known functionally-active sites, and demonstrate the utility of disorder prediction as a feature in ML-based effector prediction.

To investigate the relationship between effectors and disorder, we compared disorder characteristics and amino acid properties within disordered regions of fungal effectors and non-effectors. Comparing full-length sequences of known effectors and fungal secreted non-effectors (50-314 aa) we observed an overall enrichment of cysteines in effectors. The number and proportion of disordered regions in effectors was lower compared to secretomes, however within them an enrichment of flexible, polar/charged amino acids was observed. When superimposing combinations of disorder and charge over 3D structures of known effectors, we observed that known active sites required for virulence and/or lipid-binding often occurred in protruding disordered regions carrying a net positive charge.

We applied a suite of disorder-related features to develop DisEffected - a multi-label machine-learning classifier - to predict fungal, oomycete, (selected) bacterial effectors, as well as effector sub-families (ToxA-like, MAX, RALPH, and AvrLm6-like) where possible. We also included a number of non-effector classes with effector-like characteristics (e.g. small, secreted, disordered) including anti-microbial peptides, defensins, and hydrophobins. Preliminary findings indicate that amino-acid frequencies and properties within disordered regions are a useful feature in distinguishing effectors from secretomes, prototype classifiers and feature-sets reach 75-95% accuracy.

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P57: The role of BoiA, a Boi1-like protein, in fungal-plant symbiosis

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Reactive oxygen species (ROS) produced by the fungal NADPH oxidase (Nox) complex are essential for many differentiation and developmental processes, including regulation of beneficial and detrimental plant-fungal interactions. However, the mechanism by which this complex is regulated remains unclear. In plant and mammalian systems, lipid signaling plays an important role in both Nox complex assembly and activation. Based on analogy with these systems, it is hypothesised that fungal Nox complex assembly occurs through targeted recruitment of cytosolic Nox components to the hyphal tip via interaction between lipid-binding domains and specific phosphoinositides enriched at the tip. The fungal NoxA complex consists of membrane-bound NoxA and NoxD components and cytosolic subunits NoxR, RacA, BemA and Cdc24. Although BemA contains a putative phosphoinositide-binding PX domain, this is dispensable for membrane localisation in *Neurospora crassa*. We hypothesise that recruitment of BemA occurs via interaction with the phosphoinositide-binding Boi1-like protein, which is known to interact with Bem1 in yeast. *E. festucae* contains two Boi1-like proteins, designated BoiA and BoiB. Phenotypic analysis of Δ*boiA* deletion mutants revealed loss of hyphal cell-cell fusion in culture, and a similar defective plant-interaction phenotype to Δ*bemA* mutants. Plants infected with Δ*boiA* were hypertillered and had a significant reduction in tiller length compared to wild-type. Hyphal biomass *in planta* was also increased and intrahyphal hyphae were frequently observed. In contrast, plants infected with Δ*boiB* mutants were indistinguishable from wild-type. These results show that BoiA is required for establishment of a mutualistic symbiotic interaction between *E. festucae* and *L. perenne*. Experiments to investigate interactions between BoiA and BemA are in progress.

P58: Biomimetic materials for understanding plant-microbe interactions

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Surface interfaces play a vital role in plant-microbe interactions. Environmental microbes need to first adhere to, and then interact with plant surfaces, whether they be leaves, roots or other structures, to engage in symbiotic or pathogenic relationships. Plant fungal pathogens, for example, grow and infect based on perception of physical and chemical cues presented at the leaf cuticle[1]. Model surfaces have a good potential for investigating how the perception of inductive signals trigger infection; however, it has been difficult to construct well-defined materials that can disentangle intertwined variables (such as surface hydrophobicity and inductive chemicals). This has led to confusion about the relative importance when multiple inductive signals are present[2]. Thus, more sophisticated surface models are required if we are able to identify, and then definitively tie the physical properties of leaf surfaces to molecular pathways leading to infection.

We approach this problem from the bottom up with materials-led research aimed towards developing advanced biomimetic models. Using nanofabrication and novel surface coating technologies, we construct materials that have well-defined topologies, and present biologically-relevant physical and chemical interfacial modifiers. These models will first lead to the identification of the key inductive signals that drive infection that can then be assembled into more intricate tissue models. Such biomimetic models aim to 1) understand, from the pathogen side, the molecular biology underlying adhesion and virulence and to 2) understand, from the plant side, how inductive signalling presented on surfaces could be silenced to prevent infection. We are keen to collaborate with groups who could use these tools to understand the molecular biology underlying plant-microbe interactions at surfaces.

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2. Liu, W.; Zhou, X.; Li, G.; Li, L.; Kong, L.; Wang, C.; Zhang, H.; Xu, J.-R. *Multiple Plant Surface Signals are Sensed by Different Mechanisms in the Rice Blast Fungus for Appressorium Formation*. PLoS Pathog. **2011,** *7* (1), e1001261.

P59: Regulation of *Epichloë festucae* secondary metabolite genes by CclA, KdmB and H3K4 trimethylation.

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Trimethylated H3K4 (H3K4me3) is a histone mark typically associated with euchromatin regions where gene transcription is active. However, at subtelomeric regions H3K4me3 is involved in gene repression. Two major secondary metabolite (SM) clusters, the *LTM* and *EAS* clusters, are located 10 kb away from the telomeres of *Epichloë festucae*. These clusters each contain 11 genes that are active only when the fungus is growing *in planta* and not in axenic culture. We have studied the role of H3K4 trimethylation in SM cluster gene regulation by analysing mutants of *cclA*, a member of the H3K4 methyltransferase complex, and *kdmB*, the H3K4me3-specific demethylase.

We found that deletion of *cclA* or overexpression of *kdmB* specifically reduced global H3K4me3 levels in the fungus. In culture where the SM genes are silent, loss of *cclA* permitted a modest activation of the genes. H3K4me3 levels at the promoters were unchanged or marginally reduced, suggesting that CclA likely regulates these genes via other means. On the other hand, overexpression of the demethylase in culture had a minimal effect on the expression of the SM genes. *In planta* however, loss of *kdmB* reduced the expression of these genes by up to 7-fold, this was accompanied by increased levels of H3K4me3 at some of the gene promoters.

Therefore, our results suggest that CclA and H3K4me3 play a role in silencing the *LTM* and *EAS* clusters in culture, while *in planta*, removal of H3K4me3 as well as possibly other demethylase-independent functions of KdmB are important for the specific activation of the clusters. Interestingly, these changes in the expression of the symbiotically-relevant SMs were not accompanied by other changes in the symbiotic interaction at the whole plant level, suggesting that these proteins are specific regulators of SM in *E. festucae*

P60: Molecular organization of long lasting wheat stem rust resistance gene *Sr26* introgressed from *Thinopyrum ponticum*

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Multiple rust resistance gene combinations are considered as a practical solution for providing durable rust resistance and preventing resistance breakdown arising from single gene deployment. The stem rust resistance locus *Sr26*, originally derived from *Thinopyrum ponticum* and introgressed into wheat as a chromosome translocation, is one of the very few genes conferring resistance for almost 40 years to all known races of stem rust including the highly virulent stem rust race Ug99 (TTKSK) and its derivatives (Dundas *et al.* 2015).
To understand the underlying mechanisms of its unusual long-term effectiveness and to explore allelic diversity in different *Th. ponticum* accessions for other functional alleles that may offer new sources of resistance, we used comparative genomics and gene capture techniques (RenSeq) ascomplementary strategies for isolating the target gene (Steuernage *et al.* 2016). We generated mutagenized population for *Sr26* using EMS (Ethyl methanesulfonate). *Sr26* region was first mapped using NB-LRR (Nucleotide-binding site and leucine-rich repeat) sequences from the orthologous gene members located on the long arm of chromosome 6 from *Aegilops tauschii* reference genome (the D-genome donor of wheat). Subsequently, we revealed a cluster of NB-LRR sequences located at the distal end of the *Th. ponticum* introgression segment that was deleted in the smallest interstitial *Sr26* deletion mutant. Based on these findings we substantially narrowed down the genetic interval for *Sr26* and made progress towards identifying potential *Sr26* gene candidates. Along with comparative genomics, we subjected the mutant population to RenSeq pipeline. A candidate gene of *Sr26* has been successfully identified to be a NBS-LRR type resistance gene that encodes 935 amino acids. Validation of the gene candidate by complementation studies are currently in process and gene specific markers are being screened.

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P61: Metagenomics and culturing to investigate the diverse fungi associated with cabbage and their bio-control potential

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Endophytic fungi are increasingly used in biological control against various pests and diseases as agricultural producers attempt to reduce their environmental footprint.
In this study, putative endophytic fungi were isolated from cabbage (*Brassica oleracea* var. *capitata*) grown in 3 regions in New Zealand. Different approaches were assessed during the isolation of the fungi. Patterns in fungal communities will be described using metagenomic data acquired by high-throughput sequencing and compared with data on direct isolation through culturing. The study will highlight the differences between the culturable component of the plant endophytic community compared to that detected via high-throughput screening. Selected fungal isolates were tested in bioassays to determine their potential for growth promotion or biological control of disease and insect pest of cabbage.
This work aims to describe fungal communities associated with cabbages and identify isolates with bio-control or growth promotion potential.

P62: Identification and characterization of the *Bcvic1* and *Bcvic2* vegetative incompatibility genes in *Botrytis cinerea*

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*V*egetative *i*ncompatibility (VI) is a fungal non-self recognition system characterized by the inability of genetically distinct conspecific fungal cells to form a viable heterokaryon, and is controlled by multiple polymorphic *v*egetative *inc*ompatibility loci (*vic*). We report here the first *vic* genes that have been genetically identified and characterized in the economically important plant pathogenic, necrotrophic fungus *Botrytis cinerea.*

We generated near isogenic lines of *B. cinerea* that differed at a single *vic* locus and employed a bulk segregant analysis approached coupled with whole genome Illumina sequencing to map a 60-kb genomic region containing the *vic* locus. Within that locus, we identified two adjacent highly polymorphic open reading frames encoding predicted proteins that contain domain architectures implicated in VI in other filamentous fungi: *Bcvic1* and *Bcvic2*. Deletion of *Bcvic1* and *Bcvic2* individually did not have any effect on vegetative incompatibility. However, deletion of the region containing both *Bcvic1* and *Bcvic2* resulted in gene deletion lines that showed loss of VI. Complementation of these mutants by ectopic expression restored the VI phenotype indicating that *Bcvic1* and *Bcvic2* are interacting and controlling VI at this *vic* locus. These are the very first vegetative incompatibility genes that have been identified and characterized in *Botrytis cinerea.*

P63: Analysis of secondary metabolite gene clusters in recently described endophytic and pathogenic *Epicoccum* (Ascomycota, *Didymellaceae*) species

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Recent molecular taxonomy revisions have led to a massive expansion in the number of recognised species in many Ascomycota families, including *Didymellaceae*. The genus *Epicoccum* being almost monotypic to *E. nigrum* by the end of 20th century, now has 17 species[1]. Most of them were reclassified from ex-*Phoma* species, but four new species arose from partitioning of *E. nigrum*. While *E. nigrum sensu lato* is known as a widespread endophyte and saprophyte, the closely related species *E. soghinum* is a common pathogen of sorghum, wheat, maize and other species.

In order to explore the genetic diversity and secondary metabolic potential among newly classified *Epicoccum* species, we have sequenced, assembled and annotated genomes of three New Zealand strains of *E. italicum* and three strains of *E. layuense* isolated from New Zealand and Chile[2]. Before this year both species were considered as highly diverged clades of *E. nigrum*[3]. Very little is known about their ecological niches. For comparison, an *E. sorghinum* genome sequence was obtained from GenBank and annotated using the same pipeline (*funannotate 1.0)*[4].

Both genomic similarity, assessed by raw reads cross-mapping, and variation of secondary metabolite gene repertoires strongly support the definition of *E. italicum* and *E. layuense* as separate species, quite distant from *E. sorghinum.* The substantial difference between secondary metabolite gene clusters of *ex-nigrum* species and *E. sorghinum* can be explained by evolutionary divergence and ecological specialisation. Very few secondary metabolite clusters discovered in *Epicoccum* species can be attributed to known compounds.

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