

A 'genome to paddock' approach to control plant disease

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Pathogenic fungi evolve in concert with their plant hosts to invade and overcome defence responses. A detailed knowledge of these processes is essential for successful disease management strategies. Blackleg caused by the fungus, *Leptosphaeria maculans*, is the major disease of canola worldwide. In this lecture I describe how field data, such as disease incidence and severity, coupled with information about the biology, molecular genetics and genomics of the blackleg fungus has been exploited to control this important disease.

Field populations of *Leptosphaeria maculans* can evolve and overcome disease resistance bred into canola within three years of commercial release of a cultivar. The risk of breakdown of resistance can be determined by monitoring disease severity of canola cultivars and changes in virulence of fungal populations using high throughput molecular assays that are based on sequences of avirulence genes. Farmers can avoid a predicted epidemic by sowing canola cultivars with different resistance genes in subsequent years. This strategy has been exploited in Australia and has averted substantial yield losses due to disease.

NOTES:

Linking molecules to morphology: fruit fly integrative taxonomy

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Containing over 5,000 species, Tephritidae is a diverse fly family notorious for containing some of the world's most destructive fruit pests, including the Mediterranean fruit fly, Oriental fruit fly, South American fruit fly, and Queensland fruit fly. As for most insects, traditional taxonomy has relied primarily on morphological characters to discriminate, define, and describe new species. This has failed to fully resolve some of the most important pest groups, especially those belonging to species complexes, leading to significant debates among the tephritid community. We argue that an integrative approach, correlating independent datasets to the same individual voucher specimens, represents the future of tephritid systematics, taxonomy, and diagnostics. This is particularly important for problematic groups of high biosecurity concern at higher risk of diagnostic confusion.

The banana wilt fungus *Fusarium oxysporum* f. sp. *cubense* is even more diverse than previously anticipated

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Fusarium oxysporum f. sp. *cubense* (Foc) is a soil-borne fungus that causes Fusarium wilt of banana, a lethal disease that can result in devastating economic losses to banana production worldwide. The fungus, which originated in Asia, has a complex evolutionary history and taxonomic composition. It consists of three races, which are inadequately defined, and at least 24 vegetative compatibility groups (VCGs) that are separated by single to multiple sequence differences in the loci governing vegetative compatibility. VCG complexes are regularly formed between closely related groups. Extensive surveys of diseased banana plants in Asia resulted in the collection of pathogenic isolates of *F. oxysporum* that did not fit into any of the known FocVCGs. These isolates were, therefore, properly characterized and assigned to new FocVCGs. In the process, five multiple-member and eight single-member VCGs were assigned. Isolates previously assigned as new genotypes of Foc paired with the known VCGs 01221, 01222 and the VCG complex 0128/01220. Phylogenetic analyses indicated that the new FocVCGs were closely related. The results obtained in this study suggest that more VCGs exist than originally anticipated, but that Foc is probably phylogenetically less diverse than reflected by the number of VCGs.

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Sexual reproduction between *Pyrenophora teres* f. *teres* and *Pyrenophora teres* f. *maculata* is rare in barley field populations

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Pyrenophora teres f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*) are foliar fungal pathogens of barley that respectively cause the net and spot forms of net blotch disease. Both pathogens co-exist on crop residue and reproduce sexually, potentially leading to hybridisation between them and the evolution of new virulent pathotypes that could overcome sources of resistance in current commercial barley varieties. To date only few studies have reported *Ptt* x *Ptm* hybrids in the field, but reproduction between *Ptt* and *Ptm* has been successfully induced under laboratory conditions.

To investigate the incidence of sexual recombination between *Ptt* x *Ptm* in the field, susceptible barley varieties were infected with *Ptt* and *Ptm* isolates of opposite mating types at each of three sites to facilitate hybridisation. To test for *Ptt* x *Ptm* hybrids, 723 single conidia were isolated from infected leaf samples. Sequence-specific PCR markers amplified across DNA of each isolate indicated that none of the isolates were hybrids. Furthermore DArT (Diversity Array Technology) marker analysis indicated that recombination had taken place among *Ptt* and among *Ptm* isolates. These findings suggest that sexual hybridisation between *Ptt* x *Ptm* is rare and that there is preference for sexual recombination among isolates of the same form. Further investigations are needed to determine the conditions under which hybridisation may occur in the field.

Host specificity in net form of net blotch of barley and barley grass caused by *Pyrenophora teres* f. *teres*

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The interaction between hosts and pathogens and the severity of epidemics, depends heavily on the interactions between their genetic diversity, population size and transmission ability. Theory predicts that genetically diverse hosts will select for higher virulence and more diverse pathogens than hosts with low genetic diversity. Cultivated hosts typically have lower genetic diversity and thus small effective population sizes, but can potentially harbour large pathogen population sizes. On the other hand, hosts, such as weeds, which are genetically more diverse and thus have larger effective population sizes, usually harbour smaller pathogen population sizes. Large pathogen population sizes may lead to more opportunities for mutation and hence more diverse pathogens. Here we test the predictions that pathogen neutral genetic diversity will increase with large pathogen population sizes and host diversity. We assessed and compared the diversity of a fungal pathogen, *Pyrenophora teres* f. *teres*, on weedy barley grass (which have a large effective population size) and cultivated barley (low genetic diversity) using microsatellites and pathogenicity to assess the importance of weeds in the evolution of this pathogen. The findings indicated that the large barley acreage and low host diversity maintains higher pathogen neutral genetic diversity and has a higher effective population size. Thus, pathogen census population size is a better predictor for neutral genetic diversity than host diversity. However, the pathogen populations on the two hosts are distinct based on clustering of their microsatellite alleles and most importantly, do not show cross host pathogenicity. Thus, although weeds enhance the evolution of aggressiveness in some other pathosystems, here barley grass poses no threat to increased aggressiveness evolution of *Pyrenophora teres* f. *teres* on barley.

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Genotyping of potato cyst nematode in Victoria, Australia, and comparison with populations from Europe and the Americas

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Potato cyst nematodes (PCN) are damaging soil-borne quarantine pests of potatoes in many parts of the world. There are two recognised species, *Globodera pallida* and *G. rostochiensis*, of which only *G. rostochiensis* is present in Australia. PCN was first discovered in Australia in 1986 in a suburb of Perth, Western Australia, was subsequently eradicated and area freedom reinstated. In Victoria, PCN was first detected in February 1991 in Wandin, east of Melbourne. Since then it has been found in small pockets in Gembrook (1991), Emerald, Keysborough and Boneo (1992), Kooweerup/Cora Lynn (2003) and Thorpdale (2008), also east of Melbourne. Strict quarantine controls have been in place since each detection. In 2007, it was speculated that there may have been up to 7 separate introductions of PCN into Victoria. In this study, we utilised the PCN cyst reference collection held by Crop Health Services, Agriculture Victoria, to examine the genetic variability of Victorian PCN populations to investigate potential historical origins and subsequent changes in the populations that might inform patterns of spread. DNA was extracted from single larvae dissected from cysts and screened using nine polymorphic microsatellite markers in two multiplex PCR assays, and the ITS region. A hierarchical sampling strategy was used, comparing variability of larvae (265) within cysts (54), within paddocks (33) and between regions (4). Considerable variation was detected in the Victorian populations and the results will be presented at the Conference.

NOTES:

Point-of-Contact Diagnostics: Fast Biosecurity Decisions in the Genomic Age

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Accurate and fast identification is critical to maximising biosecurity response opportunities. Accuracy relies on both the precision of the differential diagnostic process and the robustness of the reference database, irrespective of the technology (e.g. microscope/LUCID key or PCR/GenBank). Time is critical in biosecurity decisions: first-find, border or trade decisions have substantial economic (and political) consequences. Additionally, both false positive and false negative identifications undermine confidence in the biosecurity system. As knowledge of microbial diversity increases, the boundaries that differentiate taxa have become harder to define: horizontal gene transfer, phage activity and non-chromosomal diagnostic loci have all contributed to inaccurate identifications, taxonomic uncertainty and biosecurity conundrums.

Massively parallel and other recent sequencing technologies, combined with sophisticated analytical software, underpin the genomic age of diagnostics. Comparative genomics has revealed new loci, in particular from unculturable taxa, as potential targets for differential diagnostics. Importantly, many of these loci discriminate at the sub-species level. Bioinformatic-informed analyses have identified hypothetical protein coding regions as useful differential diagnostic loci. Many genomes contain hypothetical proteins: putative protein coding regions with no known analogues or predicted function. Ten of the 15 genomic loci identified for sub-species differentiation between clades of *Ca. Liberibacter solanacearum* were located within hypothetical proteins encoded in the bacterial genomes.

Using these new identification assays in isothermal-based assays provides both fast and accurate *in situ* identification (point of contact diagnostics). LAMP and RPA are just two of the isothermal techniques available for in-field/orchard or at-border identification of biosecurity pests. Definitive results are available within 25 min of sampling, using battery-powered, communication-enabled isothermal instruments. Depending on the biosecurity mandate, available decisions range from pre-determined response-initiation to limiting site access whilst a sample is prioritised for diagnosis at an accredited laboratory. Biosecurity diagnostics is entering a new phase: accurate and fast point-of-contact identification is the new paradigm.

On-site detection of *Potato spindle tuber viroid* in a disease outbreak using loop-mediated isothermal amplification (LAMP)

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Loop-mediated isothermal amplification (LAMP) is a technique that uses a DNA polymerase which requires only a single temperature incubation and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA for nucleic acid amplification. Due to its simplicity and robustness, LAMP has the potential to be used under field conditions, which is particularly useful for surveillance, plant quarantine and infectious disease diagnosis. We report the first use of LAMP assay in Australia for the successful on-site detection of *Potato spindle tuber viroid* (PSTVd), a high priority emergency plant pest, in tomatoes. The LAMP assay was easy to use and the positive detection of PSTVd (from sampling to test result) was obtained in less than 40 minutes. It is envisaged that the LAMP assay validated in this study can be used by quarantine and surveillance officers for fast on-site detection of PSTVd in the near future.

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An internet-based bioinformatics toolkit for Plant Biosecurity diagnosis and surveillance of viruses and viroids

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Detection and preventing entry of exotic viruses and viroids at the border is critical for protecting plant industries trade worldwide. Existing post entry quarantine screening protocols rely on time-consuming biological indicators and/or molecular assays that require knowledge of infecting viral pathogens. Plants have developed the ability to recognise and respond to viral infections through Dicer-like enzymes that cleave viral sequences into specific small RNA products. Here we optimise the assembly of viral sequences by using specific small RNA subsets. We sequenced the small RNA fractions of 21 plants held at quarantine glasshouse facilities in Australia and New Zealand. Benchmarking of several *de novo* assembler tools yielded SPAdes using a kmer set of 15,17 and 19 to produce the best assembly outcomes. We also found that *de novo* assembly using 21-25 nt small RNAs can result in chimeric assemblies of viral sequences and plant host sequences. Such non-specific assemblies can be resolved by using 21-22 nt or 24 nt small RNAs subsets. Among the 21 selected samples, we identified contigs with sequence similarity to 18 viruses and 3 viroids in 13 samples. Most of the viruses were assembled using only 21-22 nt long virus-derived siRNAs (viRNAs), except for one Citrus endogenous pararetrovirus that was more efficiently assembled using 24 nt long viRNAs. All three viroids found in this study were fully assembled using either 21-22 nt or 24 nt viRNAs. Optimised analysis workflows were customised within the Yabi web-based analytical environment. We have implemented an automated viral surveillance and diagnosis (VSD) bioinformatics toolkit that produces improved viruses and viroid sequence assemblies. The VSD toolkit provides several optimised and reusable workflows applicable to distinct viral pathogens. We envisage that this resource will facilitate the surveillance and diagnosis viral pathogens in plants, insects and invertebrates.

A new bioinformatics pipeline for the rapid detection of plant viruses using next generation sequencing data

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Rapid detection of viruses in plants is essential to avoid the large negative economic impact caused by these pathogens. Currently, common methods to identify viruses rely on biological indicators and molecular assays, which are limited to one or group of viruses they are designed to detect. Because of this, next generation sequencing (NGS) techniques, such as smallRNA- and RNA-seq, are being rapidly adopted in the field. These techniques allow the unbiased detection of multiple viruses and the identification of novel viruses, an increasingly important issue in viral diagnostics. This is particularly true in the prevention of emerging viruses at borders, as post entry quarantine systems are being put under pressure to detect new pathogens because of increases in global trade and movement.

However, using NGS methods for viral diagnosis requires lengthy and robust bioinformatics analysis. For this reason, the last few years have seen an increase in bioinformatics tools designed for viral diagnosis from mixed (host and pathogen) samples. However, most are designed for clinical samples, which render them suboptimal for the identification of viruses in plants. In addition, many tools rely on alignment methods for *de novo* assembly, and mapping of short reads to a reference, which (in addition of being slow) can lead to related viral sequence being undetected, due to high mutability rates of viral genomes.

For this reason, we have developed a new bioinformatics pipeline that takes mixed raw smallRNA- or RNA-seq reads from plant samples and produces a viral index using k-mer profiles. This method divides sequences into k-mers of specific lengths and uses exact matching. The pipeline has been developed on Galaxy, an open platform for intensive data analysis, allowing analysis to be conducted with no command line input, making it accessible to all researchers.

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The use of NGS to identify quarantine pathogens and genomic traits for breeders in tropical pulse germplasm imported by the Australian Grains Genebank.

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The Australian Grains Genebank (AGG) has the mandate to import, quarantine, store and distribute grains germplasm to breeders and researchers. A major bottleneck is the availability of information which allows breeders to make informed choices of germplasm. The use of Next Generation Sequencing (NGS) to obtain genomic information on imported seedlines and their associated microbiota and the application of bioinformatics to identify both pathogens and genetic traits, provides a streamlined new approach for breeders and quarantine scientists.

We have used NGS successfully as a diagnostic tool for viruses and have detected and correctly identified a range of viruses in plant samples in Post-entry Quarantine (PEQ), from the field and from 30 year old stored samples, highlighting the robustness of the technique.

A review of the tropical pulse germplasm held in the AGG has identified approximately 140 species in the collection, the most important being of the genera *Arachis*, *Glycine*, *Phaseolus* and *Vigna*. These genera are host to well in excess of 100 viruses, including many that are seedborne and of quarantine significance worldwide but currently no post-entry quarantine virus-screening of imported lines is required by DAWR. The information will be used to identify risks, develop new PEQ protocols for tropical legumes and facilitate the safe movement of pulse germplasm.

The large number of tropical pulse species in the collection indicates that NGS would be an appropriate diagnostic tool for efficiently detecting a potentially large range of microbiota in PEQ.

NOTES:

Standing on the shoulders of giants: Vavilov's wheat collection and sources of resistance

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Nikolai Ivanovich Vavilov was a renowned Russian botanist and geneticist, best known for his theory relating to “*the centres of origin of cultivated plants*”. Inspired by his idol, Charles Darwin, he travelled the world in the early 1900s collecting more seeds, tubers and fruits than any person in history. The collections, including many wheat landraces, were stored in a seed bank in Leningrad, now known as the N. I. Vavilov Institute of Plant Genetic Resources in St Petersburg, Russia. Remarkably, the unique seed collection survived the “*Siege of Leningrad*” during World War II. However, Vavilov himself faced an ironic fate – he was arrested for criticising the non-Mendelian concepts of a Soviet biologist, Trofim Lysenko, who had the support of Joseph Stalin. Vavilov was sentenced to death in July 1941, but in 1942 his sentence was reduced to 20 years imprisonment. Despite this, he died of starvation in prison in 1943.

Following in the footsteps of the Great Russian scientist and his colleagues, we performed the world's first genetic analysis of Vavilov's wheat seeds. A total of 295 diverse wheats collected from around the world were examined using 34,000 DNA markers. The genomic analysis revealed a massive array of allelic diversity that is absent in modern germplasm. We provide a summary of our ongoing efforts to discover new genes for disease resistance hidden in the Vavilov treasures. By combining several breeding technologies, we are rapidly mining new sources of resistance to major foliar diseases of wheat, including the rapidly evolving rust diseases (stripe, leaf and stem rust) and yellow spot disease.

Unlocking novel alleles for resistance to leaf rust in the Vavilov wheat collection

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Leaf rust (LR) caused by *Puccinia triticina*, is an important foliar disease of wheat (*Triticum aestivum* L.) and the deployment of resistant cultivars is the most viable control strategy. However, *P. triticina* has the ability to rapidly overcome resistance genes, thus breeders require a constant supply of new sources of resistance. In this study we evaluated a diversity panel of 295 bread wheat accessions from N. I. Vavilov Institute of Plant Genetic Resources (VIR) in St Petersburg, Russia, for LR resistance and performed genome-wide association study (GWAS) using 8,900 polymorphic DArTseq-GBS markers. The diversity panel was evaluated in 11 environments using three LR pathotypes, prevalent in Australia. GWAS identified a total of 52 significant marker-trait associations representing 34 independent quantitative trait loci (QTL). Among them, 32 QTL were associated with adult plant resistance (APR). Of the 34 QTL, 15 were considered novel and six co-located with the catalogued *Lr* genes. Further, 13 of the predicted QTLs aligned with regions reported in recent GWAS studies. One major, seedling LR resistance QTL located on chromosome 3A with high linkage disequilibrium among markers, suggested a high allelic fixation. Subsequent haplotype analysis found seven variants, of which two were strongly associated with increased LR resistance at seedling stage. Similarly, analysis of an APR QTL on chromosome 7B depicted 22 variants, of which two were associated with adult LR resistance. Furthermore, we observed that most of the tested accessions from our diversity panel carried 10 or more combined resistance-related marker alleles. Also, additive effect of favorable alleles and LR resistance was evident, highlighting the potential of allele stacking for a sustainable LR resistance improvement. The QTLs identified in this study represent a useful basis for introgression of promising new resistance factors into modern germplasm to achieve a sustained control of LR in wheat.

Selecting wheat cultivars that combine root-lesion nematode (*Pratylenchus thornei* and *P. neglectus*) resistance and *P. thornei* tolerance.

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Root-lesion nematodes (RLN; *Pratylenchus thornei* and *P. neglectus*) are widely distributed in Australian grain producing regions, can reduce the yield of intolerant wheat cultivars by >50% and cost the industry ~\$123 M/yr. Development of commercial varieties combining tolerance and resistance to manage RLN depends on pre-breeding projects producing suitable parental cultivars. To this end, BC1F2 to BC1F4 breeding populations derived from RLN-resistant CPI133872/Janz double haploids crossed with the *P. thornei* tolerant wheat cvs. EGA Wylie and/or EGA Gregory were screened in the glasshouse for *P. thornei* and *P. neglectus* resistance simultaneously using mixed species inoculum or in the field for *P. thornei* tolerance. Each trial was designed in blocks that allowed comparison between at least 256 segregating individuals and replicated check cultivars. In each generation, individuals identified as resistant or tolerant were progressed to the next generation of selection. By the BC1F5 generation, 48 putatively resistant and tolerant cultivars had been identified. These cultivars were characterised for *P. thornei* and *P. neglectus* resistance and *P. thornei* tolerance in separate replicated trials for two years. Resistance was recovered in 42 (88%) cultivars for *P. thornei*, seven (15%) for *P. neglectus* and moderate tolerance in 12 (25%) for *P. thornei*. Ten (21%) cultivars combined *P. thornei* resistance and tolerance with one (2%) of these cultivars also combining *P. neglectus* resistance. This research has shown that RLN resistance and tolerance selected during segregating generations can be carried through until genetic homozygosity is achieved in later generations. This is likely due to the polygenic and additive mode of inheritance of these traits. Providing parental cultivars with resistance and tolerance to both these important RLN species is an attractive proposition for plant breeders. Furthermore, development of commercial cultivars that effectively manage both single species or mixed species infestations will minimise yield losses caused by RLN.

Advanced genomics for improved disease resistance prediction in potato

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Potato is an important food crop for its growing consumption and uses in production, worldwide. Traditionally, potato breeding for desirable traits such as disease resistance has relied heavily on phenotypic selection and extended field trials resulting in an 8-15 year long selection process. The introduction of marker-assisted selection for progeny screening improved the rate of genetic gain and is now common practice. However, only a few resistance genes have been identified and rudimentary DNA markers developed. Furthermore, the markers currently in use cannot predict highly polygenic traits and are often only in close proximity to the gene. This can lead to dissociation between the marker and trait resulting in incorrect genotypic information being produced. To be able to genotype cultivars correctly and include highly polygenic traits, new marker systems are needed that delivers marker saturation across the entire genome to correlate the genomes effect on the trait. This in turn will allow faster selection of pathogen resistant parents, to improve plant health and ensure food security in disease prone areas.

This project has implemented high-throughput transcriptome sequencing to genotype the Australian parental germplasm collection. The resulting genotypic data has been analysed for its applicability to replace a range of conventional DNA markers for disease resistance to a universal SNP based assay that evaluates the entire genome. This universal assay has the potential to enable improvement in disease resistance as well as all other traits via genomic selection. In addition, the genotypic data has been used to identify candidate genes in genomic regions that are known to provide disease resistance and DNA variance in candidate genes has been identified that could improve selection for introgressed PVY resistance.

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Development of markers to allow incorporation of complementary resistance genes to *Rhynchosporium* into elite barley varieties

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Rhynchosporium or leaf scald, caused by the fungal pathogen *Rhynchosporium commune*, is one of the most destructive and economically important diseases of barley. Current control practices for this and other foliar pathogens of barley rely heavily on fungicides, resulting in a significant cost to farmers. Additionally, high levels of fungicide use can lead to pathogen insensitivity to effective fungicide classes. The most sustainable and cost effective method of protecting against *R. commune* and other foliar pathogens is through the production of durably resistant barley cultivars. This requires the identification of tightly linked markers to a number of complementary resistance genes.

To address this demand, multiple collections of barley genotypes including 660 UK spring barley varieties, 480 Syrian and Jordanian landraces, and mapping populations for *Rrs1* and horizontal resistance genes have been assessed for resistance to a complex natural population of *R. commune* in a field disease nursery at the James Hutton Institute in Dundee, Scotland. Application of a genome-wide association scan (GWAS) identified a number of QTL in genomic regions previously shown to contain major resistance genes against *R. commune*, in particular *Rrs1* on chromosome 3H and *Rrs2* on 7H. In addition, novel QTL on 2H and 5H were detected. Phenotyping with individual *R. commune* strains differentiating *Rrs1* and newly mapped *Rrs18* resistances, combined with advanced barley genomics resources, allowed narrowing down of the physical intervals containing *Rrs1* and *Rrs18*.

Our results indicate the importance of major resistance genes against *R. commune*, in particular *Rrs1*, in determining resistance in UK spring barley and their continued effectiveness against natural populations of *R. commune*.

The diagnostic markers for *Rrs1*, *Rrs18*, and other resistance loci will facilitate the efficient production of new varieties with durable resistance to Rhynchosporium by barley breeders following incorporation into a marker assisted breeding programme.

NOTES:

Modelling invasive species arrival and establishment

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The spread of invasive species continues to provide significant challenges to researchers and those government biosecurity agencies charged with protecting a country's borders. In an increasingly connected world, these invasive species are potentially able to spread further and more rapidly. Transport networks such as the global shipping network or tourist movement networks represent just two examples of networks that can facilitate the human mediated spread of invasive species. I will discuss some of the work I am or have been involved in, which examines and models transport networks. Examples of this work includes modelling the spread of invasive species via shipping containers, development of a real time tool for analysing ships arriving into Australia for their risk of carrying Asian gypsy moth eggs, informal trade networks in SE Asia, and tourist movement networks in New Zealand, as well as a global analysis of the threat from crop pests and pathogens.

Fighting Queensland Fruit Fly *in-silico*: spatially explicit modelling to inform management strategies

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Highly mobile and polyphagous pests, such as the Queensland Fruit Fly (*Bactrocera tryoni*, Qfly) are a major threat to agricultural systems worldwide. In order to minimize economic losses, farmers need to develop on-farm best-management practices (BMP), get engaged in area-wide management approaches (AWM) and need to get access to new technologies, such as the Sterile Insect Technique (SIT). Ecological modelling can contribute its share in informing BMP's or show what level of AWM adoption is needed for an effective suppression so that SIT can be cost effective.

In this project, we are focussed on QFly, one of the major horticultural pests in Australia. We use existing information about the biology and ecology of QFly and combine it with information about the phenology and distribution of their hosts to mimic fruit fly populations on a regional level. We can then implement different management strategies, varying levels of adoption and community engagement to look at management effectiveness. We can also look at what levels of AWM adoption are needed in a landscape to get a level of suppression that is low enough for SIT to be effective, and thus become a viable option for QFly treatment.

Our findings can be coupled with economic analyses about costs of the different management strategies and the benefits of a reduction in fly numbers. This will inform region specific guidelines and action plans and can help get people engaged in the idea of AWM.

Fighting highly mobile polyphagous pests through AWM is a complicated task that requires experts in entomology, biosecurity, economy and social science. Spatially explicit modelling is one tool to combine their existing knowledge, identify important gaps and get community groups involved.

NOTES:

Assessing the performance of landscape-based strategies to deploy major-gene resistance

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Genetically-controlled plant resistance can reduce the damage caused by pathogens. However, pathogens have the ability to evolve and overcome such resistance. This often occurs very quickly after resistance is deployed, resulting in significant crop losses and a continuing need to breed new resistant cultivars. To tackle this issue, several strategies have been proposed to constrain the evolutionary potential of pathogen populations and thus increase the durability of resistance deployment. These strategies mainly rely on using different combinations of resistance sources in time, space, or both. In time, such combination consists of crop rotations. In space, resistance sources can be deployed in the same cultivar (pyramiding), in different cultivars within the same field (cultivar mixtures) or in different fields (mosaics). However, experimental assessment of the efficiency (i.e. ability to reduce disease impact) and the durability (i.e. ability to limit pathogen evolution and delay resistance breakdown) of different deployment strategies presents a major challenge.

Therefore, we developed a spatially-explicit stochastic model to assess the epidemiological and evolutionary outcomes of the major deployment options described above when one or two major genes for resistance are present. In addition, we analysed the impact of landscape organisation (as defined by the proportion of fields cultivated with a resistant cultivar, and their spatial aggregation) and epidemiological or evolutionary parameters (e.g. dispersal abilities, mutation rate, cost of infectivity) through sensitivity analyses and polynomial regression.

The model has been parameterised for wheat qualitative resistance to rusts, caused by fungi of the genus *Puccinia*, but can be applied to many other pathosystems. Early results suggest that strategies offering the best epidemiological control of the disease are not necessarily the most durable.

Risk assessment and application of the Military Appreciation Process to involve growers in emergency response decision making

Rebecca J Laws¹, Suzy Perry¹

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The incursion of the tropical race 4 (TR4) strain of Panama disease in north Queensland in 2015 required the rapid implementation of effective risk mitigation measures to limit the spread of this serious pathogen. To ensure that the risk mitigation measures developed were practical and effective for the range of different situations that banana growers face, the growers themselves were invited to be involved in the risk assessment and development of risk mitigation measures. The military appreciation process was chosen as the tool to evaluate the response options. Biosecurity staff first stepped through the whole decision making process and came up with a preliminary pest risk assessment and draft risk management options. These documents were then distributed to growers and other industry stakeholders for their input. A workshop was held with growers where they provided information on the diversity of farming practices and setups and evaluated the risk assessment and risk management options. This process resulted in the combination of several approaches into a novel management strategy as the growers' preferred approach. The input of growers and other industry stakeholders resulted in a risk management strategy that required fewer modifications on implementation. One of the key benefits of the process to government was allowing stakeholders to experience and understand the rigour of the biosecurity decision making processes. This experience will be drawn upon to guide future interactions with stakeholders in emergency response situations.

NOTES:

Optimizing the surveillance of invading organisms: Can we improve standard surveillance strategies?

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The number of biological invasions that occur each year is increasing and these new invasions are often associated with negative impacts. To alleviate the potential negative economic and environmental impacts of invasions surveillance strategies aim to improve the likelihood of identifying invasions early, thus increasing the probability of successful eradication efforts. Therefore, we designed and evaluated statistically-based surveillance strategies to optimize the detection of three case studies; two arthropod pests, grape phylloxera (*Daktulosphaira vitifoliae* Fitch) and Mediterranean fruit fly (*Ceratitidis capitata*; Medfly) and one nematode pest, potato cyst nematode (*Globodera rostochiensis*; PCN). To best represent the basic biology and dispersal mechanisms of each pest we developed individual spread models and simulated the spread of each pest over the area of interest (e.g., field, town or region). Next we overlaid different surveillance strategies and recorded variables related to the time of detection and the total pest spread to determine the optimal surveillance strategy (i.e., the strategy with reduced spread and/or time to detection). Surveillance strategies included the surveillance strategy currently used for the pest and alternative strategies that varied survey location, frequency and density. For all case studies we determined that an alternative surveillance strategy could decrease the time to detection and/or the spread of the invasion for the same or a reduced effort. Specifically, surveillance strategies were optimized based on known habitat suitability (grape phylloxera), the location of high risk introduction sites and preferred hosts (Medfly) or increased surveillance density and/or surveys located predominantly in infested regions with limited random sampling in non-infested regions (PCN). Additionally, our findings may be applied to other biological invasions and we discuss how the results and/or models can be adapted to other regions, organisms and species.

NOTES:

Building plant health systems to improve nutrition and livelihoods worldwide

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An estimated 40% of the world's crops are lost to pests. This impacts the ability of smallholders living in poor rural communities to feed their families. But these farmers actually grow nearly 70% of the world's food so, more broadly, it affects food supply chains, international trade and achievement of the Sustainable Development Goals. We must take action if we are to achieve those goals and feed a growing world population.

Much of the information needed by farmers in the developing world is already available but not accessible to them in a format that is practically useful. This presentation will review CABI's experiences, and the lessons learned from them, in using a variety of communication tools to help smallholder farmers get the information they need. In particular it will look at the development of, and future plans for Plantwise, a global initiative led by CABI to strengthen plant health systems. The Plantwise network and data can be leveraged into this innovative new initiative using Earth observational data to predict pest outbreaks and reduce their impact by giving farmers early warning and more timely management advice.

However, bottom-up approaches to food security and plant health are not sufficient. It does not help a farmer to grow more only to find that it cannot be sold. Therefore we need to improve farmer's access to local, national and international markets. This requires a top-down approach to building biosecurity capacity in the developing world, such as that implemented in partnership with PBCRC in Africa. This not only enables them to meet the international standards of developed countries but also to reduce the risk of importing produce and planting materials contaminated with chemicals or infested with an alien pest species.

An Eco-Health approach to plant pathology in agricultural development

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2. Faculty of Medicine, The University of Sydney, Camperdown, NSW, Australia

3. Asian Market Research, Brisbane, Queensland, Australia

Supplying reliable, adequate and safe nutrition for more than 7 billion people is among the most important and complex of human responsibilities. Pre- and postharvest plant disease cause crop losses of around 20% globally, with higher losses in poorer countries. At a time when support for agricultural research and development, essential to overcoming the inadequacies of the current system, has declined markedly, the effectiveness of development programs have come under increased scrutiny.

Many development programs fail to reach expectations because they are narrowly discipline-focussed and ignore the socio-economic, gender and environmental contexts of the research and training provided. Project milestones are often framed as inputs without an adequate evaluation of impacts. For example, increasing cocoa yields requires labour to implement the necessary disease management interventions. Participatory research programs are effective at training farmers, but widespread adoption often remains low, primarily because incentives for labour are limiting due to a range of social, economic and health constraints beyond the expertise of plant pathologists. For example, poor nutrition, infections, child and maternal diseases typically account for 20-30% lost productivity in developing countries. Young, relatively healthy labourers migrate to towns and cities in search of better living conditions and higher incomes, leaving an aging farming population with increasing health problems and poor access to services.

We believe that effective programs to reduce crop losses to plant diseases require interdisciplinary teams including expertise in health, agriculture, environment, marketing and social sciences. We will illustrate our integrated approach to improving plant, animal, human and environmental health with examples from our work with smallholder cocoa farming communities in Bougainville and Sulawesi.

NOTES:

The influence of the local social profile on establishing grower and community collaboration – The case of industry-driven fruit fly area-wide management

Heleen Kruger¹

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Increasingly local Australian industries are expected to lead local collaborative efforts to address pest issues. A prime example is area-wide management (AWM), a key recommended approach to address Queensland fruit fly (QFly) (*Bactrocera tryoni*). AWM requires a critical mass of risk contributors—such as growers, backyarders, peri-urban landholders—to effectively manage the pest on their land. It is well recognised that the chances of achieving success with AWM are boosted by effective fruit fly management technologies and conducive local ecology and climate conditions. Less well understood is the influence of the local social profile of the growers and communities involved on achieving their buy-in and support. In this presentation we explore the findings from three case studies of where industry-driven QFly AWM has been achieved or is attempted. These are Central Burnett (Queensland), the Riverina (New South Wales) and Young-Harden (New South Wales). Ostrom's principles for managing a common-pool resource are applied to highlight what social factors enable and/or hinder establishing sustainable collaboration. This work shows that the social profile influences the transaction cost involved, such as the level of negotiation and the effort needed to achieve and maintain buy-in from QFly risk contributors. Such transaction cost can influence the feasibility of AWM and it is easily overlooked in standard cost benefit analysis of AWM. Key social factors identified include the level of grower heterogeneity, the level of social capital, the presence of existing social mechanisms to monitor on-farm compliance and the ratio between AWM supporters and risk contributors who have little incentive to manage the pest. Some suggestions forward are discussed.

Crop Pathology online 24/7/365 – does it deliver?

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2. Agriculture Victoria, Bendigo, Victoria

3. Cumbre Consulting, Emu Creek, Victoria

Compelling evidence has been found demonstrating the value of an online learning network in field crop diseases. eXtensionAUS facilitates a collaborative community working together in the area of field crop diseases. The community is supported by the use of evolving online tools which enables quality content development and dissemination, responding to emerging issues using the latest knowledge to engage online with the Australian grains industry.

The eXtensionAUS Field Crop Disease Community of Practice (CoP) includes over 50 members from across Australia from state based departments, universities and private industry. The eXtensionAUS initiative, including a Crop Nutrition CoP, has reached over 18000 users since its inception, with one Facebook post reaching 12,000 people alone.

The eXtensionAUS project has found that a collaborative learning network which informs a suite of online extension activities has proved to be both innovative and effective in reaching end users. This coordinated approach to publishing new content or curating existing resources can be proactive and reactive, reaching people on any internet device, at any time. Online mechanisms also allow communication to be two-way, providing informal surveillance through social media monitoring and engagement. The suite of tools used includes web-based articles, video, social media, Slideshare and curation tools such as Storify and Paper.li.

Evaluation has found the most valuable aspects of participating in eXtensionAUS for community members to be: expanded professional networks (72%); learning to work in new ways online (70%); learning new technical information (75%) and increasing reach to clients (54%).

eXtensionAUS is a GRDC funded project, supported by Agriculture Victoria and DPI NSW. eXtensionAUS is also working closely with RIRDC to make this way of working available to other rural industries.

NOTES:

Building resilience in indigenous communities through engagement

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2. Northern Institute, Charles Darwin University, , Darwin, Northern Territory, Australia

3. NZ Institute for Plant and Food Research, Auckland, New Zealand

Indigenous communities in both Australia and New Zealand are constantly facing biosecurity threats from both naturally occurring new incursions and the human-mediated spread of existing pests, diseases and weeds. These incursions can have disastrous effects on commercial, traditional subsistence and niche-market cropping systems, the latter often occurring in relatively remote communities. For response programmes to be implemented, it is essential that effective communication, understanding and cooperation is achieved between agencies charged with controlling the incursions and communities affected by them.

The overall aim of the project was:

To enhance the ability of indigenous communities and relevant regulatory authorities and industries to better manage social, cultural, environmental and economic impacts of biosecurity threats, and to participate in biosecurity strategies by describing and evaluating bicultural engagement models that build empowerment and ownership in indigenous communities and their response to those threats.

The project was undertaken by a multicultural team drawn from the Institute for Plant and Food Research, New Zealand and the Northern Institute, Charles Darwin University, Australia. Because traditional cultural values, behaviours and protocols were recognised as essential elements of effective engagement, the team included respected elders (kaumatua) from both countries to provide guidance, support and validation on cultural issues.

Team members in each country used participatory action research to document procedure and identify key principles to the process. The careful collection of traditional knowledge from elders in both Māori and Aboriginal communities created trust, understanding and a willingness to share details the research teams could relate to and embed in the models. This methodology has resulted in models for engagement developed by indigenous communities for indigenous communities, thereby increasing the probability of adoption and success.

The work provides a knowledge base that can be utilised and implemented as procedure by government, agencies and industry. This has not been done before.

NOTES:

What can we realistically expect from biocontrol of insect vectors of plant pathogens: is significant disease mitigation possible?

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Insects that vector pathogens that cause plant disease present a challenging problem to pest managers. In many cases, low densities of infected vectors can contribute to significant disease outbreaks, which in the absence of pathogen transmission, these pest densities may be tolerable. A dominant management tool for vector suppression are applications of pesticides which may be suitable for use in some agricultural, horticultural, and ornamental commodities grown in protected culture or outdoors. The broad spectrum nature of many insecticides enables them to kill, often rapidly, a suite of disease vectors (e.g., thrips, whiteflies, aphids, and psyllids) which may help mitigate disease spread because of reduced vector densities. However, issues of environmental contaminant, human health and safety concerns, non-target impacts, and resistance development are well documented problems associated with pesticide use. Additionally, in some cases, such as vector management in urban or wilderness areas, insecticides may not be a viable control option for pest control. Under these conditions, biological control, that is the use of host specific natural enemies that attack some life stage (e.g., eggs or nymphs) of the vector, may be the best, and possibly the only control option available for reducing vector densities to less damaging levels. This presentation will review the challenges and benefits of using biocontrol as a management tool for suppressing populations of insects that vector plant pathogens. The primary focus will be on two biological control programs targeting two hemipteran insect vectors. The first review will summarize the effects of classical biocontrol targeting the glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae), a vector of the bacterium *Xylella fastidiosa*, which causes a variety of plant maladies, of which Pierce's disease of grapes may be the best known. The second review will look at the classical biocontrol program targeting Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae), that vectors the bacterium *Candidatus Liberibacter asiaticus*, the putative causative agent of the lethal citrus disease huanglongbing. Both of these case studies will draw on work in perennial outdoor crops/urban/wilderness areas, with a primary focus on what has been accomplished in California (USA) for *H. vitripennis* and *D. citri*, and the South Pacific (*H. vitripennis* only).

NOTES:

Extracellular vesicles: An underappreciated component of the plant immune system

Roger W Innes¹, Brian Rutter¹

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Exosomes are extracellular vesicles (EVs) that play a central role in intercellular signaling in mammals by transporting proteins and small RNAs. Plants are also known to produce EVs, particularly in response to pathogen infection. The contents of plant EVs have not been analyzed, however, and their function is unknown. Recent work in the Innes laboratory has revealed that plant EVs are highly enriched in proteins involved in biotic and abiotic stress responses, and carry miRNAs. In addition, EV secretion is enhanced in plants infected with *Pseudomonas syringae* and in response to treatment with salicylic acid. These findings suggest that EVs represent an important component of the plant immune system. In this talk I will present our ongoing investigations into the possible functions of EVs, and our initial investigations into the genetic requirements for EV biosynthesis.

NOTES:

Use of epidemiological models to predict the spread and potential for control of tree diseases.

Chris Gilligan¹

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With one or two notable exceptions, tree diseases have, until recently, received little attention from epidemiological modellers. The threats to natural environments, forest and plantation crops from a range of tree pests and diseases are changing this. Scale is a particular challenge, often with multiple scales of dispersal by trade, vectors, wind and rain. Cryptic infection is another challenge, where the chances of detecting emerging infections may be low, with the result that we become aware of invading pathogens too late to intervene effectively. Mapping susceptible hosts is a third challenge; we don't often know where all the susceptible hosts are across a threatened landscape. Sometime we look for disease in the wrong places, we intervene at the wrong scale or in the wrong place. How can we learn to do better? I propose to use examples from sudden oak death in California, citrus diseases in Florida and Texas, ash dieback and ramorum disease in the UK to explore some epidemiological principles that can be used to inform surveillance, predict spread and to compare the effectiveness of different control scenarios.

A disease of French beans and soybean caused by Cowpea mild mottle virus in Queensland

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In March 2016, a disease causing pod distortion, discolouration and leaf mottling in fresh market beans (*Phaseolus vulgaris*) occurred in the Fassifern area of south-east Queensland. Disease incidence in crops which are harvested nine to ten weeks after planting was frequently 60% to 100%, resulting in losses approaching \$400,000 over a period of only two months.

In April 2016, samples of soybean (*Glycine max*) cv. Zam-1 displaying virus-like symptoms of distorted leaves and mottling were collected from a crop near Gatton, approximately 50 Km from the infected bean crops. Disease incidence in this crop was 5-10%.

Symptomatic plants from bean and soybean crops had slightly flexuous filamentous particles. Samples were negative for potyvirus when tested using a potyvirus group ImmunoStrip test and in RT-PCR using degenerate primers Poty 1 and U341. These samples were positive for Carlavirus using Carlavirus group specific primers and for Cowpea mild mottle virus using CPMMV -specific primers.

Phylogenetic analysis of sequences obtained from PCR products confirmed the identity of CPMMV from bean and soybean with the closest matches over the region obtained being 85% nt and 76% nt identity to CPMMV isolates from Brazil and India respectively.

The isolates from bean and soybean were transmitted by manual inoculation to several cultivars of bean and soybean which developed symptoms very similar to those seen in the original samples.

Both virus isolates were transmitted by *Bemisia tabaci* (MEAM1) following exposure of young healthy bean and soybean plants to whitefly previously allowed access to bean plants infected with these isolates.

CPMMV has been detected in bean crops in the Fassifern area in 2017, but at much lower levels than in 2016.

The detection of CPMMV in 2016 was the first detection of this virus in Australia and of Carlavirus infection of Fabaceae species in Australia.

NOTES:

Alternative hosts, seed transmissibility and persistent-propagative replication of *Soybean blotchy mosaic virus*

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Soybean blotchy mosaic virus (SbBMV) infection of soybean results in an economically-important disease of soybean characterized by distinct blotchy mosaic symptoms. Soybean blotchy mosaic virus, a member of the genus *Cytorhabdovirus*, has only been reported in South Africa and re-emerges in the warmer, low-lying soybean production areas early in each new season. In this study, mechanisms by which the disease may persist between soybean growing seasons, such as the presence of alternative plant hosts, seed transmissibility and replication in its leafhopper vector *Peragallia caboverdensis* was investigated. Weeds, trees and crops in close proximity to soybean fields with high soybean blotchy mosaic disease incidences were collected and tested for the presence of the virus by RT-PCR. Three putative alternative hosts of SbBMV were identified, which included two herbaceous weeds, *Lamium amplexicaule* and *Flaveria bidentis* and a perennial tree species, *Gymnosporia buxifolia*, which may serve as over-wintering hosts for SbBMV. Seed transmissibility was assayed by collecting seed from PCR tested, infected soybean mother plants, and growing them in insect-free greenhouses. A total of 2, 829 seedlings representing four commercial cultivars were evaluated, and it was concluded that SbBMV was not seed transmissible at incidences detectable in the number of seedlings tested. Lastly, replication of SbBMV in its vector *Peragallia caboverdensis* was investigated using a positive strand-specific RT-PCR directed against the replicative intermediate of SbBMV. This was demonstrated through detection of the positive strand of SbBMV. Future control strategies of the virus should focus on management of alternative hosts and the insect vector, as these function as SbBMV reservoirs during the winter months.

Modelling outbreak risk of wheat stripe rust to inform on-farm crop monitoring

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2. Plant Biosecurity CRC, Bruce, ACT, Australia

Wheat stripe rust (*Puccinia striiformis* f.sp. *tritici*) is a serious fungal pathogen affecting all major wheat-producing regions in Australia. Despite the ongoing development of resistant cultivars, this fungal disease remains a recurring and widespread problem to producers with potential yield losses of up to 80%. Early detection of infected plants is vital to enable timely treatment and prevent local disease outbreaks. However, to efficiently target crop monitoring resources, better guidance about the spatial distribution of risk is needed. We developed a risk model describing the potential for stripe rust outbreaks based on the disease's infectious cycle. The model integrated important risk factors such as wind dispersal from infested fields, cultivar resistance and climatic conditions favouring rust infection and sporulation. Spatially-explicit predictions of outbreak risk were derived for wheat growing regions in mainland Australia. Research outputs can be used to guide the timing and spatial prioritization of on-farm crop monitoring decisions.

NOTES:

Do ascospores play a role in the epidemiology of citrus black spot?

Nga Tran¹, Andrew Miles¹, Ralf Dietzgen¹, Andre Drenth¹

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Citrus black spot caused by the fungus *Phyllosticta citricarpa* is characterised by fruit blemishes and premature fruit drop, resulting in significant economic losses in tropical and subtropical growing areas worldwide. Although the disease has been known of for over 100 years, several aspects of its biology and epidemiology remain unclear, hindering development of effective disease management strategies. To improve our understanding of the disease cycle, we aim to address the following questions: i) How does the fungus undergo sexual reproduction? ii) What is the role and seasonal dynamics of ascospore release in the field? and iii) When do key infection events occur? We have recently been successful in producing ascospores in pure culture by developing a novel mating technique involving pairing of isolates of complementary *MAT* idiomorphs. Being able to produce ascospores *in vitro* has enabled us to experiment with ascospore inoculations to investigate their pathogenicity. In addition, volumetric spore trapping combined with a staggered fungicide application trial was used to determine the pattern of ascospore release, timing of infection, and the link between these two factors under field conditions. Field trial results have shown that while the peak of ascospore release was in February, the main infection events occurred in October and November, suggesting that peak ascospore release does not correspond with peak infection. Our study has confirmed heterothallism in *P. citricarpa*, but the frequency of successful mating between idiomorphs in the field, and its relationship with infection in orchards warrants further investigation. Future studies will investigate changes in fruit susceptibility and effects of environmental factors on infection to better understand the disease development and epidemiology of citrus black spot.

NOTES:

Fungal crop diseases and food security

Sarah Gurr

Abstract not available at time of printing.

Bacterial Leaf Streak: an emerging disease of wheat in the Upper Midwest

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2.

Bacterial leaf streak of wheat, caused by *Xanthomonas translucens* pv. *undulosa* (*Xtu*), has become prevalent in Minnesota, South Dakota, and North Dakota over the past eight years. Bacterial leaf streak is currently considered the second most important disease of wheat in Minnesota. Managing bacterial leaf streak is difficult due to the lack of resistant cultivars and other effective tools, especially as fungicides are ineffective against this bacterial pathogen. Over the past eight years we have demonstrated the economic importance of bacterial leaf streak; obtained useful data on the responses of regionally adapted varieties and elite germplasm to *Xtu*; refined techniques to establish bacterial leaf streak in inoculated field nurseries; and established a regional collaborative nursery to screen elite and released germplasm for response to *Xtu*.

Our screening efforts have determined that the majority of wheat cultivars and many advanced lines from the regional breeding programs are at least moderately susceptible to bacterial leaf streak and that there is generally less resistance in the regionally available germplasm than is desirable. We plan to continue using screening nurseries to test wheat lines for their response to bacterial leaf streak and to expand the materials we are examining with the intent of identifying additional and/or improved sources of resistance. Previous studies suggest that resistance to bacterial leaf streak is governed by multiple genes and quantitatively inherited.

Information obtained on the response of released varieties and elite germplasm is being utilized by regional wheat breeding programs. Information on the response of released germplasm to bacterial leaf streak has been disseminated to the benefit of regional wheat growers.

NOTES:

Using biology to develop a targeted biosecurity strategy for risk management of brown marmorated stink bug, *Halyomorpha halys*.

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The brown marmorated stink bug (BMSB), *Halyomorpha halys*, is an exotic pest of biosecurity concern for Australia and New Zealand. BMSB has a history of successful expansion from its native range in East Asia into North America and Europe. Prior to 2014, Australia's quarantine service had occasionally intercepted live BMSB adults at the border as hitchhikers on various goods, which were always directed for onshore treatment to manage this biosecurity risk. In December 2014, the number and size of BMSB detections on goods arriving from the United States increased substantially. For the first time, incidents consisting of very large numbers of live BMSB were intercepted, particularly on vehicles and machinery. To ensure that the biosecurity risk continued to be appropriately managed, emergency measures were implemented requiring these types of goods to be treated prior to loading in the United States.

Key aspects of the biology of BMSB, its changing pest status overseas, and the history of interceptions have allowed the Department of Agriculture and Water Resources to develop a targeted approach to manage the risk of BMSB arriving on goods. This includes implementing a seasonal window during which offshore measures are required, and heightened surveillance to monitor these and other pathways that may be at risk of BMSB

Ramu stunt: an important biosecurity threat to the Australian sugarcane industry

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The Australian sugar industry has supported joint research projects based at the Ramu Agri-Industries Limited (RAIL) estate in Madang Province, Papua New Guinea (PNG), for many years. The projects aim to prepare the sugar industry for potential pest and disease incursions through diagnostic test development, pest and disease surveys, developing rapid and reliable resistance screening methods and obtaining resistance ratings for both Australian and PNG sugarcane cultivars. One of the target diseases is Ramu stunt, a serious viral disease characterized by stunting, failure to ratoon and various leaf streak symptoms. The insect vector is the island sugarcane planthopper *Eumetopina flavipes*. While the distribution of Ramu stunt appears to be limited to PNG, *E. flavipes* extends from PNG throughout islands in the Torres Strait and onto the Northern Peninsula Area (NPA) of Cape York on mainland Australia.

Through viral extractions, cloning and sequencing, the cause of the disease was shown to be a virus with homology to the genus *Tenuivirus*. Verification that the tenuivirus was the causal agent was obtained from caged transmission experiments using the insect vector to transmit the disease, with confirmation by symptomology, RT-PCR, sequencing and protein analysis. A diagnostic RT-PCR test was developed and validated on the RAIL estate. Surveys have been undertaken throughout parts of PNG and when the disease has been identified, partial viral genome sequencing was performed to assess the extent of genome variability between isolates. Several of the most genetically diverse isolates are now subject to whole genome sequencing.

NOTES:

Phytoplasma disease outbreaks in multiple crops in eastern Australia

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Phytoplasma disease outbreaks were common and widespread in grain legume crops including mung bean (*Vigna radiata*), soybean (*Glycine max*) and peanut (*Arachis hypogaea*) in late 2016 and early 2017. Mung bean crops were affected in all major production areas spanning a distance of over 1,200 km from north to south with numerous crops having greater than 40% disease incidence near Dalby.

Several soybean crops from Cecil Plains were also affected by phytoplasma in late autumn 2016. Almost 100% of plants were affected in some paddocks and virtually no yield.

During the 2016/17 summer, some vegetable and pawpaw crops also had damaging outbreaks of disease. Several tomato crops in south-east Queensland had incidences of “big-bud” disease in excess of 50%. Capsicum and eggplant crops also had phytoplasma at higher than normal levels. In coastal Queensland, pawpaw (*Carica papaya*) crops had about 30% of plants affected by yellow crinkle disease but up to 80% in some younger plants.

The P1/P7 region of the 16S gene was amplified from several different crop types. Resulting sequences had close to 100% nucleotide identity to pigeon pea little leaf phytoplasma, *Candidatus* Phytoplasma aurantifolia (16Sr-II group), which has been previously reported in Australia from pigeon pea (*Canjanus cajan*) and stylosanthes.

A known leaf hopper vector of phytoplasma, *Orosius orientalis*, was collected from some affected mung bean crops but it is not certain it was associated with these disease outbreaks.

To our knowledge, this is the most significant, widespread outbreak of phytoplasma in broad acre crops to occur in this region of Australia. It is unclear what the underlying reasons are for this sudden increase in disease incidence. We are continuing further studies to determine: the diversity of phytoplasma across crop and weed hosts and geographical range, which insect species are vectors, and possible management options.

NOTES:

Grapevine leafroll disease control in white cultivars

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Grapevine leafroll disease (GLD) is associated in South Africa primarily with infection by grapevine leafroll associated virus 3 (GLRaV-3), and spread mainly by *Planococcus ficus* (vine mealybug). The disease affects all grapevine cultivars and is common in South African vineyards. It can be controlled by a combination of certified planting material, control of vector numbers and dissemination and roguing of infected vines. It has clear symptoms in red cultivars which can be used for roguing purposes. In white cultivars however no symptoms, or in some instances only mild symptoms are found, and visual assessment of disease cannot be used to identify infected vines for roguing. Control of GLD in white cultivars using ELISA to detect infected vines has achieved equivalent successful control of GLD as with red cultivars. At Vergelegen wine estate in Somerset West, control has been excellent in 15 white cultivar vineyards planted since 2003, with the highest single year of incidence of infection in one vineyard being only 0.6% infected in 2016. The highest cumulative infection in any vineyard was 3.6% over a nine-year period, while starting at an incidence of 0.76%. Control, based on the use of ELISA for virus detection however is very expensive and most wine estates do not consider this a viable option. Alternate methods of virus detection during control by roguing have been assessed. Loop mediated amplification of DNA (LAMP) to detect GLRaV-3 proved to be a very sensitive and allows for greater pooling of samples into one test, with concomitant reductions in cost and improved applicability. However, the technique is contamination-prone and has proven to be of limited large scale use. Following a successful proof of concept trial, we are currently assessing the novel application of an indicator bud system for disease detection in individual white cultivar vines.

Management of yellow spot (*Pyrenophora tritici-repentis*) in wheat through paddock selection, variety selection and in-crop management

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Yellow spot (*Pyrenophora tritici-repentis*) is an important fungal disease of wheat in Australia and internationally. It causes necrosis and chlorosis of leaf and stem tissue during the growing season, and survives on crop residue (stubble) between seasons. Despite its importance, its effect on grain yield and quality is still unclear. Some consider it a disease of seedlings, which the plant outgrows, while others regard yellow spot as an important disease that warrants fungicides for its control.

During 2016, Victoria experienced its tenth wettest spring on record, and with it a rise in severity of foliar wheat diseases. A survey conducted during 2016 found that yellow spot was the most prevalent foliar disease of wheat in the Wimmera and Mallee regions of Victoria, and was identified in all paddocks inspected. With its importance established in Victoria, studies into yield loss and best management practices were sought after by industry.

Yield loss experiments conducted in the Mallee and Wimmera regions of Victoria measured significant losses of ~21-23% in susceptible wheat cultivars compared with ~0-6% in moderately resistant cultivars, demonstrating that selecting varieties with host plant resistance reduces potential yield loss significantly.

Low rates of foliar fungicide application provided marginal control in the Mallee, whereas the fungicide trial in the Wimmera, with higher fungicide rates, showed best control was achieved with sprays at early tillering (first node detectable) and flag leaf emergence. At the Wimmera site the application of fungicide sprays at these two stages improved grain yield by ~27% above the untreated control.

NOTES:

Can reniform nematode (*Rotylenchulus reniformis*) in Australian cotton be managed by crop rotation?

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Reniform nematode is an important pest in cotton production in Central Queensland. No reniform resistant cultivars are commercially available. Rotation to resistant or non-host crops is used overseas to manage this pest. The potential of non-hosts to reduce the population density of reniform nematode was investigated in a replicated strip trial over two seasons on a commercial farm in Theodore, Qld. A sorghum plant with biofumigation properties, grain sorghum, and two corn varieties were investigated. In the second year the effect of previous crop on soil population of reniform post-harvest was determined.

In the 2014/15 season the four non-hosts significantly reduced soil nematode populations, with reductions of 98% compared with cotton in the top 15 cm of soil post-harvest. Deep coring showed that high populations of reniform were present at depth after cotton, confirming they survive deep in the soil profile. It is possible that re-colonisation of the planting zone could occur by drawing upon this population reservoir.

In the 2015/16 season, pre-plant populations in the non-host plots were significantly lower than the cotton plots. Post-harvest however, reniform populations were extremely high in the top profile for all treatments. Fumig8tor forage sorghum was the only crop that significantly reduced the population in the top 15 cm compared to cotton. Soil populations under Fumig8tor were significantly lower than commercial corn 606.

Researchers overseas have shown that a single rotation with a non-host may only suppress nematode populations, with economic thresholds being reached again by the end of the next cotton crop. Two successive rotations with a non-host crop are therefore recommended for improved production sustainability in such circumstances. Data from trial concurs, as large populations were observed after cotton for all treatments hence two years out of cotton is not sufficient to manage this pest.

Do Alternate Wetting and Drying Irrigation Technologies and Nitrogen Rates Affect Rice Sheath Blight?

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Water and nitrogen management play vital roles in rice production. However, the mismanagement of either may trigger plant disease epidemics such as sheath blight of rice, caused by *Rhizoctonia solani*, which is favoured by wet conditions, high relative humidity, and high nitrogen fertiliser levels. In order to understand how different combinations of water and nitrogen management affect sheath blight epidemics, we conducted two separate split-plot experiments with two irrigation regimes in the dry seasons of 2015 and 2016. The main plot irrigation treatments in both experiments were: flooded, maintaining 10cm standing water through the season and alternate wetting and drying (AWD), a water saving technology developed by the International Rice Research Institute (IRRI) and partners as adaptation strategy for water-scarce environments. In 2015 the split plots were nitrogen (N) applications applied as 0 kg ha⁻¹, 120 kg ha⁻¹, and 150 kg ha⁻¹ total N. In 2016 changes were made to address issues discovered in 2015. In 2016 split plots were N applications at 60 kg ha⁻¹ and 180 kg ha⁻¹ total N. Nitrogen was applied in splits in both seasons. Disease scoring was the same in both experiments using a sheath blight assessment scale for field evaluation developed at IRRI to assess the severity on infected sheaths and leaves while sheath blight incidence on tillers were counted per hill. Five and four disease assessments were made in 2015 and 2016, respectively. We were unable to detect any differences in disease in either experiment due to irrigation methods, N rates or the interaction of the two treatments in either season. Our findings indicate that farmers can safely adopt the AWD technology without risk of increasing sheath blight disease.

NOTES:

Investigating biofumigation and composted chicken manure to manage soilborne pathogens of avocado

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Avocado orchard productivity is reduced by soilborne pathogens, The principal one being root rot caused by *Phytophthora cinnamomi*, (Pc) and recently *Dactylonectria macrodidyma*(Dm) was also confirmed as a pathogen. Best management relies on implementation of several nursery and orchard practices, however, the efficacy of brassicaceous biofumigant plants to reduce soilborne disease has not been reported. *In vitro* Petri dish assays demonstrated that leaf extracts of Caliente (*Brassica juncea*) and BQ Mulch (*B. napus* + *B. campestris*) inhibited mycelial growth of Dm and Pc by 80-100%, at concentrations approximately equivalent to "field strength" (50 tonnes fresh weight/Ha). Nemat (*Eruca sativa*) inhibited growth of Dm and Pc by 66 and 36%, respectively.

An initial glasshouse study demonstrated that amendment of potting media with Caliente at concentrations approximately equivalent to 2x field strength (100 tonnes fresh weight/Ha) reduced root necrosis in avocado seedlings by nearly 40% five weeks after inoculation with *Phytophthora cinnamomi*, compared with plants that had been inoculated but received no amendment treatment. However, Caliente did not reduce root necrosis caused by Dm. Neither Nemat nor composted chicken manure reduced root necrosis caused by Pc or Dm. There were no significant differences among treatments for changes in seedling height or biomass of roots, leaves and stems. However, in the absence of pathogen, roots from the biofumigant treatments were more discoloured than those from the unamended control or chicken manure treatments. Further trials are necessary to evaluate whether biofumigation may have a role in the integrated management of avocado root rot diseases, in particular as a pre-plant treatment in orchards known to have a history of Pc and/or Dm infestation.

NOTES:

Optimising surveillance protocols using unmanned aircraft systems: the power of perspective**Brian McCornack^{1,2}, John Weiss^{2,3}, Felipe Gonzalez^{4,2}, Grant Hamilton^{4,2}, Geoff Pegg², Jonathan Kok^{4,2}, Ganesh Bhattarai^{1,2}***1. Kansas State University, Manhattan, Kansas, United States**2. Plant Biosecurity Cooperative Research Centre, Canberra, Australian Capital Territory, Australia**3. Department of Economic Development, Jobs, Transport and Resources, Victoria Government, Bundoora, Victoria, Australia**4. Queensland University of Technology (QUT), Brisbane, Queensland, Australia*

Our project focuses on modern remote sensing technologies for surveillance and monitoring organisms that threaten plant biosecurity across broad spatial scales. The future of effective and efficient biosecurity surveillance programs, and pest management in general, will require a higher level of automation and technical sophistication and an increased dependence on affordable technologies. Reliable yet effective sampling efforts are imperative to the future of plant biosecurity and food security in general. Our team investigated sensitivities and capacity of emerging, small unmanned aircraft systems (sUAS) and imaging technologies, including multi- and hyperspectral sensors, for biosecurity surveillance in viticulture, horticultural and grains industries. The overarching aim of this project is to investigate the use of these technologies to support claims of pest freedom and low pest prevalence compared to commonly deployed surveillance practices and utility to inform pest management decisions for established species. The project focuses on the use of science-centric data (e.g., using pest biology to identify areas most likely to be infested) to inform surveillance decisions (e.g., when and where to deploy a UAS equipped with multi-spectral cameras) made by biosecurity personnel and pest managers. This project also aims to determine how such technologies could improve detection rates and/or surveillance efficiency at the regional, field/orchard or plant level using case studies.

The use of UAS obtained imagery to detect early stages of pest incursions: Case study of grape phylloxera in vineyards**John Weiss^{1,2}, Kevin S Powell^{1,2}, Fernando Vanegas Alvarez³, Dmitry Bratanov³, Felipe Gonzalez^{2,3}***1. Agriculture Victoria, DEDJTR, Bundoora, VIC, Australia**2. Plant Biosecurity CRC, Bruce, ACT, Australia**3. Queensland University of Technology, Brisbane, Queensland, Australia*

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) is a serious economically important invasive insect pest of European grapevine *Vitis vinifera* L.. Although widely present in most grape-growing countries worldwide, its distribution within Australia is limited, mainly due to strict biosecurity measures in place at farm, regional and state levels. As the insect is very small, and primarily lives underground on the roots of the grapevines, it is very hard to detect until the symptoms of infestation appear (slow stunted growth and premature yellowing of leaves), usually after 2-3 years (although in some instances this can be longer).

Early detection of phylloxera within the first year, would allow vineyard managers to implement phytosanitary measures to restrict or slow the spread of the pest and reduce future costs and losses in production. Previous research, indicated that early infection of grapevines by phylloxera can be detected with hand-held spectro-radiometers and changes in leaf and canopy level reflectance were associated with changes in leaf chemistry.

This research, part of a Plant Biosecurity CRC project on “Optimising Plant Biosecurity Surveillance Protocols for Remote Sensing using Unmanned Aerial Systems”, applies these prior learnings, and evaluates air-borne RGB, thermal, multi- and hyper-spectral imagery at detecting symptoms of phylloxera infestation at two different vineyards, multi-variety grapevines, two separate time periods and under different levels of phylloxera infestation. Datasets from each imagery type will be compared to existing phylloxera detection practises; visual inspection, ground-based insect traps, soil DNA probes as well as being overlain with EM38 ground conductivity survey data. The ultimate aim of this study is to move towards a more targeted integrated approach for phylloxera detection and is the first study of its type to focus on a soil borne pest of biosecurity significance.

NOTES:

UAS Remote Sensing Efforts for Myrtle Rust Management

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Being presented by Fernando Vanegas Alvarez

Myrtle rust is an invasive fungal disease that pose a significant threat to the native Australian myrtaceae ecosystems. Original initiative to eradicate myrtle rust had been unsuccessful as the spread of the spores occur too rapidly by wind, water and physical contact. This caused the shift in biosecurity efforts to focus on management rather than eradication. Management measures begin with the identification and assessment of infected plants. This can prove difficult in environments where ground access is limited or unavailable. Remote sensing using unmanned aerial systems (UAS) are increasingly adopted for such applications. Continual developments in UAS technology have allowed the platforms and sensors to be more advanced, intelligent, compact and accurate. In this work, aerial data collection of high-resolution RGB, multispectral and hyperspectral imageries over a myrtle rust infected tea tree forest ecosystem was carried out. The high-resolution RGB imagery inherits 4mm/px resolution, which shows not only the stages of infection (transitioning from small purple spots to bright yellow spores to faded dull yellow to grey) but also the severity and spread. The multispectral imagery contains several wavelength bands (red, green, blue, red edge and near-infrared) for calculating the normalised difference vegetation index (NDVI). The NDVI provides estimates to vegetation properties such as biomass, leaf area, chlorophyll concentration, plant productivity, fractional vegetation cover, accumulated rainfall, etc. The hyperspectral imagery includes narrower and more numerous spectral bands than the multispectral, specifically, 274 bands as compared to the 5 bands in the multispectral. The continuous spectral features captured within the hyperspectral data provide measurements for the unique spectral signature of myrtle rust. The spectral signature can be used to populate spectral libraries for benefiting ongoing research and development into myrtle rust studies. UAS remote sensing has demonstrated its potentials for myrtle rust management, particularly in remote forested environments.

Optimal surveillance strategies for invasive plant pathogens using a spatial stochastic algorithmic mapping approach

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The global rise in emerging plant disease threats has led to an urgent need for improved surveillance strategies in plant health. Improved mapping and monitoring for targeted surveillance must address spatial heterogeneity whilst simultaneously incorporating relevant epidemiological processes. At more localised spatial scales, statistical inference of disease status through empirical estimation methods may surpass that of mechanistic approaches by taking the effects of local random variation better into account. However, identification of the most effective locations for disease intervention and monitoring still poses a challenge because of the dynamic complexities presented by pathogen dispersal, transmission and current spatial availability of plant hosts. These characteristics are typically the most crucial factors determining the outcome of plant disease invasions at the scale of a plantation and ultimately beyond.

Here, we apply a stochastic algorithmic mapping (SAM) approach first developed by Parnell et al (2011)* which includes key dispersal and transmission epidemiological parameters to describe the dynamic spatial distribution of an invasive plant pathogen. We consider typical scenarios where sampling and monitoring to determine disease status of plant hosts is subject to finite resources. The performance of the SAM approach is then determined in terms of standard receiver operating characteristic (ROC) curves and compared with a geostatistical interpolative mapping approach (such as kriging). Using data collected for Asiatic citrus canker in Florida, we illustrate how in practice this may better enable control and monitoring strategies to be determined under a variety of sampling design objectives. These may range from identifying the host locations of highest risk to those of greatest uncertainty to currently target.

*SR Parnell, TR Gottwald, MS Irey, W Luo and F Van den Bosch (2011). A stochastic Optimization method to estimate the spatial distribution of a pathogen from a Sample. *Phytopathology* 101(10), 1184-190.

NOTES:

Monitoring of airborne *Sclerotinia sclerotiorum* ascospores in southern New South Wales canola growing regions using quantitative PCR

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Sclerotinia stem rot caused by *Sclerotinia sclerotiorum*, is a yield limiting disease of canola in Australia. Senescent petals infested with airborne ascospores of *S. sclerotiorum* are the primary source of inoculum leading to the development of stem infection. The incidence of stem rot is common in some districts of southern New South Wales (NSW) where the intensity of canola production is high and characterised by a long flowering period and reliable spring rainfall. The severity of the stem rot varies between years and paddocks, which makes it difficult to manage disease outbreaks. As disease management relies heavily on foliar fungicide applications at 20-50% bloom stage, understanding of the release patterns of airborne inoculum and its quantity during the flowering period will aid growers in making informed decisions on the optimal spraying time and reduce unnecessary fungicide applications. To achieve this, six Burkard (Hirst-type) spore traps were deployed in 2014-2016 growing seasons across different canola growing districts located in the medium-high rainfall cropping region of southern NSW. The daily spore tape samples were analysed by nested quantitative PCR (qPCR) using *Sclerotinia sclerotiorum* species-specific primers. The study showed that the developed qPCR assay was specific and sensitive enough to detect as low as one ascospore. On most occasions a low amount of ascospores was observed daily, indicating the continual production of ascospores throughout the growing season (July – October). The peak levels of ascospore release varied across different districts and were mostly observed in late winter and early spring when weather conditions were wet and cool. High levels of ascospores during this period when plants are flowering and senescent petals are in abundance is critical as it may lead to stem infection. Thus, foliar fungicide application may be necessary during this period to reduce potential infection levels and yield loss

NOTES:

Plant Biosecurity just goes with the Territory

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There's never been a more exciting and positive outlook for plant industries in the Northern Territory (NT). Well established industries such as mangoes and melons have reached a level of maturity that sees them provide significant annual value to the NT economy. In addition, wholesale and retail nurseries, other horticulture and mixed cropping, continue to develop and form an integral part of the expansion of NT exports to existing and new trading partners.

The variety of crops grown across the NT include mangoes, melons, citrus, dates, hay, fresh vegetables, tropical fruits and on the road to recovery – bananas. The opportunities to enhance agriculture production are also immense and the Central Australian Horticultural Development Project, being implemented by Plant Industries Division, is demonstrating promising results for diversification of cropping and expansion of horticulture to include crops such as seed potatoes, onions, garlic, broccoli, cauliflower, cabbage and pumpkin.

With officers based in Darwin, Katherine and Alice Springs, the NT Plant Biosecurity Branch is responsible for maintaining the overall plant health status of the Territory, providing essential market access services and protecting primary industries, building domestic and export markets nationally and internationally. The Plant Biosecurity Branch must also ensure preparedness for effective emergency response in the event of an incursion by providing leadership, technical advice and support in the event of Emergency Plant Pest incursions.

In recent times the Territory has faced a number of exotic plant pest and disease incursions, such as Cucumber Green Mottle Mosaic Virus and Banana Freckle however, the Territory's plant industries do remain in an enviable position of being free from many of the serious pests that impact industries overseas.

Diversification of horticulture in the NT increases market access opportunities but also increases pest and disease risks by introducing new crop hosts in monoculture. Therefore, such a diverse collection of plant industries requires a strong foundation in biosecurity practices delivered through partnership with industry, community and the NT Department of Primary Industry and Resources, Plant Biosecurity Branch. The success of the plant industries of the NT are living proof this goal is entirely possible and can be achieved.

NOTES:

National Plant Pest Surveillance: An Innovative Approach to National Coordination and Knowledge Creation

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Australia's plant production system, including agriculture and forestry, is worth some \$30 billion annually. The system not only supports the livelihoods and investments of individual producers, but protects consumers in domestic and export markets, by maintaining the integrity, quality and sustainability of Australia's food supply.

It is estimated that every year between 20 and 40 percent of crops are lost to plant pests and weeds globally. To mitigate these losses through the eradication of pest incursions or management of production pests, an accurate picture of pest presence is required. This is accomplished using a mix of targeted pest management and general surveillance.

During 2016 Plant Health Australia developed an innovative cloud based system for monitoring pest activity, (AUSPestCheck). Functioning as a national virtual coordination centre, the system enables multiple providers who hold information on nominated pests to easily and securely upload surveillance data. This can be done either manually using spreadsheets or automatically through an Application Programming Interface. The information is automatically collated and a seamless integrated picture of pest status provided back to registered users and stakeholders. The system examines the data, provides automatic alerts and can present value added information with embedded user defined algorithms.

AUSPestCheck has a feature that enables integrated data to be easily exported for downstream analysis. In this paper, we will demonstrate benefits from matching exported real time aggregated pest surveillance data compiled by AUSPestCheck with advanced catchment scale land use mapping. The result has been the generation of national and regional maps that can significantly improve pest threat monitoring, assist the development of response plans and support area freedom and market access arrangements.

How robust are stable isotope and trace element signatures for the assessment of a fruit fly's geographic origin?

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To distinguish a fruit fly in a trap as either a new arrival or from a locally breeding population has significant implications for the degree of risk posed, and hence for the level and nature of the response required. Elsewhere, combined stable isotope and trace element signatures have held up to the proof of principle that they can distinguish geographic regions of origin for several insect species. However, how robust this technique is for biosecurity decision-making needs to be systematically assessed.

Here we summarise highly coordinated research to test the geographic resolution that may be usefully afforded for fruit fly surveillance in Australia and New Zealand. Signatures expressed in medfly and Q-fly reared on lemons harvested from high risk regions of Queensland, New South Wales, South Australia, Western Australia and North and South islands of New Zealand, were obtained for strontium (Sr), hydrogen (H) and oxygen (O) isotopes plus non-essential trace element concentrations. This dataset, comprising 86 lemon collection points and 7,263 fruit flies, reveals (1) that fruit fly Sr isotope signatures usefully reflect that of the underlying geology, (2) there was significant variation in the west of Australia compared with that of the east and with New Zealand, and therefore correspondingly (3) that regional distinctions are possible between fruit flies, but also possibly at the township level around the southwest Australia. In addition, H and O results will be presented; these are anticipated to enhance regional provenance determination, but not near-scale discrimination.

NOTES:

The future of the National Red Imported Fire Ant Eradication Program

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In Australia, a national eradication program for the Red Imported Fire Ant (fire ant) (*Solenopsis invicta* Buren), one of the world's most invasive species, has been in operation since 2001 when the pest was first detected in Brisbane, south east Queensland. Since that time, four separate incursions of this ant have been successfully eradicated from this country but the main south east Queensland population remains. Current estimates of the potential cost impacts of fire ant should it become widespread in Australia exceed AUD\$1.65 billion/year. An external review of the National Red Imported Fire Ant Eradication Program (the Program) in 2015 concluded that it remains technically feasible, cost beneficial and in the national interest to eradicate, but that there was only a small window of opportunity left to achieve this. A ten-year eradication plan has been developed and is presently under consideration for funding by the Program's national cost-share partners. The plan involves a significant boost in all eradication activities, significantly increasing the total area receiving treatment each year and additional resources dedicated to preventing human-assisted spread and further encouraging the industry and community to look for and report suspect fire ant infestation. A progressive 'rolling' strategy will be employed that focuses eradication activities initially in infested areas on the outer western and southern perimeter of the operational area and then shifts eradication effort inwards where there will be a staged clearance of suburbs. Mathematical modelling and quality assurance systems will be used to closely monitor the implementation and progress of the eradication and to ensure continued absence of infestation in treated and cleared areas.

Managing biosecurity risk of exotic insect pest and pathogen incursions in wheat through pre-emptive breeding

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The recent discovery of Russian wheat aphid demonstrates the potential impact of pest incursions into Australia, and the absence of resistant cultivars that can be easily deployed. Further, there is no incentive for wheat breeding companies to develop resistant varieties due to the cost burden and low return on investment. Here, we report on research undertaken at ICARDA and CIMMYT, which indicates that the Australian wheat industry is very vulnerable to damage from certain pests, especially Hessian fly (zero resistance), Karnal bunt (3.6% resistance) and Sunn pest (1.5% resistance). Although these pests and pathogen are not currently present in Australia, much of the Australian cereal cropping regions offer an acceptable climate for the pests to establish. The purpose of our research is to reduce the risk of potential future economic loss to wheat growers due to incursion by providing wheat breeders with molecular markers and parental genetic stocks that carry genes for resistance to Karnal bunt, Hessian fly and Sunn pest. Using conventional breeding methods, elite Australian breeding lines have been developed that combine resistance to the exotic pests and fungal disease with good agronomic qualities. In addition, data is being generated to determine whether any deleterious traits have transferred from landraces along with the resistance genes. To further assist uptake of the newly developed resistant germplasm, molecular markers have been developed, and validated. We have identified single-nucleotide polymorphisms in the dirigent-like protein-encoding gene (*HfrDrd*), and the wheat Hessian fly responsive proteins *Hfr-1* and *Hfr-2*, to aid pre-emptive breeding for Hessian fly resistance. In addition, we have identified a major gene locus (*Ei1*) for pre-emptive breeding against Sunn pest, and gene-based markers on chromosomes 6B and 3A for Karnal bunt resistance. The resistance data, parental genetic stocks and associated diagnostic markers are freely available to the public through online access.

NOTES:

Genome editing for Plant Defence

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A single lineage of *Nicotiana benthamiana* is widely used as a model plant and has been instrumental in making revolutionary discoveries about RNA interference (RNAi), viral defence and vaccine production. It is peerless in its susceptibility to viruses and in its amenability in transiently expressing transgene. We have been engineering the nuclear and chloroplast genomes of *N. benthamiana* by conventional breeding, RNAi, CRISPR and homologous recombination. Using these techniques and technologies we have conferred wide spectrum virus resistance, enhanced insect resistance, altered fatty acid metabolism and expressed sentinel reporter genes that measure recombination frequencies.

Trans-kingdom RNAi, which is based on making plants express double-stranded or hairpin (hp) RNA for ingestion by herbivorous pests. This RNA triggers silencing of specific genes within the pest leading to death or impaired growth. However, the efficacy of the approach appears to depend on the means of delivering the duplexed RNA. In our study, we introduced a hpRNA construct, targeting the acetylcholinesterase (ACE) gene of *H. armigera*, into the chloroplast genome of *Nicotiana benthamiana*. As the chloroplast, unlike the rest of the plant cell, lacks RNA interference machinery, the hpRNAs ingested from feeding on this plant are not pre-processed and are therefore more effective at triggering RNAi in the pest. Our transplastomic (chloroplast-transformed) lines, showed stronger protection against *H. armigera* herbivory than transgenic lines with hpRNA constructs integrated into the plant's nuclear genome. These findings suggest that chloroplast expression of hpRNA is an attractive way of providing pest resistance in crops. The successes and challenges of these technologies will be discussed.

Hunting down specific triggers of resistance to the apple scab fungus

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The scab fungus, *Venturia inaequalis*, is one of the most devastating pathogens of apple worldwide. Regular epidemics result in the need for frequent fungicide applications throughout the growing season. The appearance of fungicide resistance has heightened the need for additional control options, and resistant hosts are a potential alternative. Apple has a long breeding cycle, and resistance is often found in species with relatively poor quality traits. The difficulty of selecting cultivars with scab resistance has frequently led to the production of apple cultivars incorporating single major resistance genes. This type of resistance is usually vulnerable to breakdown, due to selection of virulence in the sexually reproducing pathogen population. Multiple types of resistance stacked in a cultivar is likely to be more durable, however breeding cultivars harbouring multiple scab resistance genes is challenging. This breeding challenge is facilitated by the identification of specific pathogen races or, even better, pathogen proteins (effectors) that trigger specific, differential resistance responses. Whilst races of *V. inaequalis* have been identified, no specific effector proteins have been characterised to date. We are taking a systems biology approach to identify specific effector proteins that trigger resistance in apple to the scab fungus.

We have sequenced the genomes of several races of *V. inaequalis* and are using comparative transcriptomics and genomics to narrow down the search for specific effectors in the apple scab fungus. Genetic crosses between pathogen races are also being used in this hunt. We have identified genes encoding proteins that are secreted by the fungus, and we are characterising specific pathogen proteins that trigger the resistance responses. We are also identifying conserved *V. inaequalis* proteins that are essential for the pathogen to infect apple and hope to identify resistance proteins that can recognise these factors, reducing the ability of the pathogen to mutate to virulence.

NOTES:

Teasing apart the differences between Asian and European pear scab disease to reveal the molecular mechanisms of host-specificity and non-host resistance.

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Pear scab disease is the major fungal disease of pears caused by two distinct species of host-specific *Venturia*: *V. nashicola* is restricted to Asian pears (*Pyrus bretschneideri*, *P. ussuriensis* and *P. pyrifolia*), while *V. pirina* exclusively infects European pears (*P. communis*). The incompatible relationships between European pears and *V. nashicola*, and Asian pears and *V. pirina*, are considered to be governed by non-host resistance. However, while thought to be a more durable form of resistance, the multi-layered molecular mechanisms of non-host resistance are poorly understood. The Asian and European pear hosts have previously been crossed to produce Eurasian pears with varying degrees of resistance to both pear scab fungi, providing another layer to the study of non-host resistance in the *Pyrus-Venturia* pathosystem. This provides a unique opportunity to dissect the genetics of host-specificity and to understand host-pathogen co-evolution from the perspective of non-host resistance. Fluorescence phenotyping has been used to characterise responses to different isolates of *V. pirina* in European, Asian and Eurasian pears. The defence transcriptome of Eurasian pears in response to *V. pirina* is being investigated to understand the dynamics of the molecular mechanisms underlying non-host resistance. Furthermore, core effector candidates shared between *V. nashicola* and *V. pirina* have been investigated in the whole genome sequences to determine their role during infection and possible contribution to the host-specific nature of these pathogens. These core effector candidates may be useful in not only identifying durable resistance genes for molecular plant breeding, but also be useful targets for the specific and sensitive detection of the Asian pear scab pathogen, *V. nashicola*.

Deciphering the molecular mechanisms of *Lr34*-mediated disease resistance in wheat

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The *Lr34/Yr18/Sr57/Pm38/Ltn1* multi-resistance locus has been effective and deployed in wheat cultivars for more than 100 years. The durability and pleiotropic nature makes *Lr34* a unique and highly valuable resource for rust resistance breeding. Despite its functional annotation as ABC transporter, the mode of action is unknown. Considering this, we aimed to decipher molecular factors and signalling components using RNA-seq of Chara resistant (*Lr34*) and Chara heavy ion irradiation (HII) mutant susceptible (*Lr34*) wheat lines. The screening of Chara and Chara HII lines with *Lr34* gene specific markers confirmed the integrity of *Lr34* in both lines, however, phenotyping confirmed rust susceptibility in Chara HII lines. Plants were grown under controlled conditions and infected with *Puccinia triticina* pathotype 76-1,3,5,7,9,10,12,13+*Lr37* at the fully expanded flag leaf stage. Flag leaves were sampled at 0, 24, 48, 72, 96 and 168 hours post inoculation (hpi) from mock and infected plants. Based on real-time PCR analysis of basal defence genes and the *Lr34* gene, we selected 72 hpi for RNA-seq with four biological replicates per condition. The samples were sequenced on Illumina HiSeq 4000 at Beijing Genomics Institute, China. A total of 9.0 Gb sequences (2.25 Gb/ library) from 16 libraries for four conditions were obtained. Differential expression analysis was performed using the 'Tuxedo' analysis pipeline with standard parameters. Analysis revealed differential expression of key defense related genes, transcription factors and disease response associated proteins involved in molecular basis of *Lr34* mediated resistance. The validation of genetic components confirm their role in the activation and regulation of *Lr34* gene and resistance pathway. This insight may improve our ability to harness *Lr34* in wheat and other crops using genetic engineering approaches.

NOTES:

Identification of candidate genes involved in resistance to *Austropuccinia psidii* (myrtle rust) in *Syzygium luehmannii* (Riberry)

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Puccinia psidii, causal agent of myrtle rust, was discovered in Australia in 2010 and has since become established on a wide range of species within the Myrtaceae. *Syzygium luehmannii* (Riberry), endemic to the Australian east coast, is becoming an increasingly valuable berry crop. Plants were screened for responses to *P. psidii* inoculation and specific resistance, in the form of localised necrosis, was determined in 29% of individuals. To understand the molecular basis for this response, mRNA was extracted and sequenced from leaf samples taken pre-inoculation, at 24 and 48 hours post inoculation from four resistant and four susceptible plants. *De novo* transcriptomes were built for all plants and gene expression profiles compared at each time point. Gene expression profiles were significantly different in resistant versus susceptible phenotypes. Resistant plants significantly up-regulated 185 genes 48 hours after pathogen exposure, while susceptible plants only up-regulated three genes. Most significantly up-regulated in resistant plants were gene homologues coding for transcription factors, receptor-like kinases and enzymes in the secondary metabolite pathway and defence. We identified the presence of putative *G-type lectin receptor-like kinase* transcripts exclusively in resistant plants and significant differential expression of two transcripts incorporating Toll/interleukin-1, nucleotide binding site and leucine-rich repeat domains.

NOTES:

Objective measures for assessment of powdery mildew on grape berries

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Powdery mildew, caused by *Erysiphe necator*, affects the sensory properties of wines and objective measures are needed for assessing grape quality. The aim of this research was to investigate mid-infrared (MIR) spectroscopy, and fatty acid and powdery mildew-specific quantitative PCR (qPCR) analyses as methods for distinguishing healthy and disease-affected berries.

Intact, fully-developed berries (n=138) were detached from 35 representative Chardonnay bunches collected at harvest maturity in the Waite Campus research vineyard (University of Adelaide). Single berries were assessed using a stereomicroscope, designated visually healthy, partly- or fully-infected, deseeded, homogenised and scanned in the MIR range. A subsample of 61 berries was selected for subsequent fatty acid analysis using gas chromatography and thin layer chromatography, and subsamples of 54 of these berries were subjected to qPCR analysis. Colonisation of berries by *E. necator* altered their chemical composition, mainly in the spectral region 1800-1185 cm⁻¹. However, analysis of fingerprint-like spectra failed to distinguish partly-infected berries from the two remaining categories. Saturated fatty acids were prevalent in *E. necator*, arachidic acid (C20:0) being the most abundant. Although total saturated and total unsaturated fatty acids (C14-C24) were similar in healthy and infected berries, the fatty acid profile in individual berries was altered by infection. Arachidic acid content changed in response to powdery mildew severity. A duplex TaqMan qPCR with limit of detection of 3 fg of pure *E. necator* DNA from conidia, detected 6 fg - 0.1 ng of pure *E. necator* DNA per 0.5 ng of total DNA in the 53 berries. Increasing *E. necator* biomass on berries (based on qPCR) was strongly related to C20:0 content (R²=0.76), suggesting that arachidic acid has potential as a biomarker for *E. necator* and powdery mildew severity.

Investigations on the spore dispersal patterns of eutypa and botryosphaeria dieback pathogens in Australian vineyards

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Eutypa dieback (ED) and botryosphaeria dieback (BD) are economically important diseases of grapevines worldwide causing cankers, dieback and eventually death of vines. These diseases persist in affected vines from season to season threatening the \$8.3 billion Australian wine industry. The spores of ED and BD pathogens that infect primarily via grapevine pruning wounds are generally dispersed by rain splash and travel distances depending on wind speed. However, there is limited data on the climatic conditions required for spore release by these pathogens in different climatic regions of Australia. This study investigated the spore dispersal patterns of ED and BD pathogens in South Australia (Barossa Valley and Coonawarra) and New South Wales (Hunter Valley and Griffith) using Burkard spore traps. Spore trap tapes were collected and replaced monthly at each site and analysed using two quantitative PCR protocols (qPCR) developed to detect Diatrypaceae and Botryosphaeriaceae spores. Preliminary data analysed to date showed sporadic release of ED and BD pathogen spores at different times of the year with the highest number of spores detected in late winter and early spring when temperatures were relatively mild and rainfall was high. However, higher number of spores were trapped in New South Wales over summer compared to in South Australia. Spore release was found to be associated with rain with as little as 0.2 mm resulting in spore release of either pathogen but not all rain events resulted in spore release. Six spore traps have been further deployed across Australian wine regions, and will be monitored for a further three years. Data generated from this study will determine the critical times of the year when ED and BD pathogen spores are abundant in vineyards and this may assist growers in making decisions on optimal timing of pruning and wound treatment.

NOTES:

Increased carbon-dioxide affects crown rot incidence, severity and pathogen colonisation in wheat reducing potential grain yield.

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Plant pathogens and the diseases they cause are estimated to cause a 10 – 16% decrease in crop yield worldwide, with an additional 6 – 12% loss attributed to postharvest spoilage. However, changes in the global climate as a consequence of rising atmospheric carbon-dioxide (CO₂) are expected to further impact these losses. Crown rot (CR) of wheat has provided an excellent model to study the effects of elevated CO₂ (eCO₂) on disease incidence, severity, pathogen colonisation and the subsequent effect on grain yield. The effect of eCO₂ on the CR pathogen, *Fusarium pseudograminearum* was assessed in controlled environment facilities (CEF), glasshouse experiments and at the Australian Grains Free-Air CO₂ Enrichment (AGFACE) field experiment near Horsham, Victoria. The influence of CO₂ on crown rot development was shown to be dependent on a number of factors: 1) The CO₂ effect on CR was dependent on temperature or growing season. 2) CR incidence and susceptibility on average increased under eCO₂, and the CO₂ effect was dependent on the genotype; 3) When CR severity was correlated with pathogen colonisation, CR severity was higher per unit of infected host tissue under elevated CO₂; 4) Yield penalty at eCO₂, due to CR, was greater in wheat grown under eCO₂. 5) CR incidence, severity and pathogen colonisation were also dependent on the host growth stages and temperature.

Our findings show that an increase in atmospheric CO₂ has the potential to increase disease incidence and severity by influencing host susceptibility while causing a reduction in the potential grain yield. These studies, therefore, show CR is likely to become more problematic for farmers unless effort is made to integrate wheat genotypes with durable CR resistance under eCO₂ into breeding programs.

NOTES:

Distribution and epidemiology of viruses infecting garlic in Australia

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Infection of commercial garlic crops by viruses is common and substantially reduces crop quality and yield. Viruses found in garlic include members of the *Potyv*-, *Carla*- and *Allexivirus* genera, and a *Tospovirus*, *Iris yellow spot virus* (IYSV). Virus spread in garlic crops is facilitated through vegetative propagation of infected bulbs, and through vectors, such as aphids, mites and thrips. Extensive international research has identified viruses infecting garlic, and their impacts on yield, however, there is limited knowledge for Australian garlic. This study evaluates the distribution of viruses in Australian field garlic and vegetative planting material across multiple growing districts located in Queensland, New South Wales, South Australia, and Victoria. Molecular screening of crop samples was carried out using genus-specific RT-PCR-based assays, followed by species-specific hybridisation assays. The samples were tested for three potyviruses, *Onion yellow dwarf virus* (OYDV), *Shallot yellow strip virus* (SYSV), and *Leek yellow stripe virus* (LYSV), three carlaviruses, *Garlic common latent virus* (GarCLV), *Garlic latent virus* (GarLV) and *Shallot latent virus* (SLV), and five allexiviruses, *Garlic viruses A, B, C and X* (GarVA, -B, -C, and -X) and *Shallot virus X* (ShVX). Virus incidence within planting material was near 100%, with plants carrying at least one virus from each of the three genera tested. The most common infections were potyviruses, OYDV and LYSV, and carlaviruses, SLV and GarCLV. The genetic diversity of local viruses was evaluated through sequencing of the viral coat protein (CP) across a selection of isolates. Increased knowledge of virus population dynamics in Australian crops, their vectors and weed hosts, contributes to our understanding of virus epidemiology and supports quarantine efforts attempting to limit the entry and spread of new viruses into Australia. Additionally, this study supports further development of virus free garlic germplasm in Australia and contributes targeted pest management strategies for industry.

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Cucumber green mottle mosaic virus and honey bee pollinators

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The first Australian report of the tobamovirus *Cucumber green mottle mosaic virus* (CGMMV) was in watermelons in the Northern Territory in 2014. At the time CGMMV was known to be moved via mechanical transmission but insects were not considered capable of transmitting the virus. The commercial viability of watermelon production in Australia is reliant on managed pollinators, specifically the European honey bee *Apis mellifera* (Hymenoptera: Apidae). These managed pollinators may be moved to multiple locations, 1000s km apart to provide pollination services. The potential of European honey bees to move CGMMV in the environment has been investigated over the past three years as part of the emergency response and then the ongoing management of the virus. Hive products were sampled for the presence and viability of the virus. This sampling has shown that the virus is viable inside bee hives and a sampling protocol for future work has been developed.

NOTES:

Implementing Australia's National Plant Biosecurity Diagnostic Strategy

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The National Plant Biosecurity Diagnostic Strategy (NPBDS), released in 2012, fulfills an essential component of the overarching National Plant Biosecurity Strategy. It publishes the agreed recommendations and actions necessary to ensure Australia has the people, infrastructure, diagnostic standards and tools to provide delivery of plant biosecurity diagnostic services. The Subcommittee on Plant Health Diagnostics (SPHD) aims to sustain and improve the quality and reliability of plant pest diagnostics in Australia, and is integral to achieving the recommendations in the NPBDS.

National Diagnostic Protocols (NDPs) are the endorsed standards for use as part of national responses to emergency plant pest incidents, providing unambiguous taxonomic identification of the pest or related group of pests. With 38 NDPs published, SPHD is focusing on the continued development of NDPs for the 40 priority plant pests or pest groups identified by the Australian Government and the 350+ priority pests identified through industry biosecurity planning processes. More recently NDPs are being enhanced by encompassing diagnostic procedures and processes that specifically support surveillance activities.

SPHD is also investigating methods to improve surge capacity in Australian diagnostic laboratories to manage the influx of samples in an incursion response, progressing the strategy for ensuring maintenance of valuable plant pest reference collections, and facilitating a proficiency testing program.

The National Plant Biosecurity Diagnostic Network (NPBDN) connects people who are integral to the plant biosecurity diagnostic system across Australia and neighboring countries, leading to improved capability and capacity. The NPBDN is a flexible, self-organizing network with SPHD providing formal oversight and regulation. Through the NPBDN, diagnosticians receive professional development in areas of strategic need, opportunities for short-term residential laboratory exchanges, and participation in an annual workshop themed around common general diagnostic techniques or issues.

Continued implementation of the NPBDS will support diagnosticians and ensure Australia's diagnostic system remains robust and effective.

Molecular identification of trapped insects: Metabarcoding for improved monitoring and surveillance of insect pests

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Traditional morphological identification of insect trap catches can be highly effective, but can be difficult to implement on large trap samples, being a relatively slow approach often limited by the availability of taxonomic expertise. Various molecular approaches (e.g. PCR-RFLP, qPCR, DNA Barcoding) have been used as alternative identification methods for identification of trapped insects, however the recent development of metabarcoding, the molecular identification of samples through high-throughput DNA barcoding, now provides an alternative approach that addresses many of the limitations of previous molecular methods.

Metabarcoding is a relatively new identification method that has been primarily used for ecological studies, particularly for assessing diversity within soil and aquatic systems. However, many other fields including agriculture and biosecurity are now beginning to utilise this approach. Recent applications of metabarcoding for the identification of trapped insect pests and vectors were reviewed. High-throughput amplicon sequencing provides a cost-effective alternative to more traditional methods of identification, with several recent applications including: 1) surveillance for insect pests, and 2) detection of insect vectors and associated pathogens.

The ability to multiplex genetic markers, samples and specimens provides the high-throughput power of metabarcoding. A number of studies and reviews have begun to explore this approach in a biosecurity / surveillance context. Metabarcoding is likely to be much more commonly used in the future, given its ability to simultaneously identify insect taxa present within large trap samples.

The application of generic amplicon next generation sequencing to determine *Illavirus* species diversity in *Prunus*.

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Ilarviruses can have significant economic impacts on stone fruit and almond production, yet their distribution and population diversity amongst commercial Australian *Prunus* species is unknown. PCR amplicon next generation sequencing (NGS) is a powerful approach to sequence a high number of virus amplicon samples in a single run and also detect populations of both high and low-level occurring plant viruses for diversity estimation.

In this study, NGS of genus-specific amplicons from a 371 bp conserved region of the *Ilarvirus* RNA2 polymerase gene was used to determine the diversity of *Ilarvirus* species infecting 61 *Prunus* trees in Australia. *Apple mosaic virus* (ApMV), *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) were each detected in at least 52 trees. *American plum line pattern virus* (APLPV) was also detected in one *Prunus* tree and this is a first report of this virus in Australia. Two novel and distinct groups of *Ilarvirus*-like RNA2 amplicon sequences were also identified in several trees by the generic amplicon NGS approach. The high read depth from the generic amplicon NGS also allowed the detection of mixed infections of ilarviruses in seven *Prunus* trees and distinct RNA2 sequence variant populations of PNRSV, PDV, ApMV, APLPV and the two novel *Ilarvirus*-like sequences.

Sanger sequencing of specific RNA1, RNA2 and/or RNA3 genome segments of each virus and total nucleic acid metagenomics NGS confirmed the presence of PNRSV, PDV, ApMV and APLPV detected by RNA2 generic amplicon NGS. However, the two novel groups of *Ilarvirus*-like RNA2 amplicon sequences detected by the generic amplicon NGS could not be correlated to the presence of sequence from RNA1 or RNA3 genome segments or full *Ilarvirus* genomes, and their origin is unclear. This work highlights the sensitivity of genus-specific amplicon NGS in detection of virus sequences and their distinct populations in multiple plant samples.

Sporeid – innovative disease monitoring and diagnostics for improved efficiency of crop production

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Current advances in technology allow us to use novel agricultural solutions, such as robots, on farm integrated weather stations, and GPS for better crop management to improve sustainability and enhance environmental benefits whilst ensuring profitability for the grower.

The aim of this project is to develop a unique disease monitoring system that would be fast, robust and cost effective. Novel diagnostic tools, crop disease modelling and yield forecasting have been linked to underpin grower decision making, and investigate the potential impact of emerging disease on sugar beet.

SporeID is designed to minimise the impact of foliar diseases on the yield of the sugar beet crop including powdery mildew, rust and other potential threats. Currently, potential yield reduction due to these pathogens is estimated to be 12, 20 or 50% for rust, mildew or cercospora respectively and, whilst current practices prevent losses of this magnitude, it is calculated that up to 10% of yield in the UK is still lost each season to foliar diseases. Climate change may also lead to increased pressure, both from existing and 'new' emerging diseases, which will require increased crop protection. Improved knowledge and decision making will optimise chemical input and offer environmental benefits through improved resistance management in future climates.

SporeID involves a fully automated technology to trap, recognise and quantify possible fungal pathogens before the disease development in the sugar beet field. Results are generated by a high volume air sampler incorporated with a molecular system, performing Loop-mediated isothermal Amplification (LAMP) and results, together with weather data, are sent out to a server, where the calculations of the risk and predictions for the disease development and consequently yield losses are estimated. Growers receive an alert of the disease pressure, supported by risk analysis, in order to take appropriate action, if needed, within the crop.

NOTES:

Development of quantitative PCR assays to detect and quantify inoculum of grapevine trunk disease pathogens from environmental samples

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Grapevine trunk diseases (GTDs) are considered a serious threat to viticulture worldwide. Eutypa dieback (ED) and Botryosphaeria dieback (BD) caused by several Diatrypaceae and Botryosphaeriaceae species are the two most important trunk diseases affecting Australian vineyards to date. The ascospores and/or conidia of these pathogens are primarily spread by rain and wind and infect susceptible pruning wounds leading to cankers, dieback and eventually death of vines. The objective of this study was to develop molecular tools to detect and quantify Diatrypaceae and Botryosphaeriaceae inoculum as part of the investigations on spore dispersal patterns of ED and BD pathogens in Australian vineyards. Different DNA extraction protocols were evaluated using artificially-inoculated tapes and Burkard spore trap tapes collected from vineyards and one protocol was found efficient and gave consistent DNA yield. Two sets of multi-species primers for quantitative PCR (qPCR) were further developed to detect and quantify Diatrypaceae and Botryosphaeriaceae spores from a single environmental sample. The multi-species primers for Diatrypaceae and Botryosphaeriaceae species were designed to amplify fragments of the ITS region of the rRNA and β -tubulin genes, respectively. Specificity tests showed the two multi-species primers were highly specific to their corresponding target Diatrypaceae and Botryosphaeriaceae species (nine each) and did not amplify any of the 20 non-target species tested. The two qPCR methods were shown to amplify genomic DNA, synthetic DNA fragments (gBlocks[®]) and mixed DNA from environmental samples. The limit of detection of the qPCR for the Diatrypaceae and Botryosphaeriaceae species were ~20 fg and ~300 fg of genomic DNA, respectively. In summary, the two qPCR assays were developed for rapid and sensitive detection of ED and BD pathogens from environmental samples. These assays are currently being used to analyse spore trap tape samples collected from different grapevine growing regions in Australia.

NOTES:

Palmageddon: The ongoing invasion problem with palm killing weevils

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Weevils in the genus *Rhynchophorus* (Coleoptera: Curculionidae) are well known palm pests in their native and invaded ranges, of which the most notorious is the highly invasive and incredibly destructive red palm weevil, *R. ferrugineus*. However, two other weevil species, *R. vulneratus* and the South American palm weevil, *R. palmarum*, have emerged as potential invasive threats, with both recently establishing in California (USA). This presentation will review the invasion problems posed by *R. ferrugineus* to palms, especially Canary Islands date palms, *Phoenix canariensis*, in urban areas, and date palms, *P. dactylifera*, in date production areas of the Middle East and the Maghreb Region of North Africa. The successful eradication of *R. vulneratus* from Laguna Beach in southern California will be reviewed, and updates on the ongoing invasion in southern California by *R. palmarum*, the vector of the pathogenic nematode, *Bursaphelenchus cocophilus*, that causes lethal red ring disease of palms, will be provided. This presentation will also cover developments in the use of drones to monitor palm mortality and consider the potential use of smart traps to remotely monitor weevil activity and report captures in near real time by emerging technologies that exploit the Internet of Things (IoT).

Phylogeny, Ecology and Amplicon Sequencing of the New Zealand Psylloidea (Hemiptera: Sternorrhyncha) reveals interesting insect-plant-bacteria interactions.

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In recent years, psyllids (Hemiptera: Sternorrhyncha) have become of increasing interest due to their capability to vector damaging bacterial plant pathogens. The arrival of *Bactericera cockerelli* (Tomato Potato Psyllid, TPP) to New Zealand and the spread of *Candidatus Liberibacter solanacearum* (Lso) also raised the risk of a future arrival to Australia. To date, studies of the New Zealand psyllid fauna have primarily focused on insect morphologies, and there was scant knowledge of their phylogeny and evolution.

After a two-year field collection, the first multi marker phylogeny of the New Zealand Psylloidea is presented here for a total of 90 psyllid species distributed across 600 locations between New Zealand and Australia. Additionally, a 16S amplicon sequence data from 60 species has been developed to describe the psyllids symbiotic microbial diversity. Using the complete dataset comprising the host plant and insect distribution, and the bacterial flora, network analysis clarifies the relationships between psyllids, host plants and vectored bacteria. This first step will be of fundamental value for biosecurity to understand and possibly help predict the psyllid-vectoring of plant pathogens.

NOTES:

The importance of yeasts in the ecology and control of the Queensland fruit fly

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Queensland fruit fly (*Bactrocera tryoni*) is a major orchard pest in Australia. Adult flies lay their eggs into ripe fruit, resulting in larval infestation and the spread of bacterial and fungal rots. The role of these microbes in fruit fly ecology is only now being elucidated, with much of the emphasis to date focusing on bacterial communities. In our study, we explored the diversity of yeast species associated with *B. tryoni* adults and larvae. We found larvae were highly associated with budding yeasts of the genera *Hanseniaspora* and *Pichia* across several fruit species. In feeding trials using two yeast species, one species was highly beneficial to larval development and survival whilst the other provided no nutritional benefit. Moreover, adult flies were deterred by the odours produced by the beneficial yeast. Although these findings appear counter-intuitive we discuss how they fit with our understanding of the ecology of this insect, and how this new knowledge could lead to improved monitoring and control of fruit flies in Australia.

Whole mitochondrial genome sequencing reveals diversity of *Bactericera cockerelli* (tomato potato psyllid) in New Zealand

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The tomato potato psyllid (TPP, *Bactericera cockerelli*) is an important plant pest as well as a vector of *Candidatus Liberibacter solanacearum*, the putative causative agent of Zebra Chip disease in potato. A region in the 3' end of the mitochondrial cytochrome oxidase 1 gene has been used to distinguish between populations of TPP in the USA and Central America. Four different biotypes have been described based on 1-17 single nucleotide polymorphisms found in this 500 base pair region. Of these, three have been shown to differ biologically. All four biotypes are found in different geographical regions of the USA, but only the Western biotype has been described as an invasive population: it is able to overwinter in California and has established populations there as well as in parts of the Pacific Northwest.

It was also the Western biotype that was introduced into New Zealand, probably due to illegal importation of infested plant material. This biotype has been able to establish itself and is now found throughout the country.

To study the population structure and genetics of TPP in New Zealand, we collected TPP from the major potato-growing regions over several growing seasons. TPP were also collected from two regions in Honduras. DNA was extracted from individual insects and sequenced via Illumina HiSeq. From these data, 198 near-complete mitochondrial genome sequences were assembled. Phylogenetic and median joining network-based analyses clustered the New Zealand samples with sequences from California and Washington State. The insects from Honduras clustered separately from the New Zealand insects. These analyses illustrate significantly higher variation in the psyllid populations than described previously. The diversity in the mitochondrial sequences suggests accompanying differences in the nuclear genome which may translate into biological differences.

NOTES:

The effect of groundcovers on survival of Banana rust thrips *Chaetanaphothrips signipennis* (Bagnall) (Thysanoptera: Thripidae).

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Banana rust thrips *Chaetanaphothrips signipennis* (Bagnall) (Thysanoptera: Thripidae) is a key pest of bananas in Australia. Larvae and adults feed on the pseudostem under leaf sheaths and on fruit between the fingers. Damage first appears as water-soaked markings then later develops into reddish-brown 'rust' areas, making fruit unmarketable. Control relies upon chemical applications targeting the bunch. The chemical products available for use against thrips in bananas are being limited by registration withdrawals and anecdotal reports of resistance. Identifying alternative cultural or biological control strategies could reduce reliance on chemicals.

Conventionally grown bananas have limited ground covers protecting the soil beneath plants. Ground covers could be used to increase mortality of the soil-dwelling pupal stages of *C. signipennis*. Ground covers may provide refugia for predators and change the soil conditions that favour other natural enemies. A field trial comparing ground cover and bare soil banana plots assessed *C. signipennis* populations and identified several possible predators. Emergence traps were used to assess *C. signipennis* survival at the soil surface, while thrips populations and damage assessments were also measured on the pseudostem and in bunches. Additionally, a study of soil invertebrates of the groundcover and bare soil plots identified naturally occurring thrips predators. While this research showed no significant difference in mortality of *C. signipennis* between the ground cover and bare soil plots, ground cover features such as density and establishment time may have resulted in lowered abundance and diversity of the natural enemies. The absence of a deep litter layer may also account for the lack of natural enemies exerting control over thrips pupae. The soil study identified seven species of predatory soil mites that may impact *C. signipennis* populations. Three species were near *Gaeolaelaps aculeifer* (Canestrini) (Mesostigmata: Laelapidae) known predator of soil-dwelling thrips pupae, used commercially in vegetable and flower crops.

NOTES:

Enhancing the Australian plant biosecurity system amidst rapid global change

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Australia's biosecurity system capitalises on our geographical isolation and climatic conditions to ensure that Australia is one of the few countries in the world to remain free from many of the world's most severe pests and diseases. However the nature and magnitude of the biosecurity risks are changing as Australia is increasingly affected by climate change and increasing global movement of goods and people.

This, together with the number and complexity of plant pests, the wide range of production and native plant hosts in Australia, and our increasing aspirations for plant exports, demands a more structured approach to optimising our plant biosecurity management. We need a shared understanding - shared concepts, language and vision - about the plant biosecurity system and its elements, so that common approaches can be developed and the priorities identified and addressed.

The biosecurity role in plant import and export regulatory processes is relatively mature. Two other biosecurity management processes are proposed to (1) identify and address significantly changing biosecurity risks and (2) to identify and respond to changing pest status. These two need further national work.

The recently developed Australian plant health prioritisation framework is a first step towards a national approach to changing biosecurity risks. A key second step has been identifying national priority exotic plant pests. These provide a platform for developing national approaches for risk scanning and triage.

Australia has a well-practised national system for emergency responses to new incursions, has optimised its national diagnostic network (within the current resources) and is working to improve its national surveillance capacity. We are less mature with regard to how we work together to manage established plant pests that have an impact on trade. The development of national frameworks for plant health trade, area freedom certification and general surveillance are our first steps towards a common national approach.

Eradicating Coconut Rhinoceros Beetle from Hawaii: Do the benefits outweigh the costs?

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2. NSF Center for Integrated Pest Management, North Carolina State University, Raleigh, NC, U.S.A.

When plant pest incursions take place there is a great deal of pressure on policy-makers to get the response right. They must make critical risk management decisions that have uncertain effects on complex agricultural, ecological and social systems despite knowing little about their resilience and adaptability to change. In this paper, we demonstrate how some of this uncertainty can be captured in a bioeconomic model to help guide response policy decisions using the example of coconut rhinoceros beetle (*Oryctes rhinoceros*), or CRB, in Hawaii.

CRB is a large beetle native to Southeast Asia capable of damaging palm species that has recently been introduced to the Hawaiian island of Honolulu. The beetle has caused severe damage to coconut palms (*Cocos nucifera*) on the island of Guam despite concerted efforts to mitigate its spread and impact there. The impact of CRB could be equally severe to native and introduced palm species in Hawaii resulting in substantial economic losses to commercial nurseries, as well as Hawaii's residents and tourists who value palm trees for their aesthetic value.

We use data from CRB spread in Guam to validate a jump diffusion model of spread that is then used to predict the insect's future distribution and impact in Hawaii under different response policies. The costs of response actions and impacts of CRB on commercial nurseries and ornamental palms is simulated over a 30-year period, and the value of resultant damages compared. We find that eradication represents a net-benefit-maximising response provided the non-market (i.e. environmental and social) impacts of CRB exceed \$6.9 million per annum.

NOTES:

The Impact of Global Regulations under the Montreal Protocol on Soilborne Disease Control, Biosecurity and the Climate

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Phase out of 95% of the key ozone depleting chemicals, including methyl bromide (MB), under the Montreal Protocol has been the greatest present day success for the environment and mankind. Additionally, last year after 7 years of debate, the Kigali amendment was ratified bringing in stepwise reductions for HFCs which means one third of anthropogenic effects on the climate will be avoided. The key success to date has been the reported phase out of 98% of MB for controlled uses which has led to 35% of the present ozone layer recovery. It has also had a huge impact on plant pathology and contributed to a major rethink of control methods for soil borne pathogens and weeds in a huge number of horticultural sectors. Approximately 80% of the 75,000t of MB used worldwide was for soil disinfection for control of a wide range of soilborne pathogens and weeds. Since then, most sectors in countries globally have moved to other chemical methods, but also some more sustainable solutions. Key chemical alternatives being used today are generally old products applied with new methods i.e. 1,3-dichloropropene, chloropicrin and metham sodium either applied alone, as mixtures or as sequential applications, however they are subject to increasing regulations and bans, e.g. new VOC regulations, increased buffer zones, township caps, etc. and their future is questioned. Several new products, dimethyl disulphide and ethane dinitrile are proving effective, whilst others have failed to gain acceptance, viz; furfural, Na azide, propargyl bromide and propylene oxide. More sustainable solutions include grafting, plant resistance and soilless culture, although the use of disease suppression is still a major challenge for plant pathologists. Some industries unfortunately are now faced with increased pressure from specific diseases, eg. *Macrophomina* on strawberry fruit, *Nacobbus* nematode on tomatoes.

Approximately 10,000 t of MB are still used for Quarantine and Preshipment uses which are exempt from phase out under the Montreal Protocol owing to countries concerns over global trade, phytosanitary issues and the need to maintain national biosecurity. The impact of these regulations on MB will be discussed.

A new approach for conducting pest risk analyses – the Group Pest Risk Analysis.

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A pest risk analysis (PRA) is the process of evaluating evidence to determine whether an organism is a pest, if it should be regulated, and the strength of any phytosanitary measures to be taken against it to manage biosecurity risk. The Australian Government Department of Agriculture and Water Resources (the department) is improving the effectiveness and consistency of PRAs. A key step in this process is the development of a 'group PRA' approach which analyses the biosecurity risk posed by groups of pests with similar biological characteristics across numerous import pathways.

Group PRAs apply the significant body of scientific knowledge available to the department including pest interception data and previous commodity based PRAs. A group PRA synthesises knowledge of groups of pests and diseases with similar biological characteristics to help manage the biosecurity risks associated with commodity movement.

Group PRAs are:

- built on a foundation of more than 18 years of PRAs by the department through an extensive process of robust scientific analysis and stakeholder consultation with state and territory authorities, industry organisations and trading partners;
- validated with scientific evidence, including interception data collected at Australia's borders, and examination of research papers on significant pests recognised internationally, in Australian industry biosecurity plans, and those identified as regional pests for Australia in consultation with states and territories;
- building blocks that can be used to review existing or prospective trade pathways for which a PRA is required, avoiding the need to 'reinvent the wheel' when analysing the risks of individual species, whilst maintaining a robust system to protect against exotic pest and disease incursions.

This presentation will provide an introduction to the 'group PRA' concept and highlight the recent progress the department has made in developing group PRAs.

NOTES:

Plant Post Entry Quarantine in New Zealand – working with industry to develop new regulations

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In 2012 the New Zealand Ministry for Primary Industries (MPI) started to review the regulatory requirements for Post Entry Quarantine (PEQ) facilities. PEQ facilities are used to hold plant material imported for propagation, ahead of it gaining biosecurity clearance to enter New Zealand. The Facility Standard, which was the subject of the review, sets out the operational and physical/structural requirements to run such a facility.

Existing regulations were over 15 years old. They did not take into account changes to the New Zealand Biosecurity Act, and were not always consistent with other MPI standards, processes and international regulations. The review aimed to address these factors, and, where possible, to provide options to manage plant germplasm in a PEQ facility in a way more commensurate with biosecurity risk.

There is a perception amongst some industry members in New Zealand that imported germplasm is a low risk pathway. In contrast, MPI (and the scientific community in general) consider it one of the highest risk pathways for the introduction of disease organisms to new geographical regions. MPI worked closely with industry when reviewing the standard, to ensure that the proposed measures were practical, and that industry had a clear understanding of why the standard needed to be updated. This was considered especially important because most PEQ facilities in New Zealand are privately operated.

The revised standard included many new requirements. The most significant was a requirement for all facility operators to undertake specific training and to prepare an operating manual describing how they would comply with regulatory requirements. In addition, a new level of facility was introduced to the new standard, with the intention of decreasing capacity constraints on the PEQ system in New Zealand.

Challenges encountered by MPI throughout the review process, and during implementation of the new standard, will be discussed.

NOTES:

Tools, technologies and transdisciplinary science of Area-wide Management of Insects

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Once an exotic pest establishes in a new location, managing the pest to minimize harm becomes the priority. Area-wide management (AWM) programs – the coordinated actions of participants in the region experiencing the problem – have a history of successfully suppressing pests at scale. At the most basic level, theoretical models from the 60s show that coordinated efforts of insecticide use by farmers have resulted in the best chance of suppressing a pest and protecting crops. Particularly successful examples incorporate state-of-the-art tools, such as the Sterile Insect Technique (SIT), that ensure on-going pest suppression; and make use of technologies such as Ecological Modelling to inform the basis of their application. More recently, focus has also expanded to include AWM of natural enemies of pests, and the role of semi-natural habitat. However, there are considerable challenges to achieving a successful AWM programs including: 1. delays in knowing the presence and location of the target pest, 2. heterogeneity of target landscapes and regions, and 3. limited support of stakeholders, the community and the public. In this plenary address, Dr Schellhorn uses examples from the literature, and a case study of QLD fruit fly, and silverleaf whitefly to examine the tools, technologies and transdisciplinary science to meet the challenges and achieve a successful AWM program of a pest incursion.

NOTES:

Interaction networks shed light on the ecology and coevolution of soil microbiomes

Linda Kinkel¹

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Soil microbes exist in highly diverse communities in which thousands of bacterial species are estimated to coexist in a single gram of soil. Competitive, antagonistic, and cooperative interactions among coexisting microbial species are crucial to maintaining microbial diversity, and significantly impact the dynamics, metabolic activities, and evolution of microbial populations. These complex networks of interaction also mediate microbial community functions, such as the suppression of plant pathogens via antibiotic production. However, we have limited insight into the structure of microbial interactions within soil communities, or the implications of network structure for the ecology, evolution, and functional characteristics of soil communities. We analyzed networks of antagonistic (antibiotic inhibition), nutrient competition, and signaling interactions among sympatric populations of soil *Streptomyces*. We characterized variation in the structure of networks among populations from diverse soil locations; identified differences in network structure between coevolved (sympatric) and random (allopatric) collections of *Streptomyces*; and explored the potential for network analyses to quantify the roles of antibiotic arms race vs. niche differentiating coevolutionary dynamics in soil communities. Our results suggest that interaction network analyses offer substantial potential for providing novel insights into the factors that generate and maintain microbial functional capacities in soil communities.

Determining the effect of strawberry production cycle and mulch type on plant health, fruit yield, and root pathogens

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Strawberries in eastern Canada are cultivated in conventional matted-rows for multiple years of fruit production, which can result in deterioration of soil quality and an increase in root diseases. Recently, producers are testing raised beds with plastic mulch systems. This study was aimed at evaluating the impact of strawberry production cycle (a newer field in first-year production and an older field in third-year production) and raised-bed mulch type (red, black, and silver plastic and straw) on plant health, fruit yield, and root pathogens. The crop-production cycle evaluations were performed in matted-row commercial field sites and mulch type evaluations were performed at KRDC experimental field sites in 2015 and 2016 seasons. The first-year production field had a 12% higher fruit yield than the third-year production field. The older field also showed comparatively higher infections of multiple root pathogens and poor plant growth compared to the newer field. *Fusarium* sp., *Rhizoctonia fragariae*, *Cylindrocarpon* sp., *Pythium* sp., and *Colletotrichum acutatum* were common pathogens isolated from these fields. These pathogens are known to be associated with black root rot complex. The microbial activity (culturable bacteria and fungi) was higher in the first-year production field compared to the third-year production field. In 2015 mulch trial, fruit yields from the silver and black plastic mulch plots were slightly higher than the straw mulch plots, and *Fusarium* and *Pythium* were common root pathogens isolated from the mulch plots. In 2016 mulch trial, fruit yield in the red plastic mulch plot was higher than the other mulch plots. Root infections were slightly lower in the plastic mulch plots compared to the straw mulch plots and *Fusarium*, *Rhizoctonia*, and *Cylindrocarpon* were common pathogens isolated from 2016 mulch plots. There were no differences in microbial activity in soil samples from such plots, but black plastic mulch had a greater population of *Trichoderma*.

NOTES:

Suppression of potato bacterial wilt with non-aerated compost tea and factors which influence efficacy

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The use of water extracts from composted organic waste to suppress crop diseases has particular potential in developing countries and small-holder agriculture where resources are limited. These extracts can be produced by low-cost, non-aerated methods to prepare “non-aerated compost tea” and previous studies have shown that application can lead to reduction of a wide range of foliar and soil borne diseases in horticultural crops.

The bacteria *Ralstonia solanacearum* is a major pathogen of potato in many regions of the world and there are few successful control methods. Glasshouse trials were conducted in Ethiopia to examine if non-aerated compost teas could reduce disease and what influence various factors had on the degree of efficacy. These included production factors (i.e. compost type and resulting microbiological and physico-chemical properties), application factors (timing of application, concentration) and the addition of adjuvants. In this experiment, compost teas made from agricultural waste compost were more effective to reduce disease severity than those made from solid municipal waste or vermicompost tea. Moreover, application of NCT at the same time that potato tubers were planted and inoculated with pathogenic *R. solanacearum* led to greater disease reduction than when applied 7 days before or after introducing the pathogen inoculum. The most effective treatment resulted in a 2.5-fold reduction in disease compared to the non-treated controls, based on the “area under the disease progress curve” parameter. In a second glasshouse trial, the addition of myrrh gum led to the greater reduction of disease severity than compost tea alone.

Next-generation sequencing of 16S and ITS genes for bacterial and fungal communities in compost tea, and physico-chemical characteristics will be presented which provide a basis to explore differences in disease suppression efficacy.

Transcriptionally active wheat rhizosphere microbiomes in fields suppressive and non-suppressive for *Rhizoctonia solani* AG 8

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Rhizoctonia root rot and bare patch disease, caused by the soilborne fungus *Rhizoctonia solani* anastomosis group (AG) 8, results in patches of stunted plants with reduced grain production. The disease causes losses of \$77 million per annum in cereal cropping in Australia. In the absence of resistant cultivars of wheat or barley and a wide host range, current disease control measures are limited. A sustainable and enduring method for disease control is needed and may lie in the enhancement of biological disease suppression, where the resident microbial community counteracts the pathogen and inhibits it from infecting the plant. The mechanism of disease suppression is not known for this pathosystem however there is evidence of effective biological control of *R. solani* at our study site in Avon, South Australia. A metatranscriptomic approach was applied to determine the taxonomic and functional characteristics of the rhizosphere microbiome of wheat plants grown in adjacent fields which are suppressive and non-suppressive for *Rhizoctonia solani* AG 8. Disease suppressive soils had a unique microbial community according to their gene expression profiles. Microbial mRNA transcripts from twelve rhizosphere samples were mapped to metabolic databases (KEGG, COG, SEED) to infer differentially expressed microbial functional genes in suppressive and non-suppressive rhizosphere soils. These differentially expressed genes provide a focus for future studies to determine precisely the molecular interplay of plant-microbe-pathogen interactions with the ultimate goal of the development of a beneficial rhizosphere microflora to reduce *R. solani* AG 8 infection of crops.

NOTES:

Engineering banana cropping systems to suppress soil borne diseases

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Banana production in Australia is currently worth \$600 million annually, but is constantly threatened by root pathogens, such as plant-parasitic nematodes and Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*). To reduce the impacts of soil borne diseases on banana production, farm management interventions are being designed to enhance organisms which suppress pathogens, while maintaining productivity. A holistic view of soil functions with an understanding of the ecological interactions is therefore required to develop productive, disease suppressive farming systems. Soil nitrogen and organic matter are two factors that have a large impact on soil organisms, and can be manipulated by farm management. Their impacts on biological processes were monitored in field trials in north Queensland using soil nematodes community structures and Community Level Physiological Profiling using MicroResp™. Nitrogen is applied to bananas to enhance vegetative plant growth and maintain production. However, high soil nitrogen inputs tended to increase bacterial domination in the soil environment and lead to an increase in root pathogens. Organic matter inputs provide carbon substrates for soil organisms. By increasing the diversity of organic matter inputs, through vegetative groundcovers, it was possible to increase soil biological diversity. Furthermore, vegetative groundcovers tended to increase the proportion of nematodes involved in organic matter recycling and reduce the proportion of plant-parasitic nematodes. The use of vegetative groundcovers also led to suppression of Fusarium wilt symptoms determined through soil bioassays. The results indicated that through the careful manipulation of nitrogen and organic matters inputs it was possible to engineer banana cropping system that suppress soil borne diseases and maintaining productivity.

NOTES:

From genomics to diagnostic development, the citrus canker story

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Xanthomonas citri subsp. *citri* is the causal agent of citrus canker, a serious disease of citrus found in over 30 countries that is currently exotic to Australia. Several incursions have been recorded in Australia, the most recent in 2004, with the eradication campaign costing ~\$17.6 million. Early and accurate detection of the pathogen increases the likelihood of a successful eradication.

The symptoms of mature citrus canker are quite distinct, though in the early stages they can be easily confused with the fungal disease citrus scab. It is at this stage that an effective molecular diagnostic tool is most effective. Within the Australian context, there are a number of endemic *Xanthomonads*, including the closely related *Xanthomonas citri* subsp. *malvacearum* which also carries the *pthA* region and are capable of generating false positive results with the Cubero & Graham 2002 protocol. Previously, a false positive occurred when these primers yielded a band for a *Xanthomonas citri* subsp. *malvacearum* present on a citrus leaf which was tested due to the presence of an early scab lesion.

Whole-genome sequencing technology has been used to sequence numerous *Xanthomonads* (176 for this analysis); including 29 *Xanthomonas citri* subsp. *citri* from previous incursions and interceptions, 13 *X. citri* subsp. *maniferaeindicae*, 67 *X. citri* subsp. *malvacearum* and 67 endemic *Xanthomonads*. Comparative genomic analyses of 176 *Xanthomonads* identified unique regions of *X. citri* subsp. *citri* for diagnostic targeting. There was approximately 2Mbp of a 5-6Mbp genome total difference between the *X. citri* subsp. *citri* and *X. citri* subsp. *malvacearum*. This comprised of 58 unique regions, which were greater than 50bp, for which 38 primer sets were designed and 16 were successful at identifying *X. citri* subsp. *citri* from the other *Xanthomonads*. These same regions have now been used for LAMP (loop-mediated isothermal amplification) assay development.

A novel *Candidatus Liberibacter* species associated with *Ctenarytaina fuchsiae*, the New Zealand native fuchsia psyllid

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Three sequences (KX768754, KX810197, KX810198), assembled from metagenomic data, were obtained by sequencing DNA from the New Zealand native fuchsia psyllid, *Ctenarytaina fuchsiae* (family Aphalaridae). Phylogenetic analysis of these three sequences from the 16S rRNA operon, the *rpA* - *rpL* region and the DNA-directed RNA polymerase α subunit, indicated the presence of a novel species of *Candidatus Liberibacter*, for which the binomial '*Candidatus Liberibacter ctenarytainae*' is proposed.

The genus *Liberibacter* includes five unculturable species assigned *Candidatus (Ca.)* status: The pathogenic *L. solanacearum*, *L. asiaticus*, *L. americanus*, *L. africanus* and the endophytic *L. europaeus*; and one culturable, non-pathogenic, species assigned full status, *L. crescens*. The five *Ca. Liberibacter* species are vectored by/associated with psyllid species in the Psyllidae and Triozidae families of the Psylloidea superfamily. In 2015, a new species, '*Ca. L. caribbeanus*', was reported from the psyllid, *Diaphorina citri* and citrus samples from Columbia based on 92-96% similarity of 16S rRNA sequence from this new species to that of other *Ca. Liberibacter* species. Further, in 2015 sequences with 99% identity to *Ca. Liberibacter* 16S rRNA sequences were found in the Australian native psyllid *Acizzia solanicola* and '*Ca. L. brunswickensis*' proposed as the name for this species.

The three *C. fuchsiae* metagenomic sequences had significant similarities to (*Ca.*) *Liberibacter* sequences: the 1.4 kbp 16S rRNA sequence was 95.6% similar to the same region of *Ca. L. europaeus*. Maximum likelihood phylogenetic analyses revealed that these new sequences were not derived from any currently known species, have distinct lineages and most likely originate from a new-to-science *Liberibacter* species. This is the first report of *Ca. Liberibacter* sequences in a psyllid species that is not a member of either the Psyllidae or Triozidae families providing initial evidence that this bacterial genus may be more widely distributed in the Psylloidea superfamily than is currently recognised.

NOTES:

Characterisation of '*Candidatus Liberibacter brunswickensis*' identified in the Australian eggplant psyllid

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6. Better Border Biosecurity, Lincoln, New Zealand

Psyllids can vector species of the *Candidatus Liberibacter* that pose a serious threat to the citrus, potato and carrot industries if introduced to Australia. The liberibacter genus was known to consist of seven phytopathogenic and endophytic species exotic to Australia; *Liberibacter crescens* (Lcr), '*Candidatus Liberibacter africanus*' (CLaf), '*Ca. Liberibacter americanus*' (CLam), '*Ca. Liberibacter asiaticus*' (CLas), '*Ca. Liberibacter caribbeanus*' (CLca), '*Ca. Liberibacter solanacearum*' (CLso, synonymous to '*Ca. Liberibacter psyllaurosus*', CLps) and '*Ca. Liberibacter europaeus*' (CLEu). Except for Lcr, species in the liberibacter genus are yet to be grown as an axenic culture.

A novel species, '*Candidatus Liberibacter brunswickensis*' (CLbr) has been detected in the native Australian eggplant psyllid, *Acizzia solanicola*. The identification of CLbr is based on nucleotide sequence of the 16S ribosomal ribonucleic acid (rRNA) region and seven additional genes using a multi locus sequence mapping approach, which phylogenetically separate CLbr into its unique branch in the liberibacter genus. CLbr has not been associated with plant disease and is yet to be grown in axenic culture. *A. solanicola* has broadened its host range to have plant-host crossover with *Bactericera cockerelli*, the tomato potato psyllid, a known vector of CLso. Additionally, the 16S rRNA region of CLbr is phylogenetically similar to, and can confound diagnostic tests, for two citrus phytopathogenic liberibacter species of concern to Australia, CLas and CLaf.

The biology of CLbr in *A. solanicola* feeding on eggplants and genomics of the liberibacter genus is explored. Understanding the microflora of Australian psyllids is important for both biosecurity preparedness and response management of exotic diseases.

Discrimination between viable and dead *Xanthomonas fragariae* on strawberry host tissue by PCR

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1. Ministry for Primary Industries, St Johns, AUCKLAND, New Zealand

Being presented by Julie Pattemore

Molecular diagnostics utilising PCR techniques are routinely used for the detection and identification of pests in quarantine and biosecurity. When faced with making biosecurity decisions based on the finding of an unwanted organism by PCR, one of the major challenges is the inability to differentiate positive results originating from viable or dead cells. A positive PCR test is not sufficient to assess organism viability and traditional culturing techniques that could verify viability can be too time-consuming (1-30 days depending on pathogen and matrix). Viability PCR (vPCR) is a technique that selectively inhibits PCR amplification of DNA derived from dead cells, through the use of a nucleic acid intercalating dye. These dyes are unable to penetrate viable cells with intact membranes but will penetrate dead cells with compromised membranes. Exposure to blue light allows cross-linking of these dyes to DNA which inhibits PCR amplification.

The bacterial pathogen *Xanthomonas fragariae* can infect the calyx tissue associated with strawberry fruit. As fresh strawberries are a highly perishable commodity and *X. fragariae* is a slow-growing organism that can take over seven days to culture, a rapid method to detect viable cells is highly desirable. To enable rapid detection of viable *X. fragariae*, a vPCR protocol to discriminate viable and dead *X. fragariae* has been optimised. PEMAX™ (a double nucleic acid intercalating dye reagent) treatment resulted in complete inhibition of PCR amplification of 10⁸-10³ dead *X. fragariae* cells on strawberry host tissue. The most important parameters to optimise were amplicon length, turbidity of the sample matrix and choice of nucleic acid intercalating dye. This protocol will support timely decisions to be made if *X. fragariae* is detected on imported fresh strawberries.

NOTES:

Diagnosing 'dieback': A protocol for determining the cause of endophyte-associated mortality in plants

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Plant 'dieback' is loosely defined by overall health decline, leaf and stem death and eventually whole plant mortality. Dieback significantly reduces populations of multiple cultivated, desired and invasive plant species globally. Early diagnosis of dieback is difficult because it can potentially be due to a complex interaction of factors including environmental conditions, pathogen community composition and plant-microbe interactions.

In instances where there is a single putative pathogen causing plant death, it is possible to verify its causal role through testing Koch's postulates. However many examples of dieback in plants are associated with the hosts' community of fungal endophytes. This has been shown for trees such as native jarrah, weeds including *Parkinsonia aculeata*, and grasses such as *Sporobolus* sp. Although dieback has been studied in multiple systems, past understanding of this phenomenon has traditionally focused on the use of culture-based techniques alone. Since endophyte communities themselves are complex and consist of hundreds of taxa, testing Koch's postulates in such contexts is difficult.

Here I outline a potential approach for investigating dieback phenomena where multiple causal agents may be involved and where diagnosing which endophytes might be pathogenic or dieback-inducing is difficult. This framework requires a sequence of methodologies beginning with traditional culture-based fungal isolations and ID, followed by next-generation sequencing of the fungal endophyte community, statistical analysis of community composition in relation to dieback occurrence and identification of pathogenic taxa likely to be involved in dieback. Subsequently, isolation strategies are designed for any remaining putative pathogens, and then pathogenicity screening is conducted in the context of environmental variables.

Adopting this approach for dieback research in forestry, environmental and agricultural systems can lead to better diagnoses of causes of plant death. This would have significant implications for enhancing the productivity of desirable plant species and suppressing populations of invasive plant species.

NOTES:

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.

The *cytb* gene structure and resistance to qoi fungicides in the grape black rot pathogen, *guignardia bidwellii*, and related species

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Quinone outside Inhibitors (QoIs) are considered as single-site of action fungicides which inhibit the electron transfer in mitochondria by binding to the cytochrome *bc1* enzyme complex. In many phytopathogenic fungi a single point mutation in codon 143 of the *CYTB* gene, encoding cytochrome b, confers complete resistance to QoI fungicides. However, in some fungi neither QoI resistance nor this mutation (G143A), have been detected so far. This was explained by the presence of an intron in the *CYTB* gene after codon 143: it was predicted that a G143A mutation would prevent the splicing of this intron and, thus, the production of functional cytochrome b proteins.

Guignardia bidwellii, the causal agent of grape black rot, is considered as a *CYTB* intron-containing species with low risk for the development of QoI resistance. We amplified and cloned *CYTB* fragments in several *G. bidwellii* strains, and also in some other *Guignardia* spp., including authentic strains of *G. citricarpa*, *G. gaultheriae*, *G. mangiferae* and *G. aesculi*, obtained from CBS, to sequence the intron located after codon 143. Surprisingly, no intron was detected in the predicted position in several *G. bidwellii* strains isolated from different grape varieties in Hungarian vineyards. Also, the intron was not found in either an authentic *G. bidwellii* strain obtained from LGC ATCC, or the *G. aesculi* and a *G. gaultheriae* strains included this study, while the intron was identified, and sequenced, in all other *Guignardia* spp. strains examined by us. *In vitro* fungicide resistance tests did not show a clear correlation between the presence/absence of the intron in *Guignardia* spp. strains and their sensibility to QoIs. This suggests that other mechanisms may also be involved in their QoI resistance; and some *G. bidwellii* strains may develop QoI resistance in the field through the G143A mutation.

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NOTES:

Effects of copper used for control plant pathogenic bacteria: the case of *Pseudomonas syringae* pv. *actinidiae* (Psa)

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Copper is widely used for control of plant pathogenic bacteria including *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of bacterial canker of kiwifruit. The main risk associated with the use of copper is that the pathogen becomes resistant to it. Copper-resistant strains are usually identified by their ability to grow on agar plates supplemented with different concentrations of copper. However, the ability to grow on a medium supplemented with copper and the concentration of copper to which bacterial strains are resistant depends on the composition of the medium used for the test. In addition, resistance to copper in the laboratory does not always correlate with a loss of control of that same pathogen in the field. To establish a correlation between ability to grow on plant treated with copper and ability to grow in presence of copper in the laboratory, different growing agar media have been modified and the growth of Psa on kiwifruit leaves treated with different level of copper has been followed. Strains of Psa which can grow in presence of copper concentrations which would otherwise be lethal have been isolated. The genetic basis for this ability has been found to be similar to that found in other bacteria (presence of genes from the *pco* and *cus* operons). Psa most probably acquired them by horizontal gene transfer from bacteria of the kiwifruit phylloplane. Some of these bacteria might also affect the ability of Psa to cause disease by interfering with bacterial communication. To understand better how copper influence those two mechanisms (acquisition of copper resistance genes and increased or decreased virulence), the effect of copper on the epiphytic microbiome of the kiwifruit leaf is being investigated. The effect of copper is being assessed using Illumina Miseq Sequencing using the V5V6 region of the ribosomal DNA as barcode.

Copper tolerance in *Pseudomonas syringae* pv. *tomato* from tomatoes in Queensland, Australia

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Bacterial speck (caused by *Pseudomonas syringae* pv. *tomato*) and bacterial spot (caused by *Xanthomonas* spp.) can cause significant crop damage and loss in tomato and pepper. There are limited pesticide control options available for these diseases, with copper based bactericides currently the basis of bacterial crop management programs in Australia. Copper bactericides are used frequently over the entire crop cycle and are also used on many other horticultural crops. Copper tolerant isolates of bacterial speck and spot have been identified worldwide. In Queensland, both bacterial speck and spot occur on tomato and bacterial spot on pepper. There are three reports of copper tolerance in bacterial spot and speck in Australia, with tolerance identified in bacterial spot isolates from pepper in Queensland and in bacterial speck isolates from tomatoes in New South Wales and Victoria. Queensland produces approximately 60% of Australia's fresh market tomatoes with climatic conditions facilitating year round production in some areas. These producers are increasingly reporting a reduction in the efficacy of copper based sprays for bacterial disease control in tomatoes. Despite this there have been no studies undertaken to determine if copper tolerant *P. syringae* pv. *tomato* are present on tomatoes in Queensland. In this study copper tolerance screening methodology for *P. syringae* pv. *tomato* were analysed and tested, isolates from tomato producing regions in Queensland and some other Australian states were screened for tolerance, multiplex diagnostic testing for tolerance was explored and genome sequence analysis of Australian *P. syringae* pv. *tomato*. The findings of this study have important implications for Queensland tomato producers and highlights the need to adapt bacterial disease management strategies to control copper resistance development.

NOTES:

Biological control of Botrytis cinerea in grapes

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Grey Mould disease caused by *Botrytis cinerea* is generally the most severe decay during the ripening of grapes and costs Australian grape growers around \$30 million each year including \$8 million on control measures. The disease infects flowers to establish latent infections which later become active decays as the fruit ripen. *B. cinerea* has conventionally been managed using chemical fungicides, but over use has resulted in resistance to many chemical groups, and most effective chemicals cannot be applied after berries are the size of a pea, due to restrictions on chemical residues in export wine markets.

The environment of the ripening grapes is a challenging “infection court” for biological control with a rich diversity of microbes, abundant natural yeasts and increasing phyllosphere sugar levels. Studies by the author have suggested that the microbial diversity increases as the season progresses and natural antagonists of *B. cinerea* are not common in the grape flowers or the developing fruit, but small populations of fungi such as *Trichoderma* spp. begin to appear in the canopy at about the time of veraison.

A variety of *Trichoderma* spp. collected from vineyard environments were screened for the ability to suppress *B. cinerea* in culture and in vivo assays. Two isolates, *Trichoderma koningii* (race Td67) and *Trichoderma harzianum* (race Td81b) were collected which stood out for reducing infection. The *T. koningii* Td67 race has affinity for colonisation of the grape stigma and pollen receptacle and reducing latent flower infection by *B. cinerea*. The *T. harzianum* Td81b race is specifically selected for ability to grow and cause lysis of *B. cinerea* on the ripening fruit. The two have potential to be used as an integrated spray program and in trials provide reductions in the order of 80% relative to untreated controls.

NOTES:

Impact of fruit flies on international market access and what the scientific community can do to help

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Fruit flies are the world's most economically significant plant pests affecting horticulture, with a high economic impact on horticultural production, control costs and domestic and international market access. Consequently, sustainable management of fruit flies is of central concern to Australia's horticultural industries.

Fruit flies impact all aspects of the Australian government's pre-border, border and post-border biosecurity work related to the horticultural industries. The Australian Government's role includes:

- Contributing to international harmonisation of fruit fly management and treatment standards (including the coordination of research activities to support treatment standards and market access).
- Communicating Australia's claims of country freedom from exotic fruit fly species and regional status of endemic fruit flies.
- Providing advice on the requirements for international market access, including fruit fly monitoring systems.
- Negotiating appropriate treatment options that support Australian industries.
- Working with state and territory governments, the horticulture industry and research institutions to identify priorities, research needs and coordination mechanisms to improve fruit fly management and support export aspirations.

The ability of the Australian government to negotiate international market access for fruit fly host commodities is dependent on having scientifically robust data and information across the production system, including pre-harvest management, post-harvest treatments and an understanding of biological, physiological and ecological aspects.

The scientific community can play a key role not only in contributing to export ready data packages for market access negotiations, but also in communicating research in a manner that supports the Australian government's role in international negotiations. The sensitivities associated with international market access and the potential mechanisms to improve coordination with the scientific community to deliver improved market access opportunities will be discussed.

Knowing what you have: the benefits and challenges of maintaining national plant pest lists

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Developing and maintaining national plant pest lists help fulfill international obligations for trading countries, and also provides a foundation for national biosecurity systems. However, national lists remain rare, and analyses that might help guide biosecurity policy are few. We compiled and analysed a national plant pest list for Bhutan, a relatively small but agriculturally diverse country.

Key features of the database included: i) comprehensive referencing of records to help knowledge retention, an important challenge in most countries; ii) categorising taxonomic uncertainty for each pest record; and iii) categorising each pest according to their current importance.

Analyses revealed several challenges that are likely to be common to most countries. Confidence in species identifications reduced dramatically since the initial aid-funded, pest-documentation phase (1985-8). Furthermore, 38% of species identified by 1988 had subsequent name changes, highlighting the need for continued effort and expertise to maintain lists. Most recent records lacked voucher specimens, and those for older material are mostly located internationally, making revisions challenging.

The database did provide many benefits. All damaging pests (14% of 604 species) were already present in neighbouring India, which is also the major trading partner, and 73% occur beyond Asia, suggesting a regional approach to biosecurity would greatly benefit Bhutan. Very few new damaging pests have entered Bhutan since 1988, suggesting risks are manageable.

National pest lists are critical, but to be of greatest value they need to focus on damaging pests. For most countries their development, maintenance and use will require ongoing regional and global co-operation to overcome taxonomic impediments, and to maximise their benefits in terms of facilitating trade and guiding domestic biosecurity activities.

Postharvest market access treatments for horticultural commodities: Developing pre-approved and standardised quarantine treatments for fruit flies

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9. Auckland University, Auckland, New Zealand

The disruption to trade caused by plant pests is a major issue for the horticulture industry in Australia and New Zealand. There is an urgent need to review and improve current disinfestation methods and to develop new export protocols. This project utilises postharvest disinfestation knowledge to underpin Australasian horticulture market access. In a review a range of disinfestation treatments and uses for horticulture were summarized, including over 30 fumigants and several alternative methods of control including energy treatments (microwave, radio frequency, irradiation), controlled atmosphere treatments and physical treatments (heat, cold, pressure, pest removal). The review concluded that market access treatments will have to rely less on fumigants and more on alternative methods of control, such as irradiation, controlled atmospheres and heat and cold.

At workshops with industry stakeholders current market access was discussed and priorities for developing export phytosanitary protocols for fruit and vegetables were identified which could be implemented in the supply chain. The top priorities for Phase two of this project were: firstly, develop a data package to negotiate pre-approved cold treatments for four fruit fly species before they establish in New Zealand or parts of Australia to enable on going trade during an incursion and eradication; secondly, develop shorter controlled atmosphere cold treatments to enable more perishable commodities to be air-freighted to market. These treatments are most likely to deliver the effectiveness required for exports while maintaining the integrity and quality of the produce.

In preparation for developing cold treatment protocols, the team is collating and generating critical cold disinfestation data for four fruit fly species. This data can be used by regulators in negotiations to prevent the closure of export markets or minimize the impacts while an eradication response is attempted.

Low-dose methyl bromide fumigation as a quarantine treatment against Queensland fruit fly to improve exports of Australian capsicums

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Capsicums (*Capsicum annuum*) were fumigated with methyl bromide (MB) at a nominal treatment dose of 18g m⁻³ at 18°C for 5 hours as a quarantine disinfestation treatment against *Bactrocera tryoni*, the Queensland fruit fly. Three large scale trials were conducted against each of the four immature lifestages, eggs and first, second and third instars. There were no survivors from the estimated 35,551 eggs, 53,720 first instars, 40,371 second instars and 43,901 third instars treated in capsicums, thereby resulting in an efficacy of >99.99% mortality at the 95% confidence level for each lifestage.

Commercial fruit were also fumigated under the same conditions and then held at 6°C for 16 days or 6°C for 10 days followed by 10°C for 6 days. This was to simulate transport at 6°C followed by retail display at 10°C. Fruit quality parameters of weight loss, total soluble solids and external quality including, visual appearance, skin wrinkling, skin pitting, and incidence and severity of rots were assessed and no significant adverse treatment effects were present.

This research aims to develop a new protocol for the export of Australian capsicums to New Zealand as an alternative to the current protocol of 40g m⁻³ MB for 2 hours at 17°C. The new protocol uses half the concentration of fumigant but applied for double the treatment time to maintain the efficacy against Queensland fruit fly a significant quarantine pest in Australia. Whilst the reduction in quantity of MB used is environmentally beneficial, the research was primarily undertaken to improve out-turn quality of the treated capsicums. The existing protocol impacts fruit quality while the lower dose tested in our research had no significant effects on the range of fruit quality attributes tested.

Cost-benefit analysis of a risk profiling tool to optimise biosecurity inspections for the Asian gypsy moth

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The spread of invasive species continues to provide significant challenges to those government biosecurity agencies charged with protecting a country's borders. In an increasingly connected world, these invasive species are potentially able to spread further and more rapidly. Human mediated pathways such as ships and airlines are the most obvious ways in which invasive species can be spread. Direct routes from one port to another are currently monitored, but indirect pathways, in which a ship picks up an invasive species and then travels to a number of different locations before arriving at the final destination, present more challenging scenarios. For the Australian Government Department of Agriculture, one particular concern is for ships arriving into Australia carrying viable eggs of the Asian gypsy moth (*Lymantria dispar*). We are developing a real time tool that will analyse the pathways for incoming ships and determine the likelihood the ship could be carrying viable eggs. This strategy is likely to deliver significant benefits to the Australian Government Department of Agriculture and the wider shipping industry. In this paper, we present an ex ante cost-benefit analysis of the proposed policy. We discuss the implications, and the further work required.

NOTES:

The risks and costs of exotic pests to Australia's forest industry, conservation forests and amenity trees

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Australia's forest products industry contributes \$21 billion to the national economy and employs around 120,000 people across the value chain, and is a vital part of rural and regional communities. Australia's 123 million ha of native forest and woodlands provide a wide range of benefits, including social (recreation), environmental (conservation) and commercial (e.g. forestry, tourism, honey). What is often overlooked is the ecological, economic and social value of our amenity forests and trees in urban and peri-urban environments, and these trees are often the first point of establishment of exotic pests. Here we investigate the risk of exotic pests to Australian forests and calculate the economic costs when they establish. A review of current biosecurity activities and survey of industry revealed that forest biosecurity in Australia is currently under-resourced, but there are opportunities for greater involvement from industry, government and other stakeholders. Border interception data was analysed along with trade data (commodities that vector pests) to gain an understanding of the risk of exotic pests arriving to Australia. We found an increase in interceptions of important pests over the past 15 years, concomitant with an increase in trade. From the list of interceptions, we identify key pests that threaten plantation forests, conservation forests and amenity trees. We then conducted an exotic pest incursion scenario, using pine wilt disease, to explore the financial impact of exotic pests establishing in softwood plantations in Australia, and the benefits of conducting biosecurity activities to lower the chance of them establishing. The outcomes from this project will be used to get greater stakeholder engagement and involvement in forest biosecurity in Australia.

Myrtle rust – impact on native Myrtaceae and associated plant communities

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Austropuccinia psidii (myrtle rust) has long been considered a significant threat to Australian plant industries and ecosystems. *A. psidii* was detected for the first time in Australia in April 2010, on the central coast of New South Wales. The distribution of *A. psidii* continues to expand, with detections extending from Tasmania, along the eastern coast of Australia to Cape York Peninsula, and most recently in the Tiwi Islands and Northern Territory. The current host list for Australia includes 347 species from 57 genera. *A. psidii* severely affects key species in natural ecosystems, including threatened species with restricted natural ranges as well as widespread species with a broad native range (e.g. *Rhodomyrtus psidioides* and *Rhodamnia rubescens*).

Our studies have demonstrated severe impacts of myrtle rust on plant communities and the potential for *A. psidii* to negatively affect Australia's biodiversity in the short- and long-term. *A. psidii* has caused significant disturbance in wet sclerophyll environments, where Myrtaceae dominate the rainforest understorey, and regeneration of coastal heath following wildfire. Significant dieback caused by repeated *A. psidii* infection has seen once dominant species in severe decline with little evidence of potential for regeneration. Impacts on keystone species such as *Melaleuca quinquenervia* include tree death, decline in tree vigour and reduced flowering rates, with additional decline associated with interactions with insect damage.

Continued monitoring programs are required to identify species and plant communities at greatest risk. The implementation of a disease screening and tree breeding program may be required for some species as without human intervention regaining lost genetic diversity within these species populations may not be possible. Already, as a direct result of myrtle rust impacts, two species have been recommended for legislative listing as being critically endangered.

NOTES:

Dominant Australian native vegetation communities in danger

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Austropuccinia psidii (previously *Puccinia psidii*, commonly known as myrtle rust) is an invasive fungus native to South America that was introduced to Australia in 2010. Since its arrival it has spread throughout the east coast, with dispersion facilitated by windborne spores. It infects young growing tissues of the Myrtaceae family, including leaves, flowers and fruits, and can cause defoliation, reduced reproductive fitness and death depending on species' susceptibility. Myrtaceae is the dominant family in Australia, with approximately 2,250 species. Susceptibility status is only known for 380 species, with 363 showing susceptibility. We chose 15 species whose distribution overlaps with myrtle rust, for susceptibility testing in order to increase our management capability of this biosecurity pest. Tested species were *Triplarina nowraensis* and *T. imbricata* (a genus that has not previously been tested), ten *Eucalyptus* species

(*E. amplifolia*, *E. camphora*, *E. castrensis*, *E. copulans*, *E. largeana*, *E. macarthurii*, *E. magnificata*, *E. pachycalyx* (subsp. *pachycalyx* and *waajensis*), *E. parvula*, and *E. scoparia*), *Callistemon megalongensis*, *Melaleuca irbyana*, and *Kardomia prominens*. A first inoculation was performed and eight species showed susceptibility to myrtle rust, with different levels of disease severity (*E. camphora*, *E. castrensis*, *E. copulans*, *E. largeana*, *E. macarthurii*, *E. parvula*, *E. scoparia* and *C. megalongensis*). A second inoculation will be performed to confirm that all the plants were tested at a time when they presented potentially susceptible new growth. As these species all have an overlapping distribution with myrtle rust, it is a matter of time until they become infected in their native communities. It is necessary to assess the impacts and severity levels of these affected plants in order to guide management decisions to maximise their persistence in the wild. Monitoring of susceptible species' populations in the wild and seed collection for seed banking would be the first steps towards their conservation.

Mapping and Trapping - Protecting Queensland's Pine Resource.

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Queensland's softwood plantations cover almost 150,000 ha, principally located along the coastal lowland strip from Beerburrum to Maryborough. The threat to this resource from exotic pests is real and increasing. Rapid movement and greater volumes of international freight heightens the risk of introduction of exotic forest pests. Imported goods are increasingly opened away from entry ports, dispersing risk to urban environments whose proximity to the pine estate poses a significant threat to this resource. Strategic post-border urban surveillance allows new incursions to be detected early enough for eradication and containment. Surveillance at this point in the biosecurity continuum delivers better outcomes and a much higher benefit:cost.

Trapping at high risk sites in the port cities of Brisbane and Gladstone targets taxa groups rather than individual species, through the use of a generalist lure for timber boring beetle species. Recent mapping activities have refined the trapping program, ensuring traps are strategically located in terms of proximity to both high risk sites and exotic pine hosts. Trapping over the last ten years has identified over 70 species from the target taxa, including natives, already established exotics and a number of new records. As well as providing evidence of area-freedom for specific pests this trapping delivers important background information on the occurrence of different species, enabling more rapid recognition of new exotics and therefore prompt and targeted eradication efforts. A range of lures and traps are now on-hand for immediate response to exotic incursions. Queensland's trapping program supports efforts towards a national, interagency forest biosecurity surveillance strategy.

NOTES:

***Cyclaneusma minus* morphotypes and their distribution in *Pinus radiata* forests in New Zealand**

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Cyclaneusma needle-cast (CNC) is a disease of *Pinus radiata* caused by the ascomycetous fungus *Cyclaneusma minus*. This disease affects plantations in both New Zealand and Australia, although the deployment of less susceptible genotypes of pine has reduced its impact over time. It has been long known that morphological variation exists within the population and two morphotypes have been characterised; *C. minus* "simile" and *C. minus* "verum". A recent molecular analysis has shown that these two morphotypes are most likely different species. It is not known if one or both of these morphotypes are responsible for disease in *P. radiata* plantations in New Zealand. New tools for molecular differentiation of these morphotypes showed that *C. minus* simile is more commonly isolated or detected in infected trees than *C. minus* verum, and is more common in areas where disease is most prevalent. Most of the *C. minus* simile isolates, collected from 1969 to 2008, were isolated from the North Island, whereas most *C. minus* verum isolates were isolated from the South Island of New Zealand. However, a third type of strain has also been isolated which is genetically divergent from both the verum and simile morphotypes. While this collection of isolates is very small, it is interesting to note that the majority of these isolates came from needles that appeared healthy and green and were collected at the same as needle displaying symptoms of CNC from adjacent trees in the same forest. Understanding the genetic diversity and population structure of this group of fungi will ultimately lead to better outcomes for control of CNC.

NOTES:

When, where and how to prepare for and manage emerging epidemics.

Chris Gilligan¹

1. University of Cambridge, Cambridge, United Kingdom

I shall consider emerging pathogens from an epidemiological perspective designed to advise government and other agencies on risk assessment and effective deployment of control measures. Drawing on examples of emerging pathogens of wheat, cassava, citrus, maize and sugar-beet that threaten crop production in Africa, India, US and UK, I shall discuss approaches to assess the risk of pathogens arriving, what the impacts are likely to be, where to survey, when, where and how to deploy control. Particular challenges arise in estimating dispersal parameters, allowing for uncertainty including cryptic infection and in coupling epidemiological with economic models. I propose to consider control at the regional and country-wide scales and to show that success involves matching the scale of control with the intrinsic spatial and temporal scales of epidemic spread. Grower behaviour is also important and we show how a small change in compliance can undermine the effectiveness of control strategies such as the introduction of clean seed schemes. The intention is to show that instead of starting afresh each time, there is an emerging epidemiological framework and a set of epidemiological tools that can adapted for each new pathogen.

NOTES:

Using decoys to expand the recognition specificity of plant disease resistance proteins

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Maintaining high crop yields in an environmentally sustainable manner requires the development of disease-resistant crop varieties. Resistance to specific pathogens is most often determined by dominant disease resistance genes that encode proteins containing nucleotide binding and leucine rich repeat domains (NLR proteins). The primary function of NLR proteins in plants is the detection of pathogen effector proteins present in the plant cell cytoplasm. Detection is often accomplished by an indirect mechanism in which NLR proteins sense modification of specific effector targets in the host. How modified host proteins are sensed by NLR proteins, however, is poorly understood. We have been investigating this question using the Arabidopsis NLR protein RPS5, which detects proteolytic cleavage of the protein kinase PBS1 by the *Pseudomonas syringae* effector protein AvrPphB. In published work, we have shown that RPS5 is activated by a conformational change in PBS1 that is triggered by cleavage of PBS1 within its activation loop, and that detection is dependent on a specific amino acid sequence present in an exposed loop on the opposite side of PBS1 from the cleavage site. These observations suggested that the amino acids at the cleavage site do not interact directly with RPS5, and that cleavage by any protease in the activation loop of PBS1 might be able to activate RPS5. We subsequently showed that the activation loop of PBS1 can be modified such that it can be cleaved by proteases from other pathogens, which then enables RPS5 to be activated by these proteases instead of by AvrPphB. Thus, the specificity of RPS5 can be changed simply by altering the protease cleavage sequence within PBS1. This insight suggests that we may be able to engineer resistance to many different viral and fungal pathogens using PBS1 'decoys'. We are now adapting this protease detection system for deployment in crop plants such as barley and soybean. In this talk I will present our latest work on engineering resistance to potyviruses in both crop species.

Host-induced gene silencing: application to control nematode and aphid pests

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Crop plants fail to reach their full yield potential when constrained by biotic or abiotic challenges. Two groups of plant pests which contribute to substantial reductions in crop yields are plant parasitic nematodes and aphids – both remove plant cell contents via a mouth stylet, and so are amenable to host-induced gene silencing (HIGS). Target genes for nematode or aphid control must be genes which are vital for plant parasitism or for an aspect of the pest's life cycle. In targeting both nematode and aphid pests the approach we have adopted involves RNA seq and comparative genomics to identify candidate target genes, feeding dsRNA of candidate genes to the pests to screen for those which affect some aspect of viability or reproduction, then to generate transgenic plants expressing dsRNA to the priority pest target genes. The results show that a substantial reduction in nematode or aphid reproduction (up to 90%) can be achieved with such transgenic plants. The target genes identified can also serve as candidates for development of new chemical control agents, or for ectopic delivery of dsRNA without the need to develop transgenic plants.

NOTES:

Comparative secretome analysis of *Rhizoctonia solani* isolates reveals unique secretomes and cell death inducing effectors.

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2. The Sainsbury Laboratory, Norwich, UK

3. Curtin University, Bentley

4. The John Innes Centre, Norwich, UK

Rhizoctonia solani is a fungal pathogen causing substantial damage to many of the world's largest food crops including wheat, rice, maize and soybean. Despite impacting global food security, little is known about the pathogenicity mechanisms employed by *R. solani*. To enable prediction of effectors possessing either broad efficacy or host specificity, a combined secretome was constructed from a monocot specific isolate, a dicot specific isolate and broad host range isolate infecting both monocot and dicot hosts. Secretome analysis suggested *R. solani* employs largely different virulence mechanisms to well-studied pathogens, despite in many instances infecting the same host plants. Furthermore, the secretome of the broad host range AG8 isolate may be shaped by maintaining functions for saprophytic life stages while minimising opportunities for host plant recognition. Analysis of possible co-evolution with host plants and *in-planta* up-regulation in particular, aided identification of effectors including xylanase and inhibitor I9 domain containing proteins able to induce cell death *in-planta*. The inhibitor I9 domain was more abundant in the secretomes of a wide range of necrotising fungi relative to biotrophs. A complementary proteomics approach provided insights into how the broad host range AG8 isolate adapts to different hosts and identified additional infection-related proteins including a thaumatin that enhanced susceptibility to *R. solani*. These use of these effectors and others from *R. solani* to screen germplasm for loss-of-sensitive loci may be a useful approach to build resistance in crops such as wheat, where no resistance currently exists.

A comparison of metabolites produced by the fungus *Leptosphaeria maculans* during competition with *Leptosphaeria biglobosa* and during canker formation in the stems of *Brassica napus* cv. Westar

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2. Metabolomics Australia, Melbourne, VIC, Australia

Leptosphaeria maculans, the fungal pathogen of *Brassica napus*, is the causal agent of Blackleg disease, the most devastating disease of *B. napus* worldwide. *L. biglobosa* is a closely related species which also causes yield losses to *B. napus* in Europe and can be found growing on the same plants at the same time as *L. maculans*. Although both fungi enter the leaves of *B. napus* through wounds or stomatal openings, *L. biglobosa* causes a rapid necrotic lesion whereas *L. maculans* grows asymptotically in the leaf before the onset of necrosis and only *L. maculans* forms necrotic stem cankers. The metabolites produced by these two fungi are relatively unknown apart from the secondary metabolites sirodesmin PL and phomenic acid which are made only by *L. maculans*. In order to further define the role of sirodesmin during infection of canola stems, a comparison of metabolites made by the wild type and sirodesmin mutant isolates during growth in canola stems was made. Infected tissue was either ground and extracted with ethyl acetate and metabolites detected by liquid chromatography/mass spectrometry (LC-MS) or stems were sectioned and fixed to a slide whereupon metabolites were visualised using matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI). Identical techniques were used to compare metabolites made by wild type and sirodesmin mutant isolates while grown on agar plates in isolation or adjacent to a competing *L. biglobosa* colony. Sirodesmin was localised in infected stem canker tissue and was also found to be upregulated in the presence of *L. biglobosa*. This is the first time an untargeted metabolite profile has been generated for *Leptosphaeria* species during growth in planta and in culture and may lead to identification of novel compounds important for the interaction of *L. maculans* with its host and competitors.

NOTES:

Root transcriptome analysis of resistance against *Phytophthora cinnamomi* using RNA-seq and the association of β -cinnamomin elicitin with virulence

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Phytophthora cinnamomi is a destructive oomycete plant pathogen in both natural and agricultural systems worldwide. Natural plant communities all around Australia are greatly affected by the disease caused by this pathogen. Only a very limited number of plants have been described as field resistant and that survive within areas that are affected by *P. cinnamomi*. *Lomandra longifolia* was assessed for its resistance under controlled laboratory conditions and was found to be highly resistant. We then explored potential resistance mechanisms in roots following zoospore inoculation. Microscopic analysis and quantitative studies of lignin, callose and hydrogen peroxide (H₂O₂) showed their induction in inoculated roots and genes related to their biosynthesis (*PAL*, *Callose synthase 5 and 7*, *GST* and *Chalcone synthase*) were upregulated following infection. The transcriptome of *L. longifolia* inoculated with *P. cinnamomi* was then analysed to provide an overview of defence-related gene induction. More specifically, the genes encoding multiple defence signalling proteins and pathogenesis-related proteins, phytohormone biosynthetic enzymes, phytoalexin biosynthetic enzymes and known defence-related transcription factors were highly induced in roots in response to infection.

Elicitins are 10 kDa proteins secreted by almost all *Phytophthora* species. We isolated and purified the β -cinnamomin elicitin from *P. cinnamomi* with the aim to develop an immunoaffinity purified antibody as a tool to examine virulence. The specificity of β -cinnamomin antiserum was confirmed through western-blot and isoelectric focusing analysis. The β -cinnamomin production was shown in different *P. cinnamomi* life stages and in inoculated plant roots by using immuno detection and confocal microscopy. Pre-treatment of zoospores with the β -cinnamomin antiserum and then inoculation of susceptible *Lupinus angustifolius* roots revealed a partial loss of virulence suggesting the intrinsic role of the elicitin. Exploration of resistance-related mechanisms in plants and pathogenicity factors in *P. cinnamomi* facilitate the possibility of modifying susceptible plant responses and identifying targets to prevent infection.

NOTES:

Use of genomics to improve biological control

Gavin J Ash¹

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Biological control is often classified as either a classical or inundative. Here the main emphasis will be on the use of agricultural biological products or biopesticides (an inundative approach). As these products are routinely used as an applied product in agriculture and horticulture to manage pests, they are registered through the Australian Agricultural and Veterinary Medicines Association (APVMA). Biopesticides have been variously defined, but herein will be defined as group 3 of the APVMA Agricultural Biological Products definition as containing microbial agents.

In this presentation a number of pathogen/host systems will be used to illustrate how an understanding of genomics can be used to improve our understanding of how biopesticides can be improved and used in Australian agriculture. Examples will include research on management of soil insects (using species of *Metarhizium*), invasive snail species (using *Bacillus* sp and entomopathogenic nematodes) and the selection of bacterial species to be used in the management of stem rot of canola (caused by *Sclerotinia sclerotiorum*).

The presentation will conclude with discussion around the future use of genomics (and the other complimentary 'omic sciences) to improve plant disease management using biological control in Australia.

Identification of culturable endophytes isolated from apple tissues with antagonism towards *Neonectria ditissima*

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Apple (*Malus domestica* Borkh.) is widely cultivated in New Zealand, being a significant export crop. European canker (EC), caused by the fungal pathogen *Neonectria ditissima*, is the leading cause of apple trunk disease. It reduces the growth and yield of apple trees and may cause the death of the entire tree. In this study, culturable endophytes from apple shoots were investigated as a source of potential biocontrol agents to protect apple trees from *N. ditissima* infection.

Apple shoots without disease symptoms were taken from Lincoln University (Canterbury), Nelson and Hawke's Bay orchards between 2015 and 2016 for endophyte isolation. A total of 1004 endophytic bacteria and 81 endophytic fungi (representing 33 fungal morphology groups) were screened for biocontrol activity against *N. ditissima* using dual culture assays. Eighteen bacterial and 18 fungal isolates inhibited radial growth of three *N. ditissima* isolates. Sixteen antagonistic bacteria were isolated from commercial varieties (Royal Gala, Braeburn and Jazz) in Integrated Fruit Production (IFP) managed orchards in all three regions and two from a heritage variety (Grimes Golden) from Hawke's Bay Plant and Food Research orchard. Twelve antagonistic fungi were isolated from commercial varieties in IFP managed orchards across Nelson and six from organic orchards in Hawke's Bay. The 18 bacteria and 15/18 fungi were from low EC infection blocks (infection rate $\leq 30\%$).

The isolates were identified using PCR sequencing, and potential pathogens eliminated from further studies leaving 16 bacterial and 15 fungal isolates. These bacterial isolates were identified as *Bacillus* and *Pseudomonas* species, and fungal isolates identified as *Epicoccum*, *Chaetomium*, *Biscogniauxia*, *Neoseptophoma*, and *Penicillium* species. Seven bacterial and 13 fungal isolates were positive for siderophore production assessed using chrome azurol S blue agar plates. This study indicated that endophytes isolated from apple tissues have the potential for use in sustainable control of *N. ditissima*.

NOTES:

Antagonistic effects of *Trichoderma* spp. against the brown root rot pathogen, *Phellinus noxius*

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The basidiomycete fungus *Phellinus noxius* is well known as a forest recycling agent, however, it has been responsible for a significant number of tree deaths in public parks and gardens in south east Queensland. Management of the pathogen in public places must rely on environmentally-friendly approaches such as biocontrol and biofungicides. Numerous *Trichoderma* spp. have been initially screened *in vitro* for their antagonism towards *P. noxius* strains isolated from dead *Ficus* sp., *Caesalpinia ferrea* (leopard tree) and *Araucaria cunninghamii* (hoop pine) trees. *Trichoderma* spp. showed various levels of antagonism against tested *P. noxius* isolates. Of the nine tested *Trichoderma* isolates, five produced volatile and non-volatile metabolites that suppressed growth of *P. noxius* by up to 46% and 90%, respectively. Data collected from dual culture assays also indicated that the five *Trichoderma* isolates adversely affected the viability of *P. noxius*, with recovery of one isolate of *P. noxius* completely inhibited. The other four *Trichoderma* isolates were only observed as strong growth competitors. Mycoparasitism of the *Trichoderma* spp. towards *P. noxius* was only occasionally observed at the *Trichoderma*-*Phellinus* interaction zones implying that this is probably not an important mode of action of the *Trichoderma* against the *P. noxius*. Further pot and field site trials are warranted for those *Trichoderma* showing promising antagonistic effects against *P. noxius*.

Pitfalls of molecular identification of biocontrol fungi used in sustainable agriculture: An alarming case study

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Fungal strains beneficial for sustainable agriculture, such as those exploited as biocontrol agents (BCAs), cannot be registered and commercialized without providing precise molecular identification data. Existing inaccuracies in DNA databases, such as NCBI GenBank, can cause serious difficulties in this process: false GenBank entries can divert risk assessment and registration processes, and can lead to spurious results concerning the environmental fate of the BCAs and their non-target effects. False GenBank data thus have a negative impact on organic and integrated agricultural programs. This is exemplified by nrDNA ITS sequences wrongly deposited in GenBank for the mycoparasitic genus *Ampelomyces*, containing well-known commercial BCAs of powdery mildews. Misleading data were published on their presence in the human body, seawater, and other inappropriate environments, where *Ampelomyces* strains do not colonise. These data were all based on mis-identifications of some common fungal strains with *Ampelomyces* spp., due to routine methods using erroneous DNA sequences from GenBank. We give examples and propose a new approach for molecular identification processes applied during the assessment of fungal BCAs, and other beneficial organisms, to contribute to their reliable use in sustainable agriculture.

NOTES:

Suppression of Fusarium Crown Rot in wheat by endophytic *Trichoderma*.

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2. Ecology Institute, Shandong Academy of Sciences, Jinan, Shandong, PR China

Strategies to effectively manage wheat crown rot, primarily caused by *Fusarium pseudograminearum*, are limited. Inoculation with *Trichoderma* strains, shown to suppress soil-borne fungal and oomycete diseases of cereals, offer potential to enhance disease management. Wheat crown rot suppressive assays were conducted in cereal cropping soils to assess rhizosphere competence and efficacies of *Trichoderma* inoculants in the presence of natural soil-borne microbial communities. *F. pseudograminearum* and *Trichoderma* colonisation were assessed in rhizosphere soil (RS), crown shoots (CS), crown roots (CR), primary roots (PR) by species-specific qPCR at 84 days post-emergence (grain fill).

F. pseudograminearum actively colonised all tissues, presence in RS being below the qPCR minimum detection threshold. *T. gamsii* strains A5MH and Tr905 and *T. harzianum* strain Tr906 significantly ($P < 0.001$) reduced (33 % – 83 %) *in planta* pathogen biomass and increased ($P < 0.05$) tiller and head numbers by up to 56% and 47%, respectively. Endophytic colonisation of CS, CR and PR by *Trichoderma* inoculants and the target pathogen was confirmed by recovery from surface sterilised tissues, qPCR and AFLP genotyping.

Assays in soil with natural crown rot inoculum resulted in significantly ($P < 0.001$) greater *in planta* pathogen biomass compared to introduced inoculum. A5MH, Tr906 and combined (A5MH+Tr906) treatments significantly reduced pathogen biomass ($P < 0.001$) and stem browning ($P < 0.01$) by up to 70 % and 86 %, respectively. *Trichoderma* colonisation of CS was significantly ($P < 0.001$) greater in the combined treatment. There was a significant ($P < 0.001$) correlation ($r = 0.71$) between pathogen biomass (qPCR) and stem browning, indicating that the qPCR assay can be used as an objective measure of crown rot suppression. Strain A5MH resulted in the greatest disease suppression, despite Tr906 having significantly ($P < 0.001$) greater tissue colonisation (48 %). This implies that A5MH may exhibit disease suppressive mechanisms other than pathogen competition.

NOTES:

Fusarium dieback in California and other ambrosia beetle associated diseases of avocado and urban forest

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Polyphagous shot hole borer (*Euwallacea* nr. *forficatus*) an invasive ambrosia beetles that form symbioses with multiple fungal species, *Fusarium euwallaceae*, *Graphium euwallaceae*, and *Paracremonium pembeum* which together cause a disease called Fusarium Dieback (FD) on trees in urban and native forests in California. Females are black colored and about 1.8 – 2.5 mm long; males are brown colored and about 1.5 mm long. In California, PSHB was first reported on black locust in 2003 but there were no records of fungal damage until 2012, when the *F.euwallaceae* was recovered from the several backyard avocado trees infested with PSHB in Los Angeles County. The aim of this study was to determine the plant host range of the beetle-fungus in two heavily infested botanical gardens in Los Angeles County. Of the 335 tree species observed, 207 (62%), representing 58 plant families, showed signs and symptoms consistent with the attack by PSHB. The *F.euwallaceae* was recovered from 54% of the plant species attacked by PSHB, indicated by the presence of the *F.euwallaceae* at least at the site of the entry hole. Trees attacked by PSHB included 11 species of California natives, 13 agriculturally important species and many common street trees. Survey results also revealed 19 tree species that function as reproductive hosts for PSHB. Since 2012, the number of reproductive hosts for PSHB has increased from 19 to 49, and includes 20 species that are native to California. The infestation has spread from a single county in 2012 to seven counties in 2017. Furthermore, the infestation was originally limited to the urban forest but it has recently spread into native forests in southern California.

Dynamics of dieback in a naturally occurring parkinsonia population

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This paper investigates the results of a seven year study monitoring plant health and movement of dieback moving through a stand of *Parkinsonia aculeata*. Two transects were established to monitor the natural progress of parkinsonia dieback through a well-established naturally occurring dieback affected site in north Queensland (Australia). This would serve to gain an understanding of this disorder in its natural state, and to generate a spatial disease model. Assessment of this site demonstrated a decline in individual plant health over consecutive years, with 98% of parkinsonia plants dying over the study period. Minimal recruitment of new plants led to an eventual collapse in the parkinsonia population.

A Markov Chain model was generated using plant health data from the first 3 years of the study. This indicated that 99.5% plants would be dead in 15 years. Comparing the model at a time point of 7 years with actual data gave a close match in plant health ratings. Isolations taken from plants showing symptoms of dieback at the commencement of the trial indicated that *Macrophomina phaseolina* (Botryosphaeriaceae) was the only species with known pathogenicity on parkinsonia present in the transect site.

Understanding the ecology of dieback, and the role it plays in the population dynamics of parkinsonia is crucial from a management perspective. This information contributes to understanding both the role that dieback can have in control strategies, and to providing an insight into the timeframe involved in this disease process from infection through to plant death. This is the first research to date to assess the temporal movement of dieback through a naturally occurring parkinsonia population.

NOTES:

The impact of *Austropuccinia psidii* (myrtle rust) on the endangered *Gossia gonoclada* in south east Queensland

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Austropuccinia psidii (myrtle rust) is a biotrophic fungal plant pathogen that quickly spread throughout the east coast of Australia after initial detection in 2010. Since its discovery, research has been undertaken to determine the levels of vulnerability of Myrtaceous hosts in Australia. However, to date, little field research has been conducted on some of Australia's most endangered species, many of which are located within vulnerable rainforest ecosystems that are already under pressure from multiple threatening processes including urbanisation, land clearing, fire and increasing climate variability. This research explores the *in-situ* impact of myrtle rust on the small fragmented population of *Gossia gonoclada* (Myrtaceae) - an endangered riparian rainforest tree only found in south east Queensland. Over three years, a categorical scoring method was used to survey myrtle rust disease incidence and severity on *G. gonoclada* trees within five different sites in Logan City, Queensland. Infection incidence and severity varied among surveyed trees at all sites and among survey years. Our results indicate that variation in climate conditions over the past three years (from flood to drought) have influenced myrtle rust incidence and severity within the population of *G. gonoclada*. Further analysis is underway to explore the role of host site and leaf phenology in explaining some of the variation in myrtle rust disease susceptibility we see. This research will give us a better understanding of the dynamics of myrtle rust disease in rare plant populations and to identify trees showing tolerance to myrtle rust for future conservation propagation.

Mapping the threat of myrtle rust to Myrtaceae in Australia

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Since its introduction to Australia in 2010, the invasive rust fungus *Austropuccinia psidii* (myrtle rust, formerly *Puccinia*) has caused severe declines in several previously common Myrtaceous species. While there have been diligent efforts of a few research groups to understand the extent and impact of myrtle rust, there are still many unknowns. To facilitate a more co-ordinated effort on managing the threat from myrtle rust, this study builds on previous work by: 1) compiling an updated Australian host list; 2) collating known occurrences of myrtle rust in Australia using records gathered from researchers, government departments and natural resource managers; 3) using this updated occurrence data to model climate suitability for myrtle rust in Australia under current and future climatic conditions with MaxENT software; 4) identifying species at risk based on range overlaps with myrtle rust, susceptibility data and current threat status.

So far, 380 native Australian Myrtaceae species have been tested for susceptibility to myrtle rust, with 366 found to be susceptible. Presently, myrtle rust appears in coastal areas of QLD, NSW, VIC, TAS and the Tiwi Islands. The models suggest that under current climatic conditions, the known occurrence data aligns well to the expected areas of climatic suitability. While previous bioclimatic modelling has shown high climate suitability for myrtle rust in south-west Western Australia, our modelling found only low climatic suitability for this region. We found 867 Myrtaceae species had ranges within the area of current climate suitability for myrtle rust and 1207 Myrtaceae species had ranges within the area of future climate suitability. The majority (65 to 73%) of these species are untested for susceptibility. Three categories of actions were created for species expected to be exposed to myrtle rust, giving priority to species with at least 90% of their range overlapping climatically suitable areas for myrtle rust.

NOTES:

Transcriptome Profiling of Broadleaf Paperbark (*Melaleuca quinquenervia*) challenged by Myrtle Rust (*Austropuccinia psidii*) Revealed Variation in Defence Responses among Resistant Individuals

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Plants have developed complex defence mechanisms to protect themselves against pathogens. A wide-host range fungus, *Austropuccinia psidii*, which has caused severe damage to ecosystems and plantations worldwide, is a major threat to Australian ecosystems dominated by Myrtaceae. In particular, the east coast wetland foundation species *Melaleuca quinquenervia* appears to be variably susceptible to this pathogen. Understanding the molecular basis of host resistance would enable better management of this rust disease. We identified resistant and susceptible individuals of *M. quinquenervia*, and explored their differential gene expression in order to discover the molecular basis of resistance against *A. psidii*. Rust screening of germplasm showed a varying degree of response with totally resistant to highly susceptible individuals. We used transcriptome profiling in samples collected before and five days after inoculation (dpi). Differential gene expression analysis showed numerous defence-related genes induced in susceptible plants at 5 dpi. Mapping reads against the *A. psidii* genome showed that only susceptible plants contained fungal-derived transcripts such as 'planta-induced rust gene'. Resistant plants exhibited an over-expression of candidate *A. psidii* defence-related genes such as receptor-like kinases (RLK) and nucleotide-binding site-leucine-rich repeat (NBS-LRR) proteins, as well as transcriptional regulators and PR-proteins. We identified large differences in gene expression of defence-related genes between biological replicates of resistant plants.

NOTES:

Farm Biosecurity – a partnership approach

Sharyn Taylor¹

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Over the last decade, governments and plant industries in Australia have been responding to the detection of several significant plant pests and diseases. These incursions have had impacts on communities, production, domestic trade and the environment and include Myrtle rust, Red imported fire ant, Tomato potato psyllid, Russian wheat aphid, Banana freckle, Panama disease TR4, Chestnut blight, Giant pine scale, Khapra beetle, Varroa mite and Asian honey bee. In 2016 alone, Australia recorded 42 new detections of pests and diseases and, while not all were significant, this figure demonstrates the continual pressure of new threats.

Biosecurity incursions can be one of the greatest business risks for agricultural, horticultural or forestry production, resulting in increased costs in management, reduced production, an impact on domestic or international market access or increased complexity of management systems. While governments have a major role in identifying and managing the risks posed by new plant pest introductions, plant industry representative bodies, growers, farming group advocates and industry personnel are a significant part of the partnership approach within Australia's biosecurity system. Plant industries know their businesses, and the business of crop production, better than anyone and are best placed to know inputs, outputs and crop management issues.

Improvements to farm biosecurity preparedness requires support to raise awareness, undertake risk assessment and develop and implement effective farm biosecurity plans. Several programs are now in place that provide this support, including the Farm Biosecurity program (an initiative for plant and animal industries), the Grains Farm Biosecurity Program, The Bee Biosecurity Program and the Vegetable Biosecurity Program.

These programs are promoting the uptake of good biosecurity principles across plant production supply chains, however implementation of biosecurity is still slow in many plant industries. The 'normalisation' of biosecurity to become part of day to day business practices generally only occurs after a major incursion – sometimes too late to prevent impact major impacts. The need for incentives for good practice and disincentives for bad practice is still an issue that plant industries and government agencies need to address as we move towards a partnership approach to managing and implementing biosecurity practices.

On-farm Biosecurity Delivers Market Access

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Nursery & Garden Industry Australia (NGIA) have developed the on-farm biosecurity program '**BioSecure HACCP**' (2015 *National Biosecurity Award*) to assist producers improve on-farm biosecurity and market access. BioSecure HACCP delivers significant on-farm biosecurity benefits to growers as well as efficiency benefits to the regulator and reduces plant pest risks to the community. This is achieved through on-farm biosecurity procedures, electronic data management, domestic market access certification and traceability with an overall pest risk reduction along the supply chain.

The program and the web based Audit Management System (AMS) is designed as a holistic plant pest, disease and weed management system for on-farm implementation by individual production nurseries (Integrated Cropping System). The program seeks to identify and manage internal and external (endemic & exotic) pest threats that may harm the integrity of a plant production process through preparedness and by positioning the production system as 'biosecurity ready' under a 'business as usual' approach within the cropping system, 24/7.

Growers implement a structured proactive crop monitoring, surveillance and inspection system that is robust, technically valid and adaptable to the on-farm production cycles of containerised cropping along with high health practices under Best Management Practice. Risk reduction of key inputs along with early detection and traceability are the critical aims of the BioSecure HACCP program whilst seamlessly fitting into a typical plant production system in a cost-effective manner.

NGIA has worked with Australian state and territory governments over the past few years to gain acceptance and recognition of BioSecure HACCP as a third legal alternative for producers to meet interstate market access obligations. NGIA has demonstrated that with the combined robust nature of the on-farm program plus an innovative electronic administration process BioSecure HACCP certified growers can be authorised to self-certify compliance to interstate biosecurity entry conditions with NGIA the administering authority.

NOTES:

CropSafe – A biosecurity surveillance network for broadacre plant diseases – Does it work?

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CropSafe is a pest and disease surveillance program for grains, which is dependent upon a voluntary 180-member agronomist network (approximately 85 per cent of Victoria's agronomists) to identify threats to the Victorian grains industry.

During the growing season advisors submit plant samples to CropSafe collected during routine crop inspections where the causal agent cannot be identified. CropSafe forwards any suspected exotic sample immediately to Crop Health Services. Diagnosis of these samples provides real-time information for emerging issues, informing industry and generating surveillance data.

However, the ability of advisors to correctly identify diseases can vary significantly, hence the diagnostic capabilities of advisors are developed via regular training opportunities with plant pathologists. This increases the probability of detecting something unusual (potentially exotic).

Apart from early detection of crop pests and diseases, the program helps assure domestic and international markets of its' Area of Freedom (AoF) status. The Chief Plant Health Officer relies on CropSafe surveillance data for market access-related AoF claims.

So, Victoria has a broadacre biosecurity surveillance network, but does it work? The recent Russian wheat aphid (RWA) incursion has highlighted the benefits of CropSafe, including improved management and industry response due to effective surveillance activities. In the case of the 2016 RWA incursion an advisor in the CropSafe network was the first to detect RWA in Victoria.

An economic analysis and evaluation of the CropSafe program has been completed with findings indicative of excellent value for money. The Expected Net Present Value (ENPV) for the early detection of exotic diseases such as Karnal bunt is substantial - \$130 million discounted at seven per cent over a period of 20 years.

NOTES:

Australia's Grains Farm Biosecurity Program – a national initiative in plant biosecurity awareness, education and training.

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Sound biosecurity systems contribute to achieving resilient and sustainable agricultural and environmental systems, reducing the threat of introducing unwanted pests and diseases. Within Australia, the Grains Farm Biosecurity Program (GFBP) is a national initiative to assist in the development and implementation of improved biosecurity practice within Australia's grain industry. Initiated in 2007, the extension focused program contributes to the grain industry's risk mitigation activities, supports continued market access and promotes a partnership approach involving governments, industry and community.

The GFBP has appointed specialized State Grains Biosecurity Officers in the five key grain growing states of Australia. The program is funded by Grain Producers Australia through grower levies in partnership with state government agencies and Plant Health Australia.

Using a variety of community engagement strategies the GFBP has developed a wide range of tools to improve the management of, and preparedness for, biosecurity risks in the Australian grains industry at the farm and industry level, by highlighting risk pathways and activities throughout the supply chain and encouraging adoption of practices and strategies to mitigate risks.

Monitoring throughout the program indicate an increased awareness of biosecurity risks and voluntary adoption of biosecurity best practices throughout the sector. Key outcomes include the development of a range of practical resources including awareness material for researchers, growers and extension specialists, farm level recommendations to identify and mitigate biosecurity risks, and biosecurity training material. The GFBP also promotes formation of industry alliances and has established surveillance programs for high priority pests.

The GFBP is Australia's flagship program for promoting farm biosecurity, with its success encouraging other industries to implement similar extension programs. The focus on biosecurity best practice through industry engagement has seen the GFBP contribute to safeguarding grains production and maintaining Australia's grain export reputation.

Quaternary ammonium based disinfectants for effective on-farm biosecurity management of Panama disease in bananas

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Panama disease caused by the soil borne fungus *Fusarium oxysporum* f.sp. *cubense* poses a serious threat to the Australian banana industry. The use of disinfectants to decontaminate vehicles, machinery, tools and equipment is a critical on-farm biosecurity practice, as it reduces the risk of spreading the disease. Quaternary ammonium (QA) based disinfectants containing 120 g/L didecyldimethyl-ammonium chloride applied at a 1% solution (1200ppm) have shown to kill fungal spores that cause Panama disease. Prior to this research growers had no means of monitoring or managing effective concentrations of QA compounds. High range test strips (0-1500ppm) which measure the concentration of active ingredient have provided consistent and accurate results, and are an effective tool for routine monitoring of QA compounds. Using the test strips as indicators, in the absence of soil and organic matter, QA compounds have remained at a 1% solution when exposed to field conditions for 4 months. In addition, infrastructure such as footbaths and wash-down facilities are vital components of effective on-farm biosecurity. When various types of metals including aluminium, galvanised, painted, unpainted steel and stainless (304) steel were submerged in 3 different QA compound disinfectants (1200ppm didecyldimethyl-ammonium chloride) for 2 months, they did not corrode the metals significantly more than water alone. An important component of on-farm biosecurity which reduces the risk of spreading Panama disease is that banana growers maintain effective concentrations of disinfecting solution and are constructing biosecurity infrastructure from materials that will withstand the test of time.

NOTES:

Novel technologies in stored product protection: The European perspective

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Although stored product protection application techniques are somewhat standardized in the developed world, there are often considerable differences among countries, arising from fundamental variations in legislation and consumers' perceptions. Currently, stored product protection is based chiefly on the use of chemicals, but their continuous and often improper use has raised human safety environmental concerns. In Europe, organic production of durable stored products is much more developed than other areas, which constitutes essential the evaluation of alternative methods to conventional insecticides. Hence, in this context, the use of different "macro" and "micro" biocontrol agents has been largely supporter by European policy makers, and there are certain commercially available formulations that are already registered or are at the pre-commercial stage. Moreover, the use of pheromones for the control of insects at the post-harvest stages of durable commodities has been widely exploited in many areas, through mating disruption applications. Extreme temperatures, especially heat treatment, has been widely adopted for empty space treatment in European processing facilities (flour mills etc.), but also directly on the commodity (e.g. infrared). Modified and controlled atmospheres are also used in numerous occasions in Europe, especially nitrogen, which is usually applied in chambers. Other techniques include reduced-risk insecticides, such as nanoparticles and bacterial metabolites, which are now registered for application on stored grains. All these techniques are now used in large-scale applications, clearly suggesting that a meta-pesticide era in stored product protection in Europe is feasible. This feasibility, apart from the insecticidal efficacy, is also related to the fact that the cost of most of these approaches and techniques is directly comparable with the cost of conventional methods that are currently in use.

Challenge of Insect Control in Australian Grain Industry

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Storing grain in Australia has its unique challenges due to range of climatic conditions, types of storages and the requirement of no detection of live insects when delivering to domestic or export customers.

Increase in the resistance level of stored grain insects to grain protectants and phosphine has led the industry to develop a Phosphine Resistance Strategy.

Understanding the ecology of these insects and the adoption of Profume will assist in slowing the spread and speed of resistance.

NOTES:

Integrated approach to manage strong resistance to phosphine (PH₃) in rusty grain beetle, *Cryptolestes ferrugineus*

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Populations of rusty grain beetle, *Cryptolestes ferrugineus*, a cosmopolitan grain insect pest, have recently developed a very high-level of resistance (1300×) to fumigant phosphine in Australia, threatening the country's international grain market worth of 8 billion per year. Due to the failure of the registered rates of phosphine, industry urgently needed alternative strategies to manage this new resistance. To address this, an integrated resistance management strategy (IRM) was developed with three core components: 1. *regular monitoring and characterization of resistance*, 2. *developing effective phosphine protocols*, and 3. *exploring alternative fumigants and treatments*. Resistance was monitored across Australia in pest samples collected from farms and central storages using a modified FAO method as well as quick knockdown tests. Our results indicated that high-level resistance in *C. ferrugineus* is prevalent in eastern Australia, particularly in Queensland and New South Wales, particularly in the bulk handling sector. Time to population extinction was assessed in field-derived reference resistant strains using higher dosages (*c*) and exposure times (*t*) and the effective *c × t* regimes were validated in the field for the use by the grain industry. A phosphine concentration of 720 ppm (1 mg L⁻¹) over 18 days was found to be effective in controlling all life stages of phosphine-resistant *C. ferrugineus* at 25°C. In a parallel research, the suitability of sulfuryl fluoride (Profume™), as an alternative fumigant to phosphine was assessed through laboratory and field efficacy trials. Our results showed that phosphine-resistant insects are highly susceptible to sulfuryl fluoride and a CT of 750 g hm⁻³ (5.2 mg L⁻¹) over 6 days at 25°C would be sufficient against all phosphine-resistant pests including *C. ferrugineus*. While sulfuryl fluoride has promising potential as a “phosphine resistance breaker” the team is currently exploring ways of co-treating phosphine and sulfuryl fluoride, in order to reduce the overall selection pressure against these two fumigants in insects.

Australia's On-Farm Grain Storage Extension Project – a national initiative improving stored grain pest management and maintaining phosphine fumigation efficacy on-farm for the Australian grains industry.

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Phosphine is a cost effective and widely used fumigant to control grain insect pests in on-farm and central storage systems in Australia. Phosphine's continued use is threatened through increased resistance in both frequency and strength in target insect pests. Effective fumigation combined with best practice integrated pest management is essential to the sustainability of grain biosecurity and market access for Australian post-harvest grain systems. The National Stored Grain Extension Program (NSGEP) is the industry funded initiative developed to facilitate best practice grain storage practices, involving growers, agribusiness and industry.

The NSGEP aims to improve grain storage management practices and the efficacy of phosphine fumigation within Australia's grains industry.

The NSGEP uses a multi approach engagement strategy aimed at increasing awareness and knowledge to build capacity and support to enable farmers and industry to manage their grain storage systems and meet best practice requirements. These include: workshops, field days, industry forums, information, multi-media and website development and building networks with grower groups, government agencies and agribusiness.

In southern Australia, over 50 training workshops for farmers, advisors and agribusiness on best practice fumigation and grain storage principles are delivered, using a variety of adult learning principles and training techniques.

Various evaluation methods have shown that awareness and adoption of best practice in on-farm grain storage management has increased. The NSGEP contributes to the positive on-going changes observed in Australia's on-farm grain storage systems. It plays an instrumental role in building capacity and maintaining phosphine fumigation efficacy in the Australian grains industry.

NOTES:

Enhancing surveillance for exotic pests of stored grain using a partnership approach with industry and government.

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Exotic plant pests threaten production, market access and sustainability of Australian plant production systems. For the grains industry, over 600 exotic pests have been identified of which 54 are considered high priority pests (HPPs), posing a significant threat. Despite Australia's geographical isolation and strong quarantine systems, increasing levels of travel and trade continues to place pressure on our biosecurity systems, emphasising the need for improving our efforts in prevention, preparedness and surveillance. Verifying freedom from HPPs such as Khapra beetle and Karnal bunt is critical to supporting and maintaining access for Australian grain producers to domestic and international markets.

Within Australia, the Grains Farm Biosecurity Program (GFBP) a national initiative to assist in the development and implementation of improved biosecurity practice, plays an instrumental role in the education and awareness of exotic pests. The GFBP places significant importance on the role of surveillance and reporting by industry stakeholders in order to detect an incursion early, increasing the likelihood of eradication or containment and reducing its impact on industry and community.

The GFBP has recently undertaken a targeted surveillance monitoring program for stored product pests using a range of target sites and potential risk groups (e.g. privately-owned farming enterprises, grain millers, seed distributors, importers of dry goods and agricultural re-sellers) primarily aimed at strengthening evidence of absence and building industry knowledge in grain storage surveillance within the regions.

Different approaches for implementation were used across the three grain regions of Australia (southern, western and northern zones), depending on pre-existing collaborators, state surveillance activities and industry alliances. Khapra beetle (*Trogoderma granarium*) was the main target, with the deployment of - pheromone traps and other sample methods including vacuuming and visual inspection of grain and other host materials. These regionally specific methods for development and enhancement of surveillance efforts are discussed.

NOTES:

Diffuse symbioses: Competition, coevolution, and pathogen suppression in the rhizosphere**Linda Kinkel¹***1. University of Minnesota, Minnesota, United States*

Microbes and plants exist within complex networks of interacting plant and microbial species. Our work explores the roles of plant community diversity, plant host, and fungal and bacterial species interactions in determining the pathogen-suppressive potential and composition of soil microbiomes, and the consequences for plant productivity. Unraveling the complex interactions among plants and soil microbiomes can suggest novel insights for active management of soil microbes to support plant productivity. Our results show that rhizosphere *Streptomyces* associated with the same plant host are significantly more pathogen-suppressive when the host grew in monoculture vs. within a high-diversity plant community. In contrast, populations of *Streptomyces* in the rhizosphere of plant hosts growing in high-diversity communities are more niche-differentiated than populations associated with the same host in monoculture. These data suggest that plant community diversity plays a critical role in determining the likelihood of antagonistic arms race coevolution vs. niche differentiation among sympatric soil populations, with significant implications for plant disease suppression. *Fusarium* populations also show evidence of coevolutionary interactions with *Streptomyces*. *Fusarium* are significantly better at inhibiting sympatric than allopatric populations of *Streptomyces*, and inhibition is positively correlated with niche overlap between sympatric, but not allopatric, *Fusarium* and *Streptomyces*. This work illustrates how diffuse networks of species interactions over diverse spatial scales contribute to determining the antagonistic potential of indigenous soil microbes, and suggests specific management approaches targeting species interactions that may offer potential for sustainable disease control.

NOTES:

Molecular-based strategies for combating Fusarium wilt disease of legumes

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Fusarium wilt disease caused by pathogenic *F. oxysporum* isolates are major constraints to global legume production, accounting for losses up to 90% annually, and are significant biosecurity threats to Australia's major legume industries, particularly chickpea worth ~\$1B (2016). Substantial crop losses, no curative treatments and persistence of pathogen spores in soil ranks *F. oxysporum* high on the list of globally important plant pathogens. Strict quarantine regulations prevent import of legume-infecting isolates into Australia for research purposes. To reduce the probable impact of this pathogen on Australian legume industries we developed a model pathosystem using the model legume *Medicago truncatula* and a *F. oxysporum* f. sp. *medicaginis* isolate (*Fom*) with the aim of providing resources and expertise to combat disease incursion. To dissect pathogenicity mechanisms we generated a high quality *Fom* genome assembly including almost near complete assembly of its predicted pathogenicity chromosome. This latter scaffold sequence contains all *Fom* homologs of the tomato *F. oxysporum* f. sp. *lycopersici* Secreted In Xylem (SIX) pathogenicity-associated genes. Using genomic location and relative increase in *in planta* expression as an indicator of key roles in pathogen attack, high-depth RNA-sequencing of the *Fom* transcriptome at various stages of host infection and *in vitro* confirmed expression of candidate SIX effectors and facilitated prediction of novel candidate effectors. Further novel machine-learning based effector prediction algorithms developed in CSIRO were applied to determine a *Fom* pathogenicity effector set which assessed against the genomes of other *Fusaria* and those pathogenic on legumes (e.g. *F. oxysporum* f. sp. *ciceris*; chickpea infecting), identified 5 candidate effectors highly conserved amongst legume-infecting isolates. These effectors may be useful tools for pre-emptive breeding of Australian chickpea cultivars to protect against bioinvasion of globally destructive *F. oxysporum* f. sp. *ciceris* isolates.

1. Williams AH, Thatcher LF, Sharma M, Azam S, Hane JK, Kidd BN, Anderson JA, Ghosh R, Garg G, Lichtenzveig J, Kistler HC, Shea T, Young S, Buck SA, Kamphuis LG, Saxena R, Pande S, Ma LJ, Varshney RK, Singh KB (2016) Comparative Genomics of Legume-infecting *Fusarium oxysporum* formae speciales Informs Prediction of Lineage-specific Sequences Related to Pathogenicity. BMC Genomics. 17: 191 DOI: 10.1186/s12864-016-2486-8
2. Thatcher LF, Williams H Garg G, Sally-Anne Buck G, Karam B. Singh B. (2016) Transcriptome analysis of the fungal pathogen *Fusarium oxysporum* f. sp. *medicaginis* during colonisation of resistant and susceptible *Medicago truncatula* hosts identifies differential pathogenicity profiles and novel candidate effectors. BMC Genomics DOI 10.1186/s12864-016-3192-2

Investigating the molecular mechanisms of pathogenicity of the banana pathogen, *Fusarium oxysporum* f.sp. *cubense*

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Banana (*Musa* spp.) is one of the most significant horticultural crops due to its economic and social value to communities across the globe. A major constraint to the worldwide cultivation of banana is the disease Fusarium wilt or Panama disease, which is caused by the soilborne fungal pathogen, *Fusarium oxysporum* f.sp. *cubense* (*Foc*). Currently, the molecular mechanisms underpinning the pathogenicity of *Foc* remain largely unknown. In this study, we sought to investigate the genetic mechanisms regulating pathogenicity in *Foc*. In initial studies, we identified the distribution and sequence diversity of a class of fungal effector genes termed the secreted in xylem (SIX) genes in a collection of isolates representing the various genetic lineages of *Foc*. This work demonstrated that the repertoire of SIX genes within a *Foc* lineage is highly conserved however isolates of *Foc* from genetically distinct lineages carried unique ranges of SIX genes. A systematic analysis of the SIX gene homologues identified in *Foc* also supports the hypothesis that this class of fungal effectors has been horizontally acquired in the lineages of *Foc*. Currently, it is hypothesised that small, secreted proteins, such as the SIX proteins, confer the host- and cultivar-specific pathogenicity of the lineages of *Foc*. We further investigated this hypothesis by attempting to alter the cultivar-specific pathogenicity of *Foc* by modifying the complement of SIX genes through genetic transformation. Ongoing studies are being performed to investigate the genomic organisation and evolution of *Foc* and the localisation of the SIX genes in the genome of *Foc* using long-read sequencing technology. This work has particular significance for understanding how *Fusarium oxysporum* has evolved and adapted to a crop system as well as investigating the molecular interaction between pathogens and their hosts.

How Queensland is fighting the battle against *Fusarium oxysporum* f. sp. *cubense*.

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In March 2015, Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4), was detected on a single farm in Australia's major banana growing area in north Queensland. This area accounts for 95% of Australian banana production, valued at approximately AU\$600 million annually. The soil borne pathogen produces chlamydospores that can survive for decades. It is commonly spread by movement of infected plant material, infested soil and water. There is no cure or treatment for Panama disease tropical race 4.

Biosecurity Queensland, part of Queensland's Department of Agriculture and Fisheries (DAF), in partnership with the Australian Government and the Australian Banana Growers' Council (ABGC), mounted a major emergency response. The objectives of the response were to delimit disease distribution in Queensland, prevent disease spread from the affected property, and to encourage active participation in the response to facilitate industry resilience, recovery and sustainability.

The infested property and a linked property remain under quarantine. Initially the affected properties were returned to trade under strict biosecurity conditions. In October 2016, both properties were purchased by the ABGC, all banana plants on the properties were destroyed and risk mitigation measures to prevent disease spread remain in place.

This presentation describes the strategies, based on rigorous scientific knowledge and Queensland's regulatory framework, being deployed in the emergency response program. The strong partnership between government and industry, with commitment from all stakeholders, gives Queensland a fighting chance to live sustainably with TR4 and minimise its impact.

Genetic dissection of Foc resistance using *Musa acuminata* ssp. *malaccensis*

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Fusarium wilt is one of the major diseases threatening banana production worldwide. The causal agent is the soil-borne fungus *Fusarium oxysporum* sp. *cubense* (Foc) which enters the roots and then colonises the vascular tissues to induce a lethal wilt. We have identified Foc resistance in the wild banana *Musa acuminata* subsp. *malaccensis*. F₂ progeny testing showed segregation of a single dominant gene conferring resistance to race 1, sub-tropical race 4 and tropical race 4. Genotyping-By-Sequencing was performed on resistant and susceptible progeny to identify SNPs on a genome-wide scale. A strong level of association between SNP zygosity and resistance was identified in a region on chromosome 3. Using SNP based markers, this region was fine-mapped. The QTL was delimited to a 157 kb physical sequence containing 28 putative candidate genes. Only 15 genes are predicted to carry non-synonymous SNPs leading to changes in the encoded amino acid sequence. Analysis of the expression profiles of these candidates are underway using Foc infected roots of resistant and susceptible plants. Transcriptional changes will be correlated with resistance. SNPs from gene markers co-segregating with the resistance locus are currently being validated for the detection of the resistance allele in other diploid banana genotypes. Identifying the gene underpinning resistance will help to understand better the mechanisms of resistance at the molecular level. The molecular markers closely linked to resistance will be useful tools in marker-assisted breeding programs.

NOTES:

Varietal screening trial to identify banana varieties highly tolerant or resistant to *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4

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Fusarium wilt (Panama disease), caused by the pathogen *Fusarium oxysporum* f.sp. *cubense* (FOC) Tropical Race 4 (TR4) is considered one of the largest threats to the national and global banana industries. TR4 is known to infect and often kill the economically and globally important Cavendish varieties. Currently, a varietal screening field trial is underway in the Northern Territory, examining 27 banana varieties, including Cavendish, FHIA and selected hybrid cultivars. This screening will determine the levels of tolerance or resistance to FOC TR4 of the tested banana varieties, with the aim of selecting superior tolerant varieties for further studies and improvement.

Trial plants were inoculated with FOC TR4 colonised millet at planting and were assessed for the first appearance of external disease symptoms which is commonly leaf yellowing and wilting and pseudostem splitting, while internal symptoms were assessed following plant death or crop harvest. Agronomic characteristics were also examined, such as bunch emergence, bunch weight and plant height. Assessments of trial plants show the Cavendish varieties Williams and DPM25 are the most susceptible to TR4. However, some FHIA, selected hybrid and other Cavendish varieties have thus far displayed strong tolerances to TR4 with no external symptoms observed with little to no internal symptoms at harvest.

NOTES:

Where do plant bacterial diseases come from?

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The basic requirement for the onset of plant disease is well defined as the plant disease triangle. For a plant disease to manifest it requires the presence of a pathogen, a susceptible host and favourable environmental conditions. Conversely, plant disease is prevented upon elimination of or change in any one of these three parameters. For plant bacterial diseases the onset of disease is further complicated by plant commensal and mutualistic bacterial species and the capacity for bacterial species to exchange DNA via horizontal gene transfer (HGT). Plants are frequently colonised and/or attacked and challenged by an array of beneficial and pathogenic microorganisms.

In the modern, global world microbes are provided with even more opportunity to spread and evolve into pathogens of significance than in previous eras. There is increased movement of people and plant products around the globe. The requirement to maximise crop yields to feed increasing populations inevitably results in modifying host crop germplasm and farming practices are continuously evolving, often in response to changing climates. It is inevitable that new pathogens will emerge that will impact significantly on agricultural production systems.

The emergence of plant diseases caused by phytopathogenic bacteria will be discussed. Four case studies (fire blight caused by *Erwinia amylovora*; Annual rye grass toxicity (ARGT) caused by *Rathayibacter toxicus*; Bacterial canker of kiwifruit caused by *Pseudomonas syringae* pathovar *actinidiae*; and ratoon stunting disease (RSD) of sugarcane caused by *Leifsonia xyli* subsp. *Xyli*) will highlight potential changes in the pathogen, plant host and/or environment that has favoured the onset of disease. The challenge for plant pathologists is to learn from past disease outbreaks and predict the emergence of new pathogens and prevent their spread.

'Candidatus Liberibacters' in indigenous Rutaceous species from South Africa

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In recent years a serious disease of citrus, Huanglongbing (HLB), associated with '*Candidatus Liberibacter asiaticus*' (Las), has spread to many new citrus production countries worldwide. South Africa remains free of HLB, but for many years has had a related bacterial species, '*Ca. Liberibacter africanus*' (Laf), associated with Citrus greening disease (CG). CG is less severe than HLB and is controlled in South Africa through planting healthy certified citrus saplings, removal of infected branches or trees, and control of the triozid vector, *Trioza erytreae*. Re-infection of new citrus orchards planted in area previously abandoned for citrus production due to CG, suggested the presence of alternate hosts of Laf. This led to the hypothesis that other indigenous Rutaceous trees may be infected with either Laf or Liberibacters related to Laf. One such tree, *Calodendrum capense*, had been shown in 2000 to host a related bacterial species '*Ca. Liberibacter africanus* spp. *capensis*' (LafC). Samples from large numbers of *Calodendrum capense*, *Vepris* spp. (*V. lanceolata* or *reflexa*), *Zanthoxylum* spp. (*Z. capense* or *Z. davyi*), *Clausena anisata*, *Teclea* spp. (*T. natalensis* or *gerrardii*) and *Oricia bachmannii* were collected, primarily from within the natural distribution of these trees in South Africa. LafC was found to be widespread in *C. capense* while unique "*Ca. Liberibacter africanus* subspecies" were found associated with each tree species with the exception of *Oricia*, at incidences of between 1 and 11% of sampled specimens. One of these subspecies, "*Ca. Liberibacter africanus* spp. *clausenae* (LafCl), was recently observed in citrus. A widely applied Las PCR system, was shown to non-specifically amplify this subspecies in samples from Uganda and Kenya, where Las was recently reported. This casts some doubt on the veracity of this report in these countries.

NOTES:

Are multiple *Phytophthora* species associated with macadamias?

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In Australia, *Phytophthora cinnamomi* is presumed as the only *Phytophthora* currently associated with diseases in macadamias trees causing trunk canker, gummosis, dieback, root rot and eventually, tree death. However, the potential of other *Phytophthora* species as significant pathogens in macadamias has not been established. New macadamia orchards are established in lands previously cultivated to other crops known to be susceptible to multiple *Phytophthora* species. Report of poor establishment is common, thus, the macadamia trees are prone to 'replant problems' due to soilborne pathogens. In order to examine the prevalence and susceptibility of macadamia to multiple species of *Phytophthora*, a survey of macadamia orchards and native ecosystems commenced in 2016. Fifty macadamia sites representing different cropping histories and habitats in Australia were surveyed. Over 250 soil samples collected from potential sources of *Phytophthora* into the orchards and root zones of symptomatic and asymptomatic macadamia trees were baited for *Phytophthora* using blue lupins seedlings (*Lupinus angustifolius*), umbrella plant leaves (*Schefflera arboricola*) and Geraldton wax needles (*Chamaelirium uncinatum*). Isolates of *Phytophthora* from infected baits were maintained on antibiotics amended V8-juice media at 25°C under 8h/16 h light/dark cycle. Species identification of each isolate was based on DNA sequences of the *ITS* and *cox1* and *cox2* genes and morphological characterisation. Preliminary results revealed nine *Phytophthora* species are associated with macadamia in Australia. *P. cinnamomi* was predominant, representing 74% of the isolates, followed by *P. cryptogea* (6%), *P. multivora* (6%), *P. citrophthora* (5%), *P. nicotianae* (4%), *P. boehmeriae* (2%) and 1 % each of *P. nagaii*, *P. sojae* and an unconfirmed *Phytophthora* taxon. Association between cropping history and the *Phytophthora* species was evident and pathogenicity tests confirmed infection of these *Phytophthora* species in macadamia. The study provides a baseline information on the potential of other *Phytophthora* species as serious pathogens to macadamia.

Evolution of *Phytophthora infestans* and disease prevention strategies

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Late blight is caused by an increasingly large number of *Phytophthora infestans* genotypes that are responsible for devastating disease losses in solanaceous crops. A national survey conducted in Canada during 2009 through 2016 produced over 500 *P. infestans* isolates from the major potato and tomato growing areas. The *P. infestans* genotype US-23 A1 mating type was first detected in Canada during 2009 and now dominates the late blight pathogen populations on both tomatoes and potatoes. Characteristics of this genotype include drought tolerance, temperature tolerance and proliferation on tomato and potato that contribute to its frequent occurrence in gardens and increasing prevalence in commercial crops. Fungicide screening showed that all *P. infestans* US-23 genotypes were sensitive to the systemic fungicide metalaxyl early in the growing season but resistance developed rapidly.

Many isolates of the *P. infestans* genotypes US-22 A2 mating type were recovered but only from tomato samples while US-8 A2 mating type, US-11 A1 mating type, and US-24 A1 mating type were only isolated from potato. In some regions of British Columbia and Ontario, both A1 and A2 mating types of *P. infestans* were found in close proximity. This raises concerns as it provides an opportunity for the production of thick walled pigmented resting spores that are capable of surviving in the absence of a host for many years and sexual recombination. Evidence of recombination was detected in some pathogen populations from those areas with both *P. infestans* mating types. Volumetric spore traps were used to measure pathogen pressures to determine the timing and reduce the need for preventative fungicide applications. This data was successfully used to predict the risk of late blight in a given area and to triangulate disease hot spots that were subsequently eliminated.

NOTES:

Diversity of root lesion nematode populations in Australian cropping regions and implications for PreDicta B

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Nematodes were sequenced from soil samples collected across Australian cropping regions to determine what *Pratylenchus* species are present in each region and whether there is genetic diversity in populations that can affect the reliability of the PreDicta B assays. DNA was extracted from single nematodes and the ITS region was sequenced and used to identify the species and check for sequence variation in the diagnostic site. More than 2500 single nematodes were isolated from 455 soil samples, including 71 samples from NSW, 81 from Qld, 64 from SA, 47 from Vic and 182 from WA. Results from 1273 sequences show that *P. neglectus* (669), *P. thornei* (230) and *P. quasitereoides* (164) were the most prevalent species with *P. neglectus* and *P. thornei* widespread across the cropping regions. *P. quasitereoides* and *P. penetrans* were almost exclusively found in WA which is consistent with previous observations. All DNA identified as from *P. neglectus*, *P. thornei*, *P. quasitereoides* and *P. penetrans* by sequencing were accurately detected by the corresponding PreDicta B assay except in 6 instances (3 *P. neglectus* and 3 *P. thornei*). In addition, none of the DNA from other *Pratylenchus* species was detected by any of the PreDicta B assays. The results show that the genetic diversity in the portion of the ITS sequence used as diagnostic target is low validating its choice as a reliable diagnostic target for *Pratylenchus* detection. Furthermore, no variant or species other than the main ones was found to be widespread and susceptible to represent a threat to the Australian grains industry indicating the current PreDicta B assays are presently sufficient to assess *Pratylenchus* risk.

NOTES:

Agro-nano innovations: game changers for crop protection**Neena Mitter¹***1. Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, 4072, QLD, Australia*

The current challenges of sustainability, food security and climate change are key drivers for agricultural nanotechnology innovations for crop protection. Even with the best crop management plans in place, viruses and insects challenge all farmers. The ongoing usefulness of chemical pesticides suffers from issues such as residual toxicity, run off, specificity and resistance. Genetic modification (GM) is not available for all crops/pathogens, and it is not the preferred choice for all producers and consumers. Nanoparticle based delivery of pesticides have the potential to provide site-specific and slow-release activity on pests and diseases of plants, with benefits in reduced input and less risk to the environment. Nanoparticles as carriers of innovative biological 'active ingredients' rather than chemical ones could be a game changer for future crop protection strategies. BioClay is one such non-toxic, non-GM, biodegradable crop protection platform that delivers pest targeting RNA interference (RNAi) as a topical application using clay nanoparticles. At present the use of RNAi for disease resistance is limited to engineering transgenic disease resistance plants. BioClay opens the huge window of opportunity to deliver the same as a transient spray application. We have shown that RNAi effectors delivered as BioClay are stable, do not get washed off and provide protection to the sprayed and unsprayed leaves against the targeted virus for up to 20 days post spray. We have further shown that the clay degrades on the surface of the leaf alleviating any concerns about residues. We are now progressing with exploring BioClay targeting multiple host/pathogen systems and validation in field trials. Globally there are 3000 registered patents for pesticides developed using nanotechnology, indicating to some extent, the potential for future commercialisation.

The potential effect of karrikinolide (KAR1) in inducing resistance against *Alternaria solani* on tomato**Aloesi A Dakuidreketi¹, Jitka Kochanek¹, Michael Furlong¹, Elizabeth Aitken¹***1. University of Queensland, St Lucia, QUEENSLAND, Australia*

Karrikinolide (KAR1) is a phytoactive compound isolated from plant-derived smoke that is responsible for eliciting an array of growth responses in seeds and plant tissues. It is reported to induce rapid and vigorous growth of some seedlings such as maize and tomato at concentrations as low as one-part-per-billion. Although the use of KAR1 has shown to be of potential for enhancing plant productivity due to effects on plant growth and development, essentially nothing is known of the role of KAR1 in plant-pathogen interactions. In this study we have assessed the effect of KAR1 on tomato growth and investigated if KAR1 has the capacity to induce resistance in tomato against early blight caused by *Alternaria solani*.

Preliminary laboratory and subsequent glasshouse dose-response experiments showed significant effects of KAR1 on the germination and seedling growth of tomato. With these promising preliminary results, experiments are ongoing to determine whether the pretreatment of tomato plants with KAR1 provides subsequent protection when challenged with *Alternaria solani*.

NOTES:

Isoprene Identified in the Volatolome of Endophytic Fungi as a Promising Phosphine Alternative

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Fungal endophytes are known to confer enhanced tolerance towards both biotic and abiotic stresses to their host plants. Recently, bioactive secondary metabolites produced by these fungi have come into focus for potential application in agriculture. In particular, fungal volatile organic compounds (VOC) have shown application as fumigants to control fungal pathogens and insect pests of stored grain. These fungal VOCs open up new avenues to overcome resistance of stored grain pests to the widely used fumigant phosphine.

Using Solid-Phase Microextraction Gas Chromatography – Mass Spectrometry, a number of potential biocidal VOCs were identified in the volatolome of native endophytic fungi – *Muscador* sp. and *Nodulisporium* sp. We evaluated these compounds in isolation and combination for their biocidal activity against major insect pests of stored grains such as *Tribolium castaneum*, *Cryptolestes ferrugineus* and *Rhyzopertha dominica*, and fungal pathogens such as *Fusarium verticillioides*. From the compounds tested, isoprene, produced by *Nodulisporium* sp, emerged as the most promising candidate as a stored grain fumigant. Small scale, mock grain silo experiments demonstrated good efficacy and low residue levels. Combining isoprene with other fungal VOCs further increased insecticidal activity, including at reduced rates.

Isoprene is highly volatile and produced commercially in large quantities as a building block for organic synthesis of rubber. It is naturally produced by many plant species, and is commonly found in low levels in many foodstuffs. It is the most abundant hydrocarbon detectable in human breath and has no acute toxicity, demonstrating its relative safety compared to other fumigants, including phosphine.

Control of *Botrytis cinerea* in industrial horticulture by pulsed UV light induced plant defences

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Grey mold, *Botrytis cinerea*, is one of the most important fungal pathogens. It is estimated to cause losses of over £88 million per year in the UK tomato and cut flower industries alone, due to high fungicide cost, reduced yield and quality of produce. Broad host range, quiescent life cycle and resistance to fungicides are main challenges of *Botrytis* control. An industrial-academic research partnership was therefore formed to develop commercially applicable, non-chemical method to control *Botrytis* in pre-harvest environments.

The method is based on the principle of hormesis, where a beneficial effect (biotic stress tolerance) results from exposure to low doses of an agent (UV light) that is otherwise toxic or lethal at higher doses. It consists of short pulses of extremely high intensity broad spectrum light (UV-VIS-IR) applied to vegetative part of tomato plant. Light treatment elicits stress responses leading to increased plant defenses in subsequent *Botrytis* inoculation and reduced disease symptoms. We have optimized the dose of pulsed light to induce maximum resistance and minimum or no damage to the plants. The disease progression in treated plants was reduced by 25-40%. We determined the longevity of light induced resistance and tested its applicability in different tomato cultivars. The method is being validated in production tomato greenhouse where *Botrytis* control and effect on yield are tested on a large scale.

To understand the molecular basis of pulsed light induced resistance we have performed transcriptome analysis (RNA-seq) of treated plants different at time points following the light treatment. Additionally, response to *Botrytis* inoculation was compared between treated and control plants. Involvement of ROS and ethylene signaling in light induced resistance was highlighted.

Here developed technique could present environmentally friendly, sustainable, zero residue alternative to *Botrytis* control in industrial horticulture.

NOTES:

Sucrase inhibitors as insecticides against sap-feeding insects

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Sap-feeding insects of the suborder Sternorrhyncha cause significant damage to agriculture by feeding on plants and transmission of plant pathogens. They encounter two main obstacles from their specialized diet: low concentrations of essential amino acids and high osmotic pressure. The latter is overcome by production of oligosaccharides from sucrose, thereby decreasing the osmotic pressure per sugar unit. Sucrase, a crucial enzyme in this process, cleaves sucrose into glucose and fructose. Previous studies have shown that feeding acarbose, a sucrase inhibitor, to pea aphids (*Acyrthosiphon pisum*) at concentrations as low as 5 μM leads to increased mortality from starvation and constant loss of water from tissue. Acarbose is approved for medical use in human patients with type 2 diabetes and its toxicity and potential impact on the environment is low: its oral LD50 in rats is 24 g kg⁻¹, several times higher than that of commercial insecticides.

Screening potential inhibitors against specific enzymes allows the identification of compounds that show high activity against a narrow group of insects, thereby minimizing the impact on non-target species (e.g. honey bees). Such inhibitors may be suitable for use as insecticides by themselves, or as additives in conventional pest-control, i.e. decreasing overall fitness and survivability of target species, thus making them susceptible to lower concentrations of commercial insecticides.

The coding sequence for the sucrase enzyme from the tomato potato psyllid *Bactericera cockerelli* was identified from transcriptome sequence data. The corresponding enzyme was isolated after expression in *Escherichia coli*. The activity and kinetics of the purified enzyme were characterized and a set of potential inhibitors were tested for their effectiveness. As a control, the effect of the compounds on α -glucosidase was also measured. Additionally, the feasibility of applying sucrase inhibitors through spraying was investigated.

NOTES:

New technologies for detection of airborne pest and diseases affecting the grains industry

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New technology is rapidly developing and creating opportunities for surveillance of airborne pest and disease threats. In response the Grains Research and Development Corporation initiated the CRC Plant Biosecurity Project 2014 'New tools for field grains surveillance and diagnostics of high priority exotic pests' lead by SARDI.

This project has developed and evaluated 'Smart' Spore and Insect Trapping systems which collect samples referenced to parameters including GPS and climate data (temp, wind direction, RH) and include wireless data transmission of digital images or improved design for downstream diagnostics such as molecular assays of pest targets. These approaches demonstrate the benefits of partnering with engineering to include automation and innovation; in this instance resulting in trapping systems with smart capabilities and capacity for high sampling frequencies. Prototypes currently under evaluation at SARDI include the Mobile Jet Spore Sampler, Sensor Moth Trap and Insect Suction Trap resulting from collaboration with engineers at USQ as well as strong linkages to Burkard Manufacturing Co. (Stuart Wili) and Rothamsted Research (Prof Jon West) in the United Kingdom.

Area-wide (network) applications of these devices are required for coordinated reporting of point of origin and dispersal dynamics of targets within fields or growing regions. This applies to both exotic and endemic threats, and is equally applicable to grains, horticulture and viticulture industries. For effective pest and disease surveillance, these innovations must be combined with robust sampling protocols, rapid detection and data interpretation for accurate interrogation of spatial data generated from a network of samplers. This is particularly important given the size and scale of Australia's agricultural industries. These technologies should improve detection efficiency and provide additional time for industry to respond to incursions of airborne pests and plant pathogens.

Evaluating different passive/ interception traps for monitoring long distance wind dispersal of insects into Australia.

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This Plant Biosecurity CRC project has identified wind-assisted long distance natural dispersal of plant pests as an under-estimated biosecurity risk to Australia. However, given that long distance dispersal is beyond our control, effective management of incursions requires early detection and intervention to reduce the chance of establishment of new pests. This project thus aims to identify new surveillance strategies to better quantify and minimise the risk by; prioritising plant pest threats, identifying high risk areas for incursions and; how and when these areas should be surveyed. Such information will assist organisations, such as NAQS, target surveillance for wind dispersed threats, and will support horticultural industries update and improve their biosecurity plans.

Four primary pathways for natural dispersal into Australia have been previously identified: from PNG into northern Queensland; from Indonesia into north-west WA and NT; from the Pacific islands into eastern Australia and; from New Zealand into south-eastern Australia. The project is investigating improved trapping techniques and bulk DNA sampling methods for rapid and improved diagnostics of a pest presence. In addition, during 2016-2018 we will be comparing different passive traps in field conditions at two locations in northern and at least one in south-eastern Australia.

The final aim is to indicate improved surveillance strategies for early detection and/or monitoring of species that use wind as a means of dispersal and are therefore prone to long distance dispersal. The output of the project will be improved surveillance systems and biosecurity preparedness for naturally dispersed pests for Australia.

NOTES:

Continuing development of a new “attract and kill” trap for surveillance and control of female Queensland fruit fly

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Insect traps baited with attractant odours are powerful surveillance technologies for detecting and killing pests before they have a chance to establish and spread. In tephritid fruit fly monitoring and control, odours in these “attract and kill” traps currently target either the male flies (using pheromone-like attractants) or virgin female flies (using protein-based attractants). There is an urgent need for an effective trap that targets mated female fruit flies, which directly damage fruits through egg laying and the spread of microbial rots. We report here on our continued development of a synthetic fruit fly lure to trap mated females of the Queensland fruit fly, *Bactrocera tryoni*. Our synthetic attractants have been designed through studies in insect olfactory neuroscience (electro-antennograms and antennal lobe imaging), analytical chemistry, microbiology, and insect behaviour. Fruit-mimic sticky traps (red balls on a yellow background) baited with our synthetic blends were hung in orchards in regional Victoria (Sunraysia and Goulburn Valley), and caught significantly more Queensland fruit fly than odourless control traps. In some (but not all) trials, these traps also proved more effective at catching flies than protein baited traps. We discuss how incorporating a range of traps targeting both male and female fruit flies might be a more effective biosecurity strategy for surveillance of established and exotic fruit fly pests.

Comparison of rooftop and field-based air samplers for early detection and population monitoring of plant pathogens

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Air sampling allows us to monitor air-dispersed plant pathogens using a range of downstream diagnostic methods including DNA-based diagnostic assays and sequencing. Recent work to investigate the timing of spore release of pathogens and to evaluate potential automated spore traps with wireless reporting has compared sampler location, whether on a rooftop or at ground level in production fields. Examples of several pathogens, including *Sclerotinia sclerotiorum*, *Phytophthora infestans*, smut fungi and rusts have been compared. Generally, rooftop spore trap samples contain greater diversity than samples from ground-based locations, reflecting greater mixing of air incorporating spores from a wider range of microenvironments in the region for the rooftop sites. Pathogens known to be present in crops and found in air samples taken above the field are also found at rooftop sites, typically with numbers reduced by dilution to between 1/10th to 1/100th of what was found in the production field. However, at early stages of epidemics, spores were frequently detected albeit in low concentrations by rooftop samplers compared to samplers in production fields that did not detect any target spores. The use of high volume spore samplers will be discussed as a way to enhance the sensitivity of rooftop sample location.

NOTES:

Development of probs and platforms for the detection and identification of captured fungal spores

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Publish consent withheld

NOTES:

Modification of plant nuclei by negative-strand RNA viruses

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Rhabdoviruses infect a wide range of hosts that includes humans, terrestrial animals/vertebrates, fish, arthropods, and plants. The genomes of the plant-adapted viruses are organized generally into seven open reading frames with the gene order 3'-N-X-P-Y-M-G-L-5', which encodes the nucleocapsid, phospho, movement, matrix, glyco, and RNA-dependent RNA polymerase proteins, respectively, except for X, which is of unknown function. In addition to its structural role in virion formation, the M protein of *Potato yellow dwarf virus* (PYDV) is capable of inducing the intranuclear accumulation of the inner nuclear membranes (INM) in transfected cells. The M protein also interacts with the nuclear import and export receptors Importin- α and Exportin 1, suggesting a role for M in transport of condensed nucleocapsids from the nucleus. Interestingly, the ability to remodel the INM is conferred by the P, but not M, protein of coffee ringspot dichorhavirus, demonstrating that functional domains within rhabdoviral proteins are portable. This variation likely contributes to the finding protein interaction and localization maps (PILMs) for each virus are unique. Efforts to map the plant nucleome, the nuclear-associated portion of the proteome, show that nucleorhabdoviruses induce dramatic changes in the localization patterns of nuclear proteins, some of which are recruited to sites of viral replication and assembly.

Differentially Expressed Genes in Response to Capsicum chlorosis virus Infection in the Capsicum Host and Thrips Vector

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Capsicum chlorosis virus (CaCV) (genus *Tospovirus*, family *Bunyaviridae*) is a serious pathogen of capsicum and tomato crops in Australia and South-east Asia. CaCV is transmitted by thrips (order Thysanoptera) in a circulative and propagative mode. Due to lack of natural resistance in commercial cultivars and emergence of thrips populations with insecticide resistance, CaCV poses a serious threat to horticultural industries. Present understanding of the molecular basis of plant - CaCV - thrips interactions is rudimentary. In this study we sequenced transcriptomes of capsicum (*Capsicum annuum*) and *Thrips palmi* in response to CaCV infection using Illumina HiSeq. We comparatively analysed transcriptomes of capsicum during compatible and incompatible interactions with CaCV when strong hypersensitive reaction was induced in a CaCV-resistant *C. annuum* x *C. chinense* breeding line 4 days after inoculation. In the incompatible interaction, 2484 genes were differentially expressed and this resistance was underpinned by strong up-regulation of defence-related genes and down-regulation of energy consuming cellular processes such as photosynthesis. We also identified 54 capsicum genes that responded to CaCV infection at an early stage of the compatible interaction. These genes appeared to be involved in disease development and repression of defence-related genes and defence signalling. To determine molecular interactions between CaCV and thrips vector, we *de novo* assembled whole-body transcriptome of adult *T. palmi*. Comparative transcriptome analyses showed that genes involved in innate immune response, salivary glands and insecticide resistance were up-regulated in viruliferous thrips. The most influenced immunity pathways were lysosome and melanisation. Our analyses also showed that CaCV may have direct effect on the fecundity of *T. palmi* due to enhanced expression of 54 vitellogenin genes. Identification of genes involved in plant-virus-vector interaction has provided potential targets for future disease control efforts.

NOTES:

Viral small RNAs that target plant genes and artificial miRNA that target plant viruses

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Plant viruses cause significant damage to crops worldwide, and once infected there are no effective treatments. Large numbers of viral-derived small RNAs (vsRNA) are generated in infected plants. We are interested in the function of vsRNA and how they contribute to symptom development. We fully sequenced an Australian strain of *Turnip mosaic virus* (TuMV), and performed deep small-RNA sequencing on TuMV infected *Arabidopsis thaliana*. Mapping revealed “hotspots” of highly abundant vsRNA. We hypothesise abundant vsRNA could target host transcripts in the plant, contributing to symptom development. *A. thaliana* transcript sequences were screened to predict targets of abundant vsRNAs. Using an optimised transient assay that combines Agro-infiltration with the quantitative dual luciferase system, we demonstrate that vsRNAs can target plant transcript sequences, reducing expression. Mutating the plant transcript target sequences restored expression levels.

Artificial microRNA (amiRNA) refers to the strategy of replacing the mature miRNA sequence within a native microRNA precursor gene, to target a different expressed sequence of interest. Artificial microRNA predominantly process one mature miRNA that will target only highly complementary expressed gene sequences. This provides an advantage over other strategies such as RNA interference, where larger numbers of short interfering RNAs are generated increasing the likelihood of off-target effects. We successfully applied an amiRNA approach to develop tomato lines resistant to *Cucumber mosaic virus* (CMV). The amiRNA was designed to directly target the RNA genome of CMV. ELISA testing of CMV inoculated T1 populations from two independent amiRNA lines confirmed a reduction in CMV levels relative to controls. Symptom severity scoring and counts of harvested quality fruits further confirmed enhanced resistance to CMV in the amiRNA lines.

Next generation sequencing of resistance breaking *Tomato spotted wilt virus* from WA and QLD

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The tospovirus *Tomato spotted wilt virus* is damaging to capsicum and tomato crops in WA, QLD and SA. Crops infected with these viruses often have reduced yield and decreased marketable fruit, and when infection is early in the growing season total crops losses can occur. In the Perth metropolitan area and southern WA, TSWV is often found infecting capsicum and tomato crops as well as lettuce and celery. In QLD, TSWV occurs in all coastal and sub-coastal vegetable production areas. The virus is a major cause of crop loss in greenhouse capsicum crops on the North Adelaide Plain. TSWV is transmitted by thrips (4 species in Australia) in a circulative and propagative manner, particularly the western flower thrips which is a very efficient vector. TSWV has a large host range, of more than 900 species of weeds, ornamentals and field crops. As such, an important part of managing this disease for the tomato and capsicum industry includes resistant varieties. In recent years it has become apparent that there are resistance breaking strains of TSWV present in Australia. This study collected 19 new isolates of both resistance and non-resistance breaking strains of TSWV from WA (10), QLD and SA (9) and subjected them to next generation sequencing on an Illumina HiSeq 2000. The genomes have been assembled and compared by phylogenetic analysis and a recombination study, the results of which will be presented here.

NOTES:

Diversity of *Brevipalpus*-transmitted plant viruses

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Only a handful of *Brevipalpus* (Acari, Tenuipalpidae)-transmitted viruses (BTV) were known until the turn of the 20th century, but since then up to 40 new cases have been reported. Molecular characterization accompanied by biological and morphological data significantly increased our knowledge on BTV. There are basically two types of BTV: (1) cytoplasmic, bacilliform particles inducing a cytoplasmic viroplasm, with a genome of (+) ssRNA either bipartite (*Cilevirus*) or tripartite (*Higrevirus*), represented respectively by *Citrus leprosis C cilevirus* and *Hibiscus green spot 2 higrevirus*; (2) nuclear, rodlike virions causing an intranuclear viroplasm, genome of (-) ssRNA, bipartite (*Dichorhavirus*), with *Orchid fleck dichorhavirus* as the type species.

In the last few years several new putative members were added to the genus *Cilevirus*: Citrus leprosis virus C2; Passion fruit green spot virus, *Ligustrum leprosis virus* and *Solanum violaefolium* ringspot virus. Similarly, in addition to the two accepted species of genus *Dichorhavirus*, *Orchid fleck dichorhavirus* and *Coffee ringspot dichorhavirus*, putative members are *Clerodendrum* chlorotic spot virus, *Cestrum* ringspot virus, a recently characterized Citrus leprosis virus N and Citrus chlorotic spot virus from Brazil, among others. Overall, the new viruses show 50-70% nucleotide sequence identity with their cognates in the two genera. Nuclear type of citrus leprosis from Mexico and Colombia was demonstrated to be caused by citrus isolate of orchid fleck virus (OFV).

So far, except for OFV which has a worldwide distribution, and HGRSV-2 found in Hawaii, all reported cases of BTV occurred in the Americas, but BTV most likely will also be present in other parts of the globe.

NOTES:

POSTER 101

Correlation between *Gonipterus* sp. herbivory and leaf oil profiles in *Corymbia* species and hybrids

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Corymbia species and hybrids are promising for industrial plantations as they suit a vast range of site conditions, showing desirable traits including good field performance and wood characteristics. However, the expansion of planted area may increase the incidence of herbivory and contribute with the emergence of new pests. A major challenge for the forest sector is to select varieties with considerable resistance to pests, while still preserving productivity and market competitiveness. Breeding programs are generally based on field observations of mature trees without considering natural plant defences, such as leaf oil, which may provide an opportunity to select resistant genotypes for industrial plantations. This research aims to improve the *Corymbia* breeding program by focusing on pest resistance in seedlings. To determine the correlation between *Gonipterus* sp. herbivory and leaf oil profile, 13 resistant and 12 susceptible taxon of *Corymbia* were evaluated. Leaf oil was extracted from each taxon with hexane in five replications and the profiles were obtained by GCMS. For the herbivory trial one leaf of each taxon were placed in a petri dish with two *Gonipterus* sp. beetles in five replications. The herbivory trial run for 7 days under controlled temperature (20 °C 14 hours light: 10 hours dark) and leaf area before and after herbivory and beetle mortality were determined. The leaf oil profiles were analyzed to see if they influenced herbivory by the *Gonipterus* sp. beetles.

POSTER 102

***Bursaphelenchus* aff. *vallesianus* / *sexdentati* – identification from dying *Pinus* spp. in Queensland**

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Through a combination of geographical isolation, effective quarantine and good fortune, Australia has remained free of many of the known species of forestry nematodes. However, with increasing volumes and sources of traded goods there is increased risk of these species gaining entry. The nematode *Bursaphelenchus* aff. *vallesianus* / *sexdentati* has been recovered from urban and plantation pine trees in Southeast Queensland. This nematode can affect pine seedlings in Europe during heat and drought, and may potentially be associated with pine-wilt disease.

The nematode was first detected in a dying tree in suburban Brisbane in July 2014. Wood samples from this tree were collected during response surveillance following emergence of adult *Monochamus alternatus* (Coleoptera: Cerambycidae) from imported timber pallets. *Monochamus alternatus* is the vector of pinewood nematode, *Bursaphelenchus xylophilus*, a major pest of *Pinus* spp. and cause of pine-wilt disease.

Wood samples were soaked for 24 hours and the extracted nematodes examined morphologically. Typical of the genus *Bursaphelenchus*, the female nematodes were slender, with a fine, but well developed stylet with small basal knobs, slightly off-set head, a large, angular median bulb, longer than wide, the oesophagus overlapping the intestine dorsally and with a posterior vulva at 70-80% of the body length. The female specimens were “hand-picked” and forwarded for molecular analysis at Australian National Insect Collection. The same nematode was subsequently found in NSW and an extensive surveillance program showed it to be widespread across a number of regions throughout the state and also at Passchendaele State Forest, near Stanthorpe, Qld. Molecular identification was confirmed by NSW DPI Plant Health Diagnostic Service.

Wood samples from unthrifty host trees will continue to be examined to contribute to our knowledge of the distribution of these nematodes and provide absence data in relation to *B. xylophilus*, the original target of the QLD surveillance.

POSTER 103

Stability and heritability of RNAi in generations of transgenic plants and nematodes

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Plant parasitic nematodes (PPNs) are important pests of crop plants. Root-knot (*Meloidogyne spp*) and cyst nematodes (*Heterodera*, *Globodera spp*) are the two most damaging genera. They are difficult to control using crop rotation, chemical nematicides or biological control, and many nematicides have now been banned. RNA interference (gene silencing) technology is a potential alternative genetic approach for nematode control. A challenge of an RNAi-based transgene approach ('Host-Induced Gene Silencing') is to generate plants that express nematode control transgenes stably over many generations. Various factors can influence transgene stability (e.g., the target selected, transgene copy number, promoter methylation, sequence similarity). The aim of this research is to study the inheritance of RNAi in plants conferring resistance to PPNS to underpin knowledge of the application of RNAi as a strategy to confer nematode resistance in crop plants.

T2 seeds of different transgenic events expressing RNAi for 12 target genes are being studied to determine the segregation patterns in subsequent generations and the stability of nematode resistance. Replicated nematode challenges of transgenic events have been undertaken for populations of T2 and T3 plants, in which there was a significant reduction in nematode reproduction. In a reciprocal study, infection assays evaluating progeny nematodes for maintenance of reproductive suppression will be done with control plants. A major question is whether knockdown of target genes is stably heritable between generations, and qRT-PCR will be used to characterise this. Next generation sequencing will be used to compare characteristics of small interfering RNAs processed by different generations of transgenic plants as part of the RNAi process. The outcomes of this study will help optimise and support an RNAi-based nematode control strategy over multiple generations: stability in RNAi- based traits is vital for this strategy to be used to reduce the economic losses caused by plant nematodes.

POSTER 104

"The difference between 'Braeburn' and 'Golden Delicious' apple fruit in antioxidant response to inoculation with grey mould, a postharvest disease."

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Apples are rich in vitamin C and other antioxidants, which is beneficial to human health. Apples are usually harvested in September - October, and then stored for many months in order to maintain a supply of high quality over a long period of time. During post-harvest storage, there are several losses as a result of disorders and diseases, such as the grey mould (*Botrytis cinerea*). The present work compares the involvement of antioxidant metabolism of two different apple cultivars 'Braeburn' and 'Golden Delicious' in their susceptibility to artificial inoculation with grey mould. Apple fruit of 'Braeburn' and 'Golden Delicious' were inoculated with artificial grey mould on both sides of the fruit and antioxidant metabolism of the two apple cultivars were measured over time in peel and flesh tissues taken both sides of the fruit. Overall, 'Braeburn' was more susceptible than 'Golden Delicious', even though 'Braeburn' had higher levels of metabolism in several antioxidants than 'Golden Delicious', such as total vitamin C content in peel, phenolic compound in flesh and superoxide (SOD) activity and flavonoid peroxidase (POX) activity in flesh tissues. In 'Braeburn', it was a clear response of antioxidant metabolism to inoculation with grey mould, involving an increase SOD activity and ascorbate peroxidase (APX) activity, and a decrease POX activity, vitamin C content and phenolic compound. In 'Braeburn', disease developed more rapidly on the shaded than on the sun-exposed side with the sun-exposed side had higher vitamin C content and metabolism enzyme activity. In 'Golden Delicious', vitamin C content and antioxidant enzyme were not significantly induced by inoculation with grey mould, suggested that 'Golden Delicious' presents a different line of defence to grey mould. Preharvest exposure to high light/high temperature stress can reduce the susceptibility of apples to postharvest pathogens, but that the response also depends on the apple cultivar.

POSTER 105

Evidence of early defence to *Ascochyta lentis* in a novel primary *Lens* genepool accession

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In plants, strong structural and biochemical barriers are shown to delay fungus entry by deterring their establishment. Additionally, these alterations induce a cascade of reactions in plant that often lead to resistance against pathogens. Nevertheless, faster kinetics and amplitude of these defence mechanisms define resistance and susceptibility. The infection process of two *Ascochyta lentis* isolates (FT14069 and FT13082) on three lentil hosts (ILWL 180, ILL 7537 and ILL 6002) using histopathology revealed significant differences in early behaviour of two isolates among three hosts. Both isolates recorded significantly lower germination, shorter germ tubes and delayed appressorium formation on the resistant genotypes (ILWL 180 and ILL 7537) compared to the susceptible genotype (ILL 6002). Further, these were more pronounced on ILWL 180 than on ILL 7537. Both isolates germinated within 6 hours post inoculation (hpi) and a significant increase in germination % was observed in ILL 7537 compared to ILWL 180 at all-time points (6, 12, 20 and 30 hpi). Isolate FT14069 germinated faster on ILL 7537 and reached a maximum of 28.26% compared to 15.49% on ILWL 180. Similarly, significantly shorter germ tube lengths were observed for both isolates on ILWL 180 (ranging from 4 µm to 17.63µm) than on ILL 7537 (ranging from 7.14 µm to 21.09 µm). Although appressorium formation was delayed on both genotypes until 20 hpi and 30 hpi for isolates FT14069 and FT13082, respectively, unlike on ILL 6002, significantly higher appressorium formation was observed on ILL 7537 (4.87 % for isolate FT14069 and 2.58 % for isolate FT13082) than on ILWL 180 (3.82 % for isolate FT14069 and 1.87 % for isolate FT13082) at 30 hpi. Although early and faster recognition of *A. lentis* might be the reason for observed resistance in ILWL 180, this initial finding requires further validation through histochemical and gene expression studies.

POSTER 106

What's the use of a phytohormone? *Leptosphaeria maculans* produces abscisic acid when colonising its host, *Brassica napus*

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Blackleg disease is one of the major diseases of *Brassica napus* (canola) worldwide resulting in yearly losses of 10 to 15% Australia-wide. This disease is caused by the pathogenic fungus, *Leptosphaeria maculans* whose lifecycle alternates between biotrophy and necrotrophy. When starting to infect canola leaves, this fungus exists in a biotrophic phase before it becomes necrotrophic and causes necrosis on the leaves. The fungus expresses different genes at each stage of its lifecycle and a comparison of gene expression levels between biotrophic and necrotrophic stages of *L. maculans* was conducted by Lowe *et al.* (*PLoS ONE* 9: e103098, 2014). In this dataset we identified a gene cluster which we refer to as the *PKS5* cluster, containing a polyketide synthase gene and six adjacent genes which were upregulated during the period of biotrophic growth. To identify the compound produced by the *PKS5* cluster, we activated the cluster artificially by introducing an extra copy of the transcription factor from the gene cluster into the genome under the control of a constitutive promoter. By this approach, we generated a strain of *L. maculans* that overexpressed the transcription factor in vitro and further examination revealed that the other six genes in the cluster (*cytochrome p450*, *transferase*, *ABA1*, *ABA2*, *ABA3*, and *PKS5* genes) were also expressed in vitro. We discovered by using liquid chromatography quadrupole-time-of-flight mass spectrometry that the over-expression strain produced abscisic acid (ABA) in vitro while the wild-type strain did not. We hypothesise that the fungus produces the plant hormone ABA to promote its biotrophic stage of infection. Strains unable to produce abscisic acid are currently being generated and these will be tested for their ability to infect *B. napus* and to further define the role of ABA during the infection process.

POSTER 107

Expression of the Bifunctional Genes of *Candidatus Liberibacter solanacearum*

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Zebra Chip disease (ZC) is caused by, '*Candidatus Liberibacter solanacearum*' (*Ca. L. solanacearum*) which is vectored by the tomato/potato psyllid (TPP). The disease results in the development of blacklines in fried potato tubers which renders them commercially unacceptable. ZC has caused significant economic losses to the New Zealand potato industry, which has resulted in a need for further understanding of the biology and aetiology of this unculturable bacterium. The completion of the *Ca. L. solanacearum* genome in 2011 (Lin et al. 2011) determined the presence of putative bifunctional genes.

The research aimed to determine whether these annotated bifunctional genes were differentially expressed in vector (TPP) and in host potato plants. Primers were developed for the bifunctional genes of *Ca. L. solanacearum* and for a number of potential housekeeping/reference genes to determine differential changes in gene expression. Results showed that the bifunctional AICAR transformylase/IMP cyclohydrolase (ATIC) was the most expressed gene in TPP and plants whilst the membrane protein, SECDF, was upregulated in plants. The results suggest that these genes could play a role in *Ca. L. solanacearum* pathogenicity. Furthermore, these genes and the proteins they encode could potentially be novel targets for the development of antibacterials as many of the current disease management strategies target the psyllid.

POSTER 108

Key defense response genes of lentil (*Lens culinaris*) through the first 24 hours of *Ascochyta lentis* infection

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Using resistant lentil cultivars, breeders will be able to control one of the most devastating lentil diseases, *Ascochyta* blight, effectively. *Ascochyta* blight caused by the fungus *Ascochyta lentis* (*A. lentis*) resulting in over \$16 million AUD annual loss in Australia. However, little is known about the genes and molecular mechanisms underlying lentil resistance against *A. lentis*. To uncover the genetic basis of lentil resistance to *A. lentis*, differentially expressed genes were profiled in the resistant 'ILL7537' and susceptible 'ILL6002' lentil genotypes at 2, 6 and 24 hours post inoculation utilizing high throughput RNA-Sequencing. Genotype and time-dependent differential expression analysis identified genes which play key roles in several functions of the defence response comprising fungal elicitors' recognition and early signalling; structural response; biochemical response; transcription regulators; hypersensitive reaction and cell death; and systemic acquired resistance. Overall, the resistant genotype displayed an earlier and faster detection and signalling response to the *A. lentis* infection and demonstrated higher expression levels of structural defence-related genes. This study presents a first-time defence-related transcriptome of lentil to *A. lentis* during the first 24 of pathogen invasion.

POSTER 109

Elucidation of molecular defence mechanism in wheat (*Triticum aestivum* L.) against root-lesion nematode (*Pratylenchus thornei*)

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Wheat (*Triticum aestivum*) is one of the major sources of food in the world. Production of wheat is under threat due to the migratory, root-lesion nematode *Pratylenchus thornei*, particularly in the northern grain region of Australia. *P. thornei* causes nutrient deficiency and water stress in wheat, which results in 38 million dollars' economic loss per year in this region. Moderately resistant wheat cultivars limit reproduction of *P. thornei*. Investigation of bio-molecular defence in resistant wheat is challenging due to the migratory nature of *P. thornei*. Latest studies suggest that the resistance in wheat root acts post penetration of the nematode. However, little is known about the molecular defence mechanisms against *P. thornei* in resistant cultivars. Hence in the present study, modern molecular biology techniques will be used to analyze the wheat root extracts and exudates to explore various phytoanticipins and phytoalexins respectively, responsible for defence in resistant wheat. Production of phenolics and allelochemicals in susceptible and resistant wheat accessions will be investigated. The nematocidal properties of such potential bio-molecules will be tested and level of expression will be studied to learn if such compounds are constitutive or induced in response to nematode invasion. Comparative histopathology, proteomics and metabolomics will be conducted to elucidate the defence mechanism. This research will complement the quantitative trait loci (QTL) mapping of the moderately resistant trait loci discovered in several populations previously. Better understanding of natural chemical response of resistant wheats will aid deployment of resistant genes in wheat cultivars to minimize yield losses.

POSTER 110

First report of secondary metabolites produced by species of Botryosphaeriaceae implicated in grapevine trunk diseases in Australia.

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Botryosphaeria dieback (BD) is a serious disease of grapevines causing considerable economic loss worldwide including in Australia. The disease is caused by several Botryosphaeriaceae species that result in symptoms such as cankers, dieback and in latter stages the death of vines. In other countries, the disease is also associated with foliar symptoms, however, these have not been reported in Australian wine grape cultivars to date. Many foliar symptoms are usually associated with the production of phytotoxic metabolites produced by the pathogen and that are translocated into the leaves. The role of secondary metabolites in pathogenicity still required further study. Thus, investigating the production of phytotoxins from Botryosphaeriaceae species in Australian vineyards could result in the role of phytotoxins in the pathogenicity being elucidated. This may also assist in field diagnosis and the development of more effective control strategies for BD in Australian vineyards. The production of phytotoxins from the most widespread and virulent Botryosphaeriaceae species associated with BD in Australia were investigated and included three isolates each of *Neofusicoccum parvum*, *Dothiorella iberica*, *Spencermartinsia viticola* and four isolates of *Diplodia seriata*. All isolates were cultured in Czapek Dox broth and their culture filtrates extracted at different pH's with ethyl acetate. All filtrates and organic extracts were assayed for phytotoxicity using grapevine leaves and tomato seedlings. The results showed that the degree of toxicity differed between isolates, species and assay conditions. The secondary metabolites from the organic extracts produced from the 13 culture filtrates were screened using thin layer chromatography (TLC). From the bioassay and TLC results, *D. seriata* (H141a), *Do. iberica* (L5) and *S. viticola* (L19) were further selected, and the isolation, chemical and biological characterisation of their secondary metabolites is in progress.

POSTER 111

The use of GFP-transformed *Fusarium oxysporum* f.sp. *cubense* Sub-Tropical Race 4 to study the pathogen's movement through entire banana plants.

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Panama disease, caused by the fungus *Fusarium oxysporum* f.sp. *cubense* (*Foc*), is one of the most important and destructive diseases in banana crops worldwide. There has been numerous studies into the infection process of this soil-borne pathogen, however, the extent of research into the movement of the pathogen through the rhizome and into the rest of the plant is limited. Furthermore, little is known about the lifecycle of the pathogen once it reaches the aerial components of the plant. In controlled PC2 glasshouse conditions, Cavendish 'Williams' (*Musa* AAA) and Lady Finger (*Musa* AAB) banana plants were inoculated using *Foc* Sub-tropical Race 4 (*Foc*STR4), which had been genetically transformed with a Green Fluorescent Protein (GFP). Every 10 days for 80 days, the plants were destructively harvested and examined using confocal laser microscopy to visualise the GFP *Foc*. All areas of the plant were examined, from the midrib of the leaves above the throat, through to the root tips. The results of this study show that the pathogen infects the plant through the roots and moves through the outer leaf sheaths of the pseudostem prior to external symptoms being presented. The movement of *Foc* was confined to both discoloured and healthy xylem vessels, and moved in an upward direction to the midrib in the apex of the leaf blade. Production of chlamydospores occurred within the air chambers of senescing leaf sheaths, while sporodochia formation and hyphal growth developed on the outside of senescing leaf sheaths. These results generate a greater understanding of the epidemiology of *Foc*, providing much needed knowledge to assist in the way infestations of Panama disease are managed in the future, as well as ongoing on-farm hygiene and biosecurity management.

POSTER 112

Molecular basis of response to (sub)lethal stresses

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With the loss of chemical control options such as Fenthion and Dimethoate for postharvest treatment of horticulture commodities susceptible to fruit fly infestation, it has become even more important to understand how stress-based control techniques such as heat, cold and irradiation can be used most effectively for disinfestation.

This project aims to explore the stress-induced molecular response of two fruit fly species of horticultural significance, Mediterranean fruit fly (*Ceratitis capitata*) and Queensland fruit fly (*Bactrocera tryoni*), by characterising the cellular pathways involved in both overall and stressor-specific responses. Three specific steps are: 1) to conduct bioassays and RNAseq across developmental stages and recovery time to identify candidate genes responding to stress or recovery, 2) to construct gene networks to identify pathway membership and relationships by bioinformatics analysis, and 3) to validate specific genes' involvement in stress response and resistance using functional assays.

To date, we have completed the heat, cold and irradiation bioassays on both *C. capitata* and *B. tryoni* larvae and eggs. RNA samples were collected, sequenced and analyzed. A group of candidate genes have been identified for molecular pathway as well as "marker" genes involved in heat and cold treatments. We aim to provide the wider research community with tephritid stress-response networks which will aid in the further development of tools to safeguard our fruit and vegetable crops.

POSTER 113

Build Better Biosecurity... for the cost of a stamp

Jordan Bailey¹

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All of the records in the Australian Plant Pest Database, almost 1.3M, are backed up by the physical specimen. This is to verify that the data served up by these collections are correct; it can be questioned, and most questions can be answered by returning to the original specimen for examination. A multitude of biosecurity related activities and research studies make use of specimens in scientific collections. And any work that has ever used these specimens was made possible by the actions of the person that submitted that specimen for accessioning.

Submitting specimens to collections used to be common practice but this effort has dwindled, despite a steep increase in collections based research. This isn't just affecting biosecurity collections, it is a trend in natural history collections the world over (Prather, 2004). Although curators and taxonomist have worked to shine light on their current collections, through digitisation and online-access, the fact still remains that scientific collections in recent decades have suffered from mass decline in specimen submission (Taylor, 2014), meaning that our current reference base is set to become outdated (Prather, 2004).

The biosecurity community is in a very unique position to make a difference to their collections. Unlike scientists working on obscure fungi only found in the depths of the Amazon jungle, we are working with a range of plant pests and diseases every day. They are delivered right to our door. All that is needed is a little bit of effort to preserve that specimen and contribute it to a biosecurity collection... for the cost of a stamp.

Visit this poster to learn more about Biosecurity Collections, how you can contribute, what we can do for you, and how the collections community is modernising this centuries old science.

POSTER 114

Capturing diversity of wheat powdery mildew in Western Australia

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Wheat powdery mildew is a fungal disease caused by *Blumeria graminis* f. sp. *tritici*. The disease can be responsible for yield losses up to 25% in Australia. In Western Australia, it has re-emerged as a disease of importance in recent wheat cropping seasons.

The epidemiology of the polycyclic biotrophic disease suggests a high likelihood of significant diversity within the population throughout wheat growing areas of Western Australia (WA). Light air-borne spores, produced prolifically, are easily transported large distances. There are numerous selection factors that can impact genetic diversity within mildew populations including environmental conditions such as temperature, resistance genes deployed in wheat varieties grown and fungicide use or over-use.

Little is known about the diversity of powdery mildew pathotypes in WA. This information is important in the development of wheat lines with effective, enduring resistance and for management strategies of existing commercial varieties.

Recent work undertaken by Golzar et al. (2016) tested a combined mixture of numerous WA pathotypes against a differential set of wheat lines with a suite of resistance genes. The genes Pm2, 3a, 3e, 4a, 13 and 27 were found to be effective in Western Australia through screening plant response at seedling and adult plant growth stage. In our current study, the same suite of differential wheat lines and a sub-set of popular commercially grown wheat varieties are being screened at seedling growth stage against individual isolates with a goal to determine any regional diversity in virulence. In 2016, 15 powdery mildew isolates were collected from locations spanning the WA wheatbelt. Initial testing has confirmed previously identified effective resistance genes, though has not highlighted the expected regional population diversity. This may be due in no small way to the dominance of the susceptible wheat variety "Mace", which is >60% of all wheat area planted in WA.

POSTER 115

Is there a correlation between virulence and VCG type in Australian *Verticillium dahliae* isolates?

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Verticillium dahliae is a soil-borne ascomycete that infects hundreds of agriculturally important crops globally, including cotton. In Australia, cotton is a continuously growing billion-dollar industry. Cotton yields have increased from 500 kg per hectare in the 1960's, to 2000 kg per hectare in 2013. Sustainability and growth of the cotton industry is reliant on improved cotton varieties, soil and water management, and weed, insect and disease management.

Verticillium dahliae, the pathogen that causes Verticillium wilt in cotton, has been described according to vegetative compatibility groups (VCGs). Disease symptoms in different plant hosts are generally associated with different VCGs, and internationally, cotton is reported to be most impacted by the defoliating VCG1A. This strain causes severe disease including defoliation of cotton plants, while VCGs 2A and 4B are non-defoliating and cause less severe disease symptoms. Until recently, VCG1A was not reported to be present in Australia, and in the 2015/2016 season was not showing the expression of symptoms observed internationally. In Australia, on a disease severity scale there have been reports of VCG2A's are causing more damage than the defoliating VCG1A.

This Australian disease conundrum raises the question of the association between VCG and pathogenicity. This is being addressed through molecular and pathogenicity assays on isolates collected from infected plants in commercial cotton fields over the past 30 years of NSW disease surveys. Through improved understanding of this pathogen and its presence in Australia we can work towards improved diagnostic detection and crop management.

POSTER 116

The *Phytophthora infestans* population causing potato late blight in Australia consists of a unique novel genotype.

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Potato late blight (PLB) has become a resurgent disease following the introduction and spread of the A2 mating type and new strains of the A1 mating type of *P. infestans* into North America and Europe. New aggressive populations of the A1 and A2 mating types have now spread around the globe with the trade in seed potatoes. In Australia, PLB occurs infrequently in NSW, South Australia, Victoria, but more commonly in northern Tasmania, during atypical summer weather patterns characterised by periods of moist air combined with stagnant or slow moving depressions.

In an early study, 20 isolates of *P. infestans* collected in Tasmania during PLB epidemics in the summers of 1998/99 and 99/2000 were characterised by RG57 probe analysis as single strain and of the A1 mating type. Diseased leaflets collected during PLB epidemics in 2010/11 and 2011/12 from NSW, Victoria and Tasmania (202 samples) were preserved as leaf squash preparations on FTA™ Classic Cards for microsatellite genotyping. Simple sequence repeat (SSR) genotypes were determined using the standard 12-plex Euroblight set of *P. infestans* SSRs. Initial analysis of 125 samples identified a single novel genotype of *P. infestans* not found anywhere else in the world and identical to the genotype of freeze-dried Australian isolates collected a decade previously. Consistent evidence of a single lineage confirms that Australia does not have the A2 mating type, or the new aggressive strains of both the A1 and A2 mating types of *P. infestans* found elsewhere in the world.

POSTER 117

Victoria's Plant Pest and Pathogen Reference Collections

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Agriculture Victoria, Bundoora, holds the most comprehensive reference collection of plant pests and pathogens in Victoria. Specimens include insects and other invertebrates, fungal diseases, nematodes, bacteria and viruses of significance to agriculture, agroforestry, biodiversity and other science disciplines. This unique collection is curated and maintained by dedicated staff within the taxonomy team, which forms part of the Invertebrate Sciences and Plant Pathology sections of the Agriculture Victoria Research Division. The reference collections consist of (1) Victorian Plant Pathogen Herbarium (VPRI), (2) Victorian Plant Parasitic Nematode Collection (VPNC) and (3) Victorian Agricultural Insect Collection (VAIC). Between them, they hold more than 250,000 specimens of agricultural significance collected between the 1890s to the present day. Most are databased and feed into the national Australasian Plant Pest Database which provides evidence of pest/pathogen status to inform Australia's market access and trade decisions.

POSTER 118

Investigating luteoviridae viruses in pulse crops in Australia reveals diversity and recombination events

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The main pulse legume crops grown in Australia are chickpea (*Cicer arietinum*), lupin (*Lupinus angustifolius*, *L. albus*), faba bean (*Vicia faba*), field pea (*Pisum sativum*), lentil (*Lens culinaris*) and mung bean (*Vigna radiata*). The main viruses infecting pulse crops include the *Poleroviruses*; Turnip yellows virus (TuYV) and Phasey bean mild yellow virus (PBMV), the *Luteoviruses*; Soybean dwarf virus (SbDV) and Bean Leafroll virus (BLRV), the *Alfamovirus*; Alfalfa mosaic virus (AMV), the *Cucumovirus*; Cucumber mosaic virus (CMV) and the *Potyvirus*; Bean yellow mosaic virus (BYMV). For many pulse crops the *Luteoviridae* viruses (includes *Poleroviruses* and *Luteoviruses*) are the most important and poorly understood.

Next Generation Sequencing (NGS) has enabled viral diversity at the whole genome level to be investigated. In this paper we focus on the diversity of TuYV and TuYV-like viruses. Analysis of samples initially thought to be TuYV from infected pulse crops, shows there is a high degree of genetic variation. A few different TuYV-like variants have been found, some of which are recombinants containing a fragment of TuYV and appear to represent distinct species.

For one new recombinant virus identified we propose the name, Chickpea red leaf recombinant virus (CRLRV). This virus has been found throughout northern New South Wales in chickpea, lentils, field pea, faba bean, and marshmallow weed (*Malva parviflora*). Analysis of CRLRV genome showed a recombination event in the intergenic non-coding region between open reading frame (ORF) 2 and ORF-3. The 3' end of CRLRV, which includes ORF-3, -4 and -5, share around 92% nucleotide identity to TuYV (Genbank: NC_003743). The 5' end, ORF-0, -1 and -2, is from a currently unidentified virus and shared approximately 66% nt identity to *Chickpea chlorotic stunt virus* (CpCSV).

Viral diversity and recombination events reiterate the need for good diagnostic techniques to ensure effective resistance breeding and disease management.

POSTER 119

Does genetic diversity of *Ceratobasidium theobromae*, the cause of vascular streak dieback disease of cocoa, explain symptom variation?

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Abstract

Vascular-streak dieback (VSD), caused by *Ceratobasidium theobromae* (Tulasnelloid; Basidiomycetes), is a new-encounter disease of cocoa with a significant impact on the industry in Indonesia, Southeast Asia and parts of the Pacific. The pathogen can defoliate mature trees and kill young trees. The typical characteristic chlorotic, green spotted leaf symptom was discovered in PNG 1960s, however new symptoms of leaf necrosis were reported across the distribution of the disease in the mid-2000s. Both symptoms can exist in the same branch. A preliminary study suggested that two pathogens may be associated with these different symptoms, but this was based on a small number of observations. The current study aims to explain why leaf symptoms vary, and to identify the as yet unidentified original host. Hierarchical sampling supported by GPS mapping collected 1,500 petioles from chlorotic and necrotic symptomatic to asymptomatic leaves across Sulawesi. DNA was extracted and pathogen presence confirmed using primers specific for basidiomycetes; forward (ITS1F: 5-CTTGGTCATTTAGAGGAAGTAA-3) and reverse primers (ITS4B: 5-CAGGAGACTTGTACAGGTCCAG-3)] then for *C. theobromae*; forward (Than ITS1: (5'-GAGTCTTGGCAGTTGCTG-3') and reverse primers (Than ITS2: 5'-AGAAGCGGTCATCTGTA-3'). The Nucleic acid sequence variation will be determined using SSR (microsatellite) analysis of ITS sequences, and variation linked to sampling location and symptom type.

POSTER 120

Population dynamics and genetic structure of the wheat crown rot pathogen *Fusarium pseudograminearum* in Western Australia

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Fusarium crown rot (FCR) caused by *Fusarium pseudograminearum* (*Fp*) is an important disease of wheat and barley. FCR can cause significant yield losses and reduce grain quality in many countries, including Australia. To examine population dynamics of this pathogen, 18 locations covering wheat-growing regions in Western Australia (WA) was surveyed in 2008 and 2015. An intensive sampling was conducted in two of the sites (Tammin and Karlgarin) with moderate to high FCR disease incidence. Survey results revealed significant increase in *Fp* frequency over the 7-year period. Over 86 % of fungi isolated in 2015 were *Fp* compared with over 58% in 2008. To assess mating type idiomorph composition, trichothecene chemotype and population genetic structure, selected *Fp* isolates from 2008 (n= 135) were compared with those from 2015 (n=165). These analyses revealed a shift from *MAT1-1* in 2008 towards *MAT1-2* mating type in 2015 and this was particularly evident in the two intensively sampled sites, Tammin and Karlgarin. A chemotype-specific PCR assay indicated that over 95% of *Fp* isolates show the 3ADON trichothecene chemotype. Additionally, seven isolates that showed 15ADON chemotype were identified for the first time in Australia. The population genetic structure of 300 isolates was determined using 21 cleaved amplified polymorphic sequences markers and revealed a shift in genotypic diversity, increasing between the 2008 and 2015 populations. This study, which examined FCR population dynamics, mating types, chemotypes, and genotypic structures in WA, will contribute to the management of this important cereal disease in Australia.

POSTER 121

Diversity and structure of *Colletotrichum tanacetii* populations in Pyrethrum

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Colletotrichum tanacetii is an emerging fungal pathogen in commercially grown Pyrethrum (*Tanacetum cinerariifolium*) fields in Australia. Whole genome assembly of *C. tanacetii* was used to develop a polymorphic microsatellite marker library of 11 loci which was then used to determine the diversity and structure of pathogen populations. Genotyping 191 isolates from hierarchical sampling conducted in 2013, from three sites in Tasmania and in 2016, from two in Tasmania and one in Victoria revealed an increase in overall haploid diversity over time. The least pairwise population distance was between two sites in the same locality; Table Cape, suggesting some level of genetic structure. However, the second least distance was between two sites from 2016 in Victoria and Tasmania. Low number of private alleles between these two sites suggested the populations may be sharing a source of inoculum causing them to have a similar genetic composition despite their geographical distribution. Analysis of molecular variance showed the presence of significant differentiation only within samples but showed no evidence of structure over year, localities or fields, supporting the null hypothesis of a single panmictic population. Linkage disequilibrium analysis revealed that recombination was a widespread phenomenon in all populations from Tasmania where populations were not significantly different from randomised hypothetical datasets, providing evidence of sexual reproduction. Even though the Victorian population appeared asexual, after clone correcting that too confirmed to be in linkage equilibrium, suggesting low level of sexual reproduction. However, the presence of only 176 original multilocus genotypes from 191 haploid individuals indicated partial clonality, hence a mixed reproduction system. Pathogen populations with less geographic differentiation and mixed reproduction strategy have higher evolutionary risk. In *C. tanacetii*, this is elevated by producing both splash dispersed and airborne spores and a heterothallic mating strategy. Therefore these findings emphasise the necessity for conducting strict management practices at planting.

POSTER 122

Pythium and *Phytophthora* diversity in a *Pinus radiata* nursery from New Zealand

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Forest nurseries frequently face on-going issues with root-borne diseases caused by oomycetes. Typically diagnostics is based on soil baiting to isolate the organisms potentially associated with disease. This method, while good for detecting viable and active members of the soil, does have limitations including long processing time, seasonal variations and culture-based isolation bias. Next generation sequencing data provides outstanding opportunities for characterisation of microbial communities and diagnostics. This study aims to develop methods for characterisation of the prevalence of oomycetes such as *Pythium* and *Phytophthora* in nursery soils and to investigate the potential for NGS in downstream diagnostics applications. Soil samples were collected from a forest nursery and baiting was used to isolate species of oomycetes using selective media. ITS and Cox2 sequences were used to identify the isolates. *Phytophthora cinnamomi*, and *Pythium mamillatum* were the most commonly isolated species from three different nursery beds. Soil samples were also analysed to determine the pH, and total carbon, nitrogen and phosphorus levels, as well as performing DNA extraction for NGS analysis. The ability to detect taxa present but only in low numbers would be beneficial for nursery-based diagnostics, development of nursery hygiene practises and overall better detection of oomycetes from environmental samples. Thus we are currently assessing the ability of soil and root NGS analysis for diagnostic purposes.

POSTER 123

***Colletotrichum* species associated with anthracnose of subtropical and tropical fruit in Australia**

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Anthracnose caused by *Colletotrichum* spp. is arguably the most serious postharvest disease of subtropical and tropical fruit crops in high rainfall production areas. Fungi within the *C. gloeosporioides* species complex, and to a lesser extent the *C. acutatum* species complex, have historically been reported as principal causal agents of anthracnose in many of these hosts. Recent taxonomic revisions of the genus *Colletotrichum* using molecular phylogenetic techniques have however delineated new species within these broad species complexes. Despite this, many *Colletotrichum* accessions held in Australian collections still remain to be verified by molecular methods.

Over 300 isolates of *Colletotrichum* species from a range of subtropical and tropical fruit hosts were selected for this study. DNA extracted from these isolates were subjected to sequencing of partial gene loci of ITS, TUB2, GAPDH, GS and CAL. A relatively high degree of heterogeneity was seen in accessions from avocado, lychee, papaya and passionfruit. These isolates were assigned to a number of different taxa including *C. siamense*, *C. alienum*, *C. fructicola*, *C. queenslandicum*, *C. gloeosporioides*, *C. karstii*, *C. theobromicola*, *C. acutatum*, *C. simmondsii* and *C. fioriniae*. Isolates from mango displayed a much lower degree of heterogeneity, and were mostly identified as *C. asianum*. All of the isolates from banana were *C. musae*. Species were also assigned to a limited number of isolates of *Colletotrichum* from rambutan, jackfruit, mangosteen, carambola and custard apple. Several putative new species were found that are the subject of ongoing taxonomic study.

POSTER 124

High prevalence of a genetic lineage of the *Ralstonia solanacearum* species complex in the Southwest Indian Ocean islands inferred by phylogenetic and genotyping approaches

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In the Southwest Indian Ocean (SWIO) islands (Comoros, Mauritius, Mayotte, Reunion, Rodrigues and Seychelles), bacterial wilt caused by the *Ralstonia solanacearum* species complex (RSSC) is hampering many sustainable and cash crop productions. To thoroughly analyze the genetic diversity and population structure of the RSSC in the SWIO, we conducted a wide sampling survey that yielded 1871 isolates, mainly from solanaceous species. Phylogenetic assignation of isolates showed 88% of phylotype I that was prevalent in each SWIO islands; 9% of phylotype II and 3% of phylotype III that were only found in Reunion. Phylotype IV (2 strains), was reported in Mauritius, representing the first report of this group in SWIO. Partial *egl* sequencing, based on a selection of 143 strains covering the geographic and host diversity, inferred 10 sequevars: I-13, I-14, I-15, I-18, I-31, I-33, IIA-36, IIB-1, III-19, and IV-10. A Multilocus Sequence Analysis (MLSA) scheme based on the partial sequencing of 6 housekeeping genes (*gdhA*, *gyrB*, *rplB*, *leuS*, *adk*, and *mutS*) and 1 virulence-associated gene (*egl*) inferred the phylogenetic relationships between these 143 SWIO strains and 91 worldwide RSSC reference strains. Phylotype I was the most genetically diverse and recombinogenic, although recombination events were detected among all phylotypes. The Multilocus Sequence Typing (MLST) scheme has identified 31 sequence types (ST) with variable geographic distributions in SWIO. Outstanding epidemiologic feature was STI-13 (sequevar I-31) that was overrepresented in the SWIO, which obviously mirror a genetic lineage that have strongly adapted to SWIO environment. To further study the population structure of RSSC in SWIO, we developed a MultiLocus Variable number tandem repeat Analysis (MLVA) scheme based on 19 VNTR markers. This work, highlighting notably genetic links between African and SWIO strains, provides the basis for epidemiological surveillance of RSSC and bacterial wilt management in SWIO.

POSTER 125

Genetic and pathogenicity risk of a highly adapted clonal haplotype within the Australian *Ascochyta rabiei* population

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The Australian *Ascochyta rabiei* population is genetically narrow with only one mating type gene detected to date, potentially precluding substantial evolution through recombination. However, a large diversity in aggressiveness exists. In an effort to better understand the risk from selective adaptation to currently used resistance sources and chemical control strategies, the population was examined in detail. For this, a total of 617 isolates were quasi-hierarchically sampled between 2013-2015 across all major Australian chickpea growing regions and commonly grown cultivars. Although a large number of haplotypes were identified (103) through short sequence repeat (SSR) genotyping, overall low population diversity ($H=0.064$) and genotypic diversity ($D=0.58$) was detected. More than 63% of the isolates assessed were of a single dominant haplotype (ARH01). Disease screening on a differential host set, including three commonly deployed resistance sources, revealed distinct aggressiveness among the isolates, with 17% of all isolates identified as highly aggressive. Almost 75% of these were of the ARH01 haplotype. A similar pattern was observed at the host level, with 46% of all isolates collected from the commonly grown cultivar Genesis090 (classified as "resistant" during the term of collection) identified as highly aggressive. Of these, 63% belonged to the ARH01 haplotype.

Isolates from ARH01 haplotype pose a significant risk to the Australian chickpea industry, being not only widely adapted to the diverse agro-geographical environments of the Australian chickpea growing regions, but also containing a disproportionately large number of aggressive isolates, indicating fitness to survive and replicate on the best resistance sources in the Australian germplasm. The highest risk isolates are provided to the national chickpea breeding program for screening breeding materials and to validate markers for resistance selection, this assists breeders to select for sustainable resistance and farmers to adopt best disease management practice necessary to maintain good crop yields and quality.

POSTER 126

Invasive Spear Thistle Weed Detection and Zoysia grass Mapping Using UAV Aerial Imagery

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Manned aircraft and satellite remote sensing are very useful for weed detection at late growing 16 stages (e.g. flowering or senescence) (Pérez-Ortiz et al., 2016, Kelly, 2013). UAV imagery on the other hand can be very useful for early weed detection (e.g. seedling) (Peña et al., 2013, Torres-Sánchez et al., 2015). Each platform has its utility according to the problem. In general, UAVs can provide higher spatial resolution at lower operational costs. This paper describes a UAV platform, sensor payload and post-processing pipeline customized to detect Spear Thistle, Black Thistle (*Cirsium vulgare*) invasive weed found in crops and unmanaged farmland. A computer vision algorithm was developed to differentiate Spear Thistle weed and Zoysia grass. The system creates GPS tabulated locations for detection weed. The results are measured based on the sensitivity and the selectivity of the algorithm. The sensitivity is the ability of the algorithm to identify and detect the true positive target while the selectivity is the capability of the algorithm to filter out the false negatives for detection targets. Results have shown a 95% sensitivity and 98% selectivity when the height above ground is 5 m, 90% sensitivity and 94.5% selectivity when the height above ground is 7 m and 80% sensitivity and 85% selectivity when the height above ground is 15 m. The detection map provides useful information as a cost-effective decision-making tool to estimate herbicide requirements and overall costs of weed management. The Methodology presented in this paper has potential benefits in the fields of precision agriculture, geospatial referencing and weed detection which could benefit from early detection.

**Molecular, biological and ecological assessment of cryptic species diversity within the whitefly
Bemisia tabaci species complex**

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Whiteflies belonging to the *Bemisia tabaci* species complex can be highly damaging pests to a wide range of field and greenhouse crops worldwide. Strategies to manage this pest complex, however, are complicated by the biological diversity that exists in this group of morphologically-cryptic, putative species. The systematic framework relies currently on a partial sequence of the mitochondrial cytochrome oxidase I gene (mtCOI) and a set of crossing experiments showing the reproductive isolation among some of the >34 putative species. A threshold of 3.5% pairwise sequence divergence of the partial mtCOI has been proposed as a method for grouping populations into species.

In our study, we investigated the species status of the highly devastating 'Mediterranean' (MED) genetic group, currently recognised as having four intra-species subclades, called Q1, Q2, Q3 and ASL. Of these, MED-Q1 and MED-Q2 have spread invasively outside their indigenous regions and are often resistant to a range of insecticides.

We performed (i) reciprocal crossing experiments to investigate reproductive compatibility among three MED subclades, (ii) molecular screening for bacterial endosymbionts, (iii) genome sequencing from single males, and (iv) bioassays to assess their host-plant range and performance.

Our results showed reproductive incompatibility and differences in host-plant performance within MED. These data will be presented in the context of how differences in endosymbiont content may influence biology and reproductive compatibility. The utility of next-generation genome sequencing in analysing the metagenome and identifying pseudogenes will also be discussed.

Our study reviews the method of species delimitation currently applied to the *B. tabaci* complex and explores the way forward in resolving systematics of cryptic species complexes. Knowledge about the number and biology of cryptic species will allow more targeted and efficient design of pest control strategies.

POSTER 128

Plant pest impacts: a common set of metrics

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Agricultural stakeholders are in need of a common set of metrics to evaluate plant pests. We propose a classification system that articulates, defines and classifies the impacts of pest species (alien and native) in plant production systems. Criteria were defined by magnitude of impact (historical, current or potential), with a focus on production specific metrics. Metrics were identified and criteria defined through consideration of economic assessment parameters, risk assessment guidance tools, discussions with pest risk assessment practitioners and recent advances in environmental impact classification schemes. Twenty metrics were identified. Host crop value, market access, feasibility of management and reversibility were identified as disruptor metrics, likely to influence overall classification by at least twice that of other metrics. Utility of the metric system is explored through application to Australian and New Zealand plant pests. The system has been designed to facilitate transparency, flexibility (in development and application) and harmonisation of pest management and prioritisation across spatial scales and jurisdictions.

POSTER 129

A Genome Wide Association Study of Resistance to Stripe Rust in the Vavilov Wheat Collection

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Puccinia striiformis f. sp. *tritici* (*Pst*), the causal pathogen of stripe rust (YR), poses a major threat to global wheat production. The emergence of highly virulent races highlights the need for new sources of genetic resistance in wheat breeding programs. Landraces and historical cultivars preserved in seed banks represent a major source of genetic variation, but relatively unexplored for novel resistances. In this study, we performed a genome-wide association study (GWAS) using 295 bread wheat accessions from the N.I. Vavilov Institute of Plant Genetic Resources (Saint Petersburg, Russia) to identify new sources of YR resistance. The diversity panel was genotyped with DArTseq GBS, and 13,333 polymorphic markers were used for analyses. Disease response was evaluated at the seedling stage in the glass house and adult stage in the field over three years using highly virulent pathotype (134E16 A+ Yr17+ Yr27), which is prevalent throughout the eastern wheat growing region of Australia. GWAS identified 96 significant marker-trait associations ($-\log_{10} P$ value >3) which represented 67 quantitative trait loci (QTL). Of these 53 QTL were associated with adult plant resistance (APR) and 10 were associated with seedling specific resistance. Moreover, four QTL were associated with all-stage resistance. A total of 12 QTL were deemed novel and were positioned on chromosomes 1D, 3A, 3D, 4A, 4B, 5A, 6B, 7A and 7D. Nine QTL were co-located with cataloged *Yr* genes while 46 QTL co-located with recently published GWAS studies. These results demonstrate that the Vavilov collection provides a rich source of new alleles which can be used to broaden the genetic base of YR resistance in modern wheat germplasm.

POSTER 130

Investigations on the resistance of *Saccharum spontaneum* and its backcross progenies on root knot and root lesion nematodes

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Plant-parasitic nematodes are a major constraint to sugarcane production in Australia. Previous research has found that the wild canes *Erianthus arundinaceus* and some of its crosses with commercial sugarcane have resistance to both root-knot nematode (*Meloidogyne javanica*) and root lesion nematode (*Pratylenchus zaei*). No information was available on *Saccharum spontaneum* clones, a close relative of modern sugarcane, on their resistance to sugarcane nematodes. Thirteen wild clones of *S. spontaneum* screened two times against root knot nematodes and one time against root lesion nematodes in 2012 and 2013 in a glasshouse at SRA Woodford Pathology farm. Further, in 2016 to 2017, 20 basic *S. spontaneum* clones from SRA Meringa germplasm collection were screened against both types of nematodes. Nematode reproduction (both *M. javanica* and *P. zaei*) were significantly lower on basic *S. spontaneum* clones compared to backcross progenies and commercial standards in all trials. Some progenies from *S. spontaneum* and commercial varieties also had low nematode reproduction. This study provided a valuable information on future development of nematode resistance variety for the Australian sugar industry. Details of the results will be presented.

POSTER 131

Delivering novel genetic solutions to control yellow spot in wheat

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Yellow spot or tan spot is a serious disease of wheat crops globally. It is caused by *Pyrenophora tritici-repentis* (*Ptr*) which produces three major necrotrophic effectors (i.e. ToxA, ToxB, ToxC) that interact with specific sensitivity genes in wheat (i.e. *Tsn1*, *Tsc2*, *Tsc1*, respectively). In Australia, *Ptr* Race 1 (ToxA and ToxC) is widespread infecting the majority of modern wheat cultivars, in which *Tsn1* is prevalent. The absence of effector-host-gene interactions is correlated with resistance, but still, there is considerable phenotypic variation in response to *Ptr*. This implies the presence of other genetic factors that may contribute to quantitative forms of resistance, similar to other cereal-pathosystems. Here, we search for novel sources of genetic resistance to *Ptr* Race 1 using 300 diverse wheat accessions originally sourced from the Vavilov Institute. We report a rapid phenotyping protocol utilising accelerated growth conditions (constant light and controlled temperature), suitable for evaluating adult plant response to *Ptr*. The rapid phenotyping technique, ToxA bioassay, and field assessments were performed to screen the diverse wheat accessions. Using DArT-seq DNA markers, genome-wide association studies identified novel genomic regions conferring resistance. Interestingly, some accessions carried *Tsn1*, yet displayed a high degree of resistance to ToxA-producing races. To further examine these genetic factors, a recombinant inbred line (RIL) mapping population was generated, which was fixed for *Tsn1*, yet segregating for resistance. The RIL was genotyped using DArT-seq and evaluated by *Ptr* inoculations and ToxA bioassay. Further analysis of this key population will hopefully improve our understanding of the genetic mechanisms that may be harnessed in the presence of *Tsn1*. Thus, in addition to removing major sensitivity loci that are widespread in elite wheat germplasm, we propose manipulation of host loci that are dominant over *Tsn1* resistance could offer an alternative genetic solution for control of yellow spot disease.

POSTER 132

Characterisation of quantitative trait loci for resistance to two species of root-lesion nematode (*Pratylenchus thornei* and *P. neglectus*) on chromosome 2BS in wheat

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Root-lesion nematodes (RLN) *Pratylenchus thornei* and *P. neglectus* are microscopic, vermiform, migratory, endoparasitic eelworms that cause root damage in wheat (*Triticum aestivum* L.) in many countries. In Australia, high populations of *P. thornei* and *P. neglectus* can cause up to 85% and 27% yield loss in intolerant wheat varieties with annual loss in national wheat production estimated at \$50 million and \$73 million, respectively. Furthermore, mixed RLN populations are common with both species present in 28% of fields surveyed in the Northern region. The most efficient strategy for improving on-farm management of RLN relies on providing wheat growers with cultivars with higher levels of resistance to both species of RLN. Quantitative trait loci (QTL) on chromosome 2BS, conferring dual resistance to both *P. thornei* and *P. neglectus* will be fine mapped in a population derived from the synthetic hexaploid wheat CPI133872. Markers flanking the 2BS QTL will be used to screen 1,000 F2 individuals, using DNA extracted from half seeds. The remaining half seeds of selected recombinant genotypes will be grown for phenotypic evaluation. Resolving the map position of the 2BS target QTL is an important step towards identifying the genes underlying resistance to *P. thornei* and *P. neglectus*. Introgression of genes responsible for dual nematode resistance into commercially used cultivars will not only be economically and practically significant to breeders, but it will also enrich diversity of RLN resistance in Australian wheat cultivars. Furthermore, candidate gene analysis of this genome region will give insights into biochemical mechanisms of resistance to RLN, and potentially lead to development of novel control strategies for both species of RLN.

POSTER 133

Root lesion nematode, *Pratylenchus neglectus* and *P. thornei* pulse resistance ratings: A national approach

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A national approach to the assignment of root lesion nematode (*Pratylenchus thornei* and *P. neglectus*) resistance ratings in winter pulses was implemented. Growers can use these ratings to select rotational crops with resistance, and therefore reduce nematode densities in the field, and the yield loss in following intolerant cereal crops.

Varietal resistance/susceptibility ratings are communicated to industry through publication in disease guides. In pulses, prior to a national rating system, individual states released separate crop/variety ratings. These ratings were often based on only a few experiments, either in the glasshouse or field. Recently, a system similar to the system used in cereals has been adopted in pulses. This has collated nematode resistance data from field or controlled environment experiments nationally, with resistance ratings applied to individual varieties by pathologists. Breeders are then consulted on the ratings, with the consensus ratings released to industry. National ratings have been developed for field pea, lentil, chickpea and faba bean.

This national approach to resistance ratings has accelerated their release and improved accuracy. The process highlighted variation in resistance to root lesion nematodes within each crop, and the importance of ongoing screening of varieties to root lesion nematodes. Varieties that will provide control of root lesion nematodes to growers were identified, allowing growers to select varieties that will reduce nematode densities and yield losses. The collated data also allows breeders and pre-breeders to identify and use sources of resistance in their breeding programs.

POSTER 134

Improving resistance to tan spot (yellow spot) through pyramiding new resistance genes

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Although good progress has been made internationally to understand tan spot (TS) (syn. yellow spot), caused by *Pyrenophora tritici repentis*, resistance in wheat, relatively few resistance genes have been identified in Australian germplasm. Under a current national project, major effort was made to identify novel resistance genes and develop a series of fixed lines, each carrying genes from 2 or 3 resistance sources in elite Australian backgrounds that can be used as parents in resistance breeding.

Two doubled haploid (DH) mapping populations Calingiri/Wyalkatchem (C/W) (247 DH lines) fixed for the ToxA-insensitivity allele *tsn1* and IGW2574/Annuello (I/W) (97 DH lines) fixed for the ToxA-sensitivity allele *Tsn1* were used in this study. The populations were screened for TS resistance at various growth stages, environments and national sites. Frequency distributions for TS severity traits were continuous. Major quantitative trait loci (QTL) were detected on chromosomes 2A and 1A. Each QTL had effects at various growth stages and in experiments conducted at various locations in Australia. QTL on 2A explained up to 29.2% of the genotypic variation in C/W (resistance contributed by Wyalkatchem). QTL on 1A explained up to 28.1% of the genotypic variation in I/A (resistance contributed by Annuello). These resistance loci were stacked along with *tsn1* using single seed descent and marker assisted selection. Thirty two F₅ lines were developed which are triple homozygotes at the three TS resistance loci and are also fixed for the *vrn-1* locus.

Sixteen of these F₅ lines were screened for TS resistance at various growth stages and environments in Western Australia. Lines expressed significantly higher resistance than parents and grandparents at both seedling and adult plant stages that was effective in various environments. These lines are important resources that can be used by breeders for rapid development of varieties with high levels of resistance.

POSTER 135

Quantitative trait loci for resistance to *Stagonospora nodorum* blotch in wheat

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Stagonospora nodorum blotch (SNB) is an important fungal disease of wheat in Western Australia. Many resistance donors are in poor quality backgrounds. It is crucial to continue search for additional genes for effective improvement of resistance.

This research focussed on different resistant sources in five doubled haploid mapping populations: IGW2471/Wyalkatchem (I/W), AGT Young/Aus33414 (Y/A), BT Schomburgk/AUS33384 (S/A), Machete/Magenta (M/M) and CPI33842/Janz (C/J). Each population was assessed at least twice during 2012 to 2015.

Trials were conducted in the field and/or controlled environment using a randomised block design with three replications. Inoculation was done using a mixture of SNB isolates and the same inoculum mixture was used in repeated trials. Plants were assessed for percentage leaf area diseased at seedling and at head emergence. Field trials for I/W were conducted in a split-plot design of infected and fungicide protected main plots and were also assessed for relative grain weight (RGW). Populations showed continuous distribution and transgressive segregation for various traits. Correlations between assessments made at the same growth stage in different years were moderate to high ($r = 0.4$ to 0.8). Correlations between assessments made at seedling and adult stages within the same trial were low to moderate ($r = 0.1$ to 0.7) indicating independent inheritance of resistance at the two growth stages.

Loci for SNB leaf resistance at the adult plant stage were detected on chromosomes 2B, 2D, 3B, 4B, 5A and 6B in I/W; on 2B, 4D, 6A and 7D in Y/A; on 1B, 2D and 6B in S/A; on 3B in M/M and on 1B in C/J. Loci for SNB seedling resistance were detected on 2B in I/W; on 5D in Y/A; on 5B in M/M and on 3B in C/J. Loci for SNB resistance measured as RGW were detected on 2B, 3A, 5B and 7B in I/W.

POSTER 136

Quantitative trait loci for resistance to *Blumeria graminis* f. sp. *tritici* in wheat

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Powdery mildew (*Pm*) caused by *Blumeria graminis* f. sp. *tritici* (syn. *Erysiphe graminis* f. sp. *tritici*) is an important foliar disease of common wheat (*Triticum aestivum* L.) worldwide. The extensive cultivation of susceptible wheat varieties over the years combined with favourable environmental factors has favoured *Pm* and its economic importance has become more evident in recent years in Western Australia (WA). Deployment of resistant genes and gene pyramiding for resistance to *Pm* is desirable to combat rapid changes in pathogen virulence.

The aim of this research was to find QTL for *Pm* resistance in two double haploid mapping populations (Machete/Magenta and Calingiri/Wyalkatchem). Each population was phenotyped twice during 2012 to 2015.

Trials were conducted under a controlled environment using a randomised complete block design in duplicate. *Pm* isolates were collected from various locations across WA and a composite inoculum was used for inoculation. The composite of *Pm* isolates varied for the two phenotyping assessments. Plants were assessed at head emergence for percentage leaf area diseased on flag leaf and the leaf below. Frequency distribution of individuals within each population for various levels of *Pm* resistance was continuous and a few lines within each population showed higher levels of resistance than both parents indicating additive gene effects. Correlations between assessments made in different years were low to moderate ($r = 0.2$ to 0.5) possibly due to different inoculum compositions used in different years.

Genetic and QTL analysis showed loci for *Pm* resistance on chromosomes 2A and 2B in population Machete/Magenta and on 6B and 6D in population Calingiri/Wyalkatchem.

POSTER 137

Differences in host pathogen interactions among Australian *Ascochyta rabiei* isolates and chickpea accessions

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The Australian *Ascochyta rabiei* population is diverse in its ability to cause disease on host accessions of differing known levels of resistance, with isolates ranging from low to highly aggressive. In order to strategically manage such a diverse population, information regarding the infection and invasion processes of the pathogen is required. To better understand the diversity in *A. rabiei*-chickpea interactions, an in-depth histopathology study was conducted with isolates with varying aggressiveness on the four host genotypes previously used to characterise them based on gross disease symptomology. Highly replicated microscopy observations revealed significant differences in percentages, timings and rates of conidiospore germinations among all of the isolates and on all of the hosts assessed. In general, the previously characterised highly aggressive isolates germinated and penetrated faster than the low aggressive isolate. However, there were significance differences in these rates, indicating that some highly aggressive isolates are able to germinate and invade the host much faster than others within the first 12 hours of contact. This difference in rates continued through to the development of disease symptomology where it appeared earlier and most severe for aggressive isolates on PBA Hatria and Kyabra. All isolates germinated faster and produced longer germ tubes on the susceptible accession (Kyabra) than on the best resistant accession (ICC3996). Such knowledge may lead to the biological targeting of specific chemical controls and/or aid in decisions around farming practice changes in the presence of this ubiquitous pathosystem

POSTER 138

Taking full advantage of nematode resistance testing for wheat cultivars over multiple experiments

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Root-lesion nematodes (*Pratylenchus thornei* and *P. neglectus*) are microscopic eel-worms that feed on plant roots and can reduce the yield of intolerant wheat cultivars by more than 50% and cost the Australian industry approximately 123 M/yr. A plant is said to be resistant if it has the ability to inhibit nematode multiplication in its roots. In the period from 1996 to 2015, the resistance or susceptibility of more than 900 wheat cultivars was evaluated in glasshouse experiments. The results of these experiments were used to provide comparative ratings of cultivar performance to industry. In total, 22 pot experiments for *P. thornei* and 18 for *P. neglectus* were conducted, where resistance was determined by counting the final number of nematodes in the roots and soil of each pot after 16 weeks growth. The objective of this study was to combine multi-experiment data for resistance testing to deliver improved predictions of cultivar performance relative to separate analyses of individual experiments. The statistical analysis implemented a factor analytic mixed model approach, which was found to provide accurate predictions of the cultivar effects as well as a parsimonious and informative model for the cultivar by experiment interaction. The information obtained from this analysis gave insight into the consistency of cultivar resistance across experiments for each nematode species. It resulted in both an overall measure of nematode-resistance by species, as well as a measure of stability to environmental change, allowing for the selection of stable check cultivars with resistant or susceptible performance. Further research will be focused on measuring the improvement of the accuracy under this model as well as determining the minimum number of years of testing required for reliable cultivar rankings.

POSTER 139

Reaction of sunflower genotypes to downy mildew, *Plasmopara halstedii*, under controlled conditions

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Sunflower downy mildew is one of the important diseases of the main crop production areas in Iran. The use of resistant varieties and hybrids is an effective method to avoid its damage. The soil samples containing the pathogen isolates, *Plasmopara halstedii*, were collected, refreshed and mass-produced on susceptible variety Record, using whole seedling immersion method. Based on existence of a dominant race (race 100) of the pathogen in sunflower cropping areas, 97 sunflower genotypes including F3 off-springs of cultivars Chernanka and Gabor were evaluated against the disease under controlled conditions. Fifteen days post inoculation; the treated plants were evaluated based on six qualitative disease characteristics including damping-off, sporulation on cotyledons, sporulation on leaves and cotyledons, stunt, leaf mosaic or chlorosis and deformation. They were then converted to quantitative means based on their importance in disease development. Totally, 8 genotypes were categorized as resistant and the rest were identified as moderate-resistant (2), moderate-susceptible (2), susceptible (33), and highly susceptible (52).

POSTER 140

Reaction of sunflower genotypes to *Sclerotinia sclerotiorum* under greenhouse conditions

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Sclerotinia sclerotiorum attacks many dicot plants including oilseed rape, soybean, tobacco and sunflower worldwide. The disease stem rot of sunflower caused by the fungal pathogen in Iran is one of the most destructive diseases and causes economic yield lost annually, in particular North West of Iran. Despite lack of resistance to the disease, the different genotypes demonstrate different reactions against the disease. To evaluate the reaction of oilseed sunflower restorer lines, 3-week-old plants grown and incubated in greenhouse, were inoculated employing mycelia discs of the fungal pathogen. The sclerotia of the fungus were collected from Khoy area located in North West of Iran. A single sclerotium was cultured and mass-produced on potato dextrose agar medium. Then three-day-old mycelium plugs with 7 millimeter diameter were used for inoculation. The symptoms of the disease including cotyledon infection and necrosis, stem infection and necrosis, and damping-off were measured and scored 1, 2, and 3 based on disease progress, respectively. A majority of inoculated plants were scored three, based on sever damping-off. The effect of disease was significant on the genotypes and all 21 restorer lines demonstrated susceptibility to the pathogen.

POSTER 141

Evaluation of sunflower genetic resources to charcoal rot caused by *Macrophomina phaseolina*

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Sunflower charcoal rot is considered as one of the most important diseases on crops and infects more than 400 plants worldwide. In the current investigation, to achieve tolerant sources of sunflower, among restorer lines, the seeds were sown in research field located at research complex fields of Seed and Plant Improvement institute, Karaj, Iran. The pathogen, *Macrophomina phaseolina*, was collected from infected sunflower plants during last cropping season at the same area. It was first isolated and sub-cultured using single hypha technique and then mass-produced on potato dextrose agar medium for three days. The culture medium plugs with mycelium and some micro sclerotia produced by the pathogen were provided from the edge of Petri plates as 5 mm diameter round plugs. The stems of treatment plants were injured at seed filling stage and then the plugs were put onto which were covered with a small piece of wet cotton wool to provide humidity. The complex was covered finally with two layers of Parafilm to keep the humidity for infection progress. The lesion length produced was measured at days 7, 14, and 21 post-inoculation. For greenhouse experiments, 75-day-old oil type sunflower restorer plants were inoculated by the same technique used for field evaluation. To monitor the disease process, the lesion length on inoculated plants was measured at days 9 and 14 post inoculation. The analysis of data resulted in significant differences between genotypes showing reaction to the disease, and also days of measurements. On the basis of field evaluations, the lines R-140 and R-201 demonstrated resistance reaction to the pathogen. In greenhouse experiments, four lines including R-133, R-139, R-140, and R-141 showed the same reactions.

POSTER 142

Differential host-pathogen interactions of *Malus* and *Neonectria ditissima*

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The fungus *Neonectria ditissima* causes European canker of apple. Apple accessions vary in their resistance responses, but to date no immunity to European canker has been demonstrated. Similarly, pathogen strains vary in aggressiveness, but no race structure has been reported for this pathogen. To study the host-pathogen interactions, 14 accessions were inoculated with between four and ten single-spore isolates.

Abundant variation in both isolate pathogenicity and host susceptibility was observed, with significant isolate and accession effects. In a reduced data set, three isolates with very low pathogenicity on all accessions and accessions that were tested against low numbers of isolates were removed. In this data set of six accessions inoculated with four to seven isolates, the interaction between accession and isolate effect was significant.

A strong indication of a differential interaction was observed in a partially resistant progeny (AJ102) from an 'M9' x 'Robusta 5' family, where four isolates caused few or no lesions, while a fifth isolate (RS509c) caused lesions in 40% of the inoculated wounds. Isolate RS509c was less aggressive (16% incidence) on a more susceptible accession from the same family (AJ80), on which six other isolates were aggressive (30 to 60% incidence). Another accession (AJ167) from the same family developed few lesions with four isolates, but did develop lesions when inoculated with three other isolates. One of the three isolates that was aggressive on AJ167, caused few lesions on AJ102, while another was RS509c, demonstrating differential accession-isolate interactions.

Our research contributes to an improved understanding of the host-pathogen interactions in the *Malus-N. ditissima* pathosystem, which may include differential interactions.

POSTER 143

Effect of potato variety on *Verticillium* infection of petioles and yield at harvest. A one year field evaluation

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Verticillium dahliae is a major and persistent soil pathogen known to cause verticillium wilt in potato (*Solanum tuberosum*). Host resistance is one of the most economical, environmental and efficient management practices for this disease. Little is known about the resistance of commercial Australian potato genotypes. In glasshouse variety screening trials using 5×10^4 spores/mL, varieties Denali showed moderate to high resistance and Catani and Desiree moderate resistance to infection by *V. dahliae*. The aim of this study was to evaluate 11 varieties (Atlantic, Catani, Coliban, Desiree, Kennebec, Nicola, Pike, Ranger Russet, Russet Burbank, Shepody and Trent) under field conditions with a natural infestation. The trial was conducted in a Koo Wee Rup soil with *V. dahliae* DNA of 36 pg/g soil. Three high health mini tubers were planted per plot with 25 cm spacing in November with 3 replicate plots for each variety. In January, 6 petioles per plot per variety were collected for *V. dahliae* isolation and in April, tubers were dug by hand and yield per plant determined. Variety did not significantly affect the percentage of petioles infected with *V. dahliae* and there was no significant effect on yield. Catani which is considered moderately resistant produced consistent yield even with 50% petiole infection. Kennebec considered resistant with only 15% petiole infection did impact yield. Due to the small sample size in this study, we did not find a relation between varietal *V. dahliae* infection and yield.

POSTER 145

Influence of high and low levels of potassium in field soil on common scab symptom development – a glasshouse experiment

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Common scab, caused by pathogenic strains of *Streptomyces scabies*, is an important disease in potato worldwide that affects the quality of the potato by producing superficial, raised and deep pitted lesions on the tuber surface. Anecdotal evidence from potato growers suggests that soil that has had a high level of dairy manure deposited in holding yards near a dairy shed and areas of field that have had wood piles burnt get increased common scab. Both these scenarios produce elevated levels of potassium in soils. We identified a field in the Koo Wee Rup region with a common scab hotspot. To better understand why some potato fields get common scab and others do not, we sought to characterise the soil inside and outside the hotspot. Soil nutrient analysis found elevated levels of potassium in the hotspot (13%) compared with 4.5% outside the hotspot. Field soil was collected and a glasshouse trial was established to reduce the level of K in the hotspot soil by treatment with either gypsum (CaSO₄) or lime (CaCO₃), and increase the level of K in soil collected from outside the hotspot with the addition of murate of potash (KCl). We demonstrated that the addition of inoculum at planting produced common scab in all soils except those with low K + KCl. DNA analysis of harvest soil found pathogen DNA in all inoculated treatments. Common scab was significantly reduced in hotspot (high K) soil by the addition of gypsum and lime. Further studies are required to understand the mechanism of control.

POSTER 146

Investigating soilborne nectriaceous fungi impacting avocado tree establishment in Australia

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Black root rot, caused by soilborne fungal pathogens in the Nectriaceae family, is a severe disease of avocado nursery trees and orchard transplants. Rapid decline and death of young trees within a year after planting is typical, with symptoms including black, rotten and necrotic roots, tree stunting, leaf wilt, chlorosis, necrosis, and leaf drop prior to tree death. This study identified the nectriaceous fungal genera and species associated with black root rot of avocado, investigated the phylogeny of the genera and determined pathogens to avocado. Fungal isolates were collected from symptomatic avocado roots from orchards and nurseries in all growing regions in Australia, and from other hosts. The identities of 153 isolates were confirmed with Bayesian inference and Maximum likelihood phylogenetic analyses of concatenated ITS, β -tubulin and histone H3 gene loci. The nectriaceous fungi found associated with black root rot include *Calonectria illicicola*, *Dactylonectria macrodyma*, *D. anthuriicola*, *D. novozelandica*, *D. pauciseptata*, *D. vitis*, *Gliocladiopsis peggii*, *G. forsborgii*, *G. whileyi*, *Cylindrocladiella pseudoinfestans*, *Ilyonectria* sp. and *Mariannaea humicola*. Comprehensive phylogenetic analyses revealed additional putative new species and species complexes in each genus. Glasshouse pathogenicity tests confirmed *Ca. illicicola* isolated from avocado, papaya, peanut and custard apple, and *Calonectria* sp. from blueberry, caused significant root rot in avocado cv. Reed seedlings within 5 weeks of inoculation. *Dactylonectria macrodyma*, *D. novozelandica*, *D. pauciseptata* and *D. anthuriicola* were confirmed as pathogens, causing black root rot within 9 weeks. *Cylindrocladiella pseudoinfestans*, *G. peggii* and isolates of *Ilyonectria* sp. were non-pathogenic.

POSTER 147

Pre-breeding efforts for resistance to Ascochyta blight and Botrytis Grey Mould in pulses

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Pulses are important protein rich foods for millions of people in South Asian countries and are increasing in importance in Australian cropping systems; not only as a break crop but also providing high farm-gate returns in many regions. Fungal diseases cause major losses in pulse production especially in conducive environmental conditions in susceptible varieties. Ascochyta blight (AB) in field peas and Botrytis Grey Mould (BGM) in chickpeas and lentils consistently cause yield losses. A dedicated pre-breeding effort is being undertaken to develop new and resistant varieties for Australian growers. Reliable, high-throughput, controlled environment screening methods for these diseases have been developed to select for new sources of resistance. Over 1000 landraces and wild relatives of chickpea and lentil germplasm have been screened. In both crops, several lines were identified with partial resistance equal to the best performing current varieties. To further improve resistance, an intercrossing and recurrent selection strategy is being adopted.

In Australia, AB is caused by a complex of up to four organisms, with *Peyronella pinodes* and *Phoma koolunga* being most common. There is very little resistance for the complex in field pea germplasm with the best performing lines scored as moderately susceptible. However this is a step up from early lines that were considered susceptible. A multi environment analysis (MET) of disease scores over several years highlighted two major rankings of germplasm corresponding to trials in either Western Australia or eastern Australia. This has facilitated the selection of germplasm for intercrossing to improve overall levels of resistance and stability. Resistance x susceptible crosses were also made for the genetic characterization of resistance to this disease. Furthermore, microscopic studies were conducted to analyse differences between the most common pathogens and found that *P. pinodes* is more aggressive than *P. koolunga* under controlled environment conditions.

POSTER 148

Cross Sectoral Plant Biosecurity R,D&E Opportunities

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Plant biosecurity R,D&E is conducted by a wide range of organisations across Australia. Previously there was no overarching framework to coordinate the agricultural research being carried out in Australia. Agricultural Ministers endorsed the National Primary Industries R,D&E Framework and in 2013, the National Plant Biosecurity R,D&E Strategy was developed and endorsed. This strategy is one of 22 sector specific and cross-sectoral strategies that have been developed under the framework. Oversight of these strategies is through the Agriculture Senior Officials Committee. The plant biosecurity strategy aims to better harmonise the roles of the organisations involved in R,D&E related to plant biosecurity, ensuring that all stakeholders work together to maximise benefits of R,D&E for Australia.

A strategy implementation committee was formed in 2014 to implement the strategy. The committee includes representatives from the Commonwealth, both the policy and research arms of state jurisdictions, research and development corporations, CSIRO, the Council of Rural RDCs, the Plant Biosecurity CRC and Plant Health Australia.

As a part of implementing the strategy, a forum on plant biosecurity priorities was held in July 2016 and two targeted workshops have been run. The first of these workshops covered R,D&E needs for preparedness for whiteflies and their transmitted viruses and the second was targeted policy makers to discuss the policy opportunities and risks of new technology in diagnostics R&D. The outcomes of these workshops will be presented in this session.

POSTER 149

Fire ants in Australia: eradication tools, strategies, and success stories

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The Red Imported Fire Ant (*Solenopsis invicta*) was officially identified in Brisbane in 2001 and has been the subject of a nationally funded eradication program ever since. As the program moves into a long-term strategy to eradicate the final south-east Queensland population, we highlight the program's success in eradicating three fire ant populations from Queensland and one from New South Wales. The tools used to achieve this success include ground surveillance by staff and odour detection dogs, remote sensing surveillance using thermal signatures of active fire ant mounds, genetic analysis at both the nest and population level, and varying treatment methods combining both individual mound treatments and broadcast baiting. We also highlight how genetic analysis can inform management decisions, which was critical for defining the origin of subsequent detections at previously cleared areas. With the knowledge gained from the last decade and a half, these tools will only become more relevant as fire ants and other invasive ant species continue to spread as the result of global trade.

POSTER 150

Soil quality indicators to detect suppression of Fusarium wilt in bananas in the Philippines

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Banana is a major export commodity in the Philippines with exports valued at US\$1000 million in 2013. However, the incursion of Fusarium wilt Tropical Race 4 (TR4) (*Fusarium oxysporum* f. sp. *cubense*) has seriously jeopardised the export banana industry. To reduce the impacts of TR4 on banana production, field and greenhouse pot experiments were established to investigate the standard production methods of Grand Naine with the use of resistant clones GCTCV 218 in modified production systems. In the greenhouse experiment, Grand Naine inoculated with TR4 was grown with four groundcovers species alone or in combinations and compared with GCTCV 218, GCTCV 219, and bare soil. The field experiment was established on a commercial banana plantation where GCTCV218 was compared with Grand Naine grown in bare soil or with *Arachis pinto* groundcover and with the application of a biocontrol product. Disease incidence, severity and plant growth parameters were determined at biweekly intervals in the greenhouse and monthly intervals in the field experiment. To understand changes in soil microbial activity, soil enzyme activity and Community Level Physiological Profiles (CLPP) were determined in both experiments. In the greenhouse experiment, there was increased FDA enzymatic activity with GCTCV218. Similarly, in the field experiment there was greater CLPP diversity and utilisation of the organic and amino acids under the cultivar GCTCV218. GCTCV218 significantly reduced disease incidence, 7.5% relative to Grand Naine 37.3 % after 12 months. The biocontrol and groundcovers had no significant effects on disease or soil microbial activity over the course of the two experiments. The resistance of GCTCV218 could be due to changes in the microbial profile relative to Grand Naine, but this requires further investigation. However, the results indicated that the cultivar GCTCV218 could be part of a solution for small holder banana growers affected by TR4 in the Philippines.

POSTER 151

Improving on-farm biosecurity in the Philippine banana farms by reducing soil movement on footwear

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The Philippine Cavendish banana industry occupies 80,000 hectares with 60% operated by big plantations and 40% from small independent growers. In 2013, its export value was US\$1000 million, serving 320,000 direct employments. However, the incursion of *Fusarium wilt Tropical Race 4 (TR4) (Fusarium oxysporum f. sp. cubense) (Foc)* has seriously jeopardised the export banana industry, 3000 hectares were abandoned and 6000 hectares were affected in varying levels particularly small holder growers. To reduce its impacts on banana production, increased farm biosecurity is required. On-farm biosecurity within the Philippines requires the removal of soil from footwear of farm workers and use of disinfectants. An experiment using different low cost "boot scrapers" was done to determine their effectiveness in removing soil from footwear. Their effectiveness was tested over two contrasting seasonal conditions. Then, disinfectants used in banana farms were tested for their effectiveness to eliminate Foc. Finally, the concepts were validated by determining the longevity of footbath under a commercial banana production conditions. A simple wire mesh design proved to be the most effective method in removing soil from footwear under both seasonal conditions. The wire mesh was ten times effective than using coco coir. The use of Formo™ (2,2-Dibromo-3-nitrilopropionamide) at the recommended 9% rate proved to be the most efficacious product eliminating Foc. The longevity of Formo™ on the commercial banana farm was greatest when the footbath was freshly prepared. But its efficacy declined with time and was lowest after five days. Therefore, cleanliness of footbath is critical, as residues of soil and organic matter, which may contain spores of the pathogen, may also reduce product efficacy. To improve on-farm biosecurity for Philippine banana farms requires a combination of wire mesh boot scrapers, to remove mud and organic material, together with an appropriate disinfectant such as Formo™, that is regularly replaced.

POSTER 152

Effects of commercial disinfectants on the survival of *Fusarium oxysporum f.sp. cubense* Race 1 and Tropical Race 4 propagules

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Panama disease caused by *Fusarium oxysporum f.sp. cubense (Foc)* Race 1 (R1) and Tropical Race 4 (TR4) is one of the most destructive banana plant diseases worldwide. One of the best practices to manage the disease is to implement decontamination procedures using disinfectants on farms to restrict the movement of Foc. This work aimed to determine the most effective disinfectants against both FocR1 and TR4 to form part of on-farm biosecurity procedures. A total of 20 disinfectants were screened and their activity was assessed after ≤ 30 sec, 5, 30 min and 24 hrs of contact with Foc in the absence and presence of soil (0.05 g/ml). Of the disinfectants tested, the quaternary ammonium compounds that contain > 12% active ingredient (ai) of didecyl dimethyl ammonium chloride (DDAC) were found to be the most effective against both Foc races. These products when used at a 1:100 dilution, reduced Foc survival to a "zero" detectable level across all the contact times with or without soil present. Bleach (10% sodium hypochlorite) and two bio-flavonoid products diluted to this rate were also effective in eliminating Foc but longer treatment times (≥30 mins) were required. Two detergent-based products failed to eliminate propagules of both races even at a dilution rate of 1:10 and after 24 hrs of treatment, although they could suppress Foc colony development. Significantly ($P < 0.05$) greater numbers of Foc colonies developed in some treatments with soil than those without, suggesting that the presence of soil decreases the activity of certain disinfectants. Based on these findings, this study suggests that the DDAC products (> 12% ai) diluted to the rate of 1:100 can form part of decontamination procedures against both FocR1 and TR4. Excess soil needs to be washed off contaminated items before treatment to maintain the efficacy of disinfectants.

POSTER 153

Epidemiology and impact of myrtle rust (*Austropuccinia psidii*) in lemon myrtle (*Backhousia citriodora*) plantations

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Lemon myrtle (*Backhousia citriodora*) is a native Australian species whose leaves contain oil with the highest citral content (<98%) of any known plant in the world. Steam distillation is used to extract essential oil from the leaves and this is used in aromatherapy products, cosmetics and toiletries, and as a food flavouring. The leaves are also dried and milled for use in teas or as a spice. Growth of this Australian native food industry has increased substantially in recent years, in both Australian and overseas markets. However, the introduction of the rust fungus *Austropuccinia psidii* (*A. psidii*) into Australia in 2010 has jeopardized its expansion and success. Limpinwood, the widely used commercial lemon myrtle cultivar, is highly susceptible to *A. psidii* and yield losses have been estimated to be up to 70% in plantations not applying fungicides. Glasshouse studies and field trials in a commercial lemon myrtle plantation in New South Wales were undertaken to gain an understanding of: (1) factors influencing the susceptibility of lemon myrtle to infection by *A. psidii* and host colonisation; (2) the influence of climatic factors and inoculum density on the incidence and severity of *A. psidii* in lemon myrtle; and (3) the effect *A. psidii* infection has on the growth and yield of lemon myrtle, including the impact on essential oil properties. Our research will enable the development of a forecasting system for this disease so more informed, effective and economically feasible management decisions can be made, particularly regarding fungicide applications. It will also provide an economic evaluation of the impact *A. psidii* is having on the commercial production of lemon myrtle and detailed information concerning levels of resistance in cultivars of lemon myrtle.

POSTER 154

Spread and Severity of Fusarium Dieback – Polyphagous Shot Hole Borer Throughout Urban Forests in Los Angeles County

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Trees in California's urban forests remove the CO₂ equivalent of 120,000 cars and provide USD 1.0 billion annually for ecosystem services. These benefits are under threat by the polyphagous shot hole borer (PSHB; *Euwallacea* sp.) and its symbiotic fungal pathogen *Fusarium euwallaceae*, which together cause Fusarium dieback (FD) on 51 tree species that support reproduction of the beetle. The scope of the problem is limited by insufficient data on FD distribution and impacts. The objectives of this study were to: 1) determine FD occurrence in Los Angeles; 2) identify indicators for disease spread. We conducted a rapid assessment at the urban-rural interface of the San Gabriel and Santa Monica Mountains (89 km), marking the location of confirmed positive and negative hosts every 2 km with a GPS. In the San Fernando valley, we counted and marked the location of every infested and non-infested hosts within 45 randomly selected grids (65 ha each). FD was detected on 550 trees across 30 new locations along the urban-rural interface, and two new reproductive hosts (*Aesculus californica* and *Quercus chrysolepis*). *Platanus racemosa* is the most common reproductive host, suggesting it is important for FD spread. Most individuals of each rare host species were infested, suggesting potential losses in biodiversity in the landscape. FD was detected on 413 of the 5,684 trees surveyed in the San Fernando Valley. *Liquidambar styraciflua* was the most common host, but a larger proportion of sycamore trees were infested (28% versus 5%). The likelihood of PSHB arrival was a function of sycamore abundance, but grids differed in the magnitude of disease associated with sycamore and other reproductive host densities. Preliminary results suggest the beetle is using locations with sycamore for movement through landscape, but local dynamics are idiosyncratic. Monitoring sites over time will better determine local impacts.

POSTER 155

The rise of scolytid beetle activity is it just the hot weather?

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The rise in scolytid beetle activity in the last 3 seasons for orchard crops has corresponded with a significantly drier summer period during production in eastern Australia. The use of various pheromone lures and a range of flight trap designs have been looked at to assess the pest incidence and the impact they have on tree health. Lightning strikes are often a key precursor to scolytid attack on dying trees, the use of ethephon has also caused scolytid attack in some sites. The apparent expansion of some more serious pest species may lead to a rethink on the need to manage the pests more effectively especially if the plant disease vector scenario of a laurel wilt style organism is confirmed.

The range of pests encountered in the NSW and QLD macadamia and avocado production districts will be listed and seasonal patterns of some key species will be discussed.

POSTER 156

***Amblypelta* spp management for NSW and SE QLD avocado and macadamia orchards. Can we reduce the spray frequency with better timing?**

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The fruitspotting species *Amblypelta nitida* (FSB) and *A. lutescens* (BSB) are the key pest species to manage for macadamia avocado and many other subtropical crops grown on the east coast of Australia. The normal avocado spray schedules to prevent crop loss from these pests are approximately 14 days in far north Queensland, 21 days around Bundaberg, 28 days around the QLD/NSW border and roughly 42 days in the southern regions where only FSB is present. A prototype lure trap for BSB and a small trap hedge of plants for FSB have been used during the last 3 seasons to compare the incidence of bugs, the timing of adult flight generations, and how it relates to crop damage. Results of the crop loss to these pests in relation to comparative trials of spray timings based on BSB lure/hedge counts as opposed to a strict 21 day calendar spray program in avocado will be presented. Other trials in macadamia and custard apple have shown that FSB flight detection is possible and are being used to adjust the spray timings in NSW. By reducing the FSB/BSB sprays applied is there evidence that other pests species are impacting on the crops?

POSTER 157

Sustainable agrichemicals derived from the endophytic fungus *Daldinia* sp.

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Endophytic fungi have been identified as a unique source of novel biocides, and as such represent an exploitable resource for the discovery of new agrichemicals. A bioprospecting program was established at Agriculture Victoria to isolate and identify biocidal endophytic fungi, characterise their metabolome, and identify novel biocides with agricultural application. Permits and agreements were established between the relevant states (Northern Territory and Victoria) and authorities to facilitate the research.

Daldinia sp. was isolated as an endophytic fungus from *Pittosporum biocolor* (Banyalla). *In vitro* bioassays demonstrated that *Daldinia* sp. had high biocidal activity against insect pests (*Tribolium castaneum*) and fungal pathogens (*Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia cerealis*). Solid-Phase Microextraction Gas Chromatography – Mass Spectrometry identified a number of potential biocidal volatile organic compounds (VOCs) in the volatolome of *Daldinia* sp. A number of these compounds were evaluated in isolation and combination for their biocidal activity against insect pests (*T. castaneum*), bacterial pathogens (*Pseudomonas syringae*) and fungal pathogens (*Fusarium verticillioides*). From the compounds tested, isovaleraldehyde, 3-propanol and acetoin emerged as the most promising candidates for novel agrichemicals.

Isovaleraldehyde, 3-propanol and acetoin are common flavour and fragrance agents, approved for use in foods for human consumption (e.g. GRAS) by the Food and Drug Administration (FDA, USA). Hazard statements indicate that these compounds do not pose a risk to humans (no acute toxicity, carcinogenicity) or the environment (no environmental contamination). Their physical properties indicate that they are moderately volatile, with short substantivity (24 – 97 hrs). As such, these compounds represent promising candidates as topical biocides (e.g. treatment of parasites of livestock), oral biocides (e.g. treatment of scours of livestock) or disinfectants (e.g. sterilisation of dairies).

POSTER 201

Non-invasive techniques for determining *Phytophthora* root rot severity in avocados.

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The Australian avocado industry suffers millions of dollars of lost productivity each year as a result of *Phytophthora* root rot (PRR). PRR is caused by the soil-borne pathogen *Phytophthora cinnamomi* (Pc), particularly during prolonged wet soil conditions. Pc infects avocado feeder roots, the main source of the trees water and nutrient supply, resulting in gradual canopy decline, shoot die-back and eventually tree death.

To determine if the incidence of Pc could be assessed non-invasively an experiment was conducted where aerial optical and thermal imagery of an avocado orchard was compared against a visual canopy health rating scale and the number of zoospores generated per unit weight of feeder roots.

The canopy thermal characteristics of selected avocado trees were found to be better correlated to the visual disease rankings of tree health than the number of zoospores in roots. Interestingly, the number of zoospores also showed little correlation to the disease three ratings indicating that the concentration of Pc spores themselves were not the sole driver of PRR symptoms.

It is therefore suggested that PRR disease expression is a combination of Pc infection, exacerbated by additional biotic and abiotic constraints such as soil type, orchard management practices (eg. Irrigation) and rootstock susceptibility.

The findings of this initial study indicates canopy thermal characteristics of avocado tree can be used to indicate health status including the progression of decline due to PRR. Additional research will confirm whether this approach can be used to detect PRR non-invasively before it is visually apparent to the human eye, thus allowing remedial action to be implemented before tree decline.

POSTER 202

Fungi and bacteria associated with the Peanut Kernel Shivel (PKS) disease in the Bundaberg region

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Peanut Kernel Shivel (PKS) is a currently undiagnosed condition affecting peanut crops primarily in the Bundaberg growing region. It is a condition where kernels in pods on a plant approaching maturity cease filling normally, and fail to reach their full size resulting in reduced kernel size and high shell %, which reduces overall crop yield, quality/grading and price/Mt of farmer stock. Affected kernels are small and often develop a shrivelled testa with light tan colour. There are no aboveground symptoms as overall canopy growth (leaf and stem) from PKS affected plants appear healthy with no obvious signs of nutrient deficiency or disease.

Since 2011, the peanut industry in the Bundaberg region has seen a steady rate of decline in crop grade out due to the increasing impact of PKS. PKS is currently costing the industry more than \$2.5M+ p.a., and Bundaberg growers are threatening to reduce peanut plantings if a solution to PKS is not found soon.

To date, efforts to detect its cause have been unsuccessful. Results from initial investigations showed no evidence to indicate abiotic (including water quality and nutritional status) or biotic (including insect, nematodes, viruses and bacteria) cause. Some recent research results have suggested some sort of pathogen (e.g., fungi) may be involved, and possibly be associated with some sort of toxin or excess hormone production.

Work to identify possible biotic factors, focused on fungi and bacteria, is currently underway. A total of 33 fungi and 14 bacteria morphotypes have been identified from 80 symptomatic and asymptomatic plants. DNA sequencing was used to identify fungal and bacterial isolates and those consistently isolated from PKS affected plants will be used to perform Koch's postulates.

Once the cause(s) of PKS is established, the next step is to develop management strategies to minimize its incidence in commercial peanut crops.

POSTER 203

Insights into *Botryosphaeria* detections - unravelling its identity and its impact on biosecurity

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Over the past 25 years Agriculture Victoria's Crop Health Services has recorded more than 350 instances of plant diseases associated with *Botryosphaeria*-like fungi, mostly from Victoria, and some from the other Australian states. The main hosts have been woody species grown by horticultural and forestry industries. *Botryosphaeria* fungi usually cause canker lesions, wood staining and tree die-back. Leaves and fruits can also be affected. In most of the cases, *Botryosphaeria* isolates look similar on PDA media, having a distinctly dark and fluffy growth, which does not discriminate species. Before the advent of molecular diagnostics, *Botryosphaeria* isolates were rarely identified beyond genus. Molecular identification was first applied to Australian isolates in 2007 (Cunnington et al. 2007) when 30 isolates of this fungus from Victoria and NSW were identified to species, using rDNA-ITS and TEF-1 α sequences. Since then, applying these molecular tools combined with morphological traits has proven powerful in unravelling new detections.

In 2017, a *Botryosphaeria*-like fungus from an apple tree (Vic.) was identified *Dothiorella sarmentorum*, and this is a new record of this fungus for Australia. In 2016, *Diaporthe novem* was isolated from a diseased walnut tree (NSW) and *Botryosphaeria stevensii* was isolated from a horse-chestnut tree (Vic.), and these are first host records for NSW and Victoria respectively. In 2015, a *Botryosphaeria* fungus from Callistemon was identified as *Neofusicoccum australe*, and *Botryosphaeria*-like fungi from a walnut tree (Vic.) and a Magnolia tree (Vic.) were identified as *Neofusicoccum parvum*, and these two are new host records for Australia. The impact of these new detections on biosecurity have been assessed and reported to Biosecurity Victoria. In turn, Biosecurity Victoria reports to the other states where relevant and makes decisions on undertaking follow-up surveys, and containment or eradication, as appropriate.

POSTER 204

New host records for *Verticillium tricornis*, *Corynespora cassiicola*, *Aphelenchoides fragariae* and *Helicotylenchus dihystra*, associated with dieback and leaf blight of hydroponically grown sweet basil (*Ocimum basilicum*) in Victoria, Australia

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In February 2012, hydroponically grown sweet basil plants were submitted to Crop Health Services (CHS), Agriculture Victoria, for diagnosis of possible causes of brown necrotic patches on leaves, necrotic segments on stems, and shoot tip necrosis. Fungal isolation detected the fungi *Verticillium tricornis* and *Corynespora cassiicola* from the necrosis-affected stem segments. The identity of *C. cassiicola* was confirmed using ITS sequence data, and that of *V. tricornis* was confirmed using both ITS and elongation factor sequence data. The sequences were compared to reference sequences deposited in GenBank (<http://www.ncbi.nlm.nih.gov>) and were found to share the highest identities, either with *V. tricornis* (99% identity, 100% coverage), or *C. cassiicola* (100% identity, 100% coverage), respectively. *V. tricornis* has been reported from several hosts before (Taylor 1968), but is generally considered a saprophyte. However, *C. cassiicola* was previously reported to be pathogenic in a wide range of host plants, including *O. basilicum*, worldwide (Smith 2008).

Nematode extractions detected *Aphelenchoides fragariae* (foliar nematode) from the affected leaves and high levels of *Helicotylenchus dihystra* (spiral nematode) from the growth medium (21698 nematodes / 200 mL). Nematode identifications were confirmed by morphology, by comparison with reference specimens in the Victorian Plant Pathogen Herbarium (VPRI). *A. fragariae* is well documented for causing damage to leaves on many host plants (Siddiqi 1975). The presence of *H. dihystra* at such high numbers in the growth medium explained the considerable root damage observed, as *H. dihystra* is known to feed externally on roots of many host plants (Siddiqi 1972).

The detections of the fungi *V. tricornis* and *C. cassiicola*, and the nematodes, *A. fragariae* and *H. dihystra*, from *O. basilicum* are all first records from this host in Australia. The sweet basil crop was unmarketable and consequently destroyed.

POSTER 205

Causal pathogens of fruit rot in sweet cherry and weather-based infection risk

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The sweet cherry industry has grown substantially in recent years and high quality fruit is required for domestic and export markets. Incidence of rot can be high in some seasons and optimal management is not well understood. Several pathogens are known to cause rot in sweet cherry fruit, primarily including *Monilinia fructicola*, *Monilinia laxa* and *Botrytis cinerea*. The *Monilinia* spp. cause brown rot while *B. cinerea* causes grey mould, however the symptoms caused by these three pathogens can be difficult to distinguish and all result in substantial losses to fruit quality. Key times of risk are not currently well understood in Australian orchards but factors such as inoculum presence and weather-based infection likelihood can be determined. Relative abundance of rot pathogens in Australian sweet cherry orchards has not been well documented to date. During a three year project it was clearly established that *Botrytis cinerea* was the dominant rot pathogen in the orchards surveyed in southern Tasmania, while *Alternaria alternata* was a dominant cause of rot in orchards of Orange and Young in NSW. Temperature and wetness durations conducive to fruit infection (and the impact of fruit susceptibility) were modelled for *B. cinerea*, *M. laxa* and *M. fructicola*. Latent infection was quantified for flowers and developing fruit but there was no significant relationship with rot at harvest. A molecular method was developed to quantify spores of *B. cinerea*, *M. laxa* and *M. fructicola* from spore trap samples and peaks of high relative abundance occurred throughout the season from flowering to harvest.

POSTER 206

Real-time PCR detection of *Phyllosticta ampellicida*, the causal agent of black rot of grape

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Black rot of grape caused by *Phyllosticta ampellicida* (*Guignardia bidwellii*) is a destructive disease present in most grape growing regions worldwide, with the exception of Australia and New Zealand. *P. ampellicida* is a quarantine organism for New Zealand and it is important to have a fast and accurate diagnostic method for the early detection of this fungus. In this study, a real-time PCR (qPCR) assay was developed and validated for the detection of *P. ampellicida*. Multiple sets of *P. ampellicida* specific primers and probes were designed using internal transcribed spacer (ITS) DNA sequences. Optimisation experiments determined the best performing primers (Gb-IT4F and Gb-IT5R) and TaqMan probe (Gb-IT2P). The specificity of the qPCR assay was confirmed by testing against strains of *P. ampellicida* from *Vitis* sp., *P. bidwellii* from *Parthenocissus* sp., closely related *Guignardia* species, and other fungal isolates commonly associated with grapevines. The qPCR assay consistently detected *P. ampellicida* at genomic DNA concentration of 116 fg and therefore enables disease detection at low infection levels. The *P. ampellicida* qPCR assay was further developed as a duplex assay by co-amplifying the cytochrome oxidase (Cox) gene from plants for use as an internal PCR control. To our knowledge, this is the first qPCR developed for the rapid detection of *P. ampellicida*. This qPCR has the potential for use in screening imported nursery stocks and for the early detection of the pathogen during surveillance.

POSTER 207

Potato wart detection and eradication

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Presented by Jeyaseelan Baskarathevan

Synchytrium endobioticum is a non-mycelial, soilborne fungus that causes potato wart disease. It is an internationally significant quarantine pathogen and a notifiable organism in New Zealand. As the resting spores of this pathogen can remain viable for more than forty years, growers cannot plant any other crops for export purposes in areas that have been infested with potato wart. In New Zealand, this disease was first recorded in a home garden in Invercargill, Southland in 1970. Since then, the fungus has been found several times in home gardens in a few areas of Southland but never in commercial potato farms. When the disease was detected in home gardens, the fungus was eradicated every time. The Ministry for Primary Industries (MPI), the industry body and local regional council have established a potato wart public awareness programme. This programme targets home gardeners in areas where potato wart has been found previously to encourage early reporting and to reduce the risk of spreading the pathogen. Around 10 samples per annum have been sent to the laboratory for diagnosis in the past four years and all were found to be negative for potato wart. Potato wart was last found in home gardens in Invercargill in 2012 and the pathogen was eradicated by soil fumigation. Movement control restricting the movement of the soil, plants, and tools from the infested properties was put in place for the following two growing seasons, and indicator potato plants were used to ensure the success of eradication. MPI is conducting an ongoing awareness programme to support early detection should potato wart reoccur in New Zealand.

POSTER 208

Investigations of the aetiology of sugarcane yellow canopy syndrome

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Yellow canopy syndrome (YCS) of sugarcane, first discovered near Cairns, is characterized by prematurely senescing, bright orange leaves in the lower canopy of the plant. Spread of the disease from northern to southern Queensland and northern New South Wales suggests that the disease is biotic in nature, although symptoms are likely to be modulated by abiotic stresses. Previous research has shown accumulation of starch in early senescing leaves and also disruption of vascular transport, similar to other well-characterized diseases caused by bacteria and viruses. In order to provide clues as to the aetiology of the disease, a range of antimicrobial agents (streptomycin and oxytetracycline (antibiotics), metalaxyl (systemic fungicide) and prochloraz (general fungicide)) were tested on the sugarcane variety Q200 in field trials established in 'YCS hotspots' in Ingham and the Burdekin region in the 2016/17 season. Several growth parameters and symptom descriptors were measured during the course of the trials, and results of the analyses will be presented in this paper. Light microscopic examinations of vascular regions of plants in the different antimicrobial treatments were also undertaken, and some fungal and bacterial structures noticed that were abundant in symptomatic leaves. Efforts are being made to identify these microorganisms to provide a catalogue of putative pathogens associated with YCS.

POSTER 209

Development of a multiplex PCR for the detection of *Eutypa lata* and *Cryptovalsa ampelina* associated with Eutypa dieback of grapevines

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Eutypa dieback (ED) caused by the fungus *Eutypa lata* is one of the important trunk diseases affecting grapevines in Australian vineyards. The pathogen primarily infects pruning wounds and slowly colonises the vascular tissues resulting in cankers, dieback and eventually death of vines. Grapevines affected by ED also display foliar symptoms including chlorosis, necrosis, cupping of leaves and shortening of internodes which is due to phytotoxins produced by *E. lata*. Recent studies, however, showed that in addition to *E. lata*, other fungi belonging to the Diatrypaceae family are also associated with ED including *Cryptovalsa ampelina*, another prevalent species found in Australian vineyards. The objective of this study was to develop a multiplex PCR for rapid detection of *E. lata* and *C. ampelina* in a single sample. Two sets of primers specific for *E. lata* (BtEuF1 and BtEuR1) and *C. ampelina* (CampBtF1 and CampBtR1) were designed to amplify 327 bp and 215 bp fragments of the β -tubulin gene, respectively. The two sets of primers were optimised using single and mixed DNA of *E. lata* and *C. ampelina* in a single reaction. The assay was able to amplify two distinct PCR products specific to *E. lata* and *C. ampelina* in a mixed DNA sample demonstrating it is suitable for multiplex-PCR. Specificity tests also showed the two primer pairs can amplify all *E. lata* and *C. ampelina* DNA while no PCR products were observed with the DNA from seven other Diatrypaceae species and 20 other non-target fungal species associated with grapevines. The multiplex-PCR developed in this study is suitable for rapid detection of two ED pathogens in a single sample. This assay can be used for rapid diagnosis of ED from spore trap tapes collected in different grapevine growing regions in Australia and from grapevine tissue samples.

POSTER 210

First report of four *Phytophthora* species in the Lao PDR

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The tropical climate, agronomic practices and range of plant hosts in South-East Asia often provide ideal conditions for *Phytophthora* diseases to proliferate and be economically damaging. Despite this, there remain gaps in the data on the distribution of the genus, especially in least developed countries.

Throughout 2015, surveys were conducted on over 40 crops in Champasak province, with a focus on the Bolaven Plateau. This plateau is a fertile highland with volcanic soils and a major coffee, fruit and vegetable production area in the Lao PDR. Four species of *Phytophthora* were isolated directly from diseased plant tissue, and indirectly by soil baiting.

Phytophthora capsici and *P. drechsleri* were associated with chilli plants showing stem browning, root rot and wilt symptoms, growing in polyhouses on the Bolaven Plateau. *Phytophthora palmivora* was isolated from papaya trees showing root and collar rot symptoms in a garden in Pakse, as well as a papaya inter-row planted with citrus on the plateau. It was also isolated from the soil beneath durians with dieback on several small-holder farms. *Phytophthora cinnamomi* was associated with avocado dieback at a number of small-holder farms. Isolates of the four species were sent to the Centre of Phytophthora Science and Management at Murdoch University, Australia, where identification was confirmed through morphological and molecular markers, and where they were accessioned.

The results of this survey indicate the presence of important *Phytophthora* species in southern Lao PDR that are well known to occur in neighbouring countries. Workshops and in-field discussions have been held with small-holders to help with implementation of IDM strategies, including crop rotation where appropriate, the use of pathogen-free transplants and the use of phosphonate injections at avocado and durian farms. Another species, *P. infestans*, has become a serious problem in tomatoes on the plateau but has yet to be studied in detail.

POSTER 211

Pythium species Associated with Yield Decline in Processing Tomatoes in Victoria

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The Australian Processing Tomato Industry (APTI) is based primarily in the Goulburn Valley in northern Victoria, extending into southern NSW. The field-grown tomatoes are produced as an irrigated summer crop and are often grown successively in the same field for 4-5 years before being rotated for 1 year with a cereal or corn crop. The tomatoes are grown from transplants or seed with sub-surface tape irrigation following soil treatment with metham sodium. Following the 2015/16 season, the Australian Processing Tomato Research Council (APTRC) estimated that the industry had lost 10-15% of yield and considered that diseases caused by soil-borne pathogens might be a contributing factor.

In autumn 2016, a glasshouse pot bioassay was performed whereby tomato seedlings were grown in three similar types of field soil, two with a recent history of tomatoes and evidence of yield decline, and one with no history of tomatoes. After 6 weeks, root lesions had developed on the root systems of plants growing in the two soils with suspected yield decline. A *Pythium* species, confirmed by culture morphology and ITS gene sequence, was consistently isolated from diseased roots. The pathogenicity of the *Pythium* sp. to tomato seedlings was confirmed with 100% of inoculated seedlings dying from damping-off.

Five field surveys were completed across the 2016/17 season and isolations were made from root lesions, and from the discoloured inner crown tissue of stunted plants. Over 200 isolates of putative *Pythium* species were recovered, together with approximately 300 isolates of putative *Fusarium oxysporum*. *Phytophthora nicotianae*, *Sclerotinia sclerotiorum* and *Colletotrichum*

sp. were also isolated at a lesser frequency. Further studies using multigene phylogenetic analysis are underway in order to identify the *Pythium* species. Their pathogenicity is being assessed in greenhouse studies.

POSTER 212

First Report of Sclerotinia Stem Rot Caused by *Sclerotinia sclerotiorum* on Chinese chives in Korea

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Chinese chives (*Allium tuberosum* Roxb.) is an important allium crop in Korea. During winter of 2014-2015, typical symptoms of Sclerotinia stem rot were observed in commercial crops of Chinese chives 'Green Belt' in polyethylene tunnels in Iksan, Korea. Disease incidence ranged between 10 and 20%, causing considerable economic losses. Pure isolates made from symptomatic tissues were transferred onto potato dextrose agar (PDA) and consistently yielded abundant white mycelia developing sclerotia after two weeks. The pathogen was morphologically identified as *Sclerotinia sclerotiorum* (Lib.) de Bary. A voucher specimen was deposited with the Korea University Herbarium (KUS-F28606). A representative isolate was deposited with the Korean Agricultural Culture Collection (KACC47723). Genomic DNA was extracted from mycelia and the complete internal transcribed spacer (ITS) region of rDNA was amplified with the primers ITS1/ITS4 and sequenced. The resulting sequence of 558 bp was submitted to GenBank (Accession No. KJ614564). A BLASTn search revealed that the Korean isolate showed 100% identity with those of *S. sclerotiorum* (DQ329537, KF859932, KF859933, JN013184, etc.). Pathogenicity was confirmed by inoculating the lower stems of healthy Chinese chives 'Green Belt'. Plants were kept in the greenhouse at 15 to 20°C and relative humidity > 90%. Symptom expression was evident after three days with *Sclerotinia sclerotiorum* consistently re-isolated from the symptomatic tissue, fulfilling Koch's postulates. No symptoms were observed on control plants. This is the first report of Sclerotinia stem rot on Chinese chives globally as well as in Korea. Our field observations suggest that low temperature, high humidity, poor ventilation and continuous cropping cycles in non-heated plastic tunnels accelerate the incidence of Sclerotinia stem rot on Chinese chives. This crop is mostly grown with limited chemical control options; a Good Agricultural Practices (GAP) model for producing eco-friendly Chinese chives is currently under development.

POSTER 213

Alternate *Ipomoea* species as a diagnostic tool for enhancing virus detection in sweetpotato

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Research into yield decline in sweetpotato has confirmed virus infection as the most limiting factor impacting worldwide production. To improve Australian sweetpotato commercial yields, Australian researchers developed a pathogen tested seed scheme consistently providing growers with first generation, clean planting material. Production of sweetpotato in Australia has since increased by around 1700%, and the regular provision of clean planting material is now a commercial operation. Virus-infected sweetpotato plants are often asymptomatic, hindering visual virus detection. Sweetpotato sap contains inhibitors which can interfere with detection using standard virus diagnostic methods. The Australian sweetpotato pathogen testing process uses a range of complimentary diagnostic procedures to overcome these issues, the most important being the use of *Ipomoea setosa* as an herbaceous indicator plant. *I. setosa* is highly susceptible to infection by sweetpotato viruses, and though the grafting and symptom observation process is time consuming, it enables rapid increase in virus titres, enhancing the accuracy of both NCM-ELISA and PCR. The susceptibility of *Ipomoea* species to sweetpotato infecting viruses is relatively unexplored. This study aims to investigate the development and expression of common sweetpotato viruses in a range of *Ipomoea* species, using graft inoculation with known negative and positive control plants. This will provide information on the suitability of alternate *Ipomoea* species as indicator plants. The inclusion of native Australian *Ipomoea* species in this investigation will provide additional information on possible biosecurity concerns should virus incursions occur. Species assessment criteria includes virus susceptibility, timing of symptom expression, severity of symptoms, suitability for grafting, available leaf observation area and the effectiveness of NCM-ELISA and PCR detection using index plant tissue. Initial findings from this study indicate that several alternate *Ipomoea* species are susceptible to common sweetpotato viruses and may exhibit symptoms earlier than *I. setosa* enabling more rapid detection in an extended annual detection window.

A newly identified banana ampelovirus from south-east Asia.

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Plant pathogens and crops from tropical areas provide a disproportionately large number of diagnostic challenges, as they have been relatively less-studied. Bananas provide such an example, with novel pathogens regularly being discovered. Recently, during indexing of germplasm, *Closteroviridae*-like particles were identified by electron microscopy (EM) in five south-east Asian accessions.

Isolates were tested with degenerate PCR primers for three *Closteroviridae* genera. No amplification was achieved in the closterovirus and crinivirus assays. Ampelovirus primers targeting the conserved HSP70 region amplified 580 nt fragments from isolates Q6191 from Vietnam and Q6196 from Indonesia. Fragments were cloned and sequenced. Translated amplicon sequences within each isolate were 97.8-100% identical at the amino acid (aa) level across 193 residues. The two isolates were 96.6-98.3% identical. Sequences from both isolates were 52% identical to *Plum bark necrosis stem pitting-associated virus* (PBNSPaV; GenBank ID AIH06908), the best database match using BLASTp, and 86.0% identical to novel sequence we obtained for the tentative ampelovirus Sugarcane mild mosaic virus (SMMV).

In order to obtain further genomic sequence, preliminary high throughput sequencing for one isolate, Q6191, was conducted. Primers designed to sequences obtained within ORF1a allowed amplification of overlapping 1064 nt from Q6191 and 812 nt from isolate Q6230. These translated sequences were 93.7% identical, and had the best BLASTp matches of 39% and 40% identity over 353 and 270 aa residues, respectively, to PBNSPaV (AMH87475).

In EM decoration tests using SMMV antiserum, SMMV particles were heavily decorated, while individual particles from the banana accessions had moderate, little or no decoration, suggesting a diversity of ampeloviruses or strains in the banana accessions.

Additional high throughput sequencing has been undertaken for isolates from all five accessions. Identification of further ampelovirus sequence will allow the development of a molecular diagnostic assay for use in the safe importation of new banana germplasm into Australia.

POSTER 215

***Ipomoea setosa*; and effective and reliable tool for the detection of SPLCV in sweetpotato**

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The Australian sweetpotato industry has undergone remarkable growth and massive productivity improvement in the last 16 years. It is now the most intensive commercial sweetpotato production system in the world, with growers attaining the highest commercial yields per hectare. Much of this is due to the development of a pathogen tested (PT) clean seed scheme from the early 2000s, providing Australian growers with significantly improved quality planting material. Growers currently market over 90,000 tonne per annum of fresh sweetpotatoes worth over \$100 million. A key facet of the Australian sweetpotato PT program at the Department of Agriculture and Fisheries Queensland's Gatton Research Facility has been the use of herbaceous indicator plants such as *Ipomoea setosa* to screen for viral pathogens. Critical knowledge for the PT process is an understanding of endemic viruses of concern to the industry and an awareness of any potential biosecurity threats. While *I. setosa* is regarded as a reliable method to detect virus presence in sweetpotato globally it is widely considered to be non-specific for many viruses. Between 2006 and 2016, as part of routine germplasm screening, unusual symptoms such as leaf cupping, stunting and chlorosis were observed on grafted *I. setosa* plants. Sweetpotato plants used as scions were observed to be largely asymptomatic over this 10 year period. NCM-ELISA testing produced negative results. PCR testing with degenerate geminivirus primers in 2006 and Sweetpotato leaf curl virus (SPLCV) specific qPCR testing, 2014 to the present has consistently produced positive SPLCV detections in *I. setosa* plants exhibiting these specific symptoms. Sequence information correlates the observed symptoms on *I. setosa* plants to SPLCV infection. Australian results suggest that the particular symptoms observed on *I. setosa* are associated with SPLCV infection, and that experienced operators can deduce the presence of SPLCV in sweetpotato by observing grafted *I. setosa* plants.

POSTER 216

The assessment of physiological methods for early, quantifiable stress and disease detection in banana plants

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Fusarium oxysporum f. sp. *cubense* Tropical Race 4 (TR4) was detected on a banana farm in north Queensland in March 2015, presenting a major risk to the viability of the \$600 million Australian banana industry. Surveillance for infected plants continues through regular on-ground monitoring of external symptoms of TR4, which include lower leaf yellowing and pseudostem splitting. However, if detection of TR4 was possible before notable visual symptoms appeared, isolation and plant destruction could occur earlier to reduce the increase in inoculum and potential spread through soil movement. Symptoms of TR4 infection can often be confused with those caused by other forms of stress, therefore reducing the ability to accurately identify suspicious plants. The aim of this study was to use rapid plant physiological assessment methods (proline accumulation in leaf tissue, chlorophyll fluorescence, chlorophyll content and thermal imaging) to elucidate the potential of effectively detecting and differentiating plant stress prior to the appearance of visual symptoms. Furthermore, the severity of stress could be quantified offering objective measurements of leaf function. Stress treatments were applied to Cavendish banana 'Williams' (*Musa* AAA) plants in a glasshouse pot trial, which included: water logging; drought; nitrogen, potassium, and phosphorus deficiency; cold stress; and herbicide spray-drift. A 2 mM fusaric acid solution (a known toxin produced by TR4) and a control treatment were also included. Over the three month experiment the physiological tools could not differentiate between the control and stress treatments before visible symptoms were detectable and require further refinement for future work in early disease detection. However, the tools gave quantifiable measurement of the severity of stress by the loss of leaf function and provided alternatives to subjective disease assessments of bananas.

POSTER 217

Complete genome sequence of *Clerodendrum* chlorotic spot virus, a putative dichorhavirus

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Clerodendrum chlorotic spot virus (CICSV), which is considered a tentative member of the genus *Dichorhavirus* (family *Rhabdoviridae*, order *Mononegavirales*), causes non-systemic chlorotic and necrotic lesions in the leaves of several plant species of the genus *Clerodendrum*, family *Lamiaceae*. Rod-shaped particles of this virus (ca. 40 nm x 100-110 nm) occur in nuclear electro-lucent inclusions, and adjacent to the membranous systems of both the nuclear envelope and the endoplasmic reticulum of the infected cells. Leaves of a *Clerodendrum* sp. plant showing chlorotic and necrotic symptoms were collected in a residential garden in Piracicaba, State of São Paulo, Brazil, in 2015. Mites from that plant were anatomically identified as *Brevipalpus yothersi*. Presence of putative CICSV particles and the typical cytopathic effects associated to their infection were confirmed by transmission electron microscopy. CICSV RNA was detected by RT-PCR using primers that specifically amplify its *L* gene. Five hundred ng of RNA extracted from the leaf lesions were sequenced by NGS. *In silico* analyses revealed that the viral genome is split into two RNA molecules and, similar to other dichorhaviruses, it harbors six ORFs in the 3'-5' orientation. Five of these ORFs, *N*, *P*, *MP*, *M* and *G* genes are arranged in the RNA1, whereas the *L* gene is in the RNA2. CICSV RNA1 and RNA2 show 52-72% and 60-76% nucleotide sequence identity, respectively, with the cognate molecules of orchid fleck virus, coffee ringspot virus (CoRSV) and citrus leprosis virus N. Best identity scores of each ORF both at nucleotide and amino acid levels were always obtained in comparison with CoRSV. Molecular data confirm the results of previous biological tests that suggested CICSV as a putative dichorhavirus and support the assignation of this virus to a new species of the genus.

POSTER 218

Analysis of the NID domain of the capsid protein of banana streak virus and utilization for diagnostics

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The banana streak virus (BSV) capsid protein contains an N-terminal, intrinsically disordered (NID) domain that is surface-exposed on the virion and likely is multifunctional and plays important roles in viral replication and transmission. The immunodominant continuous epitopes on the virion are also located in the NID domain, and therefore this domain is of great interest from a diagnostics perspective. The BSV capsid protein is cleaved from a larger polyprotein through the action of the virus-encoded aspartic protease but the enzyme substrate sites, and hence the protein boundaries, have not yet been determined. Success has been achieved in expressing the cauliflower mosaic virus (CaMV) aspartic protease in *E. coli* using methods developed for mammalian-infecting retroviruses and enzyme activity is currently being investigated. Once methods are optimized for CaMV, then work will begin on characterizing the BSV AP.

Using chemically synthesized peptides to mimic the continuous epitopes in the BSV NID domain, antisera have been raised in rabbits, and shown to cross-react with the virus in a range of assay formats such as ELISA, immunosorbent electron microscopy and immunocapture PCR. This technology promises to provide a rapid and reproducible way of generating immunodiagnostic reagents for all plant-infecting pararetroviruses.

POSTER 219

The challenges of fungal and bacterial diagnostics in tropical north Queensland

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Tropical north Queensland is a unique environment and has its own set of challenges for fungal and bacterial diagnosticians to deal with on a daily basis. Risks or threats can be from external or internal sources and this requires staff to remain vigilant and constantly on the lookout for new and exotic plant pathogens. First and foremost is our proximity to neighbouring countries that are known to have plant diseases currently exotic to Australia. Some examples include, Eumusae leaf spot of banana (*Mycosphaerella eumusae*), coffee leaf rust (*Hemileia vastatrix*) and citrus greening disease (*Candidatus Liberibacter* spp.), these are present in Malaysia, Papua New Guinea and Indonesia respectively. Cropping in the region is highly variable and includes tropical and subtropical fruits and vegetables, pulses, pasture grasses and ornamentals (to name a few). The addition of protected and intensive cropping of blueberries in the region is a prime example whereby a new suite of diseases and insect pests have emerged. The list of hosts dealt with on an annual basis is on the increase and this diversification is a challenge in itself, requiring diagnosticians to be generalists rather than a specialist. Another challenge is the isolation from specialist groups with expertise in fields such as virology or specific molecular technologies. In these situations, we rely heavily on other regions that have the capacity to assist in these specific areas. Compared to research and development, diagnostics is not as highly valued but is still an integral component of plant pathology. Diagnostics in general can be extremely frustrating but highly rewarding at the same time.

POSTER 220

Ascochyta blight pathogens isolated from Australian native and weed legumes

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In Australia, ascochyta blight (blackspot) complex is the most common and damaging disease of field pea (*Pisum sativum* L.), causing yield losses ranging from 15 to 75%. Internationally, four fungal species are responsible for ascochyta blight of field pea; *Didymella pinodes* (syn. *Peyronellea pinodes*), *Phoma medicaginis* var. *pinodella*, *Ascochyta pisi* and *Phoma koolunga*. *P. koolunga* (previously identified as *Macrophomina phaseolina*) was found on field peas with ascochyta blight in South Australia in the early 1980s.

A survey of fungal species associated with foliar disease of native, pasture and weed legumes is in progress, with the aim of understanding the origin(s) and host range of *Phoma koolunga*. This survey may also provide information on the role and extent to which volunteer plants in cultivated or non-cultivated ecosystems are a source of pathogens in the ascochyta blight complex in Australia.

For four isolates found to date, infectivity on field pea, cv. Kaspera, was tested by spray-inoculation in a controlled environment growth room. Field pea plants treated with *P. koolunga* and sterile water served as positive and negative controls respectively.

The results revealed isolates of *Didymella pinodes* and *Ascochyta viciae*/A. *pisi* from a native *Senna* species, *Vicia cracca* (bird vetch), *Lathyrus tingitanus* (tangier pea) and *Vicia sativa* (common vetch) infected and caused typical ascochyta blight lesions on field pea. The implications of these findings are discussed.

POSTER 221

***Fusarium* species associated with summer field crops in eastern Australia**

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A number of important *Fusarium* diseases occur in grain sorghum and mungbean crops throughout eastern Australia, often resulting in serious losses in crop yields and grain quality. *Fusarium* stalk rot can cause significant losses in grain sorghum crops in eastern Australia, particularly when the disease results in lodged stalks. *Fusarium moniliforme* sensu lato has historically been reported to be responsible for *Fusarium* stalk rot, however a recent revision of the taxa has segregated it into a number of species. The first part of this study identifies the *Fusarium* species associated with *Fusarium* stalk rot and head blight in eastern Australia. A total of 523 *Fusarium* isolates were collected from eastern Australia during the 2009-2011 cropping seasons from diseased sorghum plants. Nine *Fusarium* species were isolated from symptomatic tissues and pathogenicity tests confirmed that *F. andiyazi* and *F. thapsinum* were the dominant stalk pathogens, whilst *F. thapsinum* and species within the *F. incarnatum*-*F. equiseti* species complex were most frequently associated with head blight.

Mungbean production has increased in Australia in recent years, and with that increase there has also been a reported increase in incidence and severity of *Fusarium* wilt. The second part of this study identified the species associated with *Fusarium* wilt of mungbean in Queensland. A total of 114 *Fusarium* isolates were collected from diseased mungbean plants throughout Queensland during the 2011-16 cropping seasons. A total of eight *Fusarium* species were recovered from symptomatic tissues. Species within the *F. oxysporum* and *F. solani* species complexes were most frequently recovered. The pathogenicity of species within the *F. oxysporum* and *F. solani* species complexes on mungbean was confirmed.

The findings in this study provide valuable insight into the diversity and abundance of *Fusarium* species associated with two important summer field crops in eastern Australia.

POSTER 222

Molecular characterization of viruses infecting taro in East Africa

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Taro (*Colocasia esculenta* L.) is a widely cultivated edible member of the *Araceae* family. It is one of the staple food crops in sub-Saharan Africa, with both the foliage and corms utilised as a food source. Despite its substantial contribution to the food and income security for many small-scale farmers in east Africa, the average yields are below the potential of the crop due to various factors including diminishing soil fertility, lack of improved varieties and the presence of pests and diseases. Viruses are known to be one of the most important constraints to taro production, and can cause severe yield reduction. Since the production of taro in east Africa has declined significantly over time, the identification of viruses affecting taro in the region has become a research priority. Leaf samples were collected from major taro growing areas in Ethiopia, Kenya, Tanzania and Uganda and nucleic acid was extracted. Initially, samples were screened for different RNA and DNA viruses by PCR using published virus-specific and/or degenerate primers. For RNA viruses, representative samples were also subjected to illumina MiSeq NGS sequencing and data validated using Sanger sequencing. For badnaviruses, the complete genome of representative samples was amplified using rolling circle amplification (RCA) and sequenced using a primer walking approach. To date, several viruses have been detected including *Dasheen mosaic virus* (DsMV), *Taro bacilliform virus* (TaBV) and *Cucumber mosaic virus* (CMV), with additional analysis ongoing. Results of the surveys and virus incidence/distribution will be discussed.

POSTER 223

Identification and detection of species associated with *Pythium* soft rot of ginger in Australia

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Pythium soft rot (PSR) of ginger is of a major constraint for ginger production in Australia and worldwide. In Australia, the PSR outbreaks first recorded on the two oldest ginger farms in 2007 were attributed to *Pythium myriotylum* and since then the disease has been observed on many other farms. This study was to: (1) identify if PSR of ginger associated with species rather than *P. myriotylum*; (2) assess an influence of species interactions on PSR disease severity of ginger; (3) develop an accurate and rapid detection method for the associated pathogens. A total of 173 *Pythium* isolates belonged to 11 different *Pythium* species and 15 *Pythiogeton* (*Py.*) *ramosum* isolates were recovered from PSR ginger and soil from around infected ginger. Of these species, only *P. graminicola*, *P. myriotylum*, *P. spinosum*, putative *P. zingiberis* and *Py. ramosum* were isolated directly from the PSR ginger. Koch's postulates were fulfilled with *P. myriotylum*, putative *P. zingiberis* and *Py. ramosum*. This is for the first time that *Py. ramosum* was recorded in Australia and as a pathogen on ginger. Comprehensive analyses of morphology, physiology, phylogeny and pathogenicity were allowed us to confirm species conspecificity of *P. myriotylum* and *P. zingiberis*, and *P. myriotylum* was the main pathogen of PSR of ginger in Australia. Co-inoculation of *P. oligandrum*, a well-known antagonist and *P. myriotylum* did not reduce PSR disease severity of ginger in pot trials. A PCR-RFLP of the CoxII fragment was successfully developed to detect *P. myriotylum* directly from PSR ginger sampled from pot trials.

POSTER 224

Occurrence of *Aster yomena* Sclerotinia Rot Caused by *Sclerotinia minor* in Korea

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Sclerotinia rot symptoms were frequently found on stems of *Aster yomena* cultivated in Gurye area of Korea in April 2016. The symptoms mainly appeared on the stems, and severely infected plants blighted. White mycelia spreaded over stems of the infected plants and soil surface, and formed small black sclerotia on the lesions and inside of diseased stems. Incidence of the disease was as high as 20~ 80% in the *Aster yomena* fields. On the basis of morphological, cultural characteristics and ITS gene sequences of the isolates from the lesions, the fungus was identified as *Sclerotinia minor*. This is the first report of *Aster yomena* Sclerotinia rot caused by *Sclerotinia minor* in Korea.

POSTER 225

Comparative diagnosis of viral pathogens using side-by-side trials of existing Post Entry Quarantine and small RNA next generation sequencing methods.

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Imported high-risk plants spend up to 2.5 years in Post Entry Quarantine (PEQ) facilities prior to their release to plant industries. During their time in PEQ, imported plants are screened for exotic pests including viruses, viroids, bacteria, fungi and nematodes. Existing post entry quarantine screening protocols rely on resource intensive molecular and serological tests and time-consuming biological indicators. The prolonged PEQ screening schedule impacts plant industries' capability to access new national and international market opportunities. The use of next-generation sequencing (NGS) and suitable bioinformatics pipelines to sequence and analyse small RNAs arising from the natural plant antiviral response system can accelerate the screening process of plants in quarantine. This strategy offers a number of advantages over existing PEQ protocols, namely improved accuracy, scalability, reduction in PEQ costs and screening times facilitating more rapid release of new genetics. We have recently implemented an automated viral diagnosis bioinformatics toolkit using the Yabi web-based analytical environment that enables improved detection of viruses and viroids. Here we present the results from side-by-side trials for Citrus, Prunus, Fragaria, Vitis and Solanum using traditional PEQ testing protocols and small RNA NGS method. These trials were undertaken at the Department of Agriculture and Water Resources Australia and Ministry for Primary Industries New Zealand, to confirm the sensitivity, specificity and robustness of the proposed new method.

POSTER 226

Modern Diagnostics Project: Enhanced Plant Health Diagnostics for Northern Australia

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The Modern Diagnostic initiative delivers on government priorities to improve biosecurity surveillance outlined in the Agricultural Competitiveness White Paper and the Our North, Our Future: White Paper on Developing Northern Australia. Diagnostics underpins Australia's tropical biosecurity surveillance activities. The Modern Diagnostics initiative is strengthening Australia's tropical biosecurity diagnostic capability through increasing connectivity between tropical biosecurity diagnosticians, enhancing diagnostic skills and tools across northern Australia, and improving tropical biosecurity infrastructure.

The project aims to enhance plant health diagnostic capability in northern Australia through strategic investment in diagnostic tools and training. Priorities for the investment of this funding have been identified through expert working groups (plant pathology and entomology) comprised of senior diagnosticians from the four northern jurisdictions (QDAF, NT DPIR, DAFWA and NAQS). The working groups developed gap analyses detailing what they considered to be the most significant plant health diagnostic gaps facing northern Australia.

Approximately \$480,000 per year for three years is available to address these gaps, with the project currently in its second year of funding. Significant progress against a number of high priority diagnostic gaps has been made during the project's first year of funding. The focus over the next two funding cycles is to engage with the diagnostic community to close the remaining diagnostic gaps through projects that will provide legacy against this investment.

POSTER 227

Cause of internal finger rot or finger tip rot of banana in far north Queensland

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A fruit quality problem has been identified in far north Queensland during the processing of ripe banana for pulp. The disease symptoms show an internal fruit discoloration; reddish brown to blackish centre and pulp breakdown. The incidence of disease has been estimated at 1% of ripened fruit and it is found most common in wet season. Initially it was thought to be a physiological problem, but two bacteria from the Enterobacteraceae family; *Pantoea agglomerans* and *Enterobacter cowanii* have been frequently recovered from infected fruits. Artificial inoculations of banana fruit with the bacteria were conducted at the QDAF South Johnstone Research Station to determine their pathogenic nature. Fingers on bunches at the bract-lift stage were injected with suspensions (10^8 cfu /ml) of each of the bacteria. One month after inoculation, fingers were cut open to check for disease development. Both bacteria produced similar symptoms to the fruit samples taken from the pulp processing facility. Inoculated fingers were easily distinguished from others in the bunch by their deformed shape, being out of line with other fingers and their premature yellowing and reduced fruit size. Internally, inoculated fingers showed a rusty red discoloration of the pulp with a deep gelatinous cavity. The exact disease cycle caused by these opportunistic bacteria is currently unknown. No treatment has been identified to manage the bacteria, the risk of infection may be reduced through insect pest management and maintaining the proper drainage and field sanitation during warm and humid weather conditions. Fruit with obvious symptoms of deformity (narrow at flower end or straight and out of alignment) should be discarded at the packing shed.

This study provided the first report of *Enterobacter cowanii* and *Pantoea agglomerans* causing internal finger rot or fingertip rot of banana.

POSTER 228

Phenotyping crown rot in wheat using imaging technologies

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Crown rot caused predominantly by *Fusarium pseudograminearum* significantly limits yield in Australian winter cereals. Breeding and pre-breeding for crown rot resistance and tolerance in wheat has been a national focus. While strong levels of partial resistance and tolerance exist, the complex nature of inheritance and the difficulties associated with phenotyping this disease, has resulted in slow progress of the availability of commercial cultivars with high levels of resistance and tolerance to crown rot. When screening for crown rot in field trials currently multiple tillers are rated on approximately 20 plants randomly sampled from within each experimental plot at maturity. This process is laborious and often difficult to achieve consistent results between seasons. Alternative methods of measuring the host response to *Fusarium pseudograminearum* infection are being tested in field trials in the northern region. Inoculated and non-inoculated plots of 12 wheat genotypes ranging in susceptibility to crown rot were assessed in the 2016 season.

A new method for data collection was designed using multispectral, thermal and visible cameras. Images were analysed by the wavelet and filter band methods to extract the differences between plus and minus inoculated plots under field conditions. A new index (PWDI: Phenotyping Wheat Disease Index) is proposed to detect the differences between the plus and minus inoculated plots; and rank 12 wheat genotypes into three classes such as most resistant, middle, and susceptible classes, with the accuracy from 80% to 90% when comparing with the expected crown rot ranking. Artificial intelligence and machine learning models will be developed for integration into the dataset to validate results against traditional disease assessments performed at maturity. Both researchers and breeding companies would greatly benefit from such technologies in Australia and internationally. Significant benefits would be further provided to growers to allow appropriate management strategies to be implemented on farm.

Detection of Cucumber green mottle mosaic virus (CGMMV) in seed commodities using lateral flow technology

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Cucumber green mottle mosaic virus (CGMMV) is a seed transmitted *Tobamovirus* that infects several cucurbit species and can result in significant crop losses. Recent findings of CGMMV in the United States and Australia have resulted in increased biosecurity measures for international seed trade. Most commonly, cucurbit seed is tested using ELISA and / or PCR to determine if it is contaminated with the pathogen. While ELISA and PCR are considered to be reliable testing methods, they are also laborious and take several hours to complete. In 2015 Agdia raised new monoclonal and polyclonal antibodies to CGMMV for the purpose of developing improved serological diagnostics that could be used for seed testing. One pair demonstrated superior sensitivity, particularly in a lateral flow device (ImmunoStrip®) format, and was selected for assay development. The resulting ImmunoStrip was found to be more sensitive than ELISA and demonstrated a high degree of specificity to CGMMV. It was hypothesized, based on its high degree of sensitivity, that the ImmunoStrip could offer a more sensitive method for detection of CGMMV in seed commodities. Such an assay could provide biosecurity personnel a mechanism to test seed lots in non-laboratory environments and obtain results hours faster than other methods currently in use.

To challenge this hypothesis several lots of infected seed were obtained that varied in CGMMV contamination levels. These lots were tested by the ImmunoStrip and compared to ELISA following International Seed Testing Association (ISTA) testing guidelines. The results, to be presented, provide compelling evidence the ImmunoStrip can be used for reliable detection of low level CGMMV contamination of cucurbit seed lots. To our knowledge this would be the first lateral flow test validated for detecting CGMMV in seed commodities.

POSTER 230

A novel isothermal DNA test for detection of *Xylella fastidiosa*

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Xylella fastidiosa is a gram-negative, xylem limited, bacterium that can be devastating to infected crops. Although *X. fastidiosa* infects hundreds of plant species, it is commonly known as the causal agent of Pierce's disease in grape, citrus variegated chlorosis (CVC), almond leaf scorch (ALS) disease, and olive quick decline syndrome (OQDS). In Australia, even though the pathogen is not present, strict testing and quarantine requirements have been imposed on imported host plants to mitigate the risk of its introduction. Testing for *X. fastidiosa* is commonly performed using ELISA or PCR methodologies. PCR has historically offered the greatest level of sensitivity and specificity for *X. fastidiosa* but is laborious, taking hours to complete, and must be performed in a laboratory by skilled technicians. Agdia, Inc has developed a rapid nucleic acid amplification assay, called AmplifyRP® XRT+, for detection of *X. fastidiosa* that is based on recombinase-polymerase amplification technology. The test uses two target-specific primers and one internal probe that results in specific detection of *X. fastidiosa*. The test is performed in only 20 to 40 minutes at single operating temperature of 39°C using crude sample extracts. Results can be visualized in real-time using a battery operated fluorometer or at end-point using a lateral flow strip housed inside a disposable amplicon detection chamber.

The test was validated to detect multiple *X. fastidiosa* isolates collected from the U.S., Brazil, and Europe and demonstrated equivalent sensitivity when compared to published quantitative real-time PCR methods. No false positives were observed when testing multiple host plants including grapevine, citrus, olive, blueberry and blackberry. The validation data collected strongly supports this assay could be used as a reliable alternative to qPCR while offering the benefits of time savings, ease-of-use, and portability.

POSTER 231

Digital tools to facilitate assessing powdery mildew on grape bunches

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Powdery mildew can affect wine quality so Australian wineries often use a threshold of 3-5% disease severity on bunches near harvest to support decisions about grape quality and price. The quality of visual assessment in the vineyard reflects the experience and training of assessors in recognising disease and estimating bunch surface affected. A free application (PMapp) for Apple and Android devices was developed to facilitate assessment of powdery mildew in vineyards and promote uniformity of assessment practices. PMapp comprises an assessment screen for entry of severity scores, a reference set of computer-generated images to support area assessment, a self-calibration tool for the user to check his/her accuracy and a diagrammatic key with 2% increments in the range 2-12%. Since release in Australia in December 2015 and globally in November 2016, PMapp has been downloaded over 2000 times.

A website developed to support use of PMapp (www.pmassessment.com.au) offers a stepwise guide to in-field assessment, with links to training in disease recognition and area assessment and to the diagrammatic key. The disease recognition module comprises photographs of grape bunches at veraison and close to harvest; each is presented in triplicate, with different areas outlined that might represent surface with powdery mildew. The user selects the image that has powdery mildew outlined most correctly. The area assessment module allows the user to test his/her ability to assess bunch surface area affected on computer-generated images coloured to indicate powdery mildew. Results are presented as a chart showing agreement of estimate with actual image, repeatability and time taken per image. The user can access results of his/her previous attempts.

Feedback from wine sector representatives indicates that these resources have improved the quality of in-field disease assessment. PMapp is also being used in assessing bunch rot on grapes and diseases of various crops.

POSTER 232

Molecular characterisation and diagnosis of badnaviruses infecting yams in the south pacific

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Yams (*Dioscorea* spp.) are economically important, annual or perennial tuber-bearing, tropical plants. Globally yam is ranked as the fourth most important root crop by production and provides a staple food for millions of people in Africa, South America, Asia and the Pacific. In the Pacific, production and utilisation of yams is limited by several issues including diseases and the lack of genetic diversity. An important global *in vitro* collection of yam germplasm is conserved in tissue culture by the Secretariat of the Pacific Community (SPC) in Fiji. Evaluation of this germplasm and its distribution to within the Pacific holds the key to improved production. However, similar to other vegetatively propagated crops, yam has a tendency to accumulate and perpetuate tuber-borne fungal and viral diseases. Although, tissue culture eliminates fungal pathogens, viruses remain an issue. As such, quarantine regulations prohibit the movement of yam germplasm from the SPC collection to other countries due to the risks associated with movement of untested and/or virus-infected material. Sensitive diagnostic tests are needed to enable the virus indexing of yam germplasm. Several different viruses are known to infect yams, but badnaviruses remain the least studied and difficult to diagnose. Rolling circle amplification (RCA) based approach has been used to characterise the diversity of badnavirus infecting Pacific germplasm. A subset of the germplasm conserved at the SPC has been screened and amplified episomal badnavirus DNA has been sequenced to better understand the diversity of badnavirus infecting Pacific yam germplasm. Full genomes of three species of yam infecting badnavirus have been sequenced. The characterisation of badnaviruses infecting yams will assist in the development of reliable diagnostic techniques and furnish germplasm centres with the tools to safely mobilise their yam collections, so that food and nutritional security can be achieved in the Pacific and other regions.

POSTER 233

Etiology of Parsley Summer Root Rot in Australia

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Parsley production in Australia is valued at \$10M per annum. A root and collar rot over the summer months can decimate crops causing plants to wilt and collapse. Curly leaf cultivars are particularly susceptible. Crop surveys in NSW, Queensland, Victoria and Western Australia determined that disease occurrence is widespread in both soil and soilless production systems. Previous studies of parsley root rot postulated that this summer disease was distinct from those occurring in cooler months that were attributed to species of *Pythium*. Those studies identified *Fusarium oxysporum* as a potential causal pathogen and/or an uncharacterised *Pseudomonas* sp. Diagnostic pathology on samples collected during this project produced an array of *F. oxysporum*, *F. solani*, *Pythium* spp, and bacterial isolates. These were partially characterised and used in pathogenicity experiments. Several experiments were conducted in potted parsley plants as well as soilless systems using the nutrient film technique or rockwool blocks. Inocula were applied to plants as single isolates or as combinations of different isolates or species. Only treatments that included isolates of either of two *Pythium* species were capable of inducing disease symptoms. The more commonly occurring of these was *P. sulcatum* which is an aggressive pathogen of apiaceous plants. It is known to cause the diseases cavity rot and forking of carrots as well as root rots of parsley and coriander. It has a wide temperature range for growth so could be solely responsible for parsley root rots throughout the year. However, there are some unexplained issues from our results and observations. Often early disease symptoms appear at the plant collar. Why do coriander and flat leaf parsley crops appear to be less affected? Could there be other contributing factors such as residual herbicides or fertiliser interactions? Is there a cryptic pathogen that we have not identified?

POSTER 234

Two little known fungal plant pathogens isolated from grapevine exhibiting trunk disease symptoms in Hungary

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Grapevine trunk diseases (GTDs) represent a complex, and hard to manage plant pathological problem in viticulture worldwide. Numerous xylem-inhabiting and other phytopathogenic fungi have already been detected to be associated with GTDs; among these, some species of the Botryosphaeriaceae seem to be the most important pathogens involved in this disease complex. To identify fungal species associated with GTDs in Hungarian vineyards, a total of 230 wood samples were collected from vines exhibiting typical GTD symptoms in 2013-2016. Fungal colonies isolated from these samples were identified based on their nrDNA ITS and EF1- α sequences. Pine needle medium was successfully used to induce pycnidial production in some of the non-sporulating strains. Pathogenicity tests were performed using approx. 5-month old shoots of 1.5 year old, asymptomatic potted vines produced in a greenhouse. In addition to well-known species of the Botryosphaeriaceae, and other fungi described as being associated with GTDs in different parts of the world, this work revealed the presence of two little known species, *Seimatosporium vitis* and *Dothiorella omnivora*, in grapevine exhibiting trunk disease symptoms in Hungary. All the strains of both species sporulated well on pine needle medium and infected grapevine shoots during pathogenicity tests.

This work was supported by GINOP-2.3.2-15-2016-00061 project.

POSTER 235

Defining threats of exotic bacteria to biosecurity using comparative genomics

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Bacterial populations are highly diverse on horticultural crops. Many species are comprised of both pathogenic and non-pathogenic variants, which can lead to limitations in existing taxonomic-based molecular diagnostics. This study used the kiwifruit-*Pseudomonas* pathosystem as a model to develop alternative molecular diagnostics that might assist in rapidly defining risks of bacteria at the border. Initially, a collection of pseudomonads from kiwifruit were tested for virulence. Loci that differentiated pathogenic pseudomonads from non-pathogenic strains were then identified using comparative genomics. Finally, PCR was optimized to target these loci, providing a more generic risk assessment of unknown strains. Our new knowledge of pathogen-associated loci was subsequently applied to define the biosecurity risk from *Pectobacterium carotovorum* subsp. *actinidiae* (Pca), an unrelated enterobacterium that purportedly causes summer cankers in kiwifruit orchards throughout South Korea. Future work will also examine whether pathogen-associated loci in other important genera may aid biosecurity decision making, especially in post entry quarantine, surveillance and incursion response.

POSTER 236

Neocosmospora root rot and Tobacco Streak Virus as a disease complex of peanut in central Queensland, Australia

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The incidence of a virus and fungal pathogen coexisting within the roots of the same plant is an uncommon occurrence. Tobacco streak virus (TSV) has been known to cause significant crop losses of sunflower (*Helianthus annuus*) and mungbean (*Vigna radiata*) and has been recorded in peanut (*Arachis hypogaea*) plants in the Emerald region of central Queensland, Australia. Neocosmospora root rot (*Neocosmospora vasinfecta* var. *africana*) is an emerging pathogen that has also caused extensive damage to peanut crops in this region during intermittent outbreaks. In 2016, peanut plants in a commercial crop near Emerald exhibited wilting of the main stem, leaf chlorosis and root decay with orange-reddish perithecia of *N. vasinfecta* var. *africana* developed on necrotic areas of the main taproot and lateral root system. These symptoms and signs were consistent with infection by Neocosmospora root rot of peanut. Other plants in the crop displayed typical foliar symptoms of virus infection such as stunted growth with chlorotic or necrotic leaves and shortened internodes. *N. vasinfecta* var. *africana* was isolated from the roots of 95% of plants with symptoms of Neocosmospora root rot and ELISA testing detected TSV in 80% of these roots. The virus was not detected in the leaves of these plants infected with both pathogens. Although TSV was detected in the leaves and the roots of the plants exhibiting typical foliage symptoms of virus infection, *N. vasinfecta* var. *africana* was not isolated from any of their roots. These results suggest an unexpected relationship between TSV and Neocosmospora root rot that has contributed to outbreaks of a root disease complex of peanut crops in central Queensland.

POSTER 238

Development of new collection and diagnostic methods for Sugarcane streak mosaic virus

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Sugar Research Australia's (SRA) biosecurity team works towards identifying and managing biosecurity threats to the Australian Sugar Industry. Sugarcane streak mosaic virus (SCSMV) is of high biosecurity concern to Australia and has resulted in yield losses of up to 30% in Indonesia. SCSMV is spreading throughout South East Asia, so the ability to rapidly collect and identify diseased specimens is of major importance to sugarcane production in Australia.

With funding from the Australian Centre for International Agriculture Research (ACIAR), SRA is collaborating with the Indonesian Sugar Research Institute (ISRI), Bogor Agricultural University, Indonesian Sweetener and Fibre Crop Research Institute (ISFRI) and Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) to research a number of aspects related to SCSMV. This includes various sample collection techniques, investigation of possible transmission vectors and development of a variety of diagnostic tests.

This poster will describe two new developments: a successful trial utilising RT-PCR diagnostic methods with Whatman FTATM Plantsaver cards for SCSMV infected sugarcane leaf samples which has given us a more rapid and less destructive method of importing samples to Australia; and loop-mediated isothermal amplification (LAMP) is being developed as a rapid diagnostic tool on a range of specimens. These tools will allow faster diagnosis and provide a better understanding of SCSMV.

POSTER 239

Characterisation of *Diaporthe* species causing husk rot in macadamia

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Phomopsis husk rot, an emerging disease in macadamia, is characterised by diffuse, soft, and spongy black lesions appearing on macadamia fruit pericarp. Phomopsis husk rot causes premature fruit drop and a reduction in kernel quality. Recent studies have indicated that species within the genus *Diaporthe* cause the disease, and that damage to the pericarp is a prerequisite for infection. In order to better understand disease aetiology, a thorough taxonomic analysis of the isolates of *Diaporthe* spp. from diseased macadamia pericarps is required. Success in the delimitation of species within this genus has been attributed to multi-locus DNA sequence analyses of several specific loci. The rDNA internal transcribed spacer (ITS) and beta-tubulin (TUB) regions represent the most promising targets for inferring meaningful taxonomic and phylogenetic relationships for species in the genus *Diaporthe*. In this study, we used ITS and TUB regions of isolates obtained from different macadamia cultivars in Australia, to characterise the *Diaporthe* spp. that cause Phomopsis husk rot. Pathogenicity assays with different *Diaporthe* spp. were used to assess pathogenic variation and virulence on macadamia varieties. Preliminary results indicate significant variation exists between cultivars to infection by *Diaporthe* spp. A more exhaustive range of cultivars are now being studied to elucidate any differences in susceptibility between cultivars and gain a temporal understanding of the infection process. The study revealed new information on varietal susceptibility, pathogenic variation and virulence of species of *Diaporthe* on macadamia. The scientific understanding of the genus *Diaporthe*, as well as our understanding of the aetiology of Phomopsis husk rot, underpin the development of future control strategies for the disease, leading to greater yields and higher profitability for farmers.

POSTER 240

Bacterial crown rot, a major threat to Australia's papaya industry

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Bacterial crown rot (BCR) also known as bacterial canker or bacterial decline is considered one of the most important diseases affecting papaya (*Carica papaya*) and is a serious threat to Australia's \$30 Million papaya industry. Symptoms include angular water-soaked lesions on leaves and petioles causing leaves at the top of the canopy to wilt and die. Firm water-soaked cankers develop on the stem causing the stem to collapse. Small water-soaked lesions which develop on green fruit develop into firm depressed lesions. This disease is reportedly caused by a few species of bacteria from the Enterobacteriaceae family and the genus *Erwinia*. A research project in the Philippines funded by the Australian Centre for International Agricultural Research (ACIAR) has recently identified the causal agent as *E. mallotivora*. This bacterium has also been implicated in recent outbreaks of the disease in Malaysia and Tonga with estimated yield losses on the Malaysian Peninsular of 200,000 metric tonnes equivalent to US \$58 Million.

Research has shown that high rainfall is the most important weather factor influencing BCR providing conditions necessary for the penetration and distribution of the pathogen. Fifty percent of Australia's papaya is grown on the wet tropical coast of far north Queensland which has an average annual rainfall of 3750 mm. Consequently an outbreak of BCR would be devastating for the Australian industry.

Research findings continue to enhance our knowledge of bacterial crown rot and improve our awareness and preparedness in the event of such a disease incursion into Queensland. The Australian Quarantine and Inspection Service (AQIS) now restricts the importation of *Carica papaya* seed from 24 countries where BCR is recorded, with seed being subjected to hot water treatment at 50°C for not less than 20 minutes or growth in post entry quarantine.

POSTER 241

Risk Management of Angular leaf spot in trade

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Since its eradication in 2011-2012 from Bundaberg Qld, Australia has been considered free of angular leaf spot (ALS -caused by the bacterium *Xanthomonas fragariae*). ALS can infect all vascular tissue including the crown, leaves, roots, petioles, stolons and calyx as well as daughter plants, resulting in reduced yield in infected strawberry crops. The bacterium causes the underside of leaves of strawberry plants to form water-soaked lesions which may develop into necrotic tissue and exude a bacterial ooze. However the main economic damage is often attributed to the loss of marketability of strawberry fruit through infection of the calyx. *Xanthomonas fragariae* was first identified in Minnesota, USA in 1962 and has since been reported throughout the world. We will discuss the range of risk management measures that are available through the International Standards for Phytosanitary Measures (ISPMs), in particular: area freedom under ISPM 4: *Requirements for the establishment of pest free areas* and ISPM 10: *Requirements for the establishment of pest free places of production and pest free production sites* or a systems approach using the integration of different risk management measures under ISPM 14: *The use of integrated measures in a systems approach for pest risk management*. International trade is important for the Australian economy and managing the risks associated with the importation of fresh food commodities is critical for Australia's agricultural future.

POSTER 242

Modelling for quarantine decisions

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Quarantine restrictions provide an essential tool in the response to incursions of threatening pests and pathogens. The theoretical underpinnings of quarantine decisions, however, tend to be less well developed than those for surveillance. We simulate plant pathogen spread and control over a regional scale, to identify smart quarantine decisions. Our model is based on the current Queensland incursion of Panama disease Tropical Race 4, a fungal pathogen of bananas, but maintains sufficient generality to apply to other fungal species. The model includes within-property pathogen growth, and anthropogenic and environmental spread through multiple risk networks. We model diverse quarantine approaches, from easily implementable heuristic rules, to ones that attempt to exploit the structure of risk networks. We also allow for some level of "leakage" or small-level failure of quarantine restrictions. We contrast the performance of the different quarantine approaches in the context of relative importance of the different connectivity networks. Overall we find that a mix of long-distance as well as locally-focussed quarantine interventions is necessary to control spread and minimise the long-term impact of the incursion. This echoes recent results for optimal surveillance while focussing on prevention, rather than detection, of spread.

POSTER 243

The 'whys' and 'hows' of exotic plant pest prioritisation

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The aim of prioritising exotic plant pests is to enable a more strategic approach to plant biosecurity activities within finite resources. This prioritisation process should be transparent, inclusive and systematic. Many lists of priority plant pests have been developed over recent times, for Queensland, for Australia, and for other parts of the world. Both the purpose of these lists and the methodologies used to create them have varied. Some of these 'whys' and 'hows' will be discussed and compared.

For Queensland a qualitative framework incorporating extensive stakeholder engagement was selected as best for achieving the goals. These goals were: enhancing preparedness; improving risk management strategies; improving industry and community engagement; interacting with legislation; facilitating education and awareness; and determining priorities for investment in research, development and extension. Factors considered included the likelihood of pest entry and establishment, the potential impact of the pest (social, environment and economic), and the ability for the pest to be eradicated or managed.

A stakeholder engagement plan was developed outlining preparation, implementation and review steps. This includes defining what will be delivered to each stakeholder group, who will be involved, the level of engagement for each part of the process, and potential risks. It also included retrospective evaluation of the process and articulation of what feedback stakeholders can expect and when.

The priority plant pest list for Queensland will be a relevant dynamic list. It will deliver value to stakeholders by focussing biosecurity resources, from both within and outside government, where they can best contribute to protect market access and the profitability and sustainability of Queensland's industries, and safeguard natural assets, human health and lifestyle.

POSTER 244

Identification and Prioritisation of High Impact Plant Pests

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A method for plant pest risk assessment using historical incursion data is currently being developed by CSIRO and PBCRC to identify and prioritise high impact plant pests and diseases. By analysing the biological and pathway data for historical invasions of plant health threats in Australia and New Zealand, in conjunction with knowledge of their subsequent impact, we aim to differentiate species likely to have a high impact on plant production systems from those that are likely to have a low or moderate impact in future. This will allow the current list of potential threats to be prioritised according to their expected impact. Prioritisation of plant pests and diseases, and increased knowledge of their probable invasion pathway, will facilitate effective allocation of biosecurity resources, enhancing the likelihood of early detection and prevention of the most significant plant health threats. This is particularly important for those pests predicted to be of high impact but requiring only a low propagule pressure to establish and cause this impact. Details of the methods and some preliminary results will be discussed in this presentation.

POSTER 245

Norfolk Island Biosecurity: Protecting Norfolk Island in a changing biosecurity world

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Norfolk Island differs from mainland Australia in many aspects. It is a subtropical island with high rainfall and deep volcanic soils. The Norfolk Island territory covers 3,455 hectares across three islands (Norfolk, Nepean and Philip) and is situated off the east coast of Australia approximately 1400 km due east, 800 km north of New Zealand and 600 km south of New Caledonia in the Coral Sea. The Island's isolation has led to a unique history of biological evolution. It possesses a unique flora and fauna, with a number of endemic plant and animal species found nowhere else in the world. Additionally, the Island's community depends on this relatively pest-free environment to grow most produce to support the community. These attributes make the island vulnerable to the introduction of pests, emphasising the need for specialised biosecurity assessment and measures. In addition, this situation also provides a unique opportunity to the Department to study many aspects of biosecurity science in Australia.

The Australian Department of Agriculture and Water Resources is now directly involved in the biosecurity management and assessment of new import opportunities for the island. Crucial to this assessment is understanding Norfolk's unique Island biosecurity status and applying it to this review.

Prior to 1 July 2016 Norfolk Island was a self-governing external territory of Australia and had responsibility for its own quarantine processes. As of that date the self-governing status was repealed and the Commonwealth of Australia became directly responsible for plant biosecurity on Norfolk Island. Historically Norfolk Island permitted only four plant products to be imported: potatoes, ginger, garlic and onions. With the implementation of the change in circumstances there is potential for a wider range of plant and plant-based commodities to be imported.

POSTER 246

A decision tool to determine the technical feasibility of eradicating plant pests

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Determining if a plant pest is technically feasible to eradicate is a key decision during an emergency response to a plant pest, guiding the response management actions and arrangements under Australia's Emergency Plant Pest Response Deed (EPPRD). This decision is complex and often there are many limitations and uncertainties in the data available to support decision making. The decision makers must consider a wide range of different factors that impact on the biology and ecology of the pest; environment and climate; farming practices; social practices and values. Pest management options must be legal, practical and effective. Decisions are also collaborative with both government and industry stakeholders involved decision making. We worked with decision makers in biosecurity policy, operations and science, to develop a comprehensive structured decision making process to improve the process of determining if a pest is technically feasible to eradicate. An extensive list of decision criteria were developed which was then consolidated and structured into a conceptual diagram. This was formatted into several different decision tools which were evaluated by decision makers. Six major decision criteria and 68 sub criteria were identified as influencing the decision. A hybrid between a Bayesian network and a conceptual diagram was chosen as the format for the decision tool. This tool allowed the accumulation of evidence for key decision criteria but these were not automatically predicted using probability tables, instead the decision maker had to make these individual decisions. This modification was required because the diverse range of pests and infestation circumstances made it impossible to estimate probabilities that would encompass the huge range of diverse possibilities. The tool was tested on four historical case studies which resulted in further small refinements of the tool. Feedback from decision makers on the structure and utility of the decision tool will be discussed.

POSTER 247

Threatening plant diseases and biosecurity risk management

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Exotic plant diseases can significantly damage Australia's plant production and productive agriculture industries. Continuing international trade of agricultural commodities poses threats of introducing new exotic plant diseases to Australia. These could reduce yields, lower the quality of agricultural commodities, increase production costs and more importantly make it more difficult to sell produce on international markets. The Australian Government Department of Agriculture and Water Resources (the department) through biosecurity risk analysis plays a critical role in reducing biosecurity risks to an acceptable level to protect our agricultural industries and the environment from exotic plant diseases.

Consistent with international obligations and Commonwealth legislation, the department conducts risk analyses of exotic plant pests. A risk analysis identifies pests associated with pathways (e.g. fruits) that present an unacceptable risk to Australia, and identifies pest management measures. Pest management measures are developed considering host-pathogen interactions, disease biology, mode of trade, production areas of the commodity and interception data to ensure risk is managed appropriately.

Biosecurity risk analysis for *Plum pox virus*, a most destructive disease of high-value stone fruit worldwide and a major threat to Australia's stone fruit industry, is illustrated as a case study to show Australia's biosecurity risk analysis system at work.

POSTER 249

Determination of pathway association for fungal pathogens in biosecurity risk assessments

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Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, as well as the agricultural industries that are relatively free from serious pests. The risk analysis process is an important part in the development of Australia's biosecurity policies and enables the Australian Government to formally consider the level of biosecurity risk that may be associated with the importation of plant materials which are potential carriers of pests into Australia. This is comparable with the assessments undertaken by Australia's trading partners for technical market access requests supporting Australian exports of similar commodities.

The risk assessment process identifies all biosecurity risks on the pathway, and for those that do not achieve the appropriate level of protection (ALOP), determines risk management measures to reduce the risks to an acceptable level, if possible.

This case study examines how risk assessments for market access take into consideration one of the major components of plant biosecurity risk, the likelihood of a pest entering, establishing and spreading from the export area, using key fungal pathogens as examples. The case study discusses how risk management measures are determined based on the biology of the pathogens, and considers factors contributing to decision making of pathway risks.

POSTER 250

Biosecurity Import Risk Analysis and Stakeholder Response Process of Queensland Government

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The Biosecurity Import Risk Analysis (BIRA) process contributes to the protection of Australia from exotic pest and disease associated with imported goods. Import conditions are determined by the BIRA based on the level of biosecurity risk associated with the particular product and its source. The BIRA is initially undertaken by the Department of Agriculture and Water Resources (DAWR), and includes stakeholder consultation to address biosecurity issues associated with the trade or proposed importation of agricultural goods. In the BIRA process, stakeholder consultation plays a critical role in identifying regional pest and disease status and highlighting information about the nature of the pest or industry, which may influence the risk analysis. Government and non-government stakeholders work in partnership to address biosecurity matters, which could change local primary producers, industries, and communities. Through this partnership, stakeholders can influence quarantine policies, including domestic market access.

As part of the stakeholders' consultation process, Biosecurity Queensland (BQ), within the Queensland Department of Agriculture and Fisheries (DAF), prepares a departmental response to BIRA reports. BQ first reviews draft BIRA documents, and then engages related plant industries for consultation to ensure their representation in the process. Appropriate scientific and technical experts within the DAF collate contributor comments, and coordinate meetings to discuss issues of concern with the contributors. A response is then submitted to DAWR on behalf of Queensland. The DAF response process is extensive, and often there are challenges in preparing a BIRA report. Some of the issues discussed in the presentation will include providing thorough analysis under short timelines, undertaking effective stakeholder consultation, negotiating points of contention, and addressing uncertainties.

POSTER 251

Simulation of the progression of yellow spot on wheat using a functional-structural plant model

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Functional-structural plant models (FSPMs) aim at simulating mutual interactions between plant architecture and physiological processes in plants, affected by environmental conditions. Typically, an FSPM represents processes at the scale of plant organs, such as leaves and stem segments. This kind of approach can then be combined with an epidemiological model for a foliar plant pathogen. This combination then allows us to investigate how the growing plant structure interacts with climatic conditions (rain, temperature, wind) to affect the life cycle of the pathogen, its spread through the crop canopy, and its impact on the growth and development of the plant.

In our study we couple an FSPM of wheat (*Triticum*) with a model of the life cycle and dispersal of the yellow spot pathogen (*Pyrenophora tritici-repentis*). In our model, the growth of the wheat is driven by light interception and temperature, which control photosynthesis calculation and carbon allocation among different organs, respectively. The model of yellow spot is driven by temperature, rain and wind data. It predicts maturation and release of ascospores, initiation and maturation of primary and secondary infection of leaves, as well as release and dispersal of conidia spores across the simulated canopy. The pathogen model operates at the scale of leaf segments.

The overall aim of the study is to give deeper insights into plant-pathogen-environment interactions, and to help farmers predict disease risk and manage their crops based on weather conditions. The model will be tested for different locations and weather conditions across Western Australia.

POSTER 252

The role of Crop Health Services in Victoria's

General Surveillance Program: New detections 2014 -2017

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Crop Health Services (CHS), a business within Agriculture Victoria, conducts a plant pest and disease diagnostic service involving discipline specialists in bacteriology, entomology, mycology, nematology, plant physiology and virology. Diagnoses support productivity and biosecurity outcomes for Australia's plant industries and the environment. Pests and pathogens are identified using the appropriate combination of cultural, morphological, biochemical, molecular and immunological techniques. Staff are members of the National Plant Biosecurity Diagnostics Network and participate in the National Plant Health Proficiency Testing Program. Nationally endorsed and NATA protocols are used when available.

CHS has an agreement with the Victorian Biosecurity Branch, to report suspect and confirmed exotic plant pests and pathogens and new host records to the Chief Plant Health Officer within 24 hours of detection under the State's General Surveillance project. Specimens are subsequently accessioned into the State's reference collections.

In the past 3 years CHS has conducted over 46,000 diagnoses on samples submitted by the public, growers, consultants, and State and Federal biosecurity agencies. In this time over 50 pests and pathogens detected have been new Australian pest records, new State pest records or host range extensions.

The most significant of these are first Australian records of Cherry virus A (CVA), *American plum line pattern virus* (APLPV), *Apricot vein clearing associated virus* (AVCaV) from *Prunus* spp and *Melon necrotic spot virus* (MNSV; genus *Carmovirus*) from Rockmelon (*Cucumis melo*); First Victorian record of blueberry rust *Thekopsora minima* on *Vaccinium* and of *Pantoea ananatis* from *Zea mays*; First Australian record of the hazelnut mite *Tetranychopsis horridus*, and first Victorian records of the giant pine scale *Marchalina hellenica*, cactus spine scale *Acanthococcus coccineus*, cotton mealybug *Phenacoccus solenopsis*, solanum mealybug *Phenacoccus solani*, adelgids *Adelges nordmannianae* "species complex" and *Adelges cooleyi* "species complex", leaf beetle *Paropsides calypso*, and the Russian wheat aphid *Diuraphis noxia*.

POSTER 253

Assessing dieback in woody weeds using UAV mounted multi-spectral cameras

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Field based research on dieback of woody weeds species in Australia, particularly parkinsonia (*Parkinsonia aculeata*), prickly acacia (*Vachellia nilotica*), Mesquite (*Prosopis* spp.), mimosa (*Mimosa pigra*) among others, relies upon visual assessment of indicators of disease or stress. Among these, foliage cover and extent of branch dieback assessed individually (Sacdalan 2015, Diplock 2016, Haque 2016) or in combination (Galea & Beilby 2009 & 2010) are standard approaches towards measuring dieback using categorical visual assessment classes.

The use of unmanned aerial vehicles (UAVs) and the development of lightweight and affordable multispectral cameras are finding their place in crop agronomy, providing a means for developing crop stress maps. This type of analysis can assist with problem diagnosis related to disease, insect damage, water stress and nutrient issues as well as measuring yield potential.

On-ground assessment of individual wild growing trees for both foliage cover and extent of dieback is somewhat problematic due to the effects of tree crowding and variation in light quality throughout the day. Overhead high resolution multispectral imagery provides a new opportunity to evaluate dieback induced stress; particularly in sites where a time series approach to observations are required. Simultaneous geo-located (GPS) canopy images photographed in green (550nm), red (660nm), red edge (735nm) near infrared (790nm) and full colour (RGB) are assembled into a mosaic, which when analysed, give a measure of plant stress using standardised approaches such as normalized difference vegetation index (NDVI). NDVI is a tool extensively used in satellite image and data capture methodologies; however the level of resolution offered by UAV mounted multispectral equipment provides a far greater degree of resolution, and potentially a more effective approach to studying population dieback events.

POSTER 254

The CROPSAFE program: the network of private eye agronomists looking for exotics in Victoria.

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3. Agriculture Victoria, Horsham

The Victorian exotic identification network CROPSAFE was first piloted in 2007-08 and became operational in 2009. Most Victorian crops are viewed by private agronomists and tapping into this resource was considered essential for enhanced passive biosecurity. One hundred and seven agronomists were recruited, covering a range of businesses, regions and levels of experience. Training on identification of exotics was provided with kits to submit samples of unknown crop problems for identification. The advantage to participating agronomists was "free" diagnostics for themselves and their clients of unknown issues. Agronomists were keen to sign up as they perceived a business advantage to do so, but industry good was also identified from surveys.

The primary aim of the network was early detection of exotics and hence a greater chance of eradication. A secondary aim was "proof of absence" data for interstate and overseas trade certification. Once a year, agronomists are surveyed as to their coverage of crops and areas monitored. Due to the confidential nature of the information collected from agronomy businesses, trust within the CROPSAFE collaboration has been essential. The program has now built high levels of engagement with agronomists, with area freedom coverage of over two million hectares achieved for the past two years (two thirds of the total Victorian crop area).

During the year, crop alerts are sent out when endemic pests are first discovered and summary data of diagnoses is disseminated to agronomists.

Two Agriculture Victoria agronomist staff @ (0.3 FTE each) manage the network, sending out kits, updating contact details and staff changes while the diagnostics allocation is 0.5FTE.

Other advantages of a network of private agronomists with trusted links to the department is the intelligence gained, as was the case with Beet Western Yellows Virus, Russian Wheat Aphid outbreak and emergency responses such as floods.

POSTER 255

An area wide surveillance network to monitor airborne inoculum of plant pathogens affecting the grains industry

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Burkard volumetric spore traps were deployed in a network to monitor airborne spore dispersal patterns within major South Australian grain growing regions. Stubbles infested with either yellow leaf spot (synonym: tan spot) of wheat (*Pyrenophora tritici-repentis*), net form net blotch (*P. teres f. teres*) of barley or blackspot (complex including *Didymella pinodes*) of field pea, were placed around the traps. Spore tapes retrieved every 36 days over the growing season and particles deposited on the adhesive tapes were analysed for fungal identification. Molecular assays specific to *P. tritici-repentis* and *P. teres f. teres* were developed to quantify fungal DNA and an assay for blackspot was also included. Trap samples were processed as 2-day tape segments separated by 3 trapping days to allow analysis of spore release over time. In the first two years, the trapping and diagnostic protocols were optimised and evaluated at five locations with various climates using data from the diseased stubble. Additional molecular assays were then incorporated to detect and quantify spores of white grain disorder (*Eutiarospora* spp.), eyespot (*Oculimacula yallundae*), spot form net blotch (*P. teres f. maculata*), septoria triticii blotch of wheat (*Septoria tritici*), blackleg of canola (*Leptosphaeria maculans*) and ascochyta blight of chickpea (*Ascochyta rabiei*). Traps now operate at eight strategic locations to analyse endemic spore dispersal patterns of this suite of fungal diseases over the length of the growing season. Our aim is to establish relationships between spore release patterns and climate triggers to provide valuable information on disease dynamics in a changing farming environment, as well as generate data to improve strategies in disease management and forecasting. New innovations are now being combined with this surveillance network to incorporate rapid detection and data interpretation for more accurate and timely interrogation of spatial data generated from a network of samplers.

POSTER 256

Estimation of genetic variation in *Macrophomina phaseolina* isolated from sugar beet using SSR markers

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Macrophomina phaseolina (Tassi) Goid. is a soil-borne fungus with an extremely wide geographical distribution and large host range. Recently, increased incidence of the pathogen on diverse crop species has been reported worldwide, causing the significant economic losses in many field crops, including the sugar beet (*Beta vulgaris* L.). Charcoal root rot, caused by *M. phaseolina* is favored with higher temperatures (30-35°C) and low soil moisture, which often occurs during the summer months in Serbia and other South-Eastern European countries. In the study were used 58 *M. phaseolina* isolates, 56 from sugar beet, and one from maize and soybean. The objective of our study is to assess the genetic diversity of *M. phaseolina* isolates from sugar beet within local geographical area and estimate their genetic relationship, using SSR markers. The isolates were screened using 43 SSR primer pairs, out of which 13 were polymorphic. Genetic variation will be estimated by calculating the following parameters: number of polymorphic loci and their percentage, effective number of alleles per loci, expected heterozygosity and Shannon's information index. For estimation of variance components among the isolates, analysis of molecular variance (AMOVA) will be performed. The genetic relatedness of *M. phaseolina* isolates will be established by cluster analyses performed on the matrix of genetic similarities.

POSTER 257

Honeybees as an early surveillance tool for plant biosecurity

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Incursions of plant viral pathogens are a primary concern for horticulture as they can significantly impact crop yields and require expensive management. Early detection of plant viruses is critical for effective plant biosecurity because it enables growers to respond quickly and limit their spread. However, current surveillance methods relying on ELISA and PCR testing of plant material can only operate at a limited scale. It quickly becomes unfeasible to test large numbers of samples for area-wide surveillance of multiple viruses. Recent advances in next-generation sequencing (NGS) technologies have greatly improved our surveillance capability and its value is demonstrated for screening plant material for viral pathogens. However, while NGS can improve detection, it doesn't necessarily address the issue of sampling effort needed to achieve early detection. The solution is to couple NGS with a method of constant surveillance and sampling from potentially infected plants. We propose that when managed honeybee (*Apis mellifera*) hives are brought to flowering crops to deliver essential pollination services, they can also provide valuable surveillance by effectively "sampling" entire crops as they collect pollen and nectar. Combining the ability of honey bees to create a representative sample from a large number of plants with the sensitivity of NGS offers a powerful system for early detection of plant viruses.

We present a novel approach to plant biosecurity where NGS virus detection in honeybees was found to provide earlier detection of plant virus incursions in Australia than current surveillance programs. In 2013-2014 a national virus survey of honey bees was conducted, which used NGS screening of pooled honeybee samples across Australia. This NGS data simultaneously identified multiple plant viruses in these samples and retrospectively showed the presence of cucumber green mottle mosaic virus (CGMMV) in several states of Australia before being detected from diseased plant material.

POSTER 258

Nematode interceptions in imported live plants at the border in the Sydney region

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Live plants and perishable material are imported into Australia in the form of nursery stock, fresh produce and passenger personal effects. As part of mandatory conditions of import, these goods are inspected by Biosecurity Officers in the Department of Agriculture and Water Resources, assessed for their biosecurity risk and treated accordingly if the risk exceeds an appropriate level for the intended end use. Where an unacceptable biosecurity risk is detected, mitigation options include export, destruction, fumigation or immersion in hot water. Nematode infested live plants destined for propagation pose a higher risk than fresh produce destined for human consumption.

In recent years live nematodes have been detected by the Department in a range of imported goods at the border in the Sydney region. These have included both plant parasitic and non-parasitic nematodes and once sampled from the goods, are submitted to Operational Science plant pathologists for morphological identification. The plant parasitic nematodes detected include *Aphelenchoides* sp. and *Xiphinema* sp. Non-parasitic nematodes have included *Rhabditis* sp., *Mesorhabditis* sp., *Labronemella* sp., *Sinurea* sp. and a number of other fungal and bacterial feeders and predatory nematodes which were not specifically determined.

Aphelenchoides sp. have been detected on live plants including orchids, *Yucca* sp. and *Nepenthes* sp. Fresh produce detections have included *Aphelenchoides* sp. and *Xiphinema* sp. on root vegetables. As expected, the non-parasitic nematodes were found on a wide range of plants and in soil on plant roots. Interestingly, *Labronemella* sp. was most commonly detected on taro corms. This nematode is a dorylaimid and has a stylet but feeds as an omnivore.

POSTER 259

Combining insect pheromones and fungal volatiles for improved field monitoring of the rusty grain beetle *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae)

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High levels of resistance to the fumigant phosphine have led to increased interest in the ecology of the rusty grain beetle (RGB) both within and outside the grain storage environment. The capacity to monitor flight activity and trap RGB in the field will allow predictive models to be developed to minimise risk during grain handling operations, and also provide material for traditional and DNA-based resistance monitoring.

We used Lindgren funnel traps to evaluate ten synthetic volatiles as potential attractants for RGB, and found 1-octen-3-ol and 3-octanone to both be effective. Both compounds are known to be produced by fungi and both showed a positive correlation between volatile release rates and trap captures, with 1-octen-3-ol being the most potent attractant. The CryptoLure™, the only commercial pheromone-based lure available for RGB, was significantly ($P < 0.05$) less effective than 1-octen-3-ol, however significantly more RGB were trapped when a CryptoLure™ was combined with 1-octen-3-ol on an individual trap than when 1-octen-3-ol was used alone.

Trap comparisons showed that panel traps were more effective than Lindgren funnel traps for capturing RGB with the combined attractants, and this is attributed to the higher volatilisation rate of 1-octen-3-ol from this trap design. Rubber septa impregnated with 1-octen-3-ol were equally as effective as glass dispenser vials when both were deployed with CryptoLures™ on Lindgren funnel traps over a five-day trapping period. Whilst 1-octen-3-ol can be used alone to effectively monitor RGB flight activity, a two-part attractant consisting of a CryptoLure™ and a separate rubber septum impregnated with 1-octen-3-ol will provide increased trapping efficiency.

POSTER 260

Plant growth-promoting bacteria isolated from Western Australian soils improve wheat and legume yields

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The plant rhizosphere contains a diverse array of microorganisms, some of which may help plants to adapt to challenges more rapidly than plant genetic adaptation alone. To investigate this, over 170 bacterial isolates from the rhizosphere of five types of plants from three Western Australian soils were tested for their ability to promote plant growth. Two isolates, *Burkholderia caledonica* and *Enterobacter soli*, significantly improved the grain yield of wheat in field trials in south-west Western Australia by 23% and 9% respectively. Another isolate, *Pseudomonas granadensis*, when co-inoculated with rhizobia on peas, significantly improved nodulation by up to 71% and increased the grain yields at harvest by up to 35%. Using strain specific primers developed from the 16S-23S rRNA ITS1 region, the *P. granadensis* cells were shown to be present in the nodules of field grown peas.

The *P. granadensis* isolate was also tested in field trials as a co-inoculant with rhizobia on a range of legume species on Christmas Island and significantly increased nutrient uptake, nodulation or plant biomass at low levels of applied fertilizer on soybean, mungbean and lablab compared with the rhizobia-only controls.

Full phosphorus response curve glasshouse trials demonstrated that phosphorus solubilisation by *P. granadensis* and *B. caledonica* is not the mechanism of plant growth promotion in wheat. *In vitro*, these bacteria produced the plant hormone, indole-3-acetic acid (IAA), which is known to alter plant root architecture. In growth pouch assays, root lengths were enhanced by the bacteria and the effect was reduced in the presence of an auxin inhibitor, suggesting that production of IAA by the bacteria may be partly responsible for increasing wheat seedling root lengths.

This study demonstrates that harnessing the ability of soil microbes to improve plant health and productivity may be an important factor in promoting food security in the future.

POSTER 301

Mining *Fusarium oxysporum* genome for putative effectors

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Fusarium oxysporum is a species complex of endophytes, saprophytes and phyto-pathogens. There are more than 150 formae speciales affecting more than 100 plant species of important agricultural crops such as banana, tomato, legumes amongst others and is ranked fifth out of top ten most important plant pathogens based on scientific and economic importance.

Fusarium oxysporum formae speciales are often polyphyletic, making it impossible to identify them on the basis of conserved genes. Currently there are few consistently reliable molecular ways of differentiating between pathogens, endophytes and saprophytes and no easy way of discriminating endemics from exotics. The ability of the fungus to undergo recombination is another hurdle to molecular diagnostics. Although a lot of putative effectors have been characterised, SIX genes have been studied and used more widely to discriminate formae speciales but still cannot discriminate between all, or separate pathogenic strains from others.

In order to devise a rapid robust diagnostic protocol for this important horticultural pathogen, one needs to understand the genetic basis of host specificity and virulence which can only be achieved via whole genome sequencing.

The aim of the project is to carry out whole genome sequencing of *Fusarium oxysporum* specimens of horticultural crops maintained in the Victorian Plant Pathogen Herbarium (VPRI) using a combination of Illumina HiSeq and PacBio RS platforms. Single molecule real time (SMRT) sequencing technology used by PacBio RS will be used for scaffolding, gap filling and genome sequence finishing of the sequence derived from Illumina HiSeq. Both *ab initio* and evidence-based methods will be used for effector prediction, which will be used to characterise the *Fusarium oxysporum* specimens in the herbarium.

POSTER 302

The survival of *Fusarium oxysporum* f.sp. *cubense* in plants co-habiting Australian banana farms

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Panama disease caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) is one of the most destructive banana diseases and poses a significant threat to the future of the \$600 million Australian banana industry. While symptoms of Panama disease are only expressed in banana, the pathogen is known to survive on other plant species. Field surveys and a glasshouse experiment were conducted to identify alternative hosts of *Foc* Race 1 (R1) and Subtropical race 4 (STR4), which were used as an experimental surrogate for Tropical race 4 (TR4). Surveys of north Queensland banana farms were conducted to determine the most common weed and ground covers co-habiting commercial banana farms. Roots from twenty different species collected from three banana farms infested with R1 were analysed for the presence of the pathogen as an endophyte. Race 1 (VCG 0124) was isolated from four species commonly found co-habiting north Queensland banana farms; *Cleome aculeata*, *Eleusine indica*, *Digitaria ciliaris* and *Youngia japonica*. Eighteen plant species were identified as being the most common weed and ground cover species, or regarded as high risk due to their presence on TR4 infested farms in the Northern Territory and north Queensland. A glasshouse inoculation experiment using these plant species was conducted over a three month period to determine survival of STR4 (VCG 0120). Subtropical race 4 was isolated from all of the species included in the experiment, with recovery of the pathogen found to be more consistent from some species such as *Cyperus brevifolius*, than others. The identification of weed and ground cover species that allow *Foc* to survive and persist in banana plantations is an important component for inoculum control and long-term management of Panama disease.

POSTER 303

Join the ant hunt: how to successfully engage the public to find fire ants

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The invasive Red Imported Fire Ant (*Solenopsis invicta*) has been the subject of a national eradication program in south-east Queensland since 2001. Early in the program, a strong focus was placed on engaging the Brisbane public about fire ants, which has continued throughout the life of the program. Commitment to a significant and long-term public awareness campaign means that the Brisbane public has been informed on the identification, impacts, and control of fire ants for more than a decade. The tools employed include media campaigns, mail outs of educational material and treatment notifications, school visits with odour detection dogs, industry and community training events, the use of static displays and signage in public areas, liaising with local government authorities, touring with a mobile office unit, and a strong digital profile through a website and social media. In recent years, the program has also been accepting and processing photos from the public, which has been very successful in locating new fire ant detections.

Over the last 16 years, the benefits of the program's community engagement activities are being realised, with the program enjoying strong brand awareness and a high success rate with public sample submissions. At present, the majority of new detections of fire ant infestation are being reported by the public. As the program continues, community engagement will play a crucial role in ensuring that public awareness remains high, and in turn, the program can maintain its confidence in the public's ability to find fire ants—which has been demonstrated to a high degree. We hope the investment in passive surveillance within both current and future eradication programs is recognised as worthwhile.

POSTER 304

Knowledge brokering in biosecurity: How international linkages and learnings can help us build a better system

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Early in February 2017, industry received news that the Tomato-potato psyllid (TPP) had been found in Australia. More than any other, this incursion highlighted a severe capacity crisis within industry and government. Many industry personnel in particular, were thrown into biosecurity roles that had for many years been the sole province of government. Effective incursion response requires an understanding of response mechanisms and solid networks with government and researchers. At the time of this incursion, industry resources and capabilities became stretched to the limit. It was under these circumstances that the importance of effective knowledge brokering was demonstrated.

In addition, this recent incursion and previous incursions (such as CGMMV) highlighted the importance of tapping into international expertise, use of acquired knowledge, and formation of international collaborative relationships. Such relationships may be researcher to researcher, industry to researcher, industry to industry, government to industry etc.. The list goes on.

In a context where biosecurity funding is under threat within state governments, and costs of production for industry are increasing, we are facing both a 'brain drain' and a capacity crisis. How do we become smarter and more effective in our approaches to incursion response and management of established pests? How can we tap into citizen science and incite cultural change amongst communities, government and industry? How can we avoid wheel reinvention?

These are the questions I will seek to answer during a study tour to the USA in July 2017. With funding from the Rural Industries and Regional Development Corporation (RIRDC) and support from AUSVEG, I will investigate the following topics: Industry/government relationships in biosecurity; Citizen science – its role in biosecurity; Pest management in peri-urban production regions; Surveillance initiatives - what works, what doesn't?; The processes, roles and funding during pests incursions; Pest impacts on production and trade; Farm biosecurity best practice schemes.

POSTER 305

GrowCare®, a web-tool for Australian fruit-growers to better use weather data in managing foliage diseases, especially downy mildew of grapevines.

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GrowCare® for fruit-growers, crop-specialists and industry personnel comprises: **GrowCare®web** (growcare.com.au) which provides infection alerts for the weather-driven diseases of different crops; and **GrowCare®e-news** bulletin e-mailed with timely disease, pest and vineyard management information. **GrowCare®web** focuses on supplying real-time information on grapevine downy mildew (*Plasmopara viticola*) primary and secondary infection events and oilspot incubation, and infection alerts for bunch rot (*Botrytis cinerea*) on flowers and fruit of grapes; rust and brown-rot of almonds and stonefruit; and a degree-day calculator for pests. **GrowCare®web** provides an 'easy-to-read, customised' graph of temperature, relative humidity, rainfall and leafwetness from a grower-nominated local weather station (AWS) network established by regional grape industry associations in South Australia (Riverland, Barossa and Clare Valleys) and in Victoria (Murray Valley). The user can nominate preferred outputs of an infection model (DModel..., PPQ 6(1):29-33, Magarey PA *et al.* 1991) via disease alerts on the graph, or via automatically-dispatched SMS or e-mail to a mobile device. The progression of each modelled-aspect of disease epidemiology eg the incubation of oilspots, is shown in graph-form, the latter serves as a guide to the optimum time to apply metalaxyl, a post-infection fungicide. An 'i-frame' allows users to easily switch to/from Bureau of Meteorology forecasts and rainfall radars across Australia to assess optimum times to apply pre-infection fungicides. **GrowCare®** also provides: season-lists and location maps of disease events; a library of e-News bulletins or scientific and extension papers; and *Disease Diagnosis*, a web-based, 'wordless' guide for identifying symptoms of diseases, insects and disorders in Australian and NZ vineyards. **GrowCare®** provides unprecedented precision for Australian grapegrowers to 'self-manage' diseases, to produce 'clean green' fruit of maximum quality. Being web-based, **GrowCare®** supersedes previous systems (eg CropWatch® and AusVit®) and, given the demise of plant pathologist 'disease experts', is facilitating the dissemination of epidemiological expertise.

POSTER 306

Building and maintaining awareness of electric ant in a long-term eradication program

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Since electric ant (*Wasmannia auropunctata*) was detected in Cairns in 2006, the National Electric Ant Eradication Program (the Program) has continually evolved to meet the needs of this challenging long-term eradication program. Building and maintaining awareness of electric ants across a diverse range of Far North Queensland stakeholders has been crucial to finding infestation. Nearly 40 % of all electric ant detections have been the result of public reporting. Maintaining that awareness has posed a significant challenge.

The wide distribution and variety in infestation size, ranging from a few infested pot plants up to the original 37 hectare detection at Smithfield, has provided significant challenges. Additionally, human assisted movement through plant swapping has often been under-appreciated as a means of electric ant spread by the broader community. Engaging with the diverse range of stakeholders, including high risk businesses, local government and inter-government agencies and the general public has been a significant challenge. The cryptic nature and small size of electric ants has made it difficult to explain to the general public where to find them and how to recognise them. To combat these challenges and avoid campaign tiredness and public apathy the Program has developed communication and engagement strategies targeting community good will and motivation to preserve their natural environment, economy and out-door way of life.

This presentation explores the evolution of engagement tools developed to build and maintain public awareness and motivation to support the Program. The relationship between motivation and the perception of how easy it is to help, will highlight a useful strategy for overcoming public apathy. Additionally, the evolution of engagement strategies from mass communication to a more personal social conversation is explored through social media.

POSTER 307

Delivering a point of truth to an industry faced with Panama disease TR4

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The landscape for the northern Queensland banana industry changed forever when Panama disease tropical race 4 (TR4) was detected on a farm in Tully in March 2015.

Unable to be eradicated, this soil-borne fungus had already wiped out Cavendish plantations overseas and in the Northern Territory. With no magic bullet, growers were extremely anxious and wanted answers – fast.

Biosecurity Queensland (BQ), within the Department of Agriculture and Fisheries, with support from the Australian Government and the peak industry body, the Australian Banana Growers' Council (ABGC), launched a major incident response. A key element of the response was an extensive community engagement strategy where BQ's Panama TR4 Program quickly established itself as the point of truth, rolling out a broad suite of resources and engagement activities to guide stakeholders through what has been described as 'one of the most difficult times in the industry's history'.

One of these resources, the Panama TR4 Grower Kit, armed growers with the tools to protect their plantations from this significant threat. The kit outlines a multi-tiered approach to on-farm biosecurity and includes a decontamination guide, collaborative feral pig trapping guide, comprehensive disease identification section and a description of the diagnostic process used to confirm the presence or absence of the disease.

Two years since the detection, and with the disease still confined to a single property, the Panama TR4 Grower Kit continues to be a key resource and reference point for many in the industry. Along with the kit, an accredited Panama education program and multimedia advertising campaign have both been highly successful in informing stakeholders about the risks posed by Panama TR4 and how those risks can best be managed.

The achievements of the Panama TR4 Program in communications and stakeholder engagement have established it as a model for future biosecurity incident responses.

POSTER 308

“Unlocking a Nation of Curious Minds”: a science participatory platform for the next generation of *Phytophthora* scientists.

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The “Curious Minds” program is an initiative of the central government of New Zealand, aiming to encourage and enable better engagement with science and technology across all sectors of New Zealand, starting at the school-level. The objective is to partner schools with science-delivery organisations, to develop fun and interesting science projects to which, students are able to bring their unique, and fresh perspectives, to answer real-life questions. Also, this program aims to demonstrate the relevance of science in the students’ everyday lives. “Keeping Kauri Standing” is a national, multi-agency, bicultural response to manage and control Kauri dieback. Part of the approach to active, landscape-level, surveillance of this pathogen, is the baiting of streams to capture the diversity of *Phytophthora* in waterways, downstream of infested forests. The collaborative project, provided the students with the opportunities to design a stream-based sampling strategy, using study sites which we have previously established in west-Auckland. The students invented a re-usable “bait cassette”, that they designed with material engineers using CAD for Kids and extruded on a 3D-printer. The innovative cassette has a number of advantages over the conventional technology, and enabled the students to carry out the disinfestation, dissecting and plating of the leaf baits to *Phytophthora*-selective media. The students also successfully recovered a diverse array of *Phytophthora*, *Pythium* and *Phytophythium* species, which they were able to sub-culture from their initial isolations. The vision is to extend the program within NZ to engage schools with access to kauri forests. There is also an opportunity to engage with other schools in Australia (e.g. Melbourne, Perth), South Africa and USA (e.g. Corvallis) to develop an “International Student Surveillance Network”, through which students can share their experiences in forests and the values which they are trying to preserve, in a collaborative, collegiate, multi-ethnic and multi-national environment.

POSTER 309

Measuring biosecurity practice change of grain growers in Western Australia.

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Benchmarking surveys of biosecurity practices employed by grain growers in Western Australia have been conducted almost a decade apart.

In June 2003 through the WA Department of Agriculture’s ‘GrainGuard’ project an inaugural survey of 100 growers in the state’s grain industry was carried out by staff from the then Western Australian Department of Agriculture’s Plant Health biosecurity planning group to assess the adoption of the farm biosecurity message.

In 2011/12 grower surveys were conducted during the course of Plant Health Australia’s Grains Farm Biosecurity Program (GFBP). A key outcome of the GFBP from 2010 through to 2015 was to document the management and preparedness for biosecurity risks at the farm gate level in each mainland state.

For this to be done, a baseline survey was commenced in March 2011 to gauge the extensiveness of Western Australian grain grower’s biosecurity practices and to develop a network of interested growers and grower groups for follow up awareness training and best practice improvement. This survey was completed in March 2012.

Differences in the survey methods used and the level of identification of the frequency of the practices being investigated do confound the identification of behaviour trends in the grains industry. Never the less, an understanding of the results for mapping practice change of WA grain growers can be made.

The rate of change of adoption of positive practice changes made within the WA grains industry over this time that the two surveys were conducted indicates that progress is being made albeit slowly. Some practices that are more of a routine activity in crop protection such as paddock inspections are already at a high level of practice while those such as implementing Farm Biosecurity signage and engaged people management were found to be at a lesser degree.

POSTER 310

Survey of Turnip yellows virus in field canola and biological studies in the glasshouse

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Turnip yellows virus (TuYV) is the most important virus disease of canola (*Brassica napus*) crops in Australia, where it is wide spread in all grain production regions. It is aphid transmitted with the green peach aphid (*Myzus persicae*) as the most efficient vector. The typical symptoms of TuYV on infected canola are leaf reddening, distortion or purple leaves and stunting but some cultivars show leaf chlorosis and yellowing or symptomless virus expression. In Victoria, canola crops were surveyed in 2015 and 2016 to determine the occurrence, incidence and distribution of TuYV in different regions (Mallee, Wimmera and Western District) of the state. Virus symptoms present at each site were assessed visually and one hundred samples were collected from each site for analysis. Samples were tested using tissue blot immunoassay (TBIA) and selected samples were confirmed by PCR using TuYV-specific primers. In 2015, TuYV was detected in 19 out of 20 crops and incidence ranged from 2- 70%. In 2016, TuYV was detected in 16 out of 21 crops and incidence ranged from 1-53%. The highest incidence of TuYV was found in the Mallee and Western District in 2015 and in the Mallee and Wimmera in 2016.

The host range of a TuYV isolate originally collected from canola in Deniliquin, NSW was assessed in the glasshouse on canola and turnip (Brassicaceae); chickpea, faba bean, fenugreek, field pea, lentil, narrow leaf lupin and white lupin (Fabaceae); and beetroot, silver beet, spinach and sugar beet (Amaranthaceae). Plants were germinated and maintained in insect-proof cages and inoculated using viruliferous green peach aphids. Plants were harvested approximately one month after inoculation and were tested for TuYV using TBIA. All Brassicaceae and Fabaceae species except for narrow leaf lupin were tested positive and the four Amaranthaceae species tested negative to TuYV.

POSTER 311

Interactions of waterlogging and fungal infection in lentil (*Lens culinaris*) cultivars

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Lens culinaris is a commonly grown food crop in many parts of the world however it is sensitive to waterlogging which can result in large yield losses. Waterlogging leads to root anoxia but also increased canopy humidity, therefore waterlogging stress can be compounded by foliar disease. When screening cultivars for waterlogging tolerance, previous studies have found that symptoms due to waterlogging and disease are difficult to separate. An experiment was devised to address this issue, which included 20 cultivars of lentils, both waterlogging and high relative humidity treatments, and plots with and without fungicide. Plants were grown in field soil within troughs which enabled the water level to be controlled to simulate waterlogging conditions. The 2 week waterlogging treatment was followed by a 7 week recovery period before harvest. The 2 week humidity treatment was simulated with a mister to keep the canopy moist, whilst not leading to root anoxia. Waterlogging significantly reduced plant height to between 53 and 88% of control, and reduced dry matter to between 13 and 33% of controls. It was also found that the waterlogging treatment increased prevalence, variety and severity of fungal disease in addition to reducing greenness. The two most prevalent fungi in the waterlogging treatment were *Alternaria alternata* and *Stemphylium botryosum*, identified through culturing and subsequent sequencing of the ITS region. *S. botryosum* was more commonly associated with disease-like symptoms and is implicated as a potential causal agent or aggravator of waterlogging symptoms. While we expected that common lentil pathogens *Ascochyta lentis*, *Botrytis fabae* and *Botrytis cinerea* may be present, these were not detected. Increased canopy humidity (without waterlogging) did not increase disease incidence. Tolerance to waterlogging was identified in the lentil lines Bolt, Nipper, Matador and Indianhead with reasonable root and shoot regrowth after waterlogging.

POSTER 312

Effect of polymer film on plant-pathogen interactions: A case study on damping-off caused by *Pythium irregulare* in field peas (*Pisum sativum*)

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The use of Clear Polymer Film (CPF) for field crops improves crop establishment via increased soil and air temperature, and the capture and recycling of CO₂ and water vapour. However, little is known about how CPF affects soil borne diseases. We developed a case study with *Pythium irregulare* which is a common seedling pathogen affecting field pea (*Pisum sativum*). An experiment was designed using CPF-enclosed (5 cm headspace) and ambient chambers filled with potting soil, where pea seedlings (Greenfeast cultivar) were either inoculated with *P. irregulare* or not. As expected, CPF increased the maximum temperature in both treatments and resulted in faster emergence than the ambient chambers. Plant height, and number of fully expanded leaves were significantly higher ($P < 0.05$) for plants grown in the CPF chambers. Inoculation with the pathogen led to significantly lower plant height and number of leaves for plants grown in the ambient chambers but not for those in the CPF chambers. The pathogen was re-isolated more frequently from roots of plants that were grown in ambient conditions. The study concluded that CPF decreased the impact of *P. irregulare* infection in GreenFeast pea during these experimental conditions. Field trials would be necessary to determine if these effects could be replicated in a crop environment with natural levels of inoculum. The success of CPF to reduce root infection is likely related to the optimal soil temperature required for both the plant and pathogen, and in this case the increased soil temperature may have been less favourable for pathogen growth relative to pea growth.

POSTER 313

Comparison of the growth patterns of two crown rot causing pathogens in wheat

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Crown rot of bread wheat (*Triticum aestivum*) is an economically important disease in many cereal growing regions of the world, including Australia and Turkey. The most frequent cause of the disease is infection by either *Fusarium pseudograminearum* (Fp) or *Fusarium culmorum* (Fc), with both pathogens causing disease in some southern Australian regions and in the Central Anatolian Plateau in Turkey.

Infection of bread wheat by both species is currently being compared in field trials in both Australia and Turkey (TR). Trials were located at Wellcamp (Qld), Horsham (Vic), Yozgat (TR) and Eskisehir (TR). Plots were inoculated with either Fp, Fc or co-inoculated with both. Twenty bread wheat genotypes were challenged including semi-resistant material from CIMMYT and a range of Australian germplasm. A 15cm section from the base of the primary tiller of each plant was rated for disease severity at crop maturity. Data from this study is providing comparative information on the disease response of the host genotypes to each of the pathogens and to the mixed inoculum.

Samples from five genotypes were also collected at the post milk development stage of growth. Samples consisted of two 6cm sections from the main stem of each plant which were scored for disease severity and then ground for analysis of fungal DNA using quantitative PCR (qPCR) assays specific to either Fp or Fc. Results from the qPCR are currently being analysed and compared with the corresponding visual rating scores to assess the extent of colonisation of each fungal species in each treatment. Results from these analyses and from the disease assessments at maturity will be presented.

POSTER 314

Utilising host and pathogen diversity to manage allium rust in gardens

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A garden environment is usually a more diverse ecosystem than that found in crops, providing both opportunities and complications for integrated disease and pest management. Allium rust (*Puccinia* spp.) affects a range of edible and ornamental *Allium* spp. There is considerable genetic and phenotypic variation amongst the rust pathogens, the hosts and their interactions. The potential to use the complexities of within- and between-species host-pathogen interactions to develop rust management strategies is hampered by a limited understanding of these very complexities. The UK allium rust population appears to be comprised of at least three species, with overlapping host ranges. Our results show that the two main rust populations can be split into those that infect chives but not leeks and vice versa. The role of other *Allium* spp. in disease epidemiology is not clear, although most collections of rust from ornamental alliums fit the 'leek' type, while garlic is susceptible to both. While rust is recorded on onions in the UK, it is seldom severe. At least one strain, common on an ornamental cultivar of *A. vineale*, appears to be particularly pathogenic on this host, while generally conforming to the 'leek' types. In this paper, we will present results of experiments on hosts, pathogens and the interactions between the two and discuss how this information can be used towards the integrated management of allium rust in garden. For example, it may be possible to alter planting and harvest times of host species to break the 'green bridge' which enables a rust species or strain to survive throughout the year in a home garden.

POSTER 315

Understanding the biology and epidemiology of blueberry rust (*Thekopsora minima*) to improve control measures

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Almost 90% of Australian blueberries are produced in northern New South Wales and Queensland. While the subtropical climate, extended growing season and evergreen cropping practices favour high blueberry yields, these conditions are also conducive to the development of fungal foliar diseases.

Blueberry rust, caused by *Thekopsora minima*, was first recorded in Australia in 2001 and has since spread throughout blueberry production areas in NSW and Queensland, with incursions recently reported in Tasmania and Victoria. Severe disease may result in defoliation, premature fruit drop and a late ripening crop. Severely infected plants are less productive in the following season. When disease pressure is high, lesions on fruit can affect marketability. Despite its high incidence and severity, little is known about the biology and epidemiology of *T. minima* in Australia.

We have shown that the blueberry rust fungus survives primarily in infected leaves retained on the plant, often asymptotically.. In NSW, air sampling has shown that urediniospores are present at any time of the year and are the main source of new infections. Spore viability in leaf litter declines significantly after six weeks.

Blueberry producers in NSW and Queensland currently adhere to strict guidelines regarding fungicide application based on calendar sprays, and inspection of fruit prior to shipment contributing to higher production costs. Identification of factors that favour spore release and infection can assist in targeting fungicide sprays to promote more effective control, and reduce costs for growers. Factors conducive to spore production and host plant infection identified can be used to modify spray programs to target the pathogen, reducing costs and the risk of fungicide insensitivity, and improving disease control.

POSTER 316

Effects of raceme age on the virulence and fecundity of *Sclerotinia sclerotiorum* on canola (*Brassica napus* L.).

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Sclerotinia sclerotiorum is an economically important fungal pathogen of broadacre cropping worldwide. Significant yield loss occurs in canola following the establishment of *S. sclerotiorum* on the primary raceme. As *S. sclerotiorum* grows along the canola raceme it necrotises host tissue, effectively restricting the ability of the plant to translocate nutrients. Observations made during routine stem infection assays led us to test the hypothesis that the virulence of *S. sclerotiorum* on canola is enhanced on mature racemes. We conducted a time of sowing (TOS) experiment by sowing seed of cultivar Charlton in a randomized block design at 7 day intervals over a three week period ($n = 10$ plants per TOS). Plants were propagated in 5 L pots of UWA mix (Richgro), in a temperature controlled glasshouse under natural light, with a controlled watering schedule. The developmental stage of each raceme was documented, by measuring primary raceme height (mm) every 7 days and recording the onset of flowering. All racemes were inoculated with *S. sclerotiorum* strain CU11.19 at the 6th node below the oldest floral branch at 18, 17, 16 and 15 weeks after sowing (WAS). The node width (mm) was recorded and weekly lesion length measurements (mm) were conducted for 4 weeks. Lesions that established on mature racemes (18 WAS) were significantly larger than lesions on the most immature racemes (15 WAS) (Tukey's HSD $P < 0.05$). Following natural raceme desiccation, total sclerotia were extracted and weighed. Mature racemes (18 WAS) developed 16 fold more sclerotia than younger racemes ($P < 0.001$). Together these data suggest that older, mature racemes are more easily overcome by *S. sclerotiorum*, resulting in a greater mass of sclerotial development. We hypothesize that changes in the source-sink relationship within the plant may contribute to the differences observed.

POSTER 317

Pathogenicity studies of *Macrophomina phaseolina* causing charcoal rot of strawberry in Australia

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Macrophomina phaseolina is a soil-borne pathogen that causes charcoal rot. Charcoal rot is an emerging and significant disease in Australia and in other strawberry producing countries, and associated with the phase-out of methyl bromide. *M. phaseolina* growth is favoured by temperatures above 30°C, it has a wide host range and current soil fumigants have not been effective in controlling the pathogen. Breeding for resistant cultivars against *M. phaseolina* is therefore an important focus of the national strawberry breeding program in Australia. Twelve cultivars, consisting of current and historical Australian and international genotypes were screened in a glasshouse trial by drenching the potting media with *M. phaseolina* microsclerotia inoculum. The cultivars tested varied in their response and were placed in four groups: very high resistance (Earlibrite, Earlisweet, Kabarla and Phenomenal); high resistance (Sweet Charlie, Suncoast Delight and Red Rhapsody); low resistance (Rubygem, Florida Festival and Florida Radiance); and very low resistance (Albion and Camarosa). In a subsequent experiment, a total of thirty isolates of *M. phaseolina* from strawberry, sorghum, mung bean, peanut, chickpea and watermelon were inoculated on strawberry cv. Albion. *M. phaseolina* isolates collected from strawberry plants and from sorghum in fields used for strawberry production were more aggressive towards strawberry. The findings of this study will be incorporated into the national strawberry breeding program in Australia to develop cultivars with resistance to charcoal rot.

POSTER 318

The effect of temperature, water potential and incubation time on the germination of *Sporisorium scitamineum* teliospores

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Sugarcane smut caused by the fungus *Sporisorium scitamineum* is an important disease of sugarcane. Environmental factors such as temperature and water availability are essential influencers of the germination of smut teliospores. This study aimed to determine the effect of temperature, water potential and incubation time on the germination of teliospores. Teliospores (200 µl each at 1.5×10^6 teliospores per ml) were cultured on water agar amended with various amount of potassium chloride (KCl) or sucrose to achieve water potentials of 0, -5, -15, -25, -35 and -45 bars in 9 cm Petri dishes. Subsequently the plates were incubated at various temperatures (20, 25, 30, 35 and 40°C) for 0.5, 2, 4, 6 and 8 hours. Each combination consisted of five replicates. This study revealed that teliospore germination decreased with declining water potential levels. The highest germination levels (95.76%) occurred with KCl at 30°C and a water potential of 0 bars. Furthermore, at 30°C and at the lowest water potential used (-45 bars), teliospores germination was 50%. This result suggests that smut fungus can germinate under hot and dry conditions. This experiment also showed that teliospore germination was drastically reduced at 40°C. For both media, maximal teliospore germination was observed at 30°C. This study also revealed on sucrose amended media, that teliospore germination commenced after 2 hours incubation, whereas on KCl amended media, germination commenced after 4 hours. This result suggests that the addition of a low concentration of sucrose would stimulate germination of smut teliospores.

POSTER 319

The influence of temperature and incubation time on the incidence of smut disease on sugarcane

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Sugarcane smut caused by a basidiomycete fungus, *Sporisorium scitamineum*, is a serious disease of sugarcane known to cause up to 60% yield losses in susceptible varieties. Temperature is an important factor in plant disease infection and development. This study aimed to determine the influence of temperature and length of exposure to teliospores on the incidence of sugarcane smut in the field. Sugarcane setts were inoculated by dipping them in teliospore suspension (5×10^6 teliospores /ml water) at various temperatures (15, 25, 30, 35 and 40°C) for 0, 6, 12 and 24 hours respectively. The treatments were performed in controlled environment cabinet with 5 replications consisted of 5 setts per replication. After the allotted incubation period, sugarcane setts were immersed in 2% sodium hypochlorite for 5 minutes then rinsed with tap water to neutralise un-germinated teliospores remaining on the surface. Subsequently the setts were planted in trays of vermiculite and maintained in a growth chamber at 30°C for 15 days. The germinated setts were transferred to a glasshouse for 15 days and then transplanted in the field according to a randomized block design. The incidence of smut disease was recorded every week after first detection of smut on week 4 after transplanting. The highest smut incidence (40.39%) was achieved when temperature during inoculation was 30°C. The incidence of smut significantly decreased (5.72%) at 35°C. Sugarcane setts incubated at 40°C failed to germinate. In terms of the influence of length of exposure to smut teliospore, our results showed that sugarcane setts incubated at 0 incubation time (actually 10 minutes) resulted in the lowest smut incidence (3.65%) which is significantly different from those setts incubated at 6, 12 and 24 hours (21.06, 19.63 and 24.68% respectively).

POSTER 320

Finding *Cucumber green mottle mosaic virus* in Western Australia

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Cucumber green mottle mosaic virus (CGMMV) is a *Tobamovirus* in the *Virgaviridae*. It was first detected in England. It then spread gradually to more European countries and different parts of Asia, and recently to North America and Africa. In 2014, it was detected for the first time in Australia infecting watermelon in the Northern Territory (NT). In July 2016, leaf sample was collected from a continental cucumber plant with leaf mottle and distortion symptoms growing in a commercial plastic tunnel house at Geraldton, Western Australia (WA). Diagnostics involving electron microscopy, ELISA, PCR and Next generation sequencing (NGS) were used to identify the presence of CGMMV in this sample (isolate WA-1).

When NGS was completed on isolate WA-1 the complete genome obtained had 6423 nucleotides. When subjected to BLAST analysis, the closest genomic nucleotide identities were 99.56-99.1% to genomes from Canada, Israel and India. CGMMV isolates from the other WA locations were found to have similar identities to that from Geraldton.

Delimiting surveys in key cucurbit growing areas (Geraldton, Carnarvon, Kununurra and Perth) were done between July-December 2016 to determine the extent of CGMMV spread. These included commercial cucurbit farms (field and protected cropping), roadside verges (*Cucurbitaceae* weeds) and seedling nurseries. A total of 95 locations were surveyed involving 41 properties and 54 roadside sites. Over 14,000 leaf samples were tested by ELISA and PCR. Infection with CGMMV was widespread occurring in 16 locations near Kununurra, Carnarvon, Geraldton or Perth). CGMMV was detected in cucumber, rockmelon, honeydew and watermelon crops, and single cucurbitaceous native plant and naturalized weed species.

Information workshops were held in WA cucurbit growing areas to deliver CGMMV management and biosecurity information.

Kehoe MA, Jones RAC, Coutts BA. 2017. First complete genome sequence of *Cucumber green mottle mosaic virus* isolated from Australia. *Genome Announc* 5:e00036-17.

POSTER 321

Host range investigations of *Phoma koolunga*

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Phoma koolunga is one of three species that causes ascochyta blight (blackspot) of field pea (*Pisum sativum* L.) in Australia. *Phoma koolunga* was first described in 2009 from South Australian isolates and has since been reported in Western Australia, New South Wales and Victoria. However, the origin of the pathogen and its host range remain largely unknown. The aim of this study is to determine the host range of *P. koolunga* and to explore questions on the origin(s) of ascochyta blight pathogens in Australia.

Host range experiments were conducted on a selection of Australian native legumes and naturalised pasture species found in geographical proximity to field pea crops. One isolate of *P. koolunga* was used to spray-inoculate the range of legume species, including *Pisum sativum* cv. Kaspera as control. In the same experiment a different comparator isolate of *P. koolunga* was used to inoculate the same range of legumes. Control plants were mock-inoculated with water. The inoculated plants were placed in plastic humidity tents, one tent per isolate, in the same growth room. Disease severity will be measured as a percentage of leaf area diseased (%LAD) every 3 days up to 21 days post-inoculation. Disease incidence and percent leaf area diseased will be compared among isolates and species. Experiments are in progress and results will be presented.

POSTER 322

A fresh look at the life cycle of powdery mildews infecting important crops

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Although life cycles of powdery mildews infecting important crops have long been considered as well defined processes, our studies revealed new aspects of both asexual and sexual cycles. We detected microcyclic conidiogenesis (MC), i.e. production of conidia on a spore without any, or only a minimal, involvement of hyphal growth, in more than 10 species belonging to distinct phylogenetic lineages. In powdery mildews, MC means that germinated conidia can sometimes serve as sites for conidiogenesis: conidiophores can develop directly on the germinated conidial body and produce new conidia. If this happens in a young colony, established by the germinated conidium itself, MC will contribute to a quick propagation of the fungus because new conidia are produced quicker on microcyclic conidiophores than on the hyphae of the young mycelium.

In many powdery mildews, especially in temperate climate zones, ascospores coming from sexual fruiting bodies, called chasmothecia (formerly: cleistothecia), initiate the primary infections early in the host plant growth season. Although ascospore infections have long been considered as the first step in the life cycle of the cereal powdery mildew pathogen, *Blumeria graminis*, this has been documented for the first time only very recently in our laboratory. Germinated ascospores penetrated the epidermal cells of wheat leaves and produced haustoria as known in the case of conidial infections. We confirmed earlier studies reporting that *B. graminis* chasmothecia do not contain mature ascospores in the field, only asci filled with protoplasm; ascospore development is induced by moist conditions and it is a fast process compared to other powdery mildews. Although ascospore infections are frequent in *B. graminis* in the field, a genomic analysis revealed the signs of clonal or near-clonal reproduction. Therefore, ascospores are probably more important as dormant structures than genetic recombination factors in the life cycle of *B. graminis*.

POSTER 323

Myceliogenic germination of *Sclerotinia sclerotiorum* is enhanced by imbibition of sclerotia in combination with other abiotic conditions

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Infection of *Brassica napus* (canola) by the fungus *Sclerotinia sclerotiorum* is usually mediated by airborne ascospores, which are produced when soil-borne sclerotia germinate carpogenically. However, basal infection of *B. napus* stems by mycelium derived from sclerotia that have undergone myceliogenic germination has also been observed (albeit less frequently) and can be very damaging. In this study the effects of artificial injury, hydration, incubation temperature, exogenous nutrients and conditioning temperature on sclerotia grown *in vitro* were assessed.

Four base treatments: scarred/imbibed, scarred/unimbibed, imbibed/unscarred and unimbibed/unscarred were applied to sclerotia to study their effects on myceliogenic germination. Imbibed sclerotia incubated at 15 °C on potato dextrose agar (PDA) produced more vigorous radial growth compared to unimbibed sclerotia. However, no difference in phenotype between the treatments was observed at 25 °C. Incubation of sclerotia at 25 °C increased pigmentation of colonies compared to incubation at 15 °C.

Subsequently, the four treatments were used in combination with three different conditioning temperatures: 17 °C, 4 °C and -20 °C for two weeks. After the conditioning treatments, sclerotia were placed on either 12.5% PDA or sterile moist sand, at 15 °C. When conditioned at -20 °C, imbibed/scarred and imbibed/unscarred sclerotia germinated myceliogenically after two and four days respectively, on moist sand. Myceliogenic germination on moist sand was characterised by dense white outgrowth from the scarred ends of the imbibed/scarred sclerotia and the formation of small dense white clumps on the upper surface of imbibed/unscarred sclerotia. Scarred/imbibed sclerotia conditioned at 17 °C and 4 °C germinated on moist sand after 11 days, producing radial hyphae that extended across the sterile sand. Together these results indicate that imbibing sclerotia promotes myceliogenic germination at 15 °C with or without exogenous nutrients. Myceliogenic germination on moist sand is enhanced when imbibed sclerotia are scarred and cold conditioned at -20 °C.

POSTER 324

Development of a detached leaf bioassay to determine cross-pathogenicity and pathogenic variation of *Botryosphaeriaceae* isolates on blueberry and macadamia

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Incidence of diseases associated with species in the fungal family *Botryosphaeriaceae* are increasing, and becoming a major economic concern in several horticultural tree crops worldwide. Species of *Botryosphaeriaceae* act in many ways but most commonly as endophytes, causing latent infections or are serious outright pathogens. In most cases, the fungal organism enters the host through wounds or natural openings. The resulting symptoms include branch and trunk cankers, dark lesions of the tissue beneath the bark of stems, leaf and stem blight, fruit rot, and in severe cases tree death. Due to the wide host range of many *Botryosphaeriaceae*, it is hypothesised that there is strong cross-pathogenicity of *Botryosphaeriaceae* isolates across multiple host species. In this study, we developed a rapid bioassay to compare host specificity and pathogenic variation of fungal isolates of species in the family *Botryosphaeriaceae* on different tree crops including macadamia and blueberry. Using a detached leaf inoculated at the petiole with the test isolate in a microcosm and maintained under moist, semi-sterile conditions at 25°C and 100% relative humidity, host susceptibility of blueberry and macadamia cultivars to species of *Botryosphaeriaceae* was determined. From preliminary studies it has been observed that blueberry is highly susceptible to isolates of *Botryosphaeriaceae* isolated from different plant hosts with macadamia proving to be far more resilient. This pilot study revealed that the detached leaf bioassay reflects *in planta* and stem inoculation assays and is rapid for quantitative analysis of virulence and aggressiveness of species of *Botryosphaeriaceae* on different tree hosts.

POSTER 325

Investigating the longevity and host range of *Cucumber green mottle mosaic virus* (CGMMV) in the Northern Territory

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Cucumber green mottle mosaic virus (CGMMV) is a tobamovirus that is able to infect a number of economically important cucurbits crops including melons. The first detection of CGMMV in Australia was reported in the Northern Territory (NT) in September 2014; as of January 2017 25 properties in the NT have been identified as CGMMV positive. Initially all infested properties (IP) were placed under quarantine for a period of two years, where all host plants including cucurbitaceae weeds were destroyed. Previous research has indicated CGMMV is viable for up to nine months in host free soils. Four IP with varying soil types and climates in the NT were chosen to determine the longevity of the virus 12 months into quarantine. At 12, 15 and 18 months of the quarantine period, 80 soil samples were taken from each IP at three intervals, with plant bioassays conducted to determine the viability of any remaining virus. A field trial was also conducted on all four properties at the initial soil sampling point (12 months into the quarantine period). At the initial test period of 12 months, 2 IP from the bioassay and 1 IP from the field trial tested positive for CGMMV, while all 4 IP tested positive in the 15 month bioassays. As there is potential for greater spread and crop loss due to the sustained longevity of the virus, commercially viable crops for the NT have been chosen to determine if the virus is able to infect a wider range of plant host. These potential non-host plants include; sweetcorn, capsicum, okra, snake bean, peanuts and sorghum.

POSTER 326

Importance of Charcoal Rot in the Australian Strawberry Industry Following the Phase-out of Methyl Bromide

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Charcoal rot, caused by the soil-borne fungus *Macrophomina phaseolina*, has recently become a serious disease of strawberry fruit crops in Australia, where it has caused plant losses of up to 50%. Symptoms of the disease include wilting, collapse and dark brown or reddish-brown necrotic areas in the cortex and along the vascular tissues of the crown. High soil temperatures (> 27°C) and low soil moisture favour disease development. An extensive survey conducted in 2017 of 100 strawberry farms in Victoria isolated *M. phaseolina* from diseased strawberry plants at 55% of properties. Furthermore, *M. phaseolina* was detected in soil at 75% of properties. In contrast, *M. phaseolina* was not detected in plants or soil at strawberry nursery farms.

Fumigation with methyl bromide (MeBr)/chloropicrin previously controlled charcoal rot in strawberry crops. Following the phase-out of the use of MeBr, however, charcoal rot has rapidly increased in importance as a serious disease of strawberry crops around the world, including in Australia. Field trials were conducted in Queensland and Victoria where microsclerotia of *M. phaseolina* or infected crowns of strawberry plants were buried into soil. Fumigation with MB/Pic eradicated *M. phaseolina* in infected crowns buried in soil, whereas other fumigants currently registered for use in the strawberry industry (chloropicrin and 1,3-dichloropropene) were not effective. All soil fumigants killed microsclerotia in soil. It is likely that the continued use of MB/Pic under a critical-use exemption has prevented outbreaks of charcoal rot in strawberry nursery crops in Australia, but these exemptions will not be granted for long periods into the future and there is an urgent need for suitable alternatives. Our current research is evaluating improved application techniques for currently registered fumigants (e.g. use of impermeable barrier films) or new fumigants (e.g. cyanogen) for control of charcoal rot. Results will be presented at the conference.

POSTER 327

Fusarium Yellows of Leafy *Brassica* species in Conventional and Vertical Farms in Singapore

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Singapore has over 50 leafy vegetable farms supplying the domestic market. More than 80% of the leafy *Brassica* vegetables produced are derived from covered land based soil cultivation i.e. conventional farms. To boost local production in land scarce Singapore, the Government has encouraged the use of technology and intensified land use. There are now several vertical farms producing vegetables outdoor and indoor in Singapore with or without soil. However, with intensified farming, plant pests are likely still a problem and impact may be more visible.

Fusarium oxysporum has been reported to be the causal agent for Fusarium yellows in soil based grown local leafy vegetables in Singapore. The local prevalence as determined in a 2010 - 2011 survey was about 64.2% (of a total of 246 samples). *F. oxysporum* and *F. commune* have been reported to be indistinguishable morphologically. In the same study, *F. oxysporum* was found likely to be *F. commune* when a sample size of 35 *F. oxysporum* isolates were further tested with specific primers. The local farms have been able to manage the Fusarium yellows disease with integrated measures and technical advisory from the government extension service.

Recently, a vertical farm in Singapore reported a yield loss of about 40-50% in the production of leafy *Brassica* vegetables and investigation of the production system revealed the involvement of two isolates of *Fusarium* i.e. *F. commune* isolated from diseased vegetables and soil and *F. oxysporum* isolated from irrigation water using sequencing. Morphologically, the *Fusarium* isolates appeared to be different from the isolates from the conventional farms. Koch's postulates confirmed the *Fusarium commune* isolated from the diseased vegetables and soil as the pathogen.

POSTER 328

Microscopic analysis of oil palm (*Elaeis guineensis*) infections by *Ganoderma boninense*.

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Oil Palm (*Elaeis guineensis* Jacq.) is an important agricultural commodity for many developing countries in South East Asia including Papua New Guinea and the Solomon Islands. Currently basal stem rot (BSR) disease caused by *Ganoderma boninense* is the major disease of economic importance causing severe crop losses. BSR disease has been known for over 100 years however, progress in disease control and management has been hampered by lack of understanding of *Ganoderma*-oil palm interaction which is essential in developing better strategies to control BSR incidence. To date, there are no effective control methods to manage BSR disease. The viable long term alternative would be to breed for resistant germplasm. However, this strategy is dependent on improved understanding of the factors associated with resistance/tolerance and susceptibility.

Pathogenicity assays were performed under controlled environmental conditions with oil palm seedlings inoculated at the bole (lower stem). Bright field and fluorescence microscopy was used to follow *Ganoderma* infection of oil palm seedlings. Microscopy revealed that *Ganoderma* appears to have a preference for colonising root tissue as opposed to bole tissue, even though the bole was the sight of inoculation. Hyphae were observed in the sub epidermal highly lignified corky cell layer found in primary and lateral roots. Other plant tissues colonised by *Ganoderma* included lateral roots emerging from primary roots, and the root-bole interface region and dead tissue areas associated with roots and bole. The tissue regions where *Ganoderma* has been observed did not fluoresce under fluorescence microscopy when stained with calcofluor white indicating that these were either dead or so highly lignified that these could not be stained. No specialized hyphal structures were observed during this early infection study. This study describes for the first time an account of early processes of *Ganoderma* colonisation and infection on oil palm seedlings.

POSTER 329

Outbreak of yellow crinkle disease of Papaya in Queensland

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A recent outbreak of papaya yellow crinkle (PpYC) disease Queensland has significantly affected production within the entire papaya industry. It has led to 15-80% plant loss in many plantings on the wet tropical coast between Cardwell and Mossman; 15-20% in the Mareeba and Dimbulah areas. This disease is caused by a phytoplasma and is transmitted by leafhoppers. Samples of PpYC and insects were collected from disease affected farms in Mareeba and Innisfail areas and analysed for phytoplasma using nested and Real time PCR. The phytoplasma was detected in symptomatic PpYC plants and insect samples. Two related strains; tomato big bud (TBB) and sweet potato little leaf variant (SPLL- V4) of *Candidatus* Phytoplasma australasia (16SrII-D) are commonly associated with PpYC in Australia. Further sequencing and RFLP studies are being carried out to confirm the phytoplasma group and variation among prevalent strain/s of phytoplasma in these samples. The leafhoppers collected were tentatively identified as *Balclutha incisa* (Matsumura) and *Cicadulina bimaculata* (Evans) (Cicadellidae). The planthoppers were tentatively identified as *Falcotoya aurinia* Fennah or *Toya dryope* (Kirkaldy) (Delphacidae). *Cicadulina bimaculata* (Evans) and *Toya dryope* (Kirkaldy) are known vectors of phytoplasmas. The detection of phytoplasma in these samples was of concern as it showed there was potential for further spread of the disease if environmental conditions (the drying of surrounding vegetation) favoured the insect vectors feeding in the papaya. Insect and plant samples are being collected weekly to better understand the relationship between weather conditions, insect vectors and disease incidence. Possible management strategies include, the use of border trap plants which are then sprayed with insecticide, crop netting, physical barriers to control movement of the insect vectors, barrier sprays to prevent the feeding of the vector, reflective mulches to deter the insect vector and clean planting material.

POSTER 330

Relative benefit of growing bread wheat or barley in the northern grains region in the presence of infection by *Fusarium pseudograminearum*

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Crown rot, caused predominantly by the fungus *Fusarium pseudograminearum* (*Fp*), is a major disease of wheat and barley crops in the northern grains region (NGR) of Australia. In the NGR, preliminary studies in 2007 found that crown rot caused an average yield loss of 20% in barley, 25% in bread wheat and 58% in durum. However, the relative benefit of growing barley or wheat under high crown rot pressure in the NGR has not been well established across sites and years. This study determined the relative yield of a widely-grown bread wheat variety EGA Gregory and the dominant malting barley variety Commander in the presence of high crown rot pressure in 32 small plot field experiments conducted between 2009 and 2015 using an uninoculated vs. inoculated (2 g *Fp* inoculum/m row) trial design. Additional replicated trials were conducted at Tamworth and Garah in 2014 using a wider range of barley and wheat varieties with two sowing dates. Under high crown rot pressure, Commander provided a yield benefit over growing EGA Gregory in 62% of the experiments, EGA Gregory provided a significant yield benefit over Commander in 8% of the experiments, and in 30% of the experiments there was no significant yield difference between varieties. Barley in most experiments and seasons (except the variety Oxford) had improved yield over bread wheat varieties in the presence of crown rot infection. However, late planting cancelled out this yield advantage as crown rot expression in barley is limited by the earlier maturity of most varieties which reduces exposure to evaporative stress during grain filling. Additionally, growing barley under high crown rot pressure can increase *Fp* inoculum levels as it is very susceptible to infection. Therefore, crop and variety choice should be considered as one element of an integrated management strategy to manage this disease.

POSTER 331

Pathogenicity of *Xanthomonas* species causing bacterial leaf spot in Australia

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Bacterial leaf spot in Australian solanaceous crops is caused by at least five *Xanthomonas* species. Isolates of *Xanthomonas* associated with bacterial leaf spot were identified using molecular and biochemical analyses. The species diversity revealed in this study indicates disease severity could vary with causal species and location.

Pathogenicity trials were conducted to assess the impact of the collected isolates on crops. The ability of these pathogens to overcome established host resistance genes and chemical treatments was assessed. Race and copper tolerance scores were assigned to the collected *Xanthomonas* isolates to determine potential disease severity on hosts and efficacy of current control methods. The need for varieties with the full suite of BLS resistance genes is highlighted. Determining the variation of the pathogenic capability of these species will lead to improved detection and identification of the *Xanthomonas* species associated with bacterial leaf spot in Australia. The potential for alternative control methods, future management strategies and the detection of exotic *Xanthomonas* species will be discussed.

POSTER 332

Winter cereal responses to root and crown rot pathogens

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Winter cereals can be colonized by a number of different soil-borne organisms including crown rot (CR) and common root rot (CRR) pathogens which can have a devastating economic impact on the agriculture sectors. In Australia, *Fusarium pseudograminearum* (*Fp*) and *Fusarium culmorum* (*Fc*) are the two main fungi causing CR in winter cereals. *Fusarium graminearum* (*Fg*) is an important pathogen reported to cause CR in other parts of the world. *Bipolaris sorokiniana* (*Bs*) is associated with common root rot in Australia and internationally has been grouped with *Fusarium* species as part of a root rot complex. The host reaction of these pathogens causing CR and CRR on winter cereals has not been fully investigated. Bread wheat (cv. Livingston), durum wheat (cv. Hyperno), barley (cv. Grimmer), triticale (cv. Endeavour) and oat (cv. Genie) were inoculated with two isolates each of the four pathogens (*Fp*, *Fc*, *Fg* and *Bs*) in field trials conducted at the Wellcamp Research Station QLD. The impact these isolates have on each of the hosts at tillering, flowering and maturity will be detailed. At each of the three harvest times disease severity, plant height, shoot biomass and yield characteristics were determined. Preliminary data indicates the greatest expression of disease was observed in Livingston and Grimmer, while Genie showed lower levels of disease. In addition, *Fp* had the greatest impact on germination.

POSTER 333

Detection and distribution of alfalfa dwarf disease-associated viruses infecting lucerne pastures: potential biosecurity implications for Australia

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Virus diseases can cause significant economic losses in agriculture and may pose biosecurity risks for crop production and trade. In 2010 a devastating disease of lucerne, named alfalfa dwarf disease (ADD) with an incidence greater than 70%, occurred in several commercial fields in Argentina and caused up to 30% annual yield and seed weight reduction. Next generation sequencing and field surveys of ADD-infected lucerne plants in Argentina revealed the presence of a new cytorhabdovirus, alfalfa dwarf virus (ADV), the (+) RNA viruses alfalfa mosaic virus (AMV), bean leafroll virus (BLRV) and alfalfa enamovirus 1 (AEV-1) and the DNA virus alfalfa leaf curl virus (ALCV). All these viruses appear to be transmissible by *Aphis craccivora*. Due to the economic impact of ADD in South America and its potential threat to the Australian lucerne industry, we developed diagnostic assays to determine the potential presence of ADD-associated viruses in Australia. In this study, lucerne leaves with virus-like symptoms were collected from seed crops in South Australia and fodder crops in Victoria and Queensland. A sensitive duplex RT-PCR assay was developed for simultaneous detection of ADV and AMV using a cloned non-infectious ADV RNA transcript as positive control. Similarly, the presence of BLRV and AEV-1 was determined by duplex RT-PCR and ALCV by PCR assay. None of the exotic viruses ADV, AEV-1 and ALCV were detected in any lucerne samples we tested in 2015/16. However, the endemic AMV and BLRV were detected in some samples, with a high incidence of AMV in symptomatic seed production paddocks. Symptoms in the collected samples varied widely and there was no obvious correlation between the symptoms observed in individual samples and presence of individual viruses. Future epidemiological studies in Argentina will assist in the development of a biosecurity plan to prevent ADD from occurring in Australia.

POSTER 334

Identifying the mechanism of infection and colonisation by *Verticillium dahliae* in potato

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Verticillium wilt is an important disease of potato which is primarily caused by the soilborne fungus *Verticillium dahliae*, a pathogen that is widespread across commercial potato fields in south-eastern Australia. Previous studies have shown that cultivar Denali has moderate to high resistance to *V. dahliae*, while Russet Burbank is susceptible. However, the mechanism of infection and colonisation within the plant tissues of each cultivar, and the cause of host resistance within Denali is still unknown. Glasshouse pathogenicity tests were conducted by immersing the roots of each plant in a spore suspension (5×10^4 mL⁻¹) for 5 mins prior to planting in soil in pots. The experimental control treatment consisted of dipping the plant roots in sterile distilled water. Each treatment had five replicates. Disease symptom severity was assessed weekly using a 0-5 qualitative scale, where 0 = healthy, 1 = chlorosis of leaves, 2 = moderate (30-50%) wilt with chlorosis, 3 = moderate wilt and necrosis, 4 = severe (>50%) wilt and necrosis, and 5 = plant death. Plants were destructively sampled at 2, 5 and 9 weeks after inoculation, and different host tissues were cultured on to ethanol potassium amoxycillin agar to determine the incidence of *V. dahliae*. A Taqman probe-based assay for real-time PCR was used to quantify the concentration of *V. dahliae* DNA in Denali and Russet Burbank stems at harvest. Overall, Denali consistently exhibited lower visual symptom severity than Russet Burbank. However, there appeared to be little difference in the incidence of infection of different host tissues and concentration of *V. dahliae* DNA in the stems of both cultivars.

POSTER 335

Determining the impact of Botrytis bunch rot on grape quality to inform disease management decisions.

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Management of Botrytis bunch rot of grapes often fails in growing seasons when wet weather occurs close to harvest. The absence of fungicide registration post-veraison for grapes destined for export wine further hampers effective disease control. Understanding how much Botrytis can be tolerated in a batch of grapes before a negative impact on wine quality arises can assist grape growers to make disease management decisions. To determine thresholds for bunch rot contamination, Chardonnay grape bunches from a commercial vineyard were divided in to one of five groups and scored for grey mould infection using a scale of 0 to 4 based on visual assessment. Fungal biomass of each group was determined as a function of ergosterol content using HPLC and grapes were vinified in triplicate. Grey mould infection resulted in elevated levels of 1-octen-3-ol, 1-octen-3-one and 3-octanone, compounds associated with earthy mouldy aromas. Using untargeted GC/MS analysis, increasing levels of a number of unidentified sesquiterpenes and 2,3-dimethylnaphthalene were found in grape juice with increasing infection. Levels of hexenal and other C6 compounds were diminished in Botrytis infected fruit, possibly due to degradation of wax layers on the berry surface following fungal colonisation. Sensory analysis indicated wine made from grapes with ≥ 1.05 g of dry weight of fungus / kg wet weight of grapes was notably different from wine made with uninfected grapes. Participants couldn't differentiate between wine made with 0.35g dry weight of fungus / kg wet weight of grapes from unaffected wine, suggesting that the threshold for Botrytis contamination is somewhere in the 0.35 to 1.05g dry weight / kg of grapes range. The findings have implications for Botrytis management decisions around disease control and thresholds for fruit rejection.

POSTER 336

Roles of high temperature, desiccation, and time in poor survival of *Pratylenchus thornei* in the topsoil after wheat harvest in a subtropical environment

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Root-lesion nematode, *Pratylenchus thornei*, a widespread pathogen of wheat in the subtropical grain region of eastern Australia, causes lost production of ~AU\$38Million/year. We are investigating the biology of *P. thornei* for the purpose of modelling its population dynamics in cropping systems of this region. Population densities of *P. thornei* increase exponentially during crop growth then decline exponentially during fallow in all depths in the soil profile except in the topsoil where the decline is more rapid than exponential. An experiment was conducted to determine the potential contribution of elevated temperature and desiccation during summer fallow to this rapid decline. A vertisolic soil containing a high population density of *P. thornei* after wheat growth was subject to three moisture regimes and incubated at eight temperatures from 10 to 45°C. Soil moisture regimes were (a) maintained at field capacity (pF 2.5), (b) initially field capacity allowed to dry during incubation, and (c) air-dried to pF5.6 before incubation. Survival of *P. thornei* was assessed at six intervals from 0 to 16 weeks incubation. *P. thornei* died out completely at 40 and 45°C irrespective of the moisture regime and populations were substantially reduced at 35°C and 30°C. At lower temperatures, survival over a 16-week period was better in soil maintained at field capacity than in soil allowed to dry. Death of *P. thornei* in air-dried soil was rapid during incubation at all temperatures tested. Soil that dried slowly in treatment (b) in the course of the experiment resulted in longer survival of *P. thornei* than in treatment (c). Best survival was in soil at field capacity incubated at the lower temperatures of 10 to 25°C. It is likely that elevated temperatures and desiccation during summer fallow contribute significantly to the poor survival of *P. thornei* in the topsoil to ~7 cm depth.

POSTER 337

Soil Solarisation as a method to deliver near zero level crown rot (*Fusarium pseudograminearum*) reference sites in the northern grain region of Australia.

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Crown rot is one of the biggest limiting factors of crop production, with yield losses as high as 60% being recorded in susceptible varieties. The nature of crown rot in the northern grain growing region means that it is extremely widespread, with background levels of the pathogen found in most cropping areas. Crown rot research currently focuses on developing both resistant and tolerant varieties for commercial use. Tolerance testing involves a number of advanced lines being put under plus and minus disease conditions, with the plus being inoculated with crown rot and the minus having no inoculation treatment. However as crown rot is so widespread, there is commonly a base level of the crown rot inoculum already evident within the minus plots, preventing a true zero yield potential value being determined. This dilemma has recently been identified by the GRDC as a significant area of concern for the future of crown rot research as tolerance trials expand and the availability of near zero level disease plots decreases.

Preliminary trials indicate that soil solarisation has the ability to deliver near zero level crown rot reference sites for experimental purposes. When plots were solarised for twelve weeks, a 98.7% reduction in *Fpg* DNA/g soil was observed, compared with 11.2% and 44.9% in fallow and mungbean plots. A reduction was also seen in infected wheat stubble with only 5.8% of *Fpg* colonised stubble remaining after the solarisation, compared to 61.9% and 53% in the fallow and mungbean treatments. This project will examine whether soil solarisation is more effective in decreasing crown rot inoculum load between seasons than current stubble management techniques and if it causes any detrimental effects to the following crop due to microbial or nutritional changes to the soil.

POSTER 338

Effect of Essential oil-loaded Mesoporous Silica Nanoparticles on *Pseudomonas syringae* pv *pisi* in vitro and in planta

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The application of nanotechnology in agriculture has not yet been as thoroughly explored as its application in other fields such as medicine. However, nanoparticles have the potential to be used as smart delivery vehicles for a controlled and targeted delivery of biocides to treat and control the spread of diseases affecting plants.

Mesoporous silica nanoparticles (MSNPs) are biocompatible, biodegradable, stable, safe and cheap to produce. Additionally, MSNPs can be functionalised for targeted delivery and can be customised to encapsulate particular biocides. Furthermore, MSNPs have been previously reported to have mainly beneficial effects on plants such as improving tolerance to abiotic stress, enhancing plant growth and development, and increasing yields.

This study evaluated the use of MSNPs loaded with essential oils as a potential seed treatment against *Pseudomonas syringae* pv *pisi*, a phytopathogenic bacteria that causes pea bacterial blight. Essential oils can be highly antimicrobial and environmentally-safe but their volatility, easy degradability and poor miscibility hinders their use in agriculture. Hence, the encapsulation of these volatile compounds into MSNPs would facilitate their delivery and would enable a controlled release of the biocide.

The effect of essential oil-loaded MSNPs on *P. syringae* pv. *pisi* was studied *in vitro* and *in planta*. A decrease in live bacterial cells demonstrated the efficacy of the oil as an antimicrobial and the correct encapsulation of the oil into the nanoparticle. In addition, pea seeds coated with a seed treatment containing loaded-MSNPs showed a short-term increased plant resistance against the pathogenic bacteria. The capping of the MSNPs to achieve a targeted and long-term effect is currently being evaluated. This biocide-delivery system can be further applied to encapsulate other antimicrobials to treat a wide variety of agriculturally important bacterial and fungal diseases.

POSTER 339

Mitigation Effect of High Temperature and Water Stress by *Bacillus velezensis* YP2 on Leafy Vegetables

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Drought is a major factor limiting agricultural crop productivity by decreasing water availability of plants, worldwide. In drought, it is usually presented by the simultaneous action of high temperature and drying. The aim of this study was to know the effect of drought stress tolerance by plant growth promoting rhizobacteria (PGPR) on high temperature and water shortage stress of leafy vegetables; *Angelica acutiloba* Kitagawa, *Brassica juncea* L., *Brassica oleracea* var. *alboglabra* Bailey. The YP2 strain was isolated from rhizosphere soil of tomato plant in Yangpyeong, Korea and the isolate was identified as *Bacillus velezensis* based on molecular analysis. As a result, the YP2 strain significantly promoted the growth of *A. acutiloba* seedlings in increase of about 43.3% of plant height, 69.8% of root diameter compared to control. There were also significantly increased, compared with control, 36.7% of leaf area in *Br. juncea* seedlings as well as 26.7% of plant height and 41.4% of fresh weight in *Br. oleracea* seedlings. In addition, *B. velezensis* YP2 was also found to be effective for mitigation of high temperature stress (66.8%, at 35°C) and water shortage stress (72.9%, at 14th day after non water-treatment) compared to control. These results suggest that YP2 might be used as a good method for improving quality and productivity of leafy vegetables under drought stress conditions.

POSTER 340

Cold plasma: A novel method to control *Botrytis cinerea*, the causal agent of grey mould on strawberry

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Cold plasma (CP) has been successfully used to decontaminate fresh produce from microorganisms, particularly bacteria that cause food-borne illness. There are limited reports of CP being used to control plant pathogenic fungi, and most are limited to fungi causing postharvest diseases of nuts and grains. In this study, we investigated the direct effect of CP on *Botrytis cinerea* isolated from strawberry (*Fragaria ananassa*). CP was tested for its efficacy against pure cultures and spore suspensions of *B. cinerea*, following different treatment durations ranging from 30 seconds to six minutes. Applications of greater than two minutes significantly reduced the radial mycelial growth of both freshly inoculated cultures and two-day old active cultures compared to the control. In addition, CP treatment of spore suspensions reduced germination to zero within 18 hours of the treatment. These results demonstrate the potential of CP for the control of *Botrytis cinerea* associated with strawberry.

POSTER 341

Direct application of cold plasma to *Colletotrichum* species *in-vitro* reduces their growth and germination

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Cold plasma (CP) is widely used in the biomedical field, especially in dentistry and skin care but also for surface sterilisation of medical equipment. It has also been successfully used for the decontamination of fresh produce from microorganisms, particularly bacteria that cause food-borne illness. There are limited reports of CP to control plant pathogenic fungi. In this study we investigated the direct effect of CP on three postharvest fungal plant pathogens, belonging to the *Colletotrichum* genus that we isolated from avocados (*Colletotrichum alienum* and *Colletotrichum floriniae*). CP using air as the feed gas was tested for its anti-fungal efficacy against pure cultures and spore suspensions of *C. alienum* and *C. floriniae*, following treatment for 3 or 6 minutes duration at a distance of 5 cm. The 6 minute CP treatment significantly reduced the radial mycelial growth of freshly inoculated cultures compared to the control. In addition, the 6 minute CP treatment of spore suspensions resulted in almost 100% reduction of spore germination, immediately after treatment. In contrast, the 3 minute CP treatment did not significantly reduce the radial mycelial growth of fresh or actively growing cultures, but did suppress the germination of spores after 15 hours of treatment. These results demonstrate the potential of CP for the control of *Colletotrichum* species associated with avocado.

POSTER 342

Silverleaf whitefly (*Bemisia tabaci* biotype B), an exotic pest: Success and challenges in its management in vegetable crops

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Silverleaf whitefly (SLW) (*Bemisia tabaci* biotype B), a major global pest, was first recorded in Australia in 1994. Since then it has established as a serious pest of many vegetable crops across Queensland. This polyphagous pest causes severe damage to crops through direct feeding, honeydew contamination of plants and fruit and by injecting a toxin into plants that causes physiological disorders including tomato irregular ripening and silverleaf in cucurbits. It also vectors tomato yellow leaf curl virus.

SLW arrived in Australia already resistant to most pyrethroid and organophosphate insecticides. Since 1996 pyrethroids and imidacloprid mainly have been used to control SLW in vegetable crops. Resistance monitoring of SLW in horticultural crops showed a very rapid rate in the development of resistance to insecticides. Resistance to insect growth regulators was detected after four years of use in vegetable crops in North Queensland. SLW caused severe economic losses to tomatoes, melons, green beans, pumpkin, eggplant, zucchini, cucumber and sweetpotato. Economic losses attributable to SLW are estimated at over \$500 million to Queensland vegetable industry. These losses include direct crop loss, the cost of chemical control and the loss of jobs and people's income.

Since 1996 industry funded research projects have been undertaken on SLW biology and ecology and short-term chemical control options. Recent researches/studies were targeted on long-term integrated management strategies including biological control, the use of selective insecticides, resistance management and industry engagement. In this paper we present an overview of 15 years of scientific research on SLW and the challenges faced during the implementation of research outcomes with growers and industry service providers.

POSTER 343

Control of Sclerotium Rot of chillies in Australia

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Capsicum and chilli production in Australia is currently valued at \$136M annually. Summer crops in NSW and Queensland can be affected by Sclerotium Rot caused by the basidiomycete *Athelia rolfsii* (asexual state = *Sclerotium rolfsii*). Affected plants develop a basal stem and crown rot causing them to wilt and die. Greater than 25% of plants commonly die by their harvest date. Fruit from wilting plants are unacceptable for fresh markets and only a few producers have a secondary processing market. Our aim was to develop effective controls for this disease. We conducted two field experiments in the summer of 2016-7 to evaluate chemical and biological controls for Sclerotium Rot of chillies. One trial was located on a farm in Bundaberg, Queensland that was known to be infested with *S. rolfsii*. The second trial was established at Somersby in NSW where a sclerotial inoculum was applied. At both sites untreated control treatments were compared with chemical (pyraclostrobin or a combined formulation of cyprodinil and fludioxonil) or biological control treatments which were drenched around the base of plants at three-weekly intervals commencing at transplanting. The trial at the Somersby site used a commercial formulation containing an isolate of *Pseudomonas fluorescens* while the Bundaberg site used a product of an undisclosed microbial formulation. Plots were rated for disease severity at three growth stages with a final assessment at harvest. Yield data was collected at the Bundaberg site only. Chemical treatments generally reduced Sclerotium Rot significantly at both sites. *P. fluorescens* also significantly reduced the disease whereas the microbial formulation at Bundaberg was ineffective. These trials demonstrate that there are potential chemical and biological control options for Sclerotium Rot of chillies. Further studies are also addressing potential cultural controls such as plant spacing and irrigation scheduling.

POSTER 344

Managing damping off in baby-leaf spinach in Australia

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Baby-leaf spinach production in Australia has surged in recent years. It is currently valued at \$55M annually. Year-round supply of semi-processed product requires a network of growers spanning all states except the Northern Territory. Damping off has become a significant constraint in this intensive production system where multiple crops are often grown without rotation. Diseased plants have yellow or wilted leaves which contaminate the harvested marketable product. Furthermore when plants are damped off the remaining healthy plants have a flatter habit making machine harvesting more difficult and causing many leaf blades to be cut rather than at the petiole. This leads to a reduced shelf life and sometimes rejection by the processor. This study has identified the pathogens causing damping off in different production areas and we have commenced field trials to evaluate a number of chemical, cultural and biological control options. The key pathogens determined so far are various species of *Pythium*, namely *P. aphanidermatum*, *P. ultimum* var *ultimum* and *P. irregulare*; *Rhizoctonia solani*; and *Fusarium oxysporum* f.sp. *spinaciae*. They appear to have both temporal and spatial differences in their occurrence and importance. A field trial in Richmond, Tasmania over the past summer compared drench treatments at sowing of metalaxyl-M, azoxystrobin, propamocarb, fosetyl-Al, and a commercially formulated strain of *Bacillus subtilis*. A combination of metalaxyl-M and azoxystrobin and another treatment with a combination of propamocarb, fosetyl-Al and *B. subtilis* resulted in significantly fewer diseased plants although yield differences were not significant. Both *Rhizoctonia* and *Pythium ultimum* were confirmed as the key causes of the diseased plants. Further studies are planned with chemically dressed seed and biocontrol treatments as well as assessments of the ameliorative effects of different cover crops and biofumigants.

POSTER 345

Biofumigation potential of *Brassica* crops to control *Rhizoctonia solani* infection of potato

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Biofumigation using *Brassica* crops has been used for the management of soilborne diseases caused by *Rhizoctonia solani*. However, to date, there is limited evidence for the efficacy of biofumigation for controlling *R. solani* on potato under New Zealand growing conditions. In this study, the three selected *Brassica* crops 'Caliente' mustard (Ca), brown mustard (Bm) and 'Nemat' arugula (Ne) were assessed for control of *R. solani* infection of potato under shadehouse conditions using soil from a field with a history of *R. solani* disease. Seven treatments were set up, comprising Ca, Bm, Ne, allyl isothiocyanate (allyl ITC) and fungicide (azoxystrobin for soil treatment and penicuron for seed treatment) applied to *R. solani* inoculated field soil, and a positive (untreated *R. solani* inoculated field soil) and nil (uninoculated field soil) control. *Brassica* crops were grown in planting bags for 55 days, then macerated and incorporated into the soil by hand, watered and allowed to decompose for four weeks before potato (cv. 'Russet Burbank') planting. Stem, stolon and root diseases caused by *R. solani* were evaluated at five weeks after planting. Ca, Ne and the fungicide treatments significantly reduced stem canker severity (50-58%) relative to the positive control, followed by Bm (34%). Ca significantly decreased stolon disease severity by 56% compared with the positive control treatment, but was significantly lower than the fungicide treatment (77%). Allyl ITC had no significant effect on stem and stolon canker levels compared with the positive control. The nil control had the lowest stem and stolon canker severity levels (3.9 and 7.8%, respectively) compared with other treatments. There were no significant differences in the level of root disease between treatments (0.73-1.11 disease scale). These preliminary results indicate that selected *Brassica* crops, particularly Ca, have the potential to control *R. solani* infection of potato.

POSTER 346

Investigations into alternative post-harvest treatments for crown end rot of banana.

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Crown end rot (CER) of banana affects the cushion or crown portion of complete hands or clusters of banana during the ripening process or at the point of sale. Symptoms can range from superficial fungal growth on the cut crown surface, to minor discolouration of the crown tissue, through to complete breakdown of the crown and pedicel tissue with necrosis extending down into the fingers. A range of fungi are known to cause these symptoms world-wide and in north Queensland, the organisms in order of prevalence include: *Musicillium theobromae* and *Fusarium equiseti-incarnatum* complex, *Thielaviopsis musarum* and *Colletotrichum musae*. Management of CER is centred on the use of post-harvest fungicides and two products are currently registered for use, Sportak® which contains the active prochloraz and Tecto® that contains thiabendazole, however both products have issues or limitations. Prochloraz has workplace health and safety concerns when applied under certain packing shed situations and laboratory studies have shown a reduction in sensitivity to some of the CER organisms to both products, but more so to thiabendazole. Because of the above concerns, a laboratory assay has been developed to screen a range of products that may have efficacy across the broad spectrum of causal fungi and that could be used by organic growers. The products include: fungicides, biologicals, quaternary ammonium solutions and household products (eg. vinegar and bicarb soda). Early screening results have indicated that some products have efficacy (although limited) across the range of crown rot organisms, whereas efficacy of other products was quite selective or specific to a fungus. Products that show some promise will be further evaluated to prove efficacy of on banana clusters and to ensure that the products do not have any phytotoxic effects on banana fruit.

POSTER 348

Growth promotion of Chinese cabbage in salt stress conditions by Volatile organic compounds of *Pseudomonas* sp. NBC107

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Soil salinity is a widespread problem that limits crop yield throughout the world. Moreover, the salinity stress in crops will increase due to climate change. Rhizobacteria can stimulate the germination of seeds and further the growth of many economically important crops by phytohormone production, nitrogen fixation, siderophore production, and volatile organic compound production. In this study, the rhizobacterial isolate *Pseudomonas* sp. NBC107 was selected and tested for the increase of salt tolerance and consequently stimulation of Chinese cabbage growth. The *Pseudomonas* sp. NBC107 didn't produced ACC deaminase and siderophore, which is known as the mode for inducing tolerance to salinity stress. In comparison to the controls, the vigor index and fresh weight of Chinese cabbage significantly increased by 12.1% and 7.4%, respectively, under the influence of VOCs of *Pseudomonas* sp. NBC107. When we analyzed the volatile organic compounds after growing the bacteria in screw cap vial containing LB broth, the main headspace compound (ca. 90.6% of the total area of all peaks) was indole (RT 14.756 min), and dimethyl trisulfide (ca. 1.42%, RT 8.889 min) and cyclic octaatomic sulfur (ca. 1.73%, RT 22.529 min), were only detected in relatively low amounts. The functional role of volatile organic compounds in plant growth promotion will be further studied.

POSTER 349

Testing the efficacy of urea as a treatment for the destruction of *Fusarium oxysporum* f. sp. *cubense* in infected soil.

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The confirmation of Fusarium Wilt Tropical Race 4 (TR4) (*Fusarium oxysporum* f.sp. *cubense* Race 4) (Foc) near Tully has put at risk the \$600 million per year Australian banana industry. To reduce the threat to the wider banana growing industry, effective destruction protocols of infected sites are imperative, to minimise inoculum and limit pathogen spread. Biosecurity protocols treat infected sites, plants and soil, with a high doses of urea to destroy all inoculum present. The efficacy of urea treatment has been tested by using banana field soil contained in sterile jars, and inoculated with Foc Race 1 (VCG 0124) (FocR1) at a rate of eighty conidia per gram of soil, and incubated for seven days. A urea treatment equivalent to 500 g urea m⁻² was then applied in triplicate, along with controls and incubated for twenty eight days. Pieces of banana pseudostem from a FocR1 susceptible variety were surface sterilised, placed in contact with the soil in the jars, and incubated for a further seven days. The pseudostem was then removed, surface sterilised, and twenty one pieces from each jar were placed onto *Fusarium* spp. selective medium. The plates were then assessed after five days for the presence or absence of the pathogen. FocR1 was not recovered from any of the urea treatments, but was consistently recovered from the controls. The experiment has been repeated twice, progressively reducing the rates urea. No FocR1 has been recovered from any urea treatment down to a rate of 62.5 g urea m⁻². However, a 6 % recovery rate of FocR1 was recorded when urea was applied at 31.25g urea m⁻². Further refinement of the procedure is currently underway to determine the point where urea loses its effectiveness as well as to understand which urea hydrolysis by-products have the greatest efficacy.

POSTER 350

Using *Trichoderma* to suppress Fusarium Wilt (*Fusarium oxysporum* f.sp. *cubense*) in banana cropping systems

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The confirmation of Fusarium Wilt Tropical Race 4 (TR4) (*Fusarium oxysporum* f.sp. *cubense*) (Foc) near Tully has put at risk the \$600 million per year Australian banana industry. To reduce the threat to the wider banana growing industry, the development of management techniques to reduce inoculum and limit disease movement is imperative. Twenty *Trichoderma* isolates were collected from banana growing soils which had a history of Foc race 1 (FocR1). The isolates were screened for potential to reduce growth of the pathogen. A *Trichoderma virens* isolate (BRIP65209) consistently suppressed the growth of FocR1. The *T. virens* isolate demonstrated the highest inhibition of the pathogen, rapid growth and completely overgrew the pathogen. The efficacy of the *T. virens* isolate to suppress FocR1 was confirmed by inoculating lengths of banana pseudostem with FocR1, the *T. virens* isolate or both and compared to uninoculated pseudostem. The pseudostems were maintained in sterile sealed containers for three months, before being removed, homogenised, and strained through cheesecloth. The total number of conidia and chlamydospores of Foc was then determined. The addition of the *T. virens* isolate to the pseudostem reduced the number of Foc conidia. Furthermore, when the *T. virens* isolate was added to the pseudostem with FocR1, chlamydospore production was reduced by two thirds relative to FocR1 only inoculation. A novel application method for the application *T. virens* was then tested, where the *T. virens* was injected directly into the pseudostem of recently harvested banana pants. The following suckers of the treated plants showed no reduction in growth measurements. In addition, the treated pseudostems exhibited rapid decomposition, which may limit Foc proliferation within decaying banana pseudostems. Further efficacy testing of the *T. virens* isolate is required in the field to confirm suppression of Foc, but the technique provides promise to limit losses to Fusarium wilt.

POSTER 351

Development of an effective delivery system for a woody weed bioherbicide

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“Di-Bak Parkinsonia” is a bioherbicide product developed for the management of the invasive woody weed *Parkinsonia aculeata* in rangeland situations in Australia. The product is formulated as a food grade gelatine or cellulose capsule containing various native fungi associated with naturally occurring dieback events in northern Australia. When introduced into non-symptomatic trees, these fungi are capable of colonising parkinsonia endophytically leading to the development of a naturally spreading dieback event in the population.

Effective colonisation of parkinsonia plants requires the capsule to be inserted into the stem and sealed to maintain a moist environment free of oxidation and desiccation. A mechanised delivery system powered by an 18V cordless drill allows the operator to quickly and effectively drill a hole, insert the capsule and seal the inoculation site with a plastic plug. The delivery system has undergone several developmental steps and robust field testing which has benefitted from suggestions provided by field staff experienced in woody weed control.

A series of work rate evaluations were conducted under field conditions in a parkinsonia infestation at Corporal Dam at Alexandria Station, Barkly Tablelands in the Northern Territory. Two operators each using a mechanical inoculating apparatus, and working in a team were capable of an average work rate of ranging between 101 and 145 trees per hour each. Battery performance ranged between 210 to 240 inoculations before a changeover was required. Overall work rate was considered to be as good, if not better than that which could be achieved by basal bark treatment with conventional (synthetic) herbicides using a backpack sprayer.

POSTER 352

Characterisation and screening of *Bacillus* spp. for lipopeptides and enzymes involved in biocontrol of *Leptosphaeria maculans*.

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Species of *Bacillus* have the ability to synthesise different antimicrobial peptides (AMPs) and other compounds such as cell wall degrading enzymes which may be effective against various phytopathogens. This study aimed to detect AMP and lytic enzymes genes, by using biosynthetic gene markers, in a collection of rhizospheric and endophytic *Bacillus* spp. displaying significant inhibition of *Leptosphaeria maculans* in *in vitro* assays. *Bacillus* spp. were preliminarily identified by sequencing 16S rRNA genes. AMP screening of the selected *Bacillus* spp. was carried out using 10 primers to amplify genes encoding for antibiotics (surfactin, surfactin synthetase; iturinA, iturin C, two genes for , bacillomycin D, zwittermycin, fengycin) and lytic enzymes (chitinase, β -1,3-Glucanase) through polymerase chain reaction. The majority of strains possessed four to six genes of interest and strains with seven AMP and enzymes genes were also detected, however, none of the strains were positive for all 10 genes. Iturin A and Bacillomycin D were present most frequently among the selected *Bacillus* strains whereas the Iturin C encoding gene was the least often amplified. Interestingly, strain B94 (*Bacillus amyloliquefaciens*), the best performer in the *in vitro* assays, was found to carry the highest number (8) of AMP and lytic enzymes genes. Thus, selected AMP and enzyme gene markers may be useful for selecting putative bacterial biocontrol agents of phytopathogens.

POSTER 353

Is weed biological control stable over decades? Investigations into the *Puccinia chondrillina*-*Chondrilla juncea* pathosystem in Australia

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Great efforts are taken to ensure that weed biological control agents are highly host-specific prior to their release into a new country. The genetic stability of the agent over the long term is often questioned, especially in relation to possible shifts in host-range, but rarely scientifically tested. Here, we use the *Puccinia chondrillina* (rust fungus) and *Chondrilla juncea* (skeleton weed) pathosystem in Australia to explore such issues. Three forms of *C. juncea* exist in Australia (narrow, intermediate and broad leaf forms), with *P. chondrillina* isolates specific to the narrow and intermediate forms previously released as biological control agents. One isolate, IT32, released in 1971 to target the narrow-leaf form showed exceptional impact in controlling this form. Three isolates targeting the intermediate-leaf form were subsequently released: TU21 in 1980, recorded soon after as not having persisted; IT36 in 1982, recorded as having a moderate impact; and TU788 in 1996, for which the impact is unknown. DNA sequencing of these released isolates, using DNA extracted from stored spores, and of isolates collected on both narrow and intermediate leaf forms in 2007 and 2016 is being used to determine the lineage of the contemporary isolates and identify genetic changes that may have occurred over time. Concurrently, each contemporary isolate is being tested on the three *C. juncea* forms to confirm its pathogenicity phenotype. We hypothesise that *P. chondrillina* has changed little since its introduction into Australia and that this pathosystem remains stable thereby providing evidence for the safety of biological control programs using host-specific plant pathogens.

POSTER 354

Biological Control of Rice Blast Fungus: A Study Case at Louisiana State University, United State of America

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Rice blast fungus, *Pyricularia oryzae* Cavara, is an important disease causing significant yield loss for rice production in USA as well as Thailand. To control this fungus, biological control is an important potential alternative practice according to its environmentally friendly and no risk to human health. Research on the use of antagonistic microorganisms to control rice blast disease was conducted at Louisiana State University, USA during October 2014 to September 2015. *In vitro* screening of antagonistic bacteria against *P. oryzae* using dual culture method found that *Bacillus amyloliquefaciens* RAB14R showed the highest activity of growth inhibition to the pathogenic fungus. The survival of antagonistic bacteria in bacterial formulation was highest when mixed with 5% sucrose during freeze-drying. In the field condition, antagonistic bacterial suspension was sprayed to rice plants at the tillering and heading stages. For cv. M202 (a highly susceptible cultivar), all the treatments of antagonistic bacteria suppressed the disease less effectively than fungicide application. For rice cv. CL151 (susceptible cultivar), there were no differences among the treatments for the control of leaf blast. In the control activity for panicle blast, all the treatments of antagonistic bacteria showed some disease suppression activities compared to the water control but their activities were less than that of fungicide application.

Genetic elements for the antagonistic activity of RAB14R were identified by using a Tn5 mutagenesis technique. The result indicated that two genes encoding urocanate hydratase and diguanylate cyclase were associated with the growth inhibition of *P. oryzae*.

POSTER 355

Biological control of grapevine trunk diseases using endophytic bacteria

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Grapevine trunk diseases (GTDs) are considered serious problems of grapevines worldwide. *Eutypa dieback* (ED) and *Botryosphaeria dieback* (BD) caused by species within the Diatrypaceae (primarily *Eutypa lata*) and Botryosphaeriaceae families are among the top five priority diseases in the Australian wine grape industry. Current management of these diseases is through remedial surgery and fungicide treatments of pruning wounds. However, registered fungicides for GTDs are limited and some only offer short term protection. Biological control offers an alternative management strategy against GTDs. There are limited studies on the use of biocontrol agents (BCAs) against BD and ED, and for ED varying levels of success have been achieved. Preliminary studies in our laboratory showed 11 endophytic bacteria isolated from grapevines to be antagonistic against four GTD pathogens: *Diplodia seriata*, *Neofusicoccum parvum*, *N. luteum* and *E. lata*. *In vitro* and dual culture assays of these bacterial endophytes showed varying degrees of mycelial growth inhibition for the four pathogen species tested. Of the 11 bacterial isolates, three (BCA 11, 13, and 18) that exhibited the greatest mycelial growth inhibition were selected for *in planta* assays using detached grapevine canes. Canes were artificially wounded and inoculated with *N. luteum* conidia 24 hrs after inoculation with the selected bacterial isolates. Re-isolations from canes after four weeks revealed the lowest disease incidence at 28% when canes were inoculated with bacterial isolate BCA 13. BCA 11 and 18 resulted in 89% and 100% disease incidence, respectively. Control cuttings inoculated with *N. luteum* resulted in 100% disease incidence. These preliminary results indicate that endophytic bacteria may be good candidates for biocontrol and in future may be incorporated into a GTD management strategy. Molecular identification and biochemical characterisation of these bacteria is ongoing.

POSTER 356

Assessing the biocontrol efficacy of nematode trapping fungi *Arthrobotrys oligospora* and *A. dactyloides* against root-knot nematodes in ginger

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Ginger (*Zingiber officinale*) is an important crop to Australia with an average annual production of 8000 tonnes. Root-knot nematode is one of the serious soil-borne pathogens in ginger cultivations causing noticeable yield damages. Though most growers rely on chemical control for root-knot nematodes, chemical application is no longer recommended by regulatory authorities. Hence, interest in eco-friendly control measures has been a new avenue for many researchers. Fungal biocontrol is a rapidly developing research area, and there is an evolving interest in the exploration of fungi for controlling root-knot nematodes. In this study, two nematode trapping fungi, *Arthrobotrys dactyloides* and *A. oligospora* encapsulated in kaolin-alginate granules were individually assessed for their efficacy in controlling root-knot nematode damage in Ginger. Significantly less root-knot nematodes were recovered from both *Arthrobotrys dactyloides* and *A. oligospora* incorporated soils than from control, which indicated that both fungi have biocontrol potential to suppress root-knot nematodes in both heated and un-heated soil. Compared to control treatments, granule application of both fungi in both soils, reduced nematode damage in terms of galling and presence of lesions. There was no significant difference between fungi and chemical treatment for both damage reduction and soil root-knot nematode reduction. The new rhizome yields were not significantly different among all treatments. Other vegetative parameters including above-ground plant height and fresh weight were also not significantly different among treatments. Significantly lower root-knot nematode numbers and root galls as well as significantly higher yield were observed in un-heated soil compared to heated soil. This may be due to the presence of other natural predators of root-knot nematodes such as predatory nematodes and nematode feeding soil micro-organisms. The yield difference could be explained by the healthier plant performance in the undisturbed soil food web and the lower root-knot nematode damage.

POSTER 357

Evaluating the influence of Pb(II) on the production of volatiles by endophytic *Trichoderma asperellum* LF11 against *Ganoderma boninense*

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Endophytes are a group of microorganisms inhabiting plants without harming their hosts. They have been studied for their biocontrol activities towards numerous pathogens, expressed primarily through the production of volatile and non-volatile inhibitors. This study aims to evaluate the production of volatile inhibitors by endophytic *Trichoderma asperellum* LF11 against pathogenic *Ganoderma boninense*, under the influence of Pb(II) stress. Pb(II) is a non-essential, common metal in the environment, which causes toxicity to living organisms at higher concentrations. The assay was first performed using the double-plate assay with agar supplemented with 10 and 25 ppm Pb(II). The growth inhibition of *G. boninense* was calculated and compared to cultures in control (0 ppm Pb(II)). The volatiles produced were then extracted via solid phase microextraction and profiled using the gas chromatography mass spectrometry (SPME-GCMS). Results from the double-plate assay revealed that *G. boninense* was suppressed by *T. asperellum* LF11 at 0 ppm (50.68%), and in the presence of 10 ppm (48.15%) and 25 ppm (31.17%) of Pb(II). The SPME-GCMS profiling of volatiles produced under the influence of 0 and 10 ppm Pb(II) revealed that the number of volatile compounds increased in 10 ppm (a total of 26, 13, 18 volatiles) than in 0 ppm Pb(II) (10, 7, 11 compounds) detected in plates inoculated with *G. boninense*, *T. asperellum* LF11 and *T. asperellum* LF11+*G. boninense*, respectively. The compounds 6-pentyl-2H-pyran-2-one (6-PP) and 2-pentylfuran were produced by *T. asperellum* LF11 in the presence and absence of Pb(II). These two compounds were reported to benefit hosts by restricting pathogen growth and regulating plant growth. Our findings suggest that *T. asperellum* LF11 remained effective in inhibiting *G. boninense* through the production of volatiles even under metal stress by Pb(II).

POSTER 358

Biocontrol of Ginseng Damping-off by *Bacillus velezensis* CC112

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Bacillus velezensis CC112 inhibited the mycelial growth of several plant pathogens including *Rhizoctonia solani* causing damping-off on ginseng. Control efficacy for *Rhizoctonia solani* by treatment of seed dipping with Luria-Bertani (LB) and *Bacillus*-soytone medium (BSM) broth diluted in 10 times, and mixing treatment of seed dipping and soil drenching with LB broth diluted in 10 times of *B. velezensis* CC112 was 65.8%, 67.1%, and 64.2%, respectively. Treatment of soil drenching with 100 times of the prototype of *B. velezensis* CC112 for *Rhizoctonia solani* and *Pythium* sp. revealed control efficacy of 77.3% and 65.7%, respectively. As a result, *B. velezensis* CC112 could be a prospective biofungicide for biological control of ginseng damping off.

Biocontrol of Cucumber Powdery Mildew by *Bacillus amyloliquefaciens* M27

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Bacillus amyloliquefaciens M27 isolated from the cotton-waste compost for cultivation of oyster mushroom (*Pleurotus ostreatus*). *B. amyloliquefaciens* M27 is a biocontrol agent with antagonistic activities against a wide range of fungal pathogens. The filtrate of the isolate cultured in LB media was treated to cucumber plants in July, October and December before cucumber powdery mildew occurred. When 10-fold diluted filtrate of the isolate was treated, control efficacy was 88.9~98.9% in the treated seasons. The result showed that the culture filtrate of *Bacillus* sp. is very effective to control powdery mildew of cucumber during the seasons of the disease development.