Change & Adaptation

24th APPS Conference
ADELAIDE 2023

ABSTRACT BOOK

APPS

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Change & Adaptation
24th APPS Conference
ADELAIDE 2023

Contents

Oral Abstracts

Monday 20 November: Pre-Conference Workshops

Tuesday 21 November: Conference Day One:

Contents *(continued)*

Contents *(continued)*

Friday 24 November: Post-Conference Workshops

Poster Abstracts

Disclaimer

Oral Abstracts

Monday 20 November:

Pre-Conference Workshops

Workshop 1: Breaking Ground in Australian Nematology

Presenters: Dr Mike Hodda - CSIRO, Canberra ACT; Dr Katherine Linsell - SARDI, Urrbrae SA; Dr Sarah Collins - DPIRD, Perth WA; Dr Rebecca Zwart - University of Southern QLD, Toowoomba QLD; Plus Guest Presenters

Summary

A workshop on what's breaking in nematology. We have new species to discuss (in the turf world) known species that are not what we thought (like cereal cyst nematode), new species that we definitely didn't want (Root knot nematode findings in our north) and new information on species that cause huge economic losses annually in Australian agriculture. We also have techniques for nematode extraction, inoculum in glasshouse trials and in the molecular world. Hear from those closest to these changes, discuss what they might mean, and have a look at the nematodes in question. Then finish by engaging with a panel of nematologists on where nematology is going and should concentrate in the years ahead. With panellists ranging from the recently converted to very experienced, and questions from the floor, we hope this will be a lively session to end the day!

The workshop is a series of talks by people working on most of the most damaging nematode genera, including Cyst Nematodes (genus *Heterodera*), Root-Knot Nematodes (genus *Meloidogyne*), Root-Lesion Nematodes (genus *Pratylenchus*), and turf nematodes (several genera). There will be demonstrations of each of these under the microscope where possible. After lunch, there is a series of talks on molecular methods in nematology, from routine identifications to detailed taxonomic examinations of the identity and validity of species and how these can be incorporated into diagnostic tests. Other talks in this section will discuss what can be learned from studying whole genomes and e-DNA. The final part of the workshop is a discussion of what all this might mean for nematology with a diverse panel of nematologists and questions and comments from the floor.

1. Cyst Nematodes: Dan Huston (CSIRO), Sarah Dunstan (CSIRO)

2. Root-Knot Nematodes: Wayne O'Neill & Dylan Corner (QDAF) & Guest presenter (NT DITT)

3. Root-Lesion Nematodes: Sarah Collins (WA DPIRD), **Mike Hodda (CSIRO**)

4. Turf Nematodes: Peter Ruscoe (Turf consultant).

5. Techniques: Neil Wilson (Netagen), **Dan Huston (CSIRO)**, **Akshita Jain (DJPR-Vic/Latrobe Uni)**, **Daniele Giblot-Ducray (SARDI)**, **Mike Hodda (CSIRO)**

6. **Nematology past, present and future:** Join the discussion with a diverse panel of nematologists on what developments such as those described might mean for nematode priorities, research, diagnostics, management, resistance breeding and other aspects of nematology. Having heard about new species, ups and downs in what we know about existing species and new techniques. Your questions from the floor welcomed. To answer these questions and others we have: **Ian Riley (Consultant), Dan Huston (CSIRO), Ian Riley (University of Adelaide), Kerrie Davies (University of Adelaide).**

Workshop 2: Grapevine Virology 101

Presenters: Dr Monica Kehoe - DPIRD, South Perth WA; Dr Fiona Constable - AgVic, Bundoora, VIC

Summary

During this workshop we will cover the basics of Grapevine Virology in Australia. We will cover:

- Information on the viruses themselves, what we know about them in the Australian context. Monica Kehoe, Kamalpreet Kaur and Dr Fiona Constable
    - Updates on recent research including:
        - The prevalence and diversity of grapevine Pinot gris virus in Australia. Kamalpreet Kaur
        - Molecular Epidemiology of Shiraz Disease with an Emphasis on Grapevine Virus A. Dr. Qi Wu (recorded)
        - A snapshot of vineyard health in Victoria. Dr. Cliff Kinoti
        - New technologies for virus detection. Dr. Monica Kehoe
        - An update on the status of grapevine red blotch virus in Australia. Dr. Fiona Constable, Dr Monica Kehoe, Kamalpreet Kaur
        - Establishing the National Grapevine Collection. Nick Dry
        - Vine improvement: A national network. Chris Bennett

- An introduction to the Sampling Protocol for Virus Diagnostics, including how to sample/submit to diagnostic services

- An introduction to the Australian Grapevine Virology Technical Committee

- Discussion on the latest diagnostic test development, and future directions . Fiona Constable, Robin MacDiarmid, Sharon Harvey, Cath Kidman, Nick Dry and Chris Bennett

Workshop 3: New approaches for surveillance and monitoring of plant pathogens

Presenters: Dr Rohan Kimber - Crop Sciences, SARDI SA; Prof Jon West, Protecting Crops and the Environment Group, Rothamsted Research, United Kingdom; Plus Guest Presentations/Demonstrations from Dr Walt Mahaffee, Dr Ismail Ismail, Dr Mohsen Khani, Dr Andrew Baker, Lewis Collins, and Dr Michelle Demers

Summary

This workshop will demonstrate and discuss common and innovative spore trapping systems for the surveillance of airborne plant pathogens related to plant health. It will be held at SARDI's Plant Health Surveillance laboratory in the Plant Research Centre located at the Waite Research Institute. Researchers with interests in spore trapping, digital & mechatronic technologies, diagnostic techniques and data visualisation to end-users are encouraged to attend. The agenda will comprise of presentations, hands-on demonstrations and discussion sessions with the aim to facilitate a collaborative and interactive forum to overview emerging technologies and platforms, advantages and disadvantages, and present opportunities these offer aerobiological research and the surveillance of airborne plant pathogens. This includes drawing on the expertise of Professor Jon West (Rothamsted Research), who is internationally recognised in his research on aerobiology, and will co-chair this workshop.

Tuesday 21 November:

Conference Day One

PRESIDENTIAL ADDRESS

167

Pioneering discoveries in plant virology from Australia (what lessons can we learn from history?)

Geering A[1]
[1]The University of Queensland, St Lucia, Australia

Plant pathology in Australia prior to World War II was full of challenges. According to Walter Carne, there was “an absence of colleagues of similar interests with whom to consult, …defects of libraries and equipment, …ignorance of the work going on with other States and the feeling of geographical and mental isolation”. Despite these challenges, truly world class research emerged from Australia, and the Waite Institute in Adelaide was a centre of excellence in plant pathology. In this talk I will follow the story of the discovery of tomato spotted wilt virus, which provides a microcosm of the beginnings of State and Federal collaboration in agricultural research. Research on this virus got off to a rocky start, with personality clashes between individuals but eventually great progress was made in understanding the aetiology and epidemiology of this virus. Two of the main protagonists in the tomato spotted wilt virus story, Geoffrey Samuel and Jack Bald, were eventually lured to the mother country, England, by promises of greater research opportunities, leaving behind Rupert Best as the lone virologist at the Waite Institute. Best turned his attention to biochemical characterisation of tobacco mosaic virus and was among the first to demonstrate that it was composed of protein and nucleic acid. It has been suggested by some that he should have been Australia’s first Nobel Prize winner, but instead the award went to a well-connected American, Wendell Stanley. At the conclusion of my talk, I will review what lessons can be learnt from this period of research. The adage that history repeats itself is still pertinent.

Keynote Address 1

204

Emerging technologies for surveillance and monitoring of sporadic pathogens

Professor Jon West[1]
[1] Rothamsted Research

Authors: Jonathan S. West, Gail G.M. Canning & Kevin M. King
Surveillance and monitoring of pathogens optimises applications of crop protection products and is particularly useful for sporadic pathogens that are difficult to forecast. Established and emerging technologies, include imaging from satellites, aircraft or in-field sensors, visual observations, portable diagnostic tests, weather-based models and airborne inoculum detection.
Optical sensing by spectroscopy or imaging from satellites or aircraft can cover a wide land area but often cannot differentiate between different crop stress and detection may be too late to enable control. The most promising optical sensing approach for early detection appears to be from proximal sensors using fluorescence or image analysis to identify anomalies but often needs controlled conditions.
Portable diagnostic assays are increasingly available as Lateral Flow Devices or LAMP assay kits and can be used to confirm identity of symptoms or even to detect incubating infections that would otherwise be missed by a simple visual inspection. These kits can also be used with samples from spore traps and for some diseases initiated by airborne spores, this approach is now being automated to detect pathogen inoculum before infection occurs. Alternatively, airborne spores can be detected based on optical properties of the spores themselves.
Integration of wireless reporting improves the timeliness of results from automated devices compared to samples being sent to a lab. This gives more time for application of crop protectant products and is essential for rapidly developing diseases such as potato blight. Current research is combining automated spore traps with metagenomic sequence-based technologies (e.g. minION sequencing) to enable rapid monitoring of the entire aerobiome to profile abundance of multiple pathogens and even genetic traits such as fungicide resistance.
The area represented by a single spore trap, depends on atmospheric conditions and the spore trap's height above ground but can serve as a proxy for relatively large areas.

Session 1 A: New Technologies (Artificial intelligent (AI)) and Novel Methods in Plant Pathology and Disease Control

192

Understanding pathogen populations using qPCR analyses of airborne spore samples to assist in the management of Eutypa dieback of grapevines

Kimber R[1], Hill K[1], Giblot-Ducray D[1], Sosnowski M[1], Oswald E[2], Baker A[3], Johnson S[4]
[1]SARDI, Urrbrae, Australia, [2]Treasury Wine Estates Wynns Coonawarra, Coonawarra, Australia, [3]Data Effects, Adelaide, Australia, [4]AUSVEG, Melbourne, Australia

An iMapPESTS Sentinel (www.imappests.com.au) with a cyclone spore sampler (200 l/min) was deployed at Treasury Wine Estates Wynns Coonawarra (SA) site in 2022 adjacent a Burkard spore trap (10 l/min) traditionally utilised in plant pathology. Quantification of airborne pathogens was examined using qPCR. Key pathogens within Eutypa dieback, including *Cryptovalsa ampelina*, *Eutypa lata*, and other *Eutypella* and *Cryptovalsa* species were targeted using 3 different assays. Additionally, a generic assay (modified A), was applied to target a range of species in the Xylariales order, which include the Eutypa dieback species complex (multiple genera). *Erysiphe necator* and *Botrytis cinerea* were also analysed fortnightly within traceable samples in the iMapPESTS diagnostics pipeline then compared to traditional trapping at the end of season. Results showed *Cryptovalsa ampelina* was the dominant pathogen in the Eutypa dieback complex. This contrasts with findings in other regions where *Eutypa lata* often drives this complex. Additionally, the Sentinel (2 m) detected early spore dispersal events not captured by the Burkard sampler at a lower height (50 cm), which would align with pruning activities, a vulnerable period of vines to infection by Eutypa dieback pathogens. Monitoring also revealed mass dispersal of foliar pathogens *E. necator* and *B. cinerea* mechanically liberated into the air by barrel pruning activities. These insights into pathogen population shifts offer new opportunities for airborne pathogen surveillance or monitoring dynamics for improved management of fungal diseases. DNA-based diagnostics is further amenable to additional analytics of air samples, such as fungicide resistance or pathogen virulence profiles, now being investigated at SARDI. Connected digital outputs using the iMapPESTS surveillance pipeline has provided device-to-data delivery to researchers and industry end-users. This could offer devices deployed into risk pathways or area freedom points for trade protection for Australian imports and exports and showcases innovative and sustainable solutions to Australia's biosecurity programs.

179

Next-generation spore trapping: exploring opportunities and overcoming challenges

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Fungal diseases pose a significant threat to agricultural productivity and food security worldwide. To help mitigate these challenges, early detection of pathogens is crucial for effective disease management. Traditional pathogen detection relies on manual sampling methods, which are labour-intensive and frequently fail to provide timely data, preventing early disease intervention in commercial farming operations. However, recent technological advancements offer promising opportunities for overcoming these challenges. This talk aims to overview the opportunities and challenges associated with automated fungal spore trapping for agriculture. First, an explanation will be provided as to how technologies, including artificial intelligence, machine learning, and automated microscopy, have improved how we can detect airborne fungal crop diseases and case studies on how this technology has been implemented to date. It will explore the possibilities posed by providing aerobiological and environmental data in near real-time on a localised and large geographic scale. This includes providing early warning systems for farmers to implement targeted interventions and tracking large-scale disease spread in near real-time. It will also explore several challenges that must be addressed to facilitate the successful implementation of automated fungal spore trapping. These challenges include dealing with diverse environmental conditions, evaluating the accuracy of machine-learning models, working within limits posed by detection resolution, and assessing the effects of this data on pest management in-field. By fostering knowledge exchange and interdisciplinary collaboration, we can harness the power of automation to improve disease monitoring and management strategies, paving the way for a more sustainable and resilient agricultural future in a rapidly changing world.

189

Integrated digital biosecurity systems for plant health diagnostics

Hill M[1], Arnold J[1], Grgurinovic C[1], Tran-Nguyen L[1]
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Plant Health Australia (PHA) administers a range of digital systems that are integral to national plant biosecurity, including the Pest and Disease Image Library (PaDIL), the Australian Plant Pest Database (APPD) and AUSPestCheck®. APPD and PaDIL are of strategic importance to Australia's national biosecurity system in supporting the accurate diagnosis of plant pests and diseases for regulatory and management purposes, and determination of pest status. AUSPestCheck® is the repository for national surveillance data and supports critical data from the National Plant Health Surveillance Program and from industry partners.

Each of these digital systems facilitate sharing of biosecurity information and data necessary for efficient functioning of our national biosecurity system. While these applications are aligned in purpose, there is an opportunity to think holistically across the services provided by PHA and look at ways to bring their functions and features together as integrated digital biosecurity systems.

Plant Health Australia have also led the scoping required for “National coordination of high throughput sequencing data for a connected diagnostics system”. The vast amount of data being generated by HTS technology has created a need for a secure, centralised storage database supporting the submission, sharing and analysis of plant pest genomic data. Links to the APPD and PaDIL are clear, as there is such value of having specimens linked to a reference collection of sequence data.

In this talk we will cover what each of the Digital Systems administered by PHA currently do, future enhancements, areas where they could be further aligned, and the scope for new digital systems and services to be developed and integrated into these current systems.

90

Harnessing mobile, foliar applied double-stranded RNA for effective RNAi against plant pathogens

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[1]University of Queensland, Brisbane, Australia, [2]Queensland University of Technology, Brisbane, Australia

Foliar application of double-stranded RNA (dsRNA) as RNA interference(RNAi)-based biopesticides represents a sustainable alternative to traditional transgenic, breeding or chemical-based crop protection strategies. A key feature of RNAi in plants is its ability to act non-cell autonomously, a process that plays a critical role in plant development and protection against pathogens. However, whether RNAi induced by foliar dsRNA application can act non-cell autonomously remains debated and the potential mechanisms and implications of this movement largely unexplored. Here we show that upon foliar application, unprocessed full-length dsRNA enters the leaf vasculature and rapidly moves to vegetative, reproductive and below ground tissue types in several model plant and crop hosts. Intact unprocessed dsRNA was detected in the apoplast of distal tissue types and maintained in subsequent new growth indicating apoplastic rather than symplastic transport. Furthermore, we show that mobile dsRNA is functional against root-infecting fungi and foliar viral pathogens. When used to target fungal pathogens, mobile dsRNA transfers to the fungus where it is processed by the fungal RNAi machinery to elicit gene silencing. Using a novel biochemical purification technique and small RNA sequencing we have for the first time diagnosed function siRNA species derived from foliar applied dsRNA in both in the applied and distal infected tissue types. Our mechanistic dissection of the uptake and maintained movement of intact, functional dsRNA provides crucial insights into RNAi biopesticides and stands to add significant benefit to this emerging field of plant protection.

193

Optimisation of BioClay™ formulation and application to control botrytis grey mould of lentil

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BioClay™ is a new bio-pesticide containing layered double hydroxide (LDH) microclay sheets that carry double stranded RNA (dsRNA). Using spray-induced gene silencing technology, a specific dsRNA construct incorporated in BioClay™ is applied on plants and targets pathogen growth or virulence gene/s that eventually reduces plant disease. We developed a reliable and high throughput protocol for screening BioClay™ products to control botrytis grey mould (BGM) on lentil plants. Briefly, BioClay™, or its components dsRNA and LDH, plus a penetrant were applied on plants to examine the efficacy of these products on BGM in a controlled environment room equipped with LED light. Plants inoculated with *Botrytis cinerea* received humidity after inoculation, and BGM progression was assessed on several days after inoculation (DAI), up to 21DAI. Early developed products such as raw and washed LDH, and BioClay™ caused phytotoxicity. Along with improvements to product formulation, we screened several penetrants at different rates on 2–6-week-old plants. The phytotoxicity issue was resolved using a safe rate of some penetrants such as Pulse® at 0.005% v/v, on 4-week-old plants. The best control of BGM was achieved when dsRNA was applied 5DAI, compared to a few days before inoculation (5-2DBI) or even 0-3DAI. The concentration of dsRNA DCL1/2 in BioClay™ was progressively reduced from higher rates (0.1-0.6 mg/ml) used in initial trials and optimised to 0.05 mg/ml. Several dsRNA constructs targeting different genes, clay formulations and loading ratios of dsRNA/clay were examined and eventually achieved 80% efficacy control of BGM on lentil. Employing BioClay™ is a promising option for control of BGM, and future work will focus on further optimisation of the protocol and products.

Session 1 B: Host Resistance Breeding and Pathogenomics

100

Factors contributing to the expression of blackleg disease (*Leptosphaeria maculans*) of canola in the context of breeding for quantitative disease resistance

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Blackleg disease is a serious economic constraint to canola production globally. Host resistance to yield-limiting crown canker symptoms is either qualitative or quantitative. Qualitative resistance can be rapidly overcome due to the high mutability of *Leptosphaeria maculans* and therefore, quantitative resistance (QR) is considered a more durable form of resistance. The aim was to identify factors influencing blackleg expression to improve phenotypic characterisation of canola for application to breeding. Experiments were conducted by inoculating (i) with a genetically uniform single isolate in a controlled environment (ii) with a genetically diverse blackleg population under field-like conditions and (iii) with the same set of three blackleg populations in three different field environments. Differences in disease severity were detected between cultivars at various plant growth stages. A significant isolate x host genotype interaction was consistently measured in multiple experiments inoculated with either single isolates or diverse blackleg populations. There was a significant effect of environment on blackleg severity when host and pathogen were held constant. Our findings highlight the complex interplay between host, pathogen and environment in the expression of blackleg disease in canola with implications for both characterisation of quantitative resistance and disease management. In a breeding context, screening with single isolates may be useful to detect individual QTL associated with QR but these may not contribute to QR when screened against a population. Strong QR is likely a combination of genes with additive effects requiring further breeding and characterisation to identify optimal combinations. The effect of environment should be a focus for future research with significant implications for phenotyping for breeding and identifying scenarios which lead to severe disease and yield reductions.

32

Exploring the wilds for resistance to banana bunchy top virus

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Edible bananas (*Musa* sp.) contain genome components of the wild, seeded progenitor *M. acuminata* (AA) and often also *M. balbisiana* (BB). Cultivars with a B genome component are often noted to be less susceptible to infection by banana bunchy top virus (BBTV). In the Philippines, the cv Lakatan (AAA) is very susceptible to BBTV while the cv Saba (BBB/ABB) may escape field infection for years. In this study, two wild progenitors of bananas were evaluated for disease response against BBTV. Two accessions of *M. acuminata* ssp. *errans* (AAW) and 34 of *M. balbisiana* (BBW) from the National Plant Genetic Resources Laboratory, IPB-UPLB germplasm collection were artificially inoculated with BBTV in a screenhouse using viruliferous aphids. Symptoms were monitored and plants indexed by PCR 3 and 6 months after inoculation. All *M. acuminata* ssp. *errans* plants and all 'Lakatan' controls were infected, whereas all plants of all *M. balbisiana* plants (BBW) remained uninfected. *M. balbisiana* plants subsequently transferred to a field under high BBTV inoculum pressure remained uninfected for more than five years, while all Lakatan control plants were infected after six months. To our knowledge, this is the first report of apparent immunity to BBTV and these wild *M. balbisiana* accessions will be further studied to uncover novel sources of BBTV resistance genes for functional genomics research, genome-wide association studies, and marker-assisted plant breeding applications.

108

Mining fungal pan-genomes for effector and fungicide resistance profiling for disease diagnosis and surveillance

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Fungal crop diseases are mediated by pathogen-secreted effectors, the accurate identification of which can enable long-term crop disease resistance. In this study, we apply recently improved methods for predicting novel effector candidate profiles from pan-genome data. By combining this with cultivar-specific disease phenotyping and locations, we aim to develop a novel genome-based disease diagnostic and surveillance database/toolkit for efficient surveillance of known effectors and improved discovery of novel effectors. We also incorporate our recently developed fungicide-resistance (FR) allele search tool (FRAST) to assess the fungicide-resistance allele diversity and emergence risks across fungal populations. This study examines 648 *Parastagonospora nodorum* isolates, focusing on 260 local isolates from the Western Australian (WA) wheat-belt region, which are contrasted with 388 internationally sourced isolates. We observed profiles of relevant pathogenicity-indicators including: mating type, fungicide resistance alleles, and effector haplotypes/isoforms across evolving pathogen populations at a state level. Mating types show both MAT1-1 and MAT1-2 loci are dispersed across WA, indicating meiotic reproduction and subsequent genome diversification via repeat-induced point mutations (RIP). Combining pan-genomics with FRAST we detected multiple FR mutations across WA, consistent with reports of emerging resistance. We report RIP is a major driver of genome-wide mutations, yet the majority of retained RIP-like SNPs within predicted protein-coding genes were synonymous mutations. Effector isoform profiles were variable across WA, but phylogenetically consistent with localised sub-populations. For 5 currently known effector loci of *P. nodorum*, most isoform changes/diversity were due to a small number of RIP-like non-synonymous mutations. RIP apparently drives pathogenic adaptations at a whole-genome level but is typically held in check by selection against widespread deleterious non-synonymous mutations. We present this pan-genomic study as an effective diagnostic method to support crop disease management by enabling rapid identification of region-specific pathogen effector profiles and emerging fungicide resistance.

128

Whole genome approach to population genomics of *Phytophthora cinnamomi* in Australia

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Since the first record of jarrah dieback disease in Western Australia in the 1920s, the causative agent, *Phytophthora cinnamomi* has continued to spread through south-western Australia, across much of Victoria and Tasmania and the east coast, devastating Australia's ecosystems and native plant species in its wake. The oomycete's distribution in Australia is well documented; however, knowledge of its genetic diversity within Australia is extremely limited. Given the pathogen's wide host range of over 2000 Australian plant species and the variation between the ecosystems within which *P. cinnamomi* thrives, a robust understanding of its evolution and diversity within Australia, specifically with regard to genes involved in the infection process, will help us to understand why *P. cinnamomi* is such a successful pathogen. Here we present a large-scale population genomics analysis of 70 *P. cinnamomi* isolates collected across Australia. Utilising a whole-genome sequencing approach, we will quantify the genetic diversity of the species in Australia and identify genes undergoing selective pressure. We focus on drawing possible associations between genetic diversity, geographical location and host plant species. Understanding the genetic diversity and important genomic features of *P. cinnamomi* in Australia will bolster our understanding of what makes the species such a successful pathogen. Moreover, identifying key genes involved in infection may shed new light on potential novel strategies for disease control.

Session 1 C: Diagnostics, Biosecurity

146

Utilisation of small RNA-omics and Rapid Genome Sequencing Complementary Strategy for characterisation of novel plant viruses

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Plant viruses cause considerable economic loss in agricultural industries. Rapid and accurate identification of plant viruses and viroids is crucial for preventing disease outbreaks. Whilst small RNA-omics are transforming our capacity to detect plant viruses, the characterisation of novel viruses is still challenging due to the lack of whole genome coverage at low titre. Here, we report a small RNA sequencing (sRNA-Seq) followed by the Rapid Amplification of cDNA Overlapping Regions (RACOR) strategy for whole genome sequencing and characterisation of novel plant viruses. This approach resulted in the detection and characterisation of the complete genome sequence of a novel potexvirus, tentatively named "*Adenium obesum potexvirus X*" (AobPX) isolated from an *Adenium obesum* (desert Rose) plant held at the Post-Entry Quarantine (PEQ) facility (Mickleham, DAFF). Our results suggest that this method is suitable for rapid and complete genome sequencing of positive-single-strand RNA viruses with a poly A tail from infected plants showing a disease phenotype. This method can be further improved by using random hexamers to target viruses with or without poly A tails, enhancing our capacity to resolve complete viral genomes to facilitate biosecurity risk assessment and decision making.

161

Biosecurity engagement in horticulture in the Virginian growing region

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AusVeg lead a series of grower education workshops under the "Peri-urban vegetable biosecurity pilot workshop" program in 2020-2022. Education workshops in South Australia focused on pests, diseases and growing in the northern Adelaide plains, particularly Virginia, in South Australia (SA). This area has intensive horticultural production, and growers from an abundance of nationalities, with a range in the size and complexity of operations, in this area. Consequently, significant language and cultural barriers have been found which may exacerbate the lack of engagement with biosecurity and testing for pests and diseases. These workshops were supported by diagnostic services to engage growers, inform BiosecuritySA of the pests in the area and were replicated in New South Wales and Victoria. Attendee survey results from SA supported the assumption of cultural diversity in the region, with growers and agronomists frequently indicating they spoke multiple languages. Workshops in the form of farm visits, with an introduction by a local consultant, were the most effective way that samples (38%) were collected during this project. Over the course of the project sixty-six diagnostic requests were made for 11 crop types, with capsicum (8), cucumber (12) and tomato (16) the most common. Reports were made of fungal (16), nematode (6), viral (5) and bacterial (5) pathogens, or the absence of pathogens (28), to individual growers. These reports combined with weekly surveillance data, provided by agronomists, were collated and circulated via a weekly report enabling understanding of pest and disease pressures in the region and management decisions into the future. A contribution to biosecurity surveillance was also made with the first identifications of: *Verticillium isaacii*, from tomato, and nematode *Aphelenchoides fragariae*, impacting parsnips. Cucumber Green Mottle Mosaic Virus (CGMMV) was also identified from one fruit sample; supporting the lack of area freedom claims for this virus in SA.

36

Exploring genomic insights into the whole genomes of *Heterodera* species

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Cyst nematodes form a major taxon of plant-parasitic nematodes causing a significant economic impact globally. They are characterised by the female's ability to retain hundreds of eggs in its body after the completion of its life cycle. They are notorious agricultural pests, classified into eight genera of which *Heterodera* and *Globodera* are two of the most economically important. Traditionally, detection of cyst nematodes is reliant upon morphological identification. However, this is time consuming and requires expertise, hence, there has been an increase in the use of molecular diagnostic strategies. Whole genome sequencing (WGS) provides information to identify unique molecular barcodes to distinguish species as well as underlying mechanisms of host invasion. However, WGS of cyst nematodes is difficult since it is very challenging to extract DNA from an individual juvenile. Therefore, millions of juveniles are pooled together to generate enough data to obtain a high-quality draft assemblies. To date, only six draft genomes have been sequenced which have given an insight into the diverse biological processes associated with cyst nematodes. Our study aims to sequence, assemble and annotate draft genomes of cyst nematodes belonging to the genus *Heterodera* including species native to Australia and couple of exotics. The goal of this genome sequencing effort is to expand on the existing genomic resources and provide usable data of sufficient quality to the nematology community and develop diagnostic assays for species identification and management.

98

Fusarium sp. [AF-18] - a friend and close confidant of polyphagous shot hole borer

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The first Australian detection of Polyphagous shot hole borer (*Euwallacea fornicatus*, PSHB) occurred in Perth, Western Australia in August 2021. PSHB is a significant threat to amenity and horticulture trees globally. Upon detection, the Department of Primary Industries and Regional Development (DPIRD) established a biosecurity emergency incident response, with a quarantine area now incorporating 25 local government areas (871km²). PSHB has a wide host range (>500 plant species globally) and a symbiotic relationship with Ambrosia clade *Fusarium* species. This symbiotic relationship is a devastating combination; the beetle bores a ~1mm diameter hole into its host, creates galleries, and deposits *Fusarium* spores directly into the host plant's vascular system as a food source for its larvae. The beetle galleries and the Fusarium growth disrupts the host's vascular system, often causing limb and tree death.

We optimised a fungal isolation method for beetle and host plant samples. The *Fusariums* isolated from PSHB samples were identified as *Fusarium* sp. [AF-18] by sequencing the ITS, TEF and RBP2 gene regions. Isolates are cultured, sequenced, morphologically described, and deposited into the Western Australian plant pathology Culture (WAC) collection. Other fungal cultures have also been isolated from beetle and wood samples that were submitted to DPIRD's diagnostic laboratory services for further identification at a later date.

Well defined workflows, rapid identification methods, and knowledge from incursions overseas enables the best outcome when responding to complex multi-species incursions such as PSHB and its associated *Fusarium* species. Ideally, ongoing knowledge-sharing of exotic species incursion responses between jurisdictions is required to promote early detection and maximise the likelihood of eradication of exotic species across Australia.

95

Xanthomonas euvesicatoria pv. *euvesicatoria* causes bacterial leaf spot of chili in Indonesia

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Chili (*Capsicum* sp.) is an important cash crop in the world with significant impacts in popular and traditional cultures. However, it has several serious diseases, including Bacterial Leaf Spot (BLS) which is caused by at least four *Xanthomonas* biotypes. The plant pathogen identification is crucial to provide proper management and avoid the spread of the disease. As the third biggest country producing chili, there is no information on the BLS pathogen in Indonesia. Chili samples exhibiting BLS symptoms were collected from 14 provinces in Indonesia. Symptomatic leaves were first screened with the ooze test, then isolations were made, and the resulting strains where initially characterised through amylase activity prior to sequence confirmation via the specific hypothetical protein and gyrase B loci. The bacterial pathogens were isolated and validated by Koch Postulates. One isolate was obtained, amylase test was conducted to differentiate *X. euvesicatoria* pv. *euvesicatoria* from *X. vesicatoria* and *X. euvesicatoria* pv. *perforans*. A pathogenicity test was conducted in two chili pepper cultivars which popular in Indonesia, *C. frutescens* var. *trisula hijau* and *C. annuum* var. *annuum*. The pathogenic bacteria were identified as *Xanthomonas euvesicatoria* pv. *euvesicatoria* based on the biochemical, PCR and sequencing using specific primers to this species, and pathogenicity test. This information is essential to provide a proper control on the symptom chili plants in Indonesia.

Session 1 D: Integrated Disease Management/ Biological Control

42

Gaining insight into the life cycle and mating system of the floral smut *Ustilago quitensis*, a prospective biocontrol agent against invasive pampas grass in New Zealand.

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Pampas grass, a perennial grass native to South America, is invasive in multiple regions including New Zealand, Australia, South Africa, the United States, and parts of Europe. In New Zealand, two species of Pampas grass, *Cortaderia jubata* and *C. selloana*, can form dense stands, outcompeting native plants. In 2012-2013, surveys for potential biocontrol agents were conducted within its native range. A floral smut fungus, *Ustilago quitensis*, was discovered in Ecuador and later in Chile. The fungus was imported to Manaaki Whenua – Landcare Research's plant pathogen containment facility in Auckland in 2017, where its life cycle is currently being studied. On artificial media, teliospores produce haploid sporidia that are unable to infect plants unless they fuse with sporidia of the opposite mating type. A method to determine compatible smut mating types, combining two sporidial types on charcoal media, was tested, but proved unsuccessful. To understand the mating system of *U. quitensis*, specific primers were designed to target its mating-type loci. This revealed the presence of four different mating-types, indicating that the life cycle of *U. quitensis* is regulated by two independent mating type loci, similar to *Ustilago maydis*. Three-year-old pampas seedlings were injected with a 0.5 mL spore suspension containing two different mating-types and were harvested 20 months later. Due to the difficulty of inducing pampas plants to flower under controlled glasshouse conditions, a species-specific, real-time PCR (TaqMan) assay was developed to detect *U. quitensis* in plant tissues. This assay identified the presence of the smut fungus in the stems of the “inoculated” plants. The smut was only detected in plants inoculated with the opposite mating types, confirming the effectiveness of the inoculation method to enable infection, and the specificity of the PCRs designed to determine the *U. quitensis* mating types.

15

The benefits of breeding major food crops for durable resistance: A meta-analysis of empirical evidence

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The benefits of breeding major food crops for durable resistance: A meta-analysis of empirical evidence

Abstract

A critical challenge for food security is to protect crops from damage caused by microbial pathogens. Breeding crops for disease resistance is a sustainable approach to meeting this challenge. However, pathogen adaptation, leading to the breakdown of resistance, is common and can cause damaging outbreaks of disease. While the importance of genetic, evolutionary and epidemiological factors to managing resistance breakdown are reasonably well understood, there has been little effort to understand the parallel socio-economic dimension. Consequently, incentives for individual decision-makers to invest in managing pathogen evolution are often difficult to articulate or support with solid evidence. We will present research investigating how socio-economic factors influence the management of genetic resistance and pathogen evolution. We first develop a conceptual framework that illustrates the socio-economic challenges to proactively managing resistance ineffectiveness. We extend our conceptual model with a meta-analysis of the agronomic and economic impacts of the adoption of disease-resistant crops worldwide to consolidate empirical evidence. Our assessment highlights that resistance delivers considerable economic and agronomic benefits. However, such benefits will only be fully realized if a significant effort is put into the identification of effective incentives for the adoption and uptake of resistance deployment strategies to increase resistance durability.

Keywords: Economic benefits; food security, genetic resistance; pathogen evolution; plant systems

201

Endophytic actinobacteria as a biocontrol agent of stem canker disease of royal poinciana caused by *Neoscytalidium dimidiatum*

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Twenty-seven endophytic streptomycete and non-streptomycete actinobacteria were isolated from healthy royal poinciana root tissues. *In vitro* screening revealed that the antifungal action of isolate #14 was associated with the production of cell-wall degrading enzymes; whereas with diffusible antifungal metabolites in isolate #21, albeit their production of volatile antifungal compounds. According to the 16S rRNA gene sequencing, isolates #14 and #21 were identified as *Streptomyces* spp. The two antagonists recovered from root tissues till 12 weeks after inoculation, efficiently colonized root cortex and xylem vessels, indicating that the royal poinciana roots are a suitable habitat for these endophytic isolates. At the end of the greenhouse experiments, the development of stem canker disease was markedly suppressed by 52% with the application of isolate #14 and 62% with isolate #21, confirming their potential in disease management. Results showed that the estimated disease severity indices in diseased seedlings were significantly ($P<0.05$) reduced from 4.64 (scale of 5) to 1.05 or 0.51 by either isolate #14 or isolate #21, respectively. In addition, conidial numbers of the pathogen significantly ($P<0.05$) dropped by 55% and 82% with isolate #14 or isolate #21, respectively, compared to infected seedlings with *Neoscytalidium dimidiatum* (control). Thus, the suppression of disease symptoms was superior in seedlings pre-inoculated with isolate #21, indicating that the diffusible antifungal metabolites were responsible for *N. dimidiatum* retardation in these plants. This is the first report of *actinobacteria* naturally existing in royal poinciana tissues acting as microbial antagonists against stem canker on royal poinciana.

110

Management of chickpea Ascochyta blight using cultivar resistance, fungicide strategies and agronomy

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Chickpea production is limited by Ascochyta blight (*Ascochyta rabiei*), globally. To reduce yield losses and manage disease, fungicides are essential, as there is limited cultivar resistance. To assist Australian growers managing Ascochyta blight, three sets of experiments were conducted across three seasons in Victoria: 1) Assessment of cultivar resistance with 14-20 cultivars in each experiment, 2) Fungicide strategies, with eight strategies assessed, including single and dual active fungicides (Groups M4, M5, 3, 7, 11, 12), with dual actives (3&11, 3&7) applied preventatively (before rainfall) or curatively (after disease is detected), and 3) Interrow sowing chickpea between a standing or slashed cereal stubble.

All experiments had Ascochyta blight, with varying disease severities. Grain yield losses varied across seasons and environments, with losses ranging from 5% to 95%, between cultivars in the absence of fungicides. All fungicide strategies reduced disease severity and grain yield losses, but both the severity and yield loss varied between actives and timings. An economic analysis was inconclusive in determining an optimal strategy,but did provide growers with multiple options for managing disease. Applying dual active fungicides curatively or preventatively did not provide a conclusive result either. This approach combined with cultivar resistance worked economically in drier environments, but not in wetter environments. Grain yield losses could also be reduced with sowing chickpeas between standing cereal stubble rows.

These experiments highlighted the importance of individual factors on reducing grain yield losses, including cultivar resistance, fungicide applications, and inter row sowing. However further research is required to determine the advantage of combining all these factors.

13

Taking shortcuts: modifying harvest height to prevent colonisation of cereal stubble by *Fusarium pseudograminearum*

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Higher harvest-heights (e.g., via stripper headers) and rotations with shorter stature break crops such as chickpea (*Cicer arietinum*) are being increasingly adopted with wheat production in the northern grain region of Australia (NSW and Qld). These practices have potential to affect the saprotrophic colonisation, survival and/or dispersal of *Fusarium pseudograminearum* (Fp) in cereal stubble. Thus, three-year field experiments at two sites in northern NSW were conducted to explore the effect of stubble height on saprotrophic colonisation of Fp-inoculated durum wheat stubble (2019-20 season). Dispersal of Fp inoculum following harvest of a chickpea break crop (2020 season) and any subsequent effects on FCR risk (2021) were also assessed. A combination of culturing and quantitative polymerase chain reaction (qPCR) methods was used. Cutting stubble short (13-17 cm in height) prevented any further colonisation of stubble by Fp occurring after harvest. In comparison, taller stubble (approximately 38-45 cm in height) allowed 61 to 70% more vertical progression of Fp within the infected stubble in the 6 months after harvest. The pathogen also persisted higher within the stubble for at least 12 months after harvest (compared with the height found at harvest). Significant displacement of Fp was observed in the basal (crown) portion of durum stubble from six months post-harvest, resulting in large decreases in total Fp DNA concentrations in stubble. As such, no significant differences in FCR risk (PREDICTA® B) were observed for the different stubble management scenarios. Still, this research provides important field-validation that saprotrophic colonisation of standing cereal stubble by Fp is occurring in the northern region, which can potentially be prevented via harvest height modification.

66

Elucidation of antagonistic mechanisms of *Bacillus velezensis* in biocontrol of shot-hole disease in flowering cherry

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The shot-hole disease is among the most important diseases affecting flowering cherry (*Prunus* x *yedoensis* Matsumura) trees in South Korea every year, resulting in spots and shot-holes in the leaves. Several pathogens are associated with the disease, including the bacteria *Bukholderia contaminans*, *Pseudomonas syringae* pv. *syringae*, and *Xanthomonas arboricola* pv. *pruni*, and the fungi *Mycosphaerella cerasella* and *Epicoccum tobaicum*. This study aimed to investigate the inhibitory activities of antagonistic bacteria against shot-hole pathogens both in vitro and in vivo and their bioactive compounds. Of the 403 bacterial strains isolated, two biosurfactant-producing bacterial strains, designated as JCK-1618 and JCK-1696, exhibited the best effects against the growth of both bacterial and fungal shot-hole pathogens in vitro through their cell-free culture filtrates (CFCFs). These two strains were identified as *Bacillus velezensis* based on morphological characteristics and sequence analysis of 16S rRNA (ribosomal ribonucleic acid) and gryA (DNA gyrase subunit A) gene. In addition, they strongly inhibited the growth of the pathogens via the action of their antimicrobial diffusible compounds and antimicrobial volatile organic compounds (VOCs). Crude enzymes, biosurfactants, and solvent extracts of the two strains also exhibited antimicrobial activities. Liquid chromatography/electrospray ionization time-of-flight mass spectrometric analysis of the partially purified active fractions, obtained from the CFCFs of both JCK-1618 and JCK-1696, revealed that the two antagonists produced three cyclic lipopeptides, including iturin A, fengycin A, and surfactin, and a polyketide, oxydifficidin. In a detached leaf assay, pre-treatment and co-treatment of flowering cherry leaves with the CFCFs led to a large reduction in the severity of the leaf spots caused by *E. tobaicum* and *Bu. contaminans*, respectively. To our knowledge, this is the first report of the antimicrobial activities of the diffusible compounds and VOCs of *B. velezensis* against the shot-hole pathogens and their efficiency in the biocontrol of shot-hole in flowering cherry.

Session 2 A: Molecular Plant Disease Interactions

67

Identification of *Pyrenophora teres* f. *teres* virulence QTL with multi-parental and bi-parental mapping populations

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Net-form net blotch (NFNB) is an economically important disease, causing severe yield losses in susceptible barley varieties. This foliar disease is caused by the fungal pathogen *Pyrenophora teres* f. *teres* (Ptt). Multi-parental nested association mapping (MP-NAM) and bi-parental mapping populations consisting of 399 and 305 progeny isolates of Ptt, respectively, were developed with the aim to identify quantitative trait loci (QTL) associated with Ptt virulence on the barley cultivars Prior and Skiff. The effectiveness of using a MP-NAM population to identify QTL in haploid plant fungal pathogens like Ptt was also assessed. The MP-NAM population consisted of four inter-crossed sub-populations. The four populations were developed by crossing parental isolates virulent to Prior with isolates avirulent to Skiff and vice versa. The bi-parental mapping population was developed by crossing two of the Ptt isolates also used in the MP-NAM crosses. A highly significant QTL for Prior virulence was identified on chromosome 5 in both the MP-NAM (LOD from 30.9 to 36.7) and the bi-parental (LOD 48.0) populations. The phenotypic variance explained by the QTL ranged from 33 to 63% in the MP-NAM population and 40% in the bi-parental mapping population. For Skiff virulence, a QTL was identified on chromosome 3 with LOD values ranging from 8.9 to 9.9 in the MP-NAM populations and a LOD of 22.0 in the bi-parental population. The phenotypic variation explained by the QTL in MP-NAM was 13% and bi-parental was 24%. The MP-NAM population detected eight QTL in total whilst only five QTL were detected with the bi-parental mapping population. The result of this study confirms that MP-NAM populations can be successfully used in detecting QTL in haploid fungal pathogens and are especially useful when individual crosses produce insufficient numbers of ascospores for bi-parental mapping analyses.

52

How does a broad host range necrotrophic fungal pathogen trigger host cell death?

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Sclerotinia sclerotiorum is a fungal pathogen that infects a wide range of crop species, causing significant economic damage. This pathogen produces small effector proteins to kill host cells and obtain nutrients. Our objective was to discover novel necrosis-inducing effectors and characterize their activity using transient expression in *Nicotiana benthamiana* leaves. Five intracellular necrosis-inducing effectors were identified with differing host subcellular localization patterns. Notably, we discovered that one of the effectors enters host cells using an RxLR-like motif. Additionally, we found evidence that another effector induces necrosis via an NLR protein. All five of the identified effectors are highly conserved in globally sourced *S. sclerotiorum* isolates. These findings contribute to our understanding of how *S. sclerotiorum* causes disease and reveal potential opportunities for developing genetic resistance against this damaging fungal pathogen.

175

The Effect of Endophytic *Fusarium oxysporum* on the rhizome quality of ginger (*Zingiber officinale Roscoe*)

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There are approximately 1500 species of plants belonging to the family Zingiberaceae. Many of these species are used for food, including ginger, turmeric and cardamon but some also contain compounds with medicinal properties with potential to treat illnesses such as nausea, blood conditions and cancer. Ginger (*Zingiber officinale Roscoe*) is both an important spice and a source of gingerol, an aromatic compound extensively used in traditional medicine. Ginger is grown widely in tropical and subtropical countries, including Australia. Despite the presence of antimicrobial compounds like gingerol, soil borne diseases, including *Fusarium oxysporum* f. sp. *zingiberi* (Foz) can cause significant discolouration and damage to ginger rhizomes. In our study, ginger rhizomes were found to host both pathogenic Foz and non-pathogenic fusaria. Our studies have also found that Foz carries secreted in xylem (SIX) genes, SIX7, SIX9, SIX10 and SIX12, but the non-pathogenic, endophytic fusaria in our study did not carry SIX genes. In order to better understand the role of SIX genes in host-pathogen interactions, we inoculated ginger with Foz; with *Fusarium oxysporum* f. sp*. dianthi* (Fod), a carnation pathogen reported to carry the same SIX gene profile as Foz; with *Fusarium oxysporum* f. sp. *niveum* (Fon), a watermelon pathogen with a different SIX gene profile; and a *Fusarium oxysporum* endophyte isolated from ginger. It was found that the ginger endophyte and Fon had little effect on rhizome quality, but that rhizome quality was reduced by Foz and Fod. Gingerol is produced via the phenylpropanoid pathway, a process associated with plant defence. While the presence of pathogens would be expected to increase gingerol levels, it is proposed that non-ginger pathogens could be used to stimulate gingerol production without reducing rhizome quality. Further research is required to quantify changes in gingerol production in the presence of pathogenic and non-pathogenic fusaria.

120

A cornucopia of signaling factors in R-mediated defense against viral pathogens allows new types of regulation to fit the occasion

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Dominant R-mediated defense against various plant pathogens occurs largely via effector triggered immunity (ETI). This can involve various pathways including those regulated by phytohormones (SA, JA, ET, ABA, NO, BR, and AUX), MAP kinase cascades, lipase-like regulator proteins (EDS1, PAD4, and SAG101), other regulator factors (NDR1/HIN1, and NPR1), transcription factors (WRKYs, MYBs, ERFs, and NACs), plant effector proteins (PR proteins, AGOs, IVR, and RDRs) and helper CNLs (ADR1, NRG1, and NRC2/3/4). Activation of the defense responses occurs following conformational changes in the R-proteins, mediated by one or more chaperones (HSP90, SGT1, RAR1). Analysis of the early steps in the five most characterized R gene response signaling pathways [TMV/N/*N.tabacum*(Nt); PVX/Rx/*S.tuberosum*(St); TCV/HRT/*A.thaliana*(At); TMV/Tm2²/*S.lycopersicum*(Sl); and TSWV/Sw5b/Sl] reveals numerous differences: (1) Not all three chaperones are required (TCV/HRT/At, TSWV/SW5b/Sl; unknown for TMV/Tm2²/Sl); (2) no need for EDS1 (PVX/Rx/Sl, TSWV/SW5b/Sl; unknown for TMV/Tm2²/Sl); (3) no need for NPR1 (TCV/HRT/At, TSWV/SW5b/Sl; unknown for PVX/Rx/Sl, TMV/Tm2²/Sl); (4) no need for NGR1 (TSWV/SW5b/Sl; unknown for TCV/HRT/At, TMV/Tm2²/Sl); (5) no need for ARD1 (TSWV/SW5b/Sl; necessary for PVX/Rx/Sl unknown for others); (6) need for NRC2/3/4 (PVX/Rx/Sl, TSWV/SW5b/Sl; unknown for others); (7) no need for NDR1 (TCV/HRT/At, TSWV/SW5b/Sl; unknown for others); (8) need for CRT1 (PVX/Rx/Sl, TCV/HRT/At; unknown for others); (9) need for MIP1a (TMV/N/Nt, TMV/Tm2²/Sl; unknown for others); and (10) additional factors identified for one system, but not examined in others (SPL6 in TMV/N/Nt; RanGAP2 in PVX/Rx/St; DRB1/4 in TCV/HRT/At). These observations indicate there is much variation in the regulation of early steps in the signaling pathways, as well as alternative approaches that can be used or better fitted to specific R proteins. In addition, these data show that it is not possible to predict what components from the ensemble available will be used by a specific R protein, and therefore each system needs to be examined separately.

93

The ilv2 gene, encoding acetolactate synthase for branched chain amino acid biosynthesis, is required for pathogenicity in *Leptosphaeria maculans*

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Control of blackleg disease of canola caused by the fungus *Leptosphaeria maculans* relies on strategies such as the inhibition of fungal growth with fungicides. However, growers use other chemicals during canola cultivation, including fertilizers and herbicides, that may inadvertently impact the fungus. Based on widespread use of herbicides that target the acetolactate synthase (ALS) enzyme involved in branched chain amino acid synthesis and evidence of low levels of these amino acids within leaves of *Brassica* species, here the impact of a commercial herbicide targeting ALS and mutation of the target ilv2 gene in *L. maculans* was explored. Exposure to herbicide had limited impact on growth *in vitro* but reduced lesion sizes in plant disease experiments. Mutation of the ilv2 gene via CRISPR-Cas9 gene editing rendered the fungus non-pathogenic. Hence, herbicide applications can influence disease outcome, but likely to a minor extent with current chemicals.

78

Ascochyta rabiei effector molecules in different tissue type of chickpea by using transcriptomics

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Ascochyta blight of chickpea, caused by *Ascochyta rabiei*, is one of the most important foliar diseases in chickpea, with substantial worldwide economic impact on production quality and yield. Foundational research on plant-pathogen molecular interactions have provided clues to the molecular mechanisms underlying *A. rabiei* infection in early stage of chickpea seedlings. These studies identified several signaling molecules (effectors) and transcription factors that have been suggested to play key roles in the infection and establishment of the pathogen, leading to disease. The aim of this study was to reveal the differential genomic controls and mechanisms underlying molecular mechanisms of *A. rabiei* when in contact with different tissue types (leaf and pod) of chickpea. For this, a dual RNA-Sequencing approach was chosen to compare the transcriptomic responses of pathogen while infecting the vegetative or reproductive tissues. The identified differentially and co-expressed sequences were then functionally annotated using the ME14 (NCBI accession GCA_004011695.2) *A. rabiei* genome reference sequence as well as homology and domain searches and cross-referenced to a database of *A. rabiei* predicted effector proteins using the featureCounts output count matrix using the R (v3.6.1; R Core Team, 2018) package edgeR v3.26.8 as implemented in Degust v4.2-dev. The results of this study will help to identify for novel genes/effector sequences that are differentially expressed at maturity growth stages, including on reproductive host tissue. This will aid in unraveling a better understanding of the pathogen's strategy to overcome host defences when the crop is most advanced in the field.

Session 2 B: Microbiomes and Disease Complex

165

Soilborne pathogens influence on plant-microbiome interactions: cause and effects

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Plant root-associated microbiomes in the endosphere and rhizosphere environment are integral to the phenotypic performance of host plants and pathogen interactions with plants and microbiomes in rhizosphere – root environment play an important role in the composition of root microbiome and overall functional outcome. While the root associated microbiomes are known to influence plant-pathogen interactions and disease incidence, infection of host plant by soilborne fungal pathogens can also influence the diversity and composition of root microbiomes. We investigated the changes in rhizosphere and endosphere bacterial (16S) and fungal (ITS region) community composition and diversity as influenced by fungal pathogens *Rhizoctonia solani* AG8 in wheat and *Eutypella* sp in cotton plants using group specific amplicon sequencing. Results from the beta diversity analysis of rhizosphere bacterial and fungal communities indicated significant changes in the presence of high levels of *R. solani* with responses in specific members and groups of bacteria and fungi and the changes were more dramatic with the addition of chitin substrate. Pathogen presence also decreased alpha diversity in the presence of plant. Results for *Eutypella* sp. infected cotton roots indicated significant reduction in the total fungal community diversity, with two *Eutypella* OTUs accounting for 45-99% of all sequences including displacement of other fungi including mycorrhizae. Overall, this work demonstrates that infection by fungal pathogens can cause drastic changes in root associated bacterial and fungal communities in terms of diversity and composition, directly through displacement of other microbes and/or through modulation of plant-microbiome signaling pathways.

144

Metatranscriptomics captures functional dynamics between the plant and its microbiome

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Recent advances in DNA and RNA sequencing technologies have provided an unprecedented view of the complex microbial communities that populate our bodies and surroundings. For plants, these microbial communities (microbiomes) are sources of both essential mutualistic services and devastating pathogens. Metatranscriptomics captures the active gene expression profiles of complex microbial populations and their host simultaneously, revealing dynamics in community structure (who is there?), functions (what are they doing?), and their coordination with host processes. This talk will present a metatranscriptomic case study and discuss ongoing applications of this approach to build predictive frameworks that will guide strategic agricultural practices.

177

Measuring the impacts of deep tillage on soil biological health

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Large areas of Australian agricultural land are affected by numerous soil constraints including water repellence, low pH, subsoil compaction, salinity, sodicity and low organic matter. Soil amelioration techniques, including strategic deep tillage can help alleviate these constraints to improve productivity.

Common mechanical amelioration techniques, including soil mixing (rotary spading), soil inversion (mouldboard ploughing) and deep ripping, all lead to various degrees of soil redistribution, creating changes to the soil's physical and chemical profile. These actions also mix the soil's biological organisms, but little is known about the impact on soil biological health.

It is difficult to measure soil biology due to its large size and complexity. Nematode communities are good bioindicators and are routinely used to monitor the biological status of soils. SARDI has developed a suite of 15 DNA tests that cover the five major free-living nematode (FLN) feeding types. The abundance and diversity of nematode feeding groups reflects the underlying structure of the soil microbial community and can be used to diagnostically assess soil biological health.

The impacts of deep tillage techniques on soil biological status were investigated at several field trials on deep sand and sandy gravels in Western Australia's grainbelt using FLN DNA soil tests.

The treatments (inversion, deep ripping and mixing) improved soil biological health between 10-30cm in depth with increases in all FLN taxa compared to control treatment, and these positive effects were observed up to 3 years post amelioration. At the surface 0-10cm layer, there were no significant effects on FLN communities except in the inversion treatment which decreased total FLN. At the deeper 30-40cm depth there was little to no FLN community present in any treatment.

19

Soil disease suppression in the omics world: From small RNA molecules to HiC genomics

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The rhizosphere microbiome is critical in maintaining plant health, supplying nutrients, and defence during abiotic stress. In addition, some soils possess a microbiome able to suppress soil-borne disease development. Given the importance of microbial communities in suppressing agricultural diseases, investigation of the edaphic and plant host-derived factors affecting the composition and function of these communities is essential. Thus far, root biochemical exudates have been identified as a central controller of the rhizosphere microbiome assembly. As a part of the CSIRO Microbiome for One System Health (MOSH) rhizosphere project, we are investigating how bacterial and root-secreted nucleic acids drive plant-microbiome interaction below ground. To do so, we applied multi-omics studies (sRNA profiling, Metagenomics, and Transcriptomics) to understand the assembly, composition, and function of microbiomes in disease-suppressive and non-disease suppressive agricultural soils.
Our results indicate that sRNA profiling in the rhizosphere-microbiome can enhance our understanding of plant - microbiota communication. Furthermore, sRNA datasets could predict the microbiome taxonomy profile as comparable to metagenomics assembled taxonomy. Knowledge gained through integrated metagenomics, transcriptomics, and sRNA profiling will help to develop cropping systems with disease suppressive capacities through in-situ development of suppressive communities, modification of host genetics/immunity to support disease suppression and/or development of synthetic communities conferring stable disease suppression.
Keywords: Small RNAs, microbiome, soil disease suppression, multi-omics

61

Harnessing microbial allies for successful pine growth under pathogen pressure.

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Early attempts to introduce pine plantation forestry in Australia during the nineteenth century faced failure, partly due to the absence of essential microbial allies, such as ectomycorrhizal fungi. These fungi play a crucial role in nourishing plants with vital nutrients that would otherwise be inaccessible, while also providing protection against pathogenic invaders. Recognizing the ecological significance of microbial symbiosis, it has become essential to promote beneficial microbiomes during growth of seedlings in pine nurseries. However, the full potential of diverse microbial associations in enhancing ecosystem functioning and promoting sustainable reforestation practices remains largely untapped. In collaboration with Australian pine nurseries in four states, we are investigating how young *Pinus radiata* and Southern pines treated with functionally diverse microbial inoculum perform under low fertilizer and fungicide application with or without disease pressure (*Fusarium* and *Botrytis*) as compared to routine nursery application. Our data reveal that, despite receiving reduced level of nutrition, seedlings treated with organic fertilizer and functional microbes exhibit enhanced photosynthetic rates, greater growth, and increased biomass compared to those treated using standard nursery practices. Furthermore, mycorrhizal association increases under disease pressure. With multi-omics approaches, we delve deeper in to underlying molecular mechanisms by which seedlings benefit from organic fertilization in association with microorganisms under pathogen pressure, as well as the specific roles played by each microbial species in this association and paving the way for sustainable reforestation practices.

191

One vine, two diseases: Interactions of different fungal trunk pathogens associated with Botryosphaeria dieback and Petri disease complex in Australian vineyards

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Grapevine trunk diseases (GTDs) remain a significant threat to vine yield and sustainability. Recent studies utilising microbial profiling demonstrated that the pathogens associated with two significant GTDs, Botryosphaeria dieback (BD) and Petri disease (PD), were present together in individual vines, with PD pathogens being the most abundant. The incidence and impact of PD has not been comprehensively studied in Australia, while being considered a serious disease in Europe. This study investigated the *in vitro* interactions between the pathogens associated with BD and PD disease complexes by dual inoculation. Within BD and PD complexes, mycelial plugs from the actively growing margin of the pathogens were inoculated, simultaneously, 4 cm apart onto culture media, while for between pathogen groups (BD x PD), PD pathogens were inoculated two weeks prior to co-inoculation with BD pathogens. The plates were incubated at 25° in total darkness for 7-30 days and assessed for morphological characteristics and fungal growth. Within the BD complex, a change in colour and formation of a dark-pigmented margin was observed when *Diplodia seriata* was paired with *Neofusicoccum parvum* in comparison with its control. Although morphological changes were observed in this interaction, no significant differences were observed in paired and self-paired growth rates. Between pathogen groups, a significant reduction in the average fungal growth was observed between *Phaeoacremonium al*eophilum and *N. parvum* and formation of an inhibition zone between *P. aleophilum* and *D. seriata*. The morphological changes *in vitro* revealed specific interactions between BD and PD pathogens, which require further examination *in vivo*. Investigating the interaction of GTD pathogens will provide critical data to assist in understanding disease epidemiology, forming the basis of improved management strategies.

Session 2 C: Plant Disease Management, Chemical Resistance

121

Xanthomonas campestris pv *campestris*: What have we learnt over the past four years

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Brassicas are one of the dominant vegetable crops grown in NSW with an annual value of $36.7M. Black rot, caused by *Xanthomonas campestris* pv *campestris* is considered the most devastating disease globally. This seedborne pathogen, once established in a crop, can decimate marketable yield as there are no effective treatment options. It thrives in climatic conditions of rain, humidity and temperate conditions, yet is still a problem in low temperatures. Small landholders in NSW are not able to effectively rotate crops to reduce pathogen load, giving the advantage to the pathogen. This means that the pathogen likely remains on farm perennially and is continually reintroduced with new seed stock/seedlings. As management is the main option for growers, we needed to address some fundamental knowledge gaps about this pathogen. This study investigated the genetic diversity of the historical *X. campestris* culture collection in Australia and compared their genetic diversity to isolates found globally and surveillance isolates collected over the past four years.
We then selected representative clonal types for pathogenicity assays to investigate the relationship between genetic diversity and pathogenicity in different brassica types and to develop a new assay for the specific detection of *X. campestris* pv *campestris*.
The longevity of a pathogen on a farm is another key question for a seedborne pathogen like *X. campestris*. An experiment to determine how long *X. campestris* was able to survive in plant material when buried below the soil or left as debris on top of the soil was monitored for 12 months, and the viability of the pathogen determined through pathogenic assays. These results with those from surveillance helped determine if *X. campestris* are remaining on farm or are being continually reintroduced. This study improves our understanding of this pathogen immensely and equips us with more tools in black rot management.

92

Detecting fungicide resistance mutations in *Pyrenophora teres* using a portable DNA sequencer

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Fungicide resistance remains a threat to food security with resistance to demethylase inhibitor (DMI) fungicides being particularly problematic. The gene encoding the DMI target sterol 14α-demethylase (Cyp51) has evolved an array of mutations in both the promoter and coding sequence in two closely related barley pathogens, *Pyrenophora teres* f. *teres* (Ptt) and *P. teres* f. *maculata* (Ptm). Detection of resistance mutations using allele-specific amplification-based technologies relies on one assay per mutation and all assays must be run on every sample increasing the time and cost of detection. Also, uncharacterised mutations will not be detected which requires the use of sequencing pipelines. To improve the speed of mutation detection, we developed an amplicon sequencing based approach using a combination of the Oxford Nanopore Technologies MinION portable DNA sequencer and unique molecular identifiers (UMIs) for sequencing error correction. We profiled resistance mutations in pure isolates of Ptt and Ptm and a hybrid and detected all known mutations with SNP-level accuracy with built-in chimera filtering, which is especially important for accurate genotyping of complex samples where amplicons may differ by only a few SNPs. We have expanded the utility of our pipeline by including three genes encoding the targets of succinate dehydrogenase inhibitor (SDHI) fungicides and detected mutations associated with reduced sensitivity or resistance to both modes of action directly from infected leaf samples. This method is broadly applicable to other systems where resistance is problematic including weeds, insects, and human pathogens.

173

Managing the unmanageable – understanding the role of seedborne *Ramularia collo-cygni* as a source of disease spread in New Zealand barley crops.

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The lack of any substantive genetic resistance or tolerance to Ramularia leaf spot (RLS) of barley and the emergence of reduced sensitivity to Group 7 and 11 fungicides in New Zealand's *Ramularia collo-cygni* (Rcc) population has resulted in a drive to find alternative disease control strategies.
The prevalence of seedborne Rcc inoculum was studied in surveys of post-harvest grain collected from 2017 to 2020. Assays detected Rcc DNA on almost all samples, regardless of season, location, cultivar, sowing date, rotation position, irrigation, prior fungicide programme or in-field RLS severity, suggesting seedborne Rcc inoculum could be epidemiologically important.

Despite Rcc DNA being detected in almost all surveyed grain samples, the amount of seedborne inoculum reduced more than 100,000 times from 2017 to 2020. This implied that different management strategies across the sampling period may have impacted quantities of seedborne inoculum. Subsequent field trials identified fungicide programmes that reduced the amount of Rcc transmitted to the progeny seed, which resulted in reduced disease severity on progeny plants.
Glass house studies protected from seasonal infection followed persistence of Rcc inoculum on barley seed over multiple generations. Seedborne DNA in this study was detected for three generations, confirming Rcc could persist as a source of ongoing contamination. However, the incidence and amount of detectable inoculum decreased on seed with each generation, resulting in Rcc DNA being detected in only 16% of G2 and 3% of G3 seed samples grown from the original (G0) infected seed.

Together these data suggest seedborne inoculum of Rcc can persist, creating a source of infection in subsequent crops, but use of an effective fungicide programme on the preceding barley crop can help to reduce this initial inoculum source and RLS. These programmes all included mixtures of multiple fungicide mode-of-action groups, which could reduce selection for further loss of chemistry.

213

Enhancing Hot Pepper Production in the Southwest US: Field Evaluation of Advanced Varieties for Disease Resistance

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Hot peppers (*Capsicum* spp.) are of a great ethno-botanical and economic importance in southwestern United States, particularly in Arizona, California, New Mexico, and Texas. Domestic production of hot peppers faces competition from imports from other hot pepper-production regions, and is constrained by high labor and input costs, and abiotic and biotic stresses. A multi-state project was initiated in 2019 involving Texas AgriLife and New Mexico State University, with sponsorship from the USDA-Agriculture Marketing Service, Specialty Crop Multi-State Program through Texas Department of Agriculture, to optimize production of hot pepper production in the U.S. One specific objective of the project is to monitor high-yielding advanced varieties for resistance to plant pathogens. In the growing seasons of 2020, 2021, and 2022, several advanced varieties from the Texas AgriLife Breeding Program were planted in southern New Mexico in growers' fields and monitored for development of diseases. Across all three seasons, disease types and disease pressure were variable. Diseases encountered consisted of chile wilt caused by *Phytophthora capsici*, *Verticillium dahliae*, and *Rhizoctonia solani*. The incidence of chile wilt was 1% to over 20% across fields. Other diseases found were caused by viruses (Beet curly top virus, Alfalfa mosaic virus, and Tomato spotted wilt virus), bacteria (bacterial leaf spot, caused by *Xanthomonas euvesicatoria*), and powdery mildew (caused by *Leveillula Taurica*). The incidence of viral and bacterial diseases was at 1% to over 50% across all advanced varieties. In the later part of the seasons, powdery mildew was observed in all advanced varieties, at 100% incidence (at field level, that is, all plants displayed symptoms of the disease). The severity of powdery mildew (at plant level) was greater than 30% across all varieties. None of the advanced varieties were found resistant across the spectrum of diseases encountered.

Session 2 D: Integrated Disease Management/ Biological Control

68

Biochemical changes in *Vitis vinifera* leaves and responses to *Botrytis cinerea* infection after the application of a yeast extract formulate

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Grapevine (*Vitis vinifera* L.) is one of the most economically important crops worldwide, and the increase in wine production rate demand requires changes in the agricultural processing and manufacturing practices to make them sustainable. Issues associated with the large use of agrochemicals and consumer needs for residue free products have stimulated research into new and eco-friendly tools for sustainable pest management and vine protection. The aim of the present study (supported by KWIZDA Agro GmbH) was to characterize at functional level the "indirect" protective mechanisms induced by the application of a yeast extract formulate (YE) through the induction of defense responses in *V. vinifera* cv. Sangiovese plants artificially inoculated with *Botrytis cinerea* (Bc). In YE+/Bc+ leaves, germ tubes did not elongate well, and their hyphae were slightly spread over the leaves after 2 days. The hydrogen peroxide induction (observed from 1 h post inoculation; +40% compared with YE-/Bc- ones) triggered a production of ethylene and a concomitant accumulation of jasmonic acid (7 fold-higher and +34%, respectively). These results confirm a synergistic action in the regulation of defense reactions. The absence of oxidative stress (as confirmed by the unchanged values of malondialdehyde by-products) and the early activation of a signaling pathway suggest the capability of YE to induce resistance in grapevine against necrotrophic pathogens.

103

Understanding the factors influencing the efficacy of biocontrol against plant diseases for an optimal use in the field

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The control of plant diseases using micro-organisms has been the subject of intensive study over recent decades, leading to the identification and development of biocontrol agents. These biocontrol agents can limit the development or even suppress plant pathogens, and thus protect plants by direct or indirect means. However, their protective efficacy is generally considered to vary according to growing conditions in the field.

Despite its obvious advantages, biocontrol is not without its challenges. Among these challenges, understanding their mode of action is essential to better guide their production and formulation. Another challenge is the lack of knowledge about their durability and the factors modulating their protective efficacy, in particular their interaction with plant pathogens in a changing environmental context. Knowledge of the factors influencing their efficacy is essential to better guide their use in the field, and, consequently, in their success as commercial products. Finally, the implementation of integrated protection strategies requires biocontrol to be compatible with other pest and disease control tools, as well as with cropping practices.
During the presentation, the different types of microbial biocontrol agents developed against plant diseases in Europe and their main modes of action will be presented. Development of knowledge on the identification of key factors in their protective efficacy and their use in integrated pest management strategies will be discussed. This presentation will also examine research perspectives to promote an effective and durable use of these products in practice.

43

Uncovering soil microbial interactions during the destruction of Fusarium wilt infected banana plants

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Fusarium wilt Tropical Race 4 (TR4) is a devastating fungal disease caused by *Fusarium oxysporum* f.sp. *cubense* (Foc) that affects banana plants. Foc is spread through contaminated soil and planting material. The identification and destruction of infected plants is an important measure in preventing the disease spread. This involves treating the plant and soil with urea to release of ammonia gas with designated destruction zones. The urea application may initially reduce the abundance of Foc but alter soil physicochemical properties, leading to a reduction in soil microbial community diversity and functions, and hence an eventual increase in TR4 within the destruction zone. An investigation of three destruction zones on a commercial banana farm revealed significant increases in the soil nitrate-nitrogen three months after destruction began. Using a qPCR soil test, DNA from TR4 increased during the first 6-months, before declining by 12-months. Significant changes in the soil bacterial community diversity and structure also occurred within the destruction zone. The understanding of the soil microbial interactions occurring within the destruction zone can be used to improve the efficiency in destruction of Fusarium wilt TR4 infected plants, to help slow the spread of the disease.

202

Biological control of *Pythium aphanidermatum* damping-off disease of cucumber using actinobacteria capable of producing cell-wall degrading enzymes

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Fifty-eight streptomycete and 35 non-streptomycete actinomycetes were recovered from cucumber rhizosphere soil without and with polyvalent Streptomyces phages. Using mycelial (*Pythium aphanidermatum*) fragment agar, these isolates were screened for the production of cell-wall destroying enzymes. Eighteen potential isolates were tested for their ability to colonize roots. *P. aphanidermatum*, the causal agent of post-emergence damping-off of cucumber seedlings, was effectively suppressed *in vitro* by eight isolates with outstanding rhizosphere competence. *Actinoplanes philippinensis, Microbispora rosea, Micromonospora chalcea*, and *Streptomyces griseoloalbus* generated *in vitro* ß-1,3, ß-1,4, and ß-1,6-glucanases and caused hyphae lysis in *P. aphanidermatum*. None of these produced volatile inhibitors or siderophores. Only *S. griseoloalbus* produced diffusible inhibitory metabolites, but *A. philippinensis* and *M. chalcea* parasitized *P. aphanidermatum* oospores. These four isolates were then examined singly or in combination in the greenhouse for their potential to control damping-off of cucumber seedlings in soil with or without cellulose amendment. The treatment that included all four isolates in soil modified with cellulose suppressed damping-off much better than all other treatments and was nearly as good as the metalaxyl treatment. The findings suggest that rather than fungicides, a mixture of antagonistic rhizosphere-competent actinomycetes and cellulose amendment could be used to treat this disease in the field. This is the first study to screen rhizosphere-competent non-streptomycete actinomycetes capable of generating cell-wall degrading enzymes for the control of *Pythium* infections.

186

Crop rotation reduces the outbreak of Panama disease caused *by Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 in Lao PDR

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Banana production in Lao PDR is an important export crop as well as supporting the livelihoods of smallholder banana growers. A field experiment was conducted alternating rotation crops with banana in an area with an outbreak Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (FOC TR4) in bananas. The experiment was located in longitude and latitude at N18°08´25.14´´; E 102°57´14.68´´, 137 m above sea level, located in Huay Nam Youn Village, Pak Ngum District, Vientiane on a commercial banana farm. Crop rotations with yard longbean, chilli, sticky corn, tomatoes, sweet potatoes and peanuts were used to determine the reduction in the occurrence of FOC TR4 in the following banana crop. Monthly disease incidence and severity observations were made over a 12-month period commencing in June 2021, and terminating in July 2022. The results indicated that corn had a lower percentage of Fusarium wilt incidence of 2.8%, compared with other crop rotation treatments and the untreated controls, which had disease incidence as high as 84.4%. The severity of FOC TR4 in bananas was reduced following the sticky corn to 0.6%; compared with other crop rotation treatments and the untreated untreated controls, which had a disease severity as high as 84.1%. The soil fertility in the experimental area before and after planting was acidic, with a pH value in H2O and KCl of 4.93 and 4.32. Soil for good banana cultivation and disease suppression should be closer to a neutral pH around 6.5. This experiment demonstrated that the incidence and severity of FOC TR4 can be reduced through crop rotation using corn before replanting bananas, therefore, helping smallholder banana growers to retain an income while they manage the disease.

Keynote Address 2

216

Uncovering virulence gene diversity in rust fungi

Dr Peter Dodds[1]
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Rust fungi are important pathogens of many crops, especially wheat and other cereals where they cause substantial economic losses worldwide. These fungi are biotrophic pathogens that depend on living host cells for growth and form specialised haustoria structures during infection that serve as nutrient uptake sites as well as delivering effector proteins to the host cell. We have investigated the molecular basis of rust infection and host immunity and have identified several Avr genes in *Melampsora lini* (flax rust) and *Puccinia graminis* f. sp. *tritici* (Pgt) (wheat stem rust), which encode secreted proteins that are co-ordinately expressed in haustoria structures. Using a combination of crystallographic approaches and AI-based structural prediction we have found that these Avr proteins fall into a number of novel structural families. Structure-guided functional analyses have identified key features controlling recognition of Avr proteins by corresponding immune receptors. This has revealed mechanisms of virulence evolution and potential means for engineering resistance genes with novel recognition capacities. New advances in rapid Avr gene detection raise the prospect of generating complete Avr gene atlases for cereal rusts to enhance resistance breeding and surveillance. Most cereal rust populations are clonal, since sexual reproduction requires alternate host plants generally absent from crop-growing regions. However, these fungi are dikaryotic, with two divergent haploid nuclei, and there is growing evidence for somatic nuclear exchange between clonal lineages. Using new approaches based on chromatin contact information (Hi-C) to assemble the two nuclear genomes separately, we detected extensive somatic nuclear exchange in populations of Pgt, wheat leaf rust (*P. triticina*) and oat crown rust (*P. coronata*). Thus, nuclear exchange seems to be the predominant mechanism generating diversity and the emergence of new strain in otherwise clonal rust populations.

Keynote Address 3

208

Species interactions in the plant microbiome: a riddle, wrapped in mystery, inside an enigma

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Plant-associated microbiomes play critical roles in mediating crop productivity. The promise of microbiome management for agriculture is compelling, but our understanding of the factors that mold the composition, diversity and functional capacities of plant microbiomes remains limited. While much work has focused on the roles of plant host, management, or habitat on the composition and functions of plant-associated microbiomes, we know much less about the role of microbial species interactions in mediating the ecological and evolutionary dynamics of microbiome functional capacities. Competition for resources, as well as complex, multi-species antagonistic, mutualistic, and cross-kingdom signaling interactions can all impact the both the potential for selective enrichment of beneficial or pathogenic microbes in the microbiome, as well as the real-time functional capacities of specific microbes. Moreover, species interactions within the microbiome are themselves impacted by plant host, management, and habitat. Here I will explore the roles of microbial species interactions, including within soil microbiomes and among foliar endophytes, in determining the composition and functional capacities of rhizosphere and endophytic populations. Focusing on nutrient use phenotypes, resource competition, and antagonistic species interactions among bacterial and fungal populations in the microbiome, this work will highlight the significant roles that microbial species interactions within the microbiome play in determining the functional capacity of the microbiome. Simultaneously, our work highlights the significant roles of plant host, management, and habitat in determining the trajectories of species interactions within the microbiome, thereby shedding light on practical strategies for managing microbial interactions to achieve beneficial plant outcomes.

Wednesday 22 November:

Conference Day Two

Daniel McAlpine Lecture

205

Ascochyta blight of temperate pulses; the undefeated pathogens

Dr Jennifer Davidson[1]
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Ascochyta blight diseases cause major yield loss in Australian temperate pulse crops. The causal pathogens on faba bean and lentil have teleomorphs (*Didymella fabae*, *D. lentis*) and anamorphs (*Ascochyta fabae, A. lentis*, respectively) present in Australia, increasing genetic variability in pathogen populations. The consequent evolution and selection of aggressiveness has compromised host resistance in both crop types. Conversely, for chickpea, the anamorph *A. rabiei* is the dominant form of the pathogen in Australia. Extensive testing has identified the predominance of one mating type, concluding that epidemics in Australia are driven by the asexual stage of this pathogen. Despite this, resistance has also been widely compromised in chickpea. In contrast, ascochyta blight in field pea is caused by a complex of pathogens and disease resistance is not effective. Pathogen population studies have found a wide variability in aggressiveness among isolates of A. lentis. This natural diversity, coupled with selection pressure in intensive farming systems, resulted in loss of effective resistance in popular cultivars within 4-5 years of commercialisation. The rapid loss of resistance indicates one or more major genes for resistance to *A. lentis* in these cultivars. However, there is also an apparent continuum of aggressiveness among the *A. lentis* isolates on the differential host set. This fits with the theory that resistance against necrotrophs is polygenic, and can be quantitative as well as qualitative, rather than only discreet responses as seen against many biotrophs. However, responses of Australian A. fabae isolates on a faba bean differential host set are more discriminatory, identifying 2, or possibly 3, discrete pathotypes, suggesting that major genes are more important in this system. The aggressive isolates of the pathogens identified in these studies are used to screen germplasm in national breeding programs, and in collaborative inheritance and transcriptome studies in hosts and pathogens.

Keynote Address 4

214

Using Biosecurity and Diagnostics to protect Australian cropping industries - some lessons from near-neighbour collaborations

Dr Murray Sharman[1]
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Australia and the other island nations of the Australasian region are uniquely positioned to maintain a level of biosecurity protection for our plant industries and natural environments not possible in many other regions of the world. However, this does not mean we are immune from damaging disease incursions. A rigorous combination of surveillance, diagnostics and legislative controls are needed to work cohesively across multiple agencies and state and international boundaries for this protection to be effective. In this talk, I will focus on an Australian context and will discuss my experiences with the cotton industry as a case study of a plant industry working with state, federal and international collaborators to proactively look beyond our borders for potential exotic threats for preparedness. The aphid-transmitted polerovirus, Cotton leafroll dwarf virus (CLRDV) is a high priority biosecurity threat to the Australian cotton industry and is present in many countries. Our work has demonstrated it is both genetically diverse and commonly found in Timor-Leste, a near neighbour to Australia's north. We used HTS to characterise the genomes of CLRDV isolates from four countries. There was a high level of genetic diversity between isolates. This new knowledge was used to develop a rapid diagnostic to detect that diversity. With similar symptoms in cotton, the exotic and endemic poleroviruses provide a good example of the importance of accurate and rapid diagnostics. We need to always consider how this all comes together to provide the best outcomes possible for growers, industry and the environment.

Session 3 A: Diagnostics, Biosecurity and Community/ Industry Engagement/ Extension

113

National Diagnostics and Surveillance Protocols – Endorsement process and recent updates

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National diagnostic protocols (NDPs) are an essential tool in Australia's plant biosecurity system, ensuring pest and diseases are identified in an accurate and consistent manner across diagnostics laboratories. In the NDPs, details on the pest, its host and taxonomic status and the methods to detect and identify are provided based on the best available information. A national surveillance protocol (NSP) is a technical reference guide for conducting surveillance on a specific plant pest or group of plant pests. It includes information on surveillance methodology, pest biology, taxonomy, identification, and sample processing. The facilitation of the development, review and verification to the delivery of endorsed NDPs and NSPs are coordinated by Plant Health Australia (PHA) and this project is funded by the Department of Agriculture, Fisheries and Forestry. The main objective of the project is to enhance and strengthens Australia's diagnostics and surveillance capacity and capability to identify plant pest that have an impact on plant industries, environment and the community.

In this presentation, information on NDPs and NSPs and various steps that are involved in their development and endorsement process will be provided. Further details on various resources for developing a protocol and PHA role in facilitating this process will be given. Lastly, information on current status of the protocols and upcoming future avenues for review and development will be provided.

109

Advances in the molecular identification of Australian root knot nematodes (*Meloidogyne* spp.)

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Root-knot nematodes (RKN), *Meloidogyne* spp., are considered the most important genus of plant-parasitic nematode, causing significant disease in a wide range of hosts, with yield losses being as high as 70% in crops such as tomatoes, sweet potatoes, and guava. Historically, identification to species level for RKN relied on morphological characterisation, which is difficult, time consuming, and oftentimes subjective. Multiple PCR-based techniques have been developed; however, no protocol exists for the efficient identification for the five species of Meloidogyne that threaten Queensland's broadacre and horticultural industries. Furthermore, molecular data for three of the key pest species in Australia, *M. arenaria*, *M. incognita*, and *M. javanica*, are confused, with little to no genetic variation present in the commonly sequenced gene regions (i.e., ITS, COI, COII, and NAD5). The objective of this study was to develop and evaluate a PCR-based protocol for rapid RKN identification, and to develop PCR/sequence primers that can reliably differentiate species of Meloidogyne with barcode sequence data. Accurately identifying RKN to species level in a given sample or location is crucial for determining appropriate management strategies for growers, and is a key surveillance and biosecurity tool.

148

Detection of viruses and identification of plant species from hive-stored pollen: guiding surveillance and weed management strategies.

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Cucumber green mottle mosaic virus (CGMMV) causes serious disease in cucurbit crops such as *Citrullus lanatus* (watermelon), *Cucumis melo* (melon), *Cucumis sativus* (cucumber), and *Cucurbita maxima* (pumpkin). Up to 53 plant species are reported to host CGMMV with the majority in the family Cucurbitaceae. Several weed species that commonly occurring in Australian and near cucurbit crops have also been identified as hosts. Symptoms can be harder to identify in weeds because they are asymptomatic hosts or symptoms are confounded by other biotic and abiotic factors. Determining the presence and distribution of viruses in vegetable crops and surrounding 'green-bridge' flora is challenged by the magnitude of the agricultural landscape and resourcing limitations. Therefore in this study we utilised the pollinator activities of Apis mellifera (honey bee) to address these difficulties. High throughput sequencing and metatranscriptomic analysis of hive-stored pollen samples facilitated the combined detection of CGMMV and identification of known virus hosts and local plant species in a single experiment. Full CGMMV genomes were assembled using standard bioinformatic pipelines, and plant species identification was carried out using taxonomic classification, and transcript mapping and quantification tools in conjunction with a custom sequence database. These results demonstrate the potential of this method, and future use could assist surveillance planning and identifying weeds for targeted management by growers.

130

Development of a loop – mediated isothermal amplification (LAMP) assay for the detection of Sweetpotato virus G (SPVG) in sweetpotato planting materials for commercial production in Papua New Guinea

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Commercial sweetpotato (*Ipomea batatas*) production is increasing in the Highlands provinces of Papua New Guinea (PNG). Sweetpotato viruses are a major production constraint. They are initially spread by propagating infected planting materials and can result in significant yield reductions. Sweetpotato virus G (SPVG) is common and is detected either in single or dual infections, mostly with Sweetpotato feathery mottle virus (SPFMV). The aim of this study was to develop a single step reverse-transcription loop-mediated isothermal amplification (RT-LAMP) SPVG assay to complement the current virus management protocols and support commercial production in PNG. Complete genomes of two SPVG isolates, from East Timor (KX279878.1) and Brazil (MF185716.1), were retrieved from the (US) National Center for Biotechnology Information (NCBI) Genbank. The Eiken PrimerExplorer v5 program was used to design primers and prospective primers sets were selected based on end stability evaluations of the target regions. These primer sets were further subjected to specificity checks by BLAST analysis. Three candidate primer sets designed from the Brazilian isolate were synthesized and their performance was evaluated by screening nucleic acids extracted from SPVG controls, SPFMV controls and eight PNG sweetpotato varieties that were grafted on to *Ipomea setosa* and stored on Flinders Technology Association (FTA) cards. This assay was optimized in real time by time to threshold and was found to be specific to SPVG. Preliminary results of RNA extracted from FTA cards indicate strong promise for this assay, but further field testing is required before it can be more widely deployed.

Keywords: Sweetpotato, Sweetpotato virus G assay, loop-mediated isothermal amplification

41

Protocol development for bulk and single plant screening of kauri (*Agathis australis*) in nurseries for *Phytophthora agathidicida*

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In December 2021, a National Pest Management Plan (NPMP), the strongest form of protection under the Biosecurity Act 1993, was initiated to provide a framework for protecting kauri (*Agathis australis*) from *Phytophthora agathidicida* (PA). The NPMP for PA requires that nursery stock exhibiting ill thrift, be tested for Pa before they leave the facility. To enable this testing a standardised method needs to be in place for both nurseries and testing laboratories. The purpose of this study was to develop a simple, robust, and fit for purpose protocol for PA testing of nursery stock. To find a suitable protocol methods were evaluated using PA at ~104 CFUs/L potting mix with lupin and cedar needle baits. Detection using a traditional plating method was also compared to a LAMP-based genetic assay. Baits recovered directly from the leachate and those plated onto selective media for traditional detection were tested using the LAMP assay. The method deemed most suitable was then evaluated at varying concentrations of PA in order to establish what batch sizes can be tested effectively. A combined treatment of PA and *P. cinnamomi* at a single CFU concentration was also performed to evaluate whether the colonisation of baits by *P. cinnamomi* may mask the detection of PA. LAMP testing was again conducted on baits directly from leachate and those that had been plated on media for this second phase. The development of this protocol will mitigate the spread of PA through nursery stock.

105

What is the reach of plant pathology information freely shared on the internet?

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The impact and reach of freely available information should not be underestimated. The extensionAUS website 'Field Crop Diseases Victoria (FCDVic)' hosts a comprehensive online grains-based field crop disease identification and management manual as well as articles, videos and podcasts that are relevant to the Victorian grains industry. The key aim of the site is to provide the Victorian grains industry with access to the most up-to-date crop disease management information. By publishing these resources online, in different user-friendly formats and making them freely available we have been able to track site usage and analyse the data to get a better understanding of who uses the information and how they prefer to access it.

Since the site was launched on Jul 1, 2020, FCDVic has had 38,150 users, creating over 78,000 page views. Less than 24 % of these users were from Australia and over 42% were from India. The site was also accessed by users from 170 different countries/territories during this time.

Most of the traffic is driven by Australia, India, and other developing countries, however, information access is truly global. For example, the 6th most accessed page on the website is by the Netherlands.
How content is accessed is also an important consideration. For Indian users' mobile friendly content is important as over 75% of users access is by mobile devices. For countries such as the United States or Australia most content is accessed via a desktop. The type of content accessed also changes. Users from developing countries (i.e., India) were more likely to access the online crop disease identification and management manual while Australian users accessed information written to address crop diseases specific to their region at the time.

The uptake of information globally has been remarkable given the Australian (and particularly Victorian) focus of the information.

Session 3 B: Epidemiology, Ecology, Modelling and Risk Analysis

84

Modelling the impact of reduced primary inoculum carryover on the spatiotemporal spread of Ascochyta blight in chickpeas

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Understanding the persistence of plant pathogen inoculum during periods when host plants are not present is crucial for predicting the initiation of epidemics and optimizing control strategies. Here, for the necrotrophic fungal pathogen *Ascochyta rabiei*, we report on empirical and modelling experiments investigating the persistence and management of primary inoculum in the landscape. Control of this highly aggressive pathogen of chickpeas is challenging; genetic resistance offers only partial control and has repeatedly broken down, placing a heavy reliance on fungicides. Effective integrated disease management strategies are urgently needed to ensure the long-term sustainable control of this disease. Results from field experiments quantifying pathogen load on post-harvest crop residues indicated that levels of primary inoculum contained with crop residues are influenced by in-season management choices and can decay rapidly. Using this data to parameterize an epidemiological model that captures various aspects of dispersal and inoculum decay dynamics, we investigated the potential for management interventions at landscape scales to minimize disease severity and impact. This simulation model served as a useful tool to assess the impact of various landscape deployment strategies and management interventions aimed at reducing the persistence of inoculum between seasons. The outcomes of this study can be leveraged to develop integrated and area-wide disease management strategies.

187

Seed Potatoes Certification - influencing potato disease management by flattening the curve

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Seed potato certification systems that assess the commercial quality including pest and disease disorders play a vital role in the suppression and management of diseases in commercial crops. In so doing, seed potato certification Schemes have largely relied on the visual assessment of seed potato crops for symptoms of disease, including those caused by bacterial and fungal pathogens. For many years this approach was successful in mitigating disease, however the increased occurrence of Potato Virus Y (PVY) and other asymptomatic diseases, required a different approach which has involved the routine sampling and laboratory testing of all seed potato stocks for selected viruses. Initially, this approach resulted in a higher rejection of crops submitted for seed potato certification, however after several years of continued testing the levels of virus and in particular PVY have been reduced and effectively managed below economic thresholds. This has successfully allowed the Australia industry to reduce the impact of PVY on the entire potato supply chain. Importantly, growers are able to make informed decisions in relation to the health status of seed potato stocks.

In addition to commercial quality diseases, seed potato certification also records phytosanitary data that supports area freedom status of pests and diseases. All fields used for seed potato production are soil sampled and laboratory tested for Potato Cyst Nematode (PCN). Further, targeted surveillance of seed crops using laboratory based assays has shown that Potato Spindle Tuber Viroid (PSTVd) does not occur in the areas of certified seed production. This evidence supports trade of high health seed potatoes both domestic and export markets and ensures that the industry remains free of these important diseases.

26

Identification of Eutypa dieback pathogens from eDNA collected from Australian vineyards using high-resolution melting analysis

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Eutypa dieback (ED) is considered an important grapevine trunk disease (GTD) in Australia, causing significant yield reduction and threatening the sustainability of vineyards. *Eutypa lata* and other Diatrypaceae fungi produce ascospores that infect primarily through pruning wounds resulting in cankers, dieback and eventually death of vines. Thus, understanding the prevalence and distribution of these Diatrypaceae airborne spores in vineyards will help elucidate their importance in disease spread and to develop subsequent disease management strategies in vineyards. High resolution melting analysis (HRMA) is a simple PCR-based method that has been applied widely for species identification and genotyping of plant pathogens. Thus, a HRMA protocol that can generate specific melting profiles for different Diatrypaceae species can potentially provide a simple alternative method for the identification of Diatrypaceae species in environmental DNA (eDNA) samples. This study developed a HRMA protocol to analyse eDNA collected from eight wine regions in South Australia (SA) and New South Wales (NSW), over 8 years using a Burkard spore trap. The HRMA coupled with DNA sequencing identified seven species, with *E. lata* being present in seven out of eight regions and the most prevalent species in four SA wine regions. However, *Cryptovalsa ampelina* and a newly reported species, *Diatrype stigma*, outnumbered *E. lata* in three other regions. *Eutypella citricola* and *Eu. microtheca* were also the most prevalent species in NSW but were barely detected in SA. The data generated from the eDNA samples analysed showed high species diversity of *Diatrypaceae* airborne spores released in different wine growing regions in Australia. Localised data for each region will assist growers in making decisions for optimal timing of pruning and wound protection in their vineyards.

62

Next Generation Crop-Pest Model: A case study on Blackleg disease (*Leptosphaeria maculans*) and canola (*Brassica napus*)

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Potential yield loss caused by plant disease can be better predicted with models of pest dynamics within a farming system. In recent years, integrated crop-pest models have been developed which link pest incidence/severity with seasonal conditions and management practices, allowing a more accurate prediction of how conditions and management decisions may impact yield. However, existing models account for the biophysical impact of pests often based on a yield reduction approach via external visual symptoms and their respective physiological crop mechanism counterparts which fail to capture the pest response to agro-ecological conditions. The process-based modelling approach is more suitable for capturing a realistic representation of pest-host interactions in the field as it highlights the biological modelling of the pest lifecycle.

The aim of this project was to develop an integrated pest-crop-weather-management model within the APSIM Next Generation framework using Blackleg disease (*Leptosphaeria maculans*) and canola (*Brassica napus*) as a case study. The study centred on the monocyclic phase of disease development, showing the key lifecycle stages starting from inoculum production and maturation in infected crop debris, followed by leaf lesion development (necrotrophic phase), biotrophic hyphal growth through the petiole and stem, to the development of yield-limiting canker at the crown. Data for modelling was derived from both field and controlled environment studies. Each disease development stage interacts seamlessly with the environment, crop and preventive measures imposed in the APSIM Next Generation framework. The improved knowledge on integrated crop-pest modelling exemplified by the linkage of a blackleg disease lifecycle model and a canola crop model contributes significantly to the advancement of existing farming systems models in addressing biotic constraints for crop productivity. The modelling approach used in this study has broad application to different plant pathosystems.

196

Polyphagous shot-hole borer – tracking the development of an invasive exotic beetle in Western Australia

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Polyphagous shot-hole borer (PSHB; *Euwallacea fornicatus*), an invasive exotic beetle, was detected in August 2021 on a residential property in Perth, Western Australia. PSHB lives in symbiosis with an ambrosia clade *Fusarium* species. It has a wide host range (> 500 hosts) and is significantly detrimental to amenity and horticultural trees in other regions such as Israel, California, and South Africa. Currently no chemical treatment is available and infested hosts are treated by limb reduction or removal. The Department of Primary Industries and Regional Development (DPIRD) declared a biosecurity emergency response and is working to eradicate PSHB through a nationally agreed phased response plan. A quarantine area was declared, encompassing much of the Perth Metropolitan Area, where movement restrictions are in place to prevent further spread. DPIRD is working closely with local industries, councils, and the community to inform and conduct surveillance activities. Host and trap samples are submitted to DPIRD Diagnostic Laboratory Services (DDLS) for diagnostic testing. These results uncover trends in host preferences for PSHB, movement across the landscape and contribute to evaluation of eradication efforts. DPIRD has conducted more than 1.4 million tree inspections, and maintain around 2,700 PSHB traps. More than 100 plant species have tested positive for PSHB in WA, and PSHB can reproduce in approximately half of these. Most of the infested new hosts are located in four high pest pressure areas in Perth. Preferred hosts include box elder maple (*Acer negundo*), black locust (*Robinia pseudoacacia*), poinciana (*Delonix regia*), Moreton Bay and Port Jackson figs (*Ficus macrophylla*, *F. rubiginosa*), and coral trees (*primarily Erythrina* x *sykesii*). This presentation will summarise DPIRD's PSHB response activities, examine incursion differences in Australia to elsewhere, and how we are using response and diagnostics data to inform surveillance activities and measure the success of the eradication effort.

155

Updating systematics of *Ganoderma* spp, the causal agent of Basal Stem Rot of *Elaeis guineensis* Jacq. and its spectrum of aggressiveness

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For more than 25 years, basal stem rot of oil palm is believed to be caused by 3 species of *Ganoderma* spp namely *G. boninense*, *G. zonatum* and *G. miniatocinctum*. This gave rise to the current investigation on validating and improving the systematics of Ganoderma. A total of 434 isolates of Ganoderma were isolated, visualised and preserved for an intensive morpho-taxonomy and molecular assessment. Samples were collected from mature oil palms throughout Malaysia. Sampled fruiting bodies displayed high levels of vegetative plasticity and was almost impossible to be categorised according to specific morphological characteristics. Molecular approach using ITS primers found that the isolates were predominantly identified as *G. boninense* with none being identified as *G. zonatum* and *G. miniatocinctum*. All data on the samples are accessible through a virtual system providing details of taxonomy, distribution maps, digital imagery documentation via https://ganoidmpob.arkgene.com/. A pathogenicity study ensued using some of the candidates from the same culture collection sampled from Perak, Terengganu, Selangor and Johor. The study found isolate ET61 (Terengganu) identified as the most aggressive among the tested isolates while SJ33 (Johor) as the least aggressive. Interestingly, when the demographic data of the all the hosts examined, it was found that aggressiveness heightened from isolates sampled from younger palms. Isolate ET 61 was sampled from a 22-year-old infected palm, while isolate SJ33 was sampled from a 27-year-old infected palm which was due for replanting. The isolates were also sampled from coastal soil (Terengganu and Selangor) as compared to the least aggressive isolates from Perak and Johor areas that came from inland soil. The findings from this study suggests that there is only one species of *Ganoderma* responsible for the disease in oil palm and a spectrum of aggressiveness was noted among the isolates but will require further investigation.

Session 3 C: New Diseases and Climate Change

198

Emerging diseases in macadamia: A growing concern

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Macadamia nut has gained significant economic importance worldwide. South Africa and Australia are the top two producing and exporting countries, accounting for approximately 53% of the global market. However, the macadamia industry faces significant challenges due to diseases affecting yield and orchard productivity. Several diseases caused by fungal, oomycetes, and viral species have recently emerged with increasing spread and detection in various producing regions and countries. The emergence of several pathogens can be attributed to various factors, including expanding macadamia cultivation into new regions, changing climate patterns, and intensifying production systems. The spread of new aggressive pathogens of various flower blights including *Botrytis macadamiae*¸ *Neopestalotiopsis macadamiae, N. drenthii* and *N. maddoxii*, and *Cladosporium devikae, C. macadamiae and C. proteacearum* is challenging disease management practices and the productivity of the orchards. The insurgence of new fruit and leaf diseases including Calonectria husk rot caused by *Calonectria sp*. and yellow halo leaf spot caused by *Neopestalotiopsis* spp., with a potentially detrimental impact on yield and growth, is a growing concern to the Australian and South African growers. A new virus was recently identified and has been named Macadamia ringspot-associated virus in South Africa, which causes ringspot symptoms on several major cultivars also grown in Australia. Understanding the risks associated with the extent of the spread due to abnormal vertical growth in Australia and South Africa is crucial for the choice of cultivars to plant. Tree decline and death symptoms are associated with the detection of various oomycetes - *Phytophthora* spp. *Pythium* spp., *Globisporangium* spp. and *Phytopythium* spp. This presentation provides an overview of the emerging diseases impacting macadamia. It highlights the importance of understanding their etiology, epidemiology, and the escalating challenges associated with diagnostics, emphasising the need for proactive measures to understand, monitor, and manage these evolving threats.

154

Witches' broom disease of cassava is associated with a fastidious fungal pathogen in Southeast Asia

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Cassava Witches' Broom Disease (CWBD) is a threat to cassava (*Manihot esculenta*) in mainland Southeast Asia (SEA). The prevalence of CWBD in the region, which had been low in the previous 5 years, has reached a critical point. CWBD incidence in Lao PDR, Cambodia, Vietnam, and Thailand has been reported at 75% in some cases. CWBD was previously suspected to be caused by a phytoplasma, with limited-to-no detection. There is no biological evidence linking phytoplasma and CWBD. We recently used shotgun sequencing to examine pathogen communities in CWBD-infected and healthy plants. We found that a fungus in the genus *Ceratobasidium*, 98.3-99.7%, similar to *C. theobromae*, was conspicuously present in contigs from diseased but not healthy plants. *C. theobromae* causes vascular streak disease (VSD), one of the major cacao diseases in SEA and challenging to culture in-vitro. We have developed a robust PCR-based assay based on the latest sequence data to support the multiplication of disease-free planting materials and regional-wide surveillance. Currently, we are investigating CWBD diversity, disease progression, host range, and transmission pathways to accelerate the development of integrated management options, including breeding for resistance and biochemical control.

80

Effects of drought and turnip yellows virus on canola plants

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Canola (*Brassica napus* L.) is one of the most important crops worldwide. It is affected by several pathogens which can cause significant losses and increase production costs. Aphid-borne, turnip yellows virus (TuYV, *Polerovirus*, Family: *Luteroviridae*) is one of the most damaging and difficult to control. Under future climate, for many parts of the world, more intense and prolonged drought events are predicted, adding another factor that can significantly impact the epidemiology of plant diseases. In this study, we aimed to understand the impact of drought on canola plants previously infected with TuYV. We conducted glasshouse trials, where TuYV infected and noninfected canola plants were exposed to different watering regimes as well as terminal drought. Canola plants were inoculated with TuYV at one leaf stage then at four leaf stage, water treatments were initiated. Several parameters including: height, number of leaves, growth stage and leaf area were recorded as well as chlorophyll content, water use efficiency, symptom expression and biomass, to assess the impact of virus infection and drought on canola growth. TuYV exacerbated the effects of water stress and terminal drought on biomass and other plant growth parameters when compared to non-infected plants. These results suggest that drought could aggravate the negative impact of TuYV on canola crops as a consequence of climate change.

23

Diversity and pathogenicity of *Globisporangium* and *Pythium* spp. associated with pyrethrum in Australia

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Pyrethrum (*Tanacetum cinerariifolium*) cultivation in Australia suffers from a persistent yield decline which in part is caused by a complex of soilborne pathogens. There has been no research on the relationship between oomycetes and pyrethrum yield decline. During surveys between 2018 to 2021, ten known *Globisporangium* species, two new *Globisporangium* species and three *Pythium* species were recovered from crown and root tissues of infected pyrethrum plants and from soils from 16 sites in Tasmania and Victoria, Australia. Identification of *Globisporangium* and *Pythium* spp. was based on morphological characters and multigene phylogenetic analyses using ITS, Cox1 and Cox2 sequences. *Globisporangium ultimum* var*. ultimum* was the most abundant in soils, while *G. sylvaticum* and *G. commune* sp*. nov*. were most abundant in pyrethrum plants. Seven *Globisporangium* species were pathogenic on both pyrethrum seeds (*in vitro* assays) and seedlings (glasshouse bioassays) causing pyrethrum seed rot, seedling damping-off and significant plant biomass reduction, while two *Globisporangium* species and three *Pythium* species only caused significant symptoms on pyrethrum seeds. The results suggest that *Globisporangium* and *Pythium* spp. could be contributing to yield decline in pyrethrum in Australia. This is the first report of *Globisporangium* and *Pythium* spp. as pathogens of pyrethrum globally.

132

REOCCURRING WILT, A NEW DISEASE OF COTTON IN AUSTRALIA CAUSED BY NOVEL *EUTYPELLA* SPECIES

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Reoccurring wilt is a newly described lethal disease of cotton in Australia which was first detected in the 2017/18 season as a single small patch of wilting and dying cotton plants in a field in Central Queensland. Species of Diatrypaceae were consistently recovered from the stem and roots of dying and dead cotton plants, that were distinct from, but closely related to, *Eutypella scoparia*. Phylogenetic analyses and sequence alignment of the combined ITS1 and ITS2 DNA sequence data determined there are two undescribed species of *Eutypella* present in the isolates, both quite different from *E. scoparia*. Pathogenicity of a representative isolate of *Eutypella* on healthy cotton plants was confirmed and is the first known case of *Eutypella* affecting cotton worldwide. Community profiling of diseased root samples showed that two OTUs related to E. scoparia were the most abundant fungi accounting for 45 to 99% of all sequences, indicating this pathogen excludes other fungi from colonising the roots. Annual disease surveys have determined that incidence and the number of fields, farms and regions this pathogen occurs has increased since its first detection. The disease has been confirmed in Theodore, Emerald, St George, Darling Downs and the Border Rivers. In Queensland novel species 1 has been confirmed in Theodore, Moura, St George and Border Rivers, as well as Boggabilla and Mungindi in NSW. Novel species 2 has only been detected in Central Queensland. It was observed in the field that root infection by the pathogen likely occurs, and the source of inoculum is hypothesised to be dead infected cotton trash. Four cotton cultivars were evaluated for their resistance to *Eutypella sp*. under field conditions. All were equally susceptible to the disease and therefore host resistance is not a management option at present.

Session 3 D: Taxonomy, Diversity and Evolution

56

The *Ralstonia solanacearum* species complex simplified, and what's present "down under"?

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The *Ralstonia solanacearum* species complex (RSSC), comprises a large group of strains causing bacterial wilt in over 200 plant species, in more than 50 plant families. It's considered one of the most destructive plant pathogenic bacterium worldwide, and strains vary in host range, and biology. Over the decades, the RSSC were classified in different ways confusing the identity of strains and disease causal agents. This diverse group was recently resolved into a stable taxonomic framework of three species aligning with the previously determined molecular groupings of phylotype, namely *R. solanacearum* (phylotype II), *R. pseudosolanacearum* (Phylotype I and III), and *R. syzygii* (phylotype IV) (Prior and Fegan 2005; Safni et al. 2014). Safni (2014) also separated *Ralstonia syzygii* into subspecies, *R. syzygii* subsp. *celebesensis*, *R. syzygii* subsp. *syzygii* and *R. syzygii* subsp. *indonesiensis*. Phylogenetic relationships within-species or strain identity are determined using a single marker, gene-based, sequevar system, and a whole genome-based LINs system (Prior and Fegan 2005; Sharma et al. 2022). Reconciling within-species nomenclature systems, in terms of host range, biology and identity of the strains that do, and do not occur in Australia, is of importance to Australia's biosecurity and market access. The overall objective of our current project is to determine the identity and distribution of *Ralstonia* strains in Australia. Thus far, over 160 RSSC isolates have been retrieved from Australia's culture collections. Australia's *Ralstonia* collections date back to the 1960's and originate from a diverse range of host genera including *Solanum, Capsicum, Strelitzia, Heliconia, Zingiber, Vaccinium, Lactuca, Olea, Nicotiana, Annona, Zinnia, Galphimia, Acacia, Cucurbita, Eucalyptus, Salvia, Sorghum, Synedrella, Diospyros, Archontophoenix*, and *Dahlia*. The Australian *Ralstonia* isolates have been identified using the current species concepts, and the utility of within-species or strain identity is explored.

117

Exploring the diversity of *Colletotrichum* species infecting Australian native plants

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Colletotrichum is a genus of fungi primarily comprising plant pathogens, many of which are responsible for significant losses to economically important plants. This genus currently accommodates around 340 species which have a wide host range, however, their diversity is not well studied on Australian native plants. Given that many native plants are in the vicinity of agricultural crops, there is a significant risk of disease transmission between native and agricultural systems. The aims of this study are to taxonomically identify *Colletotrichum* spp. associated with native plant hosts and to assess their risk to Australian agriculture. A collection of *Colletotrichum* cultures isolated from Australian native plants were acquired from state culture collections, VPRI (Victoria) and BRIP (Queensland), and new field collections in Victoria and Queensland. The isolates were identified using a polyphasic approach of multigene phylogenetics and morphological characterisation. Forty-two isolates were identified as members of the gloeosporioides species complex, nine isolates of the acutatum species complex, eight isolates of the boninense species complex and one isolate from each of the dematium, and spaethium species complexes. Of particular interest were two isolates of *C. pyricola*, isolated from *Banksia burdetti* and *Eucalyptus caesia*. These findings extend the known host range of *C. pyricola* from temperate and sub-tropical fruit trees to Australian native plants. Pathogenicity bioassays conducted on *E. caesia* leaves resulted in severe anthracnose symptoms at both the wounded and non-wounded inoculation sites. Evidently, *C. pyricola* presents a significant disease risk to both native and agricultural systems.

47

A multilocus sequence typing (MLST) scheme for rapid identification of *Xanthomonas citri* subspecies and pathovars

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The species *Xanthomonas citri* includes plant-pathogenic bacteria associated with a diverse range of host plant species. They have undergone substantial reclassification and currently consist of at least fourteen different subspecies/pathovars that are responsible for a wide range of plant diseases. Examples of economically important *X. citri* subspecies/pathovars are *citri*, *malvacearum*, *mangiferaindicae*, *fuscans*, *anacardii* and *glycines* causing diseases in citrus, cotton, mango, common bean, cashew and soybean, respectively. Identification of *X. citri* subspecies/pathovars has been performed using traditional molecular identification methods such as pulse field gel electrophoresis (PFGE), fatty acid analysis, detached leaf assays, enzyme-linked immunosorbent assay (ELISA) and 16S rRNA gene sequence analysis. However, they do not have enough resolution power to distinguish sometimes even at the species level. The onset of molecular analysis has seen the development of multi-locus sequence typing (MLST) schemes. These utilise six to eight genes in a bacterial species which allows identification and discrimination between subspecies/pathovars. In this study, we developed an eight-gene MLST scheme that yields 19 STs found to be highly correlated with *X. citri* subspecies/pathovars. The scheme was validated using 2,911 *Xanthomonas* genomes obtained from the National Center for Biotechnology Information (NCBI). Each of the *X. citri* genomes examined with the complete eight-gene MLST sequences were correctly grouped in one of 19 STs. This included 18 misclassified genomes from NCBI that were confirmed to be *X. citri* species by overall genome-relatedness indices, such as average nucleotide identity. Furthermore, and outside the original scope of this study, our findings indicate that this scheme may provide profiles to examine closely related *Xanthomonas perforans* and *Xanthomonas phaseoli*. This developed MLST scheme shows robustness for rapid isolate classification using whole genome sequencing (WGS) and will be used to further generate a core genome multi-level genome typing (MGT) scheme.

20

How field-based research can contribute to fungal taxonomy: detection of two undescribed *Kordyana* species in Australia during the course of a biocontrol agent release program

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During vegetation surveys conducted across New South Wales as part of the long-term release and monitoring program of the biocontrol agent, *Kordyana brasiliensis*, disease symptoms similar to those caused by *Kordyana* species were observed on two native plant species, *Anielema* sp. and *Pollia crispata*. During the two- and half-year biocontrol monitoring study, plants within the *Commelinaceae* family were routinely examined for symptoms like those caused by *Kordyana* species. Ten specimens from *Pollia crispata* were collected from locations across NSW and Southeast Queensland and four specimens from *Aneilema* sp. were collected from two regions in NSW. The detection of these specimens prior to the release of *K. brasiliensis* and in areas where this fungus wasn't yet released, along with the host-specificity data from previous studies, meant it was highly unlikely that these specimens were *K. brasiliensis*. To confirm this, several fungal specimens from both plant hosts were subject to morphological and DNA-based molecular analysis of informative barcode loci. The morphological and molecular data identified that the fungi collected on *Pollia crispata* and *Aneilema* sp. were not *K. brasiliensis*. However, these analyses did confirm that these fungal specimens are undescribed species of *Kordyana*. Thus far, *Kordyana* species have only been collected and recorded on three different *Commelina* hosts in Australia; *Kordyana celebensis* and *Kordyana* sp.

30

Diversity of orthotospoviruses in nurseries in Victoria

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Orthotospoviruses are among the most economically significant plant pathogens in the world at present. As of 2022, the genus Orthotospovirus includes 26 officially recognised species. In Australia, five species were reported, including *Orthotospovirus tomatomaculae* (tomato spotted wilt virus, TSWV), *Orthotospovirus capsiciflavi* (capsicum chlorosis virus, CaCV) and *Orthotospovirus iridimaculaflavi* (iris yellow spot virus, IYSV) which were first reported in 1915, 2002, and 2003. *Orthotospovirus impatiensnecromaculae* (impatiens necrotic spot virus, INSV) was found only in NSW in 2010 and again in 2018, and pterostylis blotch virus (PtBV), found only in Queensland, was described as a provisional species in 2022. The nursery sector in Victoria is worth over $2.5 billion and orthotospoviruses pose a significant risk, as they can cause a significant loss in yield and quality of many cultivated plants. Orthotospovirus surveillance has been conducted in Australia in the vegetable industry, but it has never been performed in the ornamental industry.
The following work describes the epidemiological study done to understand the prevalence and the diversity of Orthotospovirus species and strains in the nursery sector in Victoria. Screening for orthotospoviruses in 1636 nursery and weed plant samples collected from Victorian nurseries is underway. So far, the surveillance results demonstrate the presence of TSWV and INSV in five different nurseries in Victoria. It is the first report of INSV in Victoria. This poses a high risk to the local nursery industry, as the existence of its vectors (western flower thrips and onion thrips) and its host plants could lead to a wider spreading of the virus, which will complicate management for nurseries already trying to control TSWV. This information will be used to support nursery industry biosecurity and improve the management practices for controlling Orthotospovirus species in nursery production systems.

71

Identification of *Colletotrichum* species associated with twig dieback of citrus in Western Australia

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Up to 32 *Colletotrichum* species have been reported to be associated with pre- or post-harvest diseases of citrus globally. *Colletotrichum australianum*, *C. fructicola*, *C. gloeosporioides*, *C. karstii*, *C. siamense* and *C. theobromicola* were identified to cause citrus plant and fruit disease within Australia. Most of the *Colletotrichum* isolates examined in previous studies were isolated from fruits or leaves from eastern Australian citrus. During 2020/21, twig or shoot dieback emerged as an important disease in the citrus orchards in Western Australia. Diseased twigs showing withertip or lesions, with or without gummosis, were collected from various cultivars of orange and mandarin across several growing regions. A polyphasic approach including multi-gene phylogenetic analysis and morphological character descriptions were conducted to identify *C. gloeosporioides* sensu stricto, *C. karstii* and *C. novae-zelandiae*. *Colletotrichum gloeosporioides* was the most prevalent species associated with twig dieback in Western Australia while C. novae-zelandiae was reported for the first time in Australia. Pathogenicity tests on shoot twigs from lemon and orange trees confirmed *C. gloeosporioides*, *C. karstii* and *C. novae-zelandiae* as causing twig dieback with *C. gloeosporioides* being the most aggressive species.

Session 4 A: New Technologies (Artificial intelligent (AI)) and Novel Methods in Plant Pathology and Disease Control and Forest and Perennial Crop Diseases

150

Fungi and oomycetes associated with trunk diseases in the Australian almond industry

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Several fungal, bacterial, and oomycete species cause trunk diseases in almond trees. Trunk diseases are present in almond production regions worldwide and can significantly impact orchard profitability. Common symptoms include necrosis of the wood, discolouration of vascular tissues, extensive gumming, and dieback of limbs from the point of infection. Infection may kill the primary branches and the entire tree if left untreated. *Phytophthora* species can also cause root rot symptoms, degrading the root hairs and impeding the ability of trees to access water and nutrients. *Phytophthora* and fungal trunk diseases in almond were investigated in the 1980s and 90s. Trunk disease has more recently been reported as a problem in almond production, so further research is required to determine the current epidemiology of fungal and oomycete species in Australia. Samples collected from symptomatic trees in South Australia, New South Wales, Victoria, and Western Australia from 2018 to 2023 yielded numerous fungal and oomycete species. The identity of the oomycete isolates was confirmed by DNA sequencing of the ITS, COX-1 and/or β tubulin gene regions, and fungal isolates were identified using ITS, TEF1α, and GAPDH gene regions as required. Botryosphaeriaceae and *Phytophthora* species were the most prevalent and destructive pathogens. In contrast, *Colletotrichum acutatum*, *Pleurostoma richardsiae* and *Collophora, Cytospora*, *Phytopythium* and *Diatrypaceous* species were isolated less frequently from symptomatic samples. The distribution of the trunk disease pathogens differed among the growing regions, and there was a greater incidence of disease symptoms in areas with higher rainfall. Key differences in symptom expression caused by the trunk disease pathogens were also observed, which may help with field identification. Further studies are ongoing to determine the sources of inoculum and the abiotic and biotic factors that influence disease development and severity to gain a better understanding of the trunk disease complex.

64

First report of pathogens associated with shot-hole disease on flowering cherry trees (*Prunus* x *yedoensis*) and warning of their possible cross-infection with stone fruit trees (*Prunus* spp.)

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The shot-hole disease is among the most common and important diseases affecting flowering cherry (*Prunus* x *yedoensis* Matsumura; Somei-yoshino) trees in South Korea every year, resulting in spots and shot-holes in the leaves, premature defoliation, and reduced flowering of cherry blossoms in the following year. However, pathogens associated with the disease remain unknown, which has rendered disease management challenges. Based on multilocus sequence analysis and in planta pathogenicity tests, two pathogenic bacteria identified as *Burkholderia contaminans* and *Pseudomonas syringae* pv. *syringae* and one pathogenic fungus identified as *Epicoccum tobaicum* were shown to cause leaf spot and shot-hole on flowering cherry. These pathogens were recorded for the first time as the causative agents of shot-hole on flowering cherry trees. Further, in a detached leaf assay, these pathogens induced leaf spots and shot-hole on several stone fruit trees belonging to the *Prunus* genus, including peach (*P. persica*), plum (*P. salicina*), and apricot (*P. mume*), with peach being the most susceptible. This indicates that *B. contaminans*, *P. syringae* pv. *syringae*, and *E. tobaicum* possess a broad range of *Prunus* hosts and may also pose a threat to stone fruit production. The findings in this study will be of great importance as a reference for the effective management of shot-hole disease on flowering cherry and to warn of the potential for cross-infection between *Prunus* species in the future.

97

New solutions to a perennial problem: curing myrtle rust with double stranded RNA

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Myrtle rust (caused by *Austropuccinia psidii*) continues to spread throughout Australia. Its invasion predicts an extinction event of 16 environmentally and culturally important rainforest tree species in a single generation. The recent myrtle rust-driven closure of World Heritage site Lord Howe Island, and first-time sightings in Western Australia, have reinforced the need for an effective and multi-pronged approach to pathogen management. However, the management of rust fungi on perennial trees is a unique challenge. Due to the long generation times of perennials, a curative treatment, effective at all points of the disease cycle, will be crucial for effective management of myrtle rust and similar diseases. We previously showed that double-stranded RNA (dsRNA) significantly inhibited the infection physiology of *A. psidii* and reduced disease symptoms in planta. Here, we build on these findings to determine if dsRNA can be applied exogenously pre- and post-infection to prevent and cure myrtle rust. We investigated the preventative and curative potential of dsRNA through in planta assays in *Syzygium jambos*. Disease severity, plant health and recovery, and photosynthesis parameters were assessed following the application of dsRNA at multiple timepoints pre- and post-infection. We show that dsRNA – likely acting through RNA interference (RNAi) – can be used to prevent and cure severe myrtle rust infections on *S. jambos* at multiple points in the *A. psidii* infection cycle, by decreasing disease coverage and increasing plant growth and photosynthetic capacity. Curative RNAi provides a new solution to the catastrophic, decade-long epidemic of myrtle rust in Australia and has applications in the conservation of long-living perennial trees and at-risk species, including those listed as conservation priorities in the Myrtle Rust Action Plan. Due to its use as both a preventative and curative treatment, dsRNA may also be an effective control for myrtle rust in nursery and horticultural industries.

136

Finding the pathogen before the disease

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Airborne plant pathogens pose an existential threat to regions naïve to their impact. There are many examples where such pathogens are detected too late; when entire forests are dying (ash dieback - *Hymenoscyphus fraxineus*), stands have been decimated (chestnut blight - *Cryphonectria parasitica*), or bushland all but destroyed (myrtle rust – *Austropuccinia psidii*). Once established, pathogen eradication becomes exceedingly difficult as they continue to spread, and management resources are allocated in a reactive manner. Here, we present a surveillance technique that co-opts existing air pollution monitoring infrastructure for the early detection and monitoring of airborne plant pathogens. For this, we used *A. psidii* as a case study and verified its presence in the air of southeast Queensland, Australia using a new species-specific TaqMan qPCR assay. We also present fungal metabarcoding results from the same filters that extend beyond targeting specific plant pathogens to include a biodiversity perspective. *Austropuccinia psidii* has not established in Western Australia. Routine monitoring for spores from the eastern Australian states using the technique proposed here, will enable more efficient allocation of resources thereby enhancing our ability to predict, find and contain *A. psidii*, and other pathogens like it, before disease is established. A proactive, rather than reactive response.

209

Sniffing *Phytophthora*

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Phytophthora in plant nurseries causes significant direct losses form seedling deaths and indirect impacts resulting from the distribution of *Phytophthora*-infected planting materials for orchard and ornamental plantings, and environmental rehabilitation projects. Preventing the distribution of infected planting material relies on sensitive and accurate diagnostics to monitor the disease-free status of nurseries. Disease free nursery accreditation schemes depend on sensitive and accurate diagnosis of pathogens. For *Phytophthora* this usually requires baiting and identification based on morphology or molecular diagnostics, adding to the cost of nursery production. Trials in California and New Zealand have shown the potential for sniffer dogs to be trained to detect volatiles indicating *Phytophthora*. Our research is examining if these volatiles originate from disease-stressed plants or from the pathogen. Can sniffer dogs distinguish *Phytophthora* from other pathogens? Can they distinguish *P. cinnamomi* from other species? How cost-effective are sniffer dogs compared to conventional diagnostics? What are the most effective sampling strategies to use sniffer dogs in combination with conventional diagnostics? Sniffer dogs not only offer an opportunity to improve the detection and management of Phytophthora in nurseries and reduce further impacts, but they could be a powerful tool for public awareness raising and education.

194

Exploiting RNA-mediated silencing to induce GPGV resistance in *in-vitro* grown *Vitis vinifera* by exogenous application of dsRNA

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Grapevines are economically significant fruit crops that are vulnerable to viral pathogens, which are transmitted through vegetative propagation and insect vectors, and can significantly reduce grape quality and yield. Effective vineyard establishment and ongoing health management using virus-free planting material is crucial to manage virus-associated diseases in grapevines. Primary virus elimination methods to produce high-health virus-free grapevine planting materials include treatments such as chemotherapy and thermotherapy combined with *in-vitro* meristem isolation. RNA interference (RNAi)-based “vaccination” can silence viral replication and represents an attractive and promising alternative for controlling virus-associated diseases in plants. It has been shown to be effective against RNA viruses in some annual crops. In this study, grapevine Pinot gris virus (GPGV) is used as a model to investigate the potential of “RNAi-vaccination” as a method to produce GPGV-free grapevine material. The topical application of specific double-stranded RNA (dsRNA) targeting a specific region of the GPGV RNA-dependent RNA polymerase (RdRp) coding gene is applied *in vitro* to the leaves of whole tissue culture plantlets or the excised shoot tips, followed by measurement of the titre of viruses post application. If successful this research represents the first step into developing a broader application of “RNAi-vaccination” beyond GPGV, providing an opportunity to effectively control other grapevine viruses in vegetatively propagated tissue culture and enhance grape quality thereby significantly benefiting the viticultural industry.

Session 4 B: Epidemiology, Ecology, Modelling and Risk Analysis

122

Effects of environmental stresses on *Fusarium oxysporum* f. sp. *cubense* subtropical race 4 *in vitro*

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Fusarium wilt is one of the leading causes for loss in the global banana industry. The current primary focus is on *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) due to its high levels of devastation on Cavendish bananas. With no current viable management options and no resistance available in market-acceptable varieties, the race is on for a genetic solution. Since Cavendish is globally the main cultivated variety, subtropical race 4 (STR4) has the potential to cause economic impact, especially given the increasing occurrence of extreme weather events. Furthermore, the introduction of TR4-resistant cultivars can lead to higher susceptibility to other pathogens, including STR4. STR4 has a limited distribution and is considered pathogenic to Cavendish cultivars under predisposing conditions such as temperature stress, increased salinity levels, and altered pH levels. However, such conditions have never been rigorously assessed on banana plants, the pathogen, or their interaction. The effects of these environmental factors on the growth rate, spore production, and phenotypic structure of different STR4 isolates are still unclear. To fill this knowledge gap, we sought to answer the following questions: a) how do abiotic stresses impact the culture phenotype and growth behaviour of subtropical race 4 isolates? and b) how do these results compare with those of race 1 (R1) isolates? Results of experiments conducted to answer these questions will be presented. Other elements of this project have been devised to address the following: a) to provide a better understanding of the classification system within STR4, b) to compare virulence and competitive advantage when both STR4 and R1 are present, and c) to address diagnostic issues within STR4.

169

Lenticels: the infection portal for *Phlyctema vagabunda* causing Bull's eye rot of apple

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Bull's eye rot (BER) is a postharvest apple fruit rot disease caused by the fungus *Phlyctema vagabunda* syn. *Neofabraea alba*. The fungus latently infects apples in the field. Disease symptoms are expressed after about 12 weeks cold storage. Laboratory experiments have shown that hydration of lenticels is an important determinant for fruit susceptibility. Apple fruit were hydrated by soaking in deionised water overnight, then inoculated without wounding (Everett et al. 2017). After 20 weeks cold storage, resultant rots were measured. Rots were more severe and numerous on hydrated fruit than on unhydrated control fruit. Measurement of lenticels with an eyepiece micrometer and a light microscope on strips of apple skin removed with a razor blade showed that when lenticels are hydrated, they expand. Inoculations without wounding showed that hydrated fruit become more susceptible to infection by *P. vagabunda*. Examination of the incidence of natural infections of BER on fruit harvested at weekly intervals and inoculating detached fruit without wounding followed by comparisons with climate variables showed that wind run was an important factor influencing infection, in combination with leaf wetness. We conclude that these factors influence fruit susceptibility by modifying lenticel size and integrity.

Reference

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115

Surveillance for vector-borne diseases – a Western Australian experience

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In February 2017, *Bactericera cockerelli* (tomato-potato psyllid [TPP]) was detected in Perth. TPP feeds on Solanaceae crops, affecting plant growth and reducing crop yield. TPP also vectors the bacterium *Candidatus Liberibacter solanacearum* (CLso), causing zebra chip disease in potato. This was the first incursion of TPP on mainland Australia, prompting the Western Australian Department of Primary Industries and Regional Development (DPIRD) to call a biosecurity incident. A quarantine area was declared, and interstate market access was restricted. By April 2017, it was evident that TPP was widespread in Perth, eradication was not possible, and the incident moved into the Transition to Management (TTM) phase. During TTM, DPIRD was able to prove freedom from CLso and restore market access. However, to maintain this, continued evidence supporting absence of CLso was needed. DPIRD trapped for TPP twice a year, in spring and autumn, utilizing backyard trappers across Perth. Trapped TPP were counted and then tested for CLso using molecular methods, CLso was not detected. Using Bayesian logic, these results were used to demonstrate absence from CLso in Western Australia. Further analysis of the data demonstrated that TPP numbers are generally declining every year, with autumn populations especially low. Also, 'fluctuation' of the TPP populations is apparent, between south and west, to the eastern and northern suburbs. From 2022, trapping was integrated into a multispecies trapping program, trapping for additional exotic species (e.g., BMSB, ACP), and only taking place in spring to increase efficiencies in exotic species surveillance. Insights from the data analysis not only resulted in restored market access for Western Australia, but also aided in designing and implementing multispecies trapping trials for more efficient trapping.

168

Can genetic resistance to pathogens be a renewable resource? Impact of cultivar rotation on pathogen evolution and disease control

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In interactions between *Brassica napus* and *Leptosphaeria maculans*, host resistance breakdown often occurs quickly after deployment, resulting in increased management costs, significant crop losses and a continuing need to develop new resistant cultivars. Here I will present results from recent modelling and empirical work where we investigate how different major gene deployment strategies influence *B. napus* resistance efficacy, pathogen population genetic structure and epidemiology. Results from field experiments demonstrated that host resistance strongly influenced the pre- vs post-infection virulence profile of pathogen populations. As expected, major gene resistance strongly selected for pathogen virulence. However, strong shifts in the relative abundance of complex pathotypes, resulting in significant decreases in the frequency of unnecessary virulence genes, were also observed. These results suggest that carrying virulence genes can be costly for the pathogen when they are not required for infection. We used these data on resistance gene specificity and fitness costs to build an epidemiological and evolutionary model to investigate the potential for different cultivar rotation strategies to control disease epidemics. The modelling results suggested that the frequency of virulence pathotypes in *L. maculans* populations, and subsequent disease epidemics can be managed, at least in part, by using cultivar rotations and informed use of resistance gene diversity. From a management perspective, the results indicate that controlled deployment of resistance genes in space and time can be used to help control blackleg disease, even when resistance has already been overcome.

38

Grapevines are at risk of infection by trunk disease pathogens during spring

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Grapevine trunk diseases (GTDs) such as Eutypa (ED) and Botryosphaeria dieback (BD) are caused primarily by fungal spore infection of winter pruning wounds. Hail events during spring can cause severe damage to developing green shoots and lignified canes. Spring shoot thinning also creates wounds and is undertaken to improve airflow, increase sunlight exposure, maintain crop yield, and to reduce pruning wounds in the following winter. Extensive spore trapping in Australian vineyards has detected ED and BD pathogens throughout spring and summer in association with rainfall. In winter 2022, canes were collected randomly from two Shiraz vineyards in the Barossa Valley, South Australia that were affected by a severe hail event in the previous spring. Over 200 hail damage wounds were assessed from each vineyard for recovery of trunk disease pathogens on Petri dishes filled with potato dextrose agar (PDA). A trial was also established in the shadehouse to evaluate spring shoot thinning practices on Shiraz vines grown in pots. In spring, green shoots were either cut with secateurs leaving a smooth wound, or the whole shoot was torn off from the lignified cane, leaving a rough 'socket' wound. The wounds were inoculated with fungal spores of either *Eutypa lata* (ED) or *Diplodia seriata* (BD) with 50 replications, and in the following winter they were assessed for recovery of the pathogens on PDA. Pathogens were recovered from up to 5% of naturally infected hail damage wounds, and from 62 to 91% of inoculated shoot thinning wounds. Ongoing research is now evaluating the risk of infection in the vineyard under inoculated and natural conditions following shoot thinning activities.

21

Field release of a host-specific biotrophic plant pathogen, *Kordyana brasiliensis* in NSW, Australia for the biocontrol of an environmental weed: establishment and impacts

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Kordyana brasiliensis is a biotrophic plant pathogen that was deliberately released in Australia as a biocontrol agent to target environmental weed, *Tradescantia fluminensis*. In 2020 a two-and-a-half-year release and monitoring study was established in New South Wales across a latitudinal gradient (~ 1000 km). The study had two main aims; (1) to investigate the climatic conditions conducive to *K. brasiliensis* establishment and severe disease development and (2) to evaluate the ecological impacts of sustained disease on *T. fluminensis* and how this varied across climatic and latitudinal gradients. Sites were monitored 6, 18, 24 and 30-months post release assessing *K. brasiliensis* disease incidence (number of stems infected per plot) and severity (percentage of leaf area covered by lesions) and *T. fluminensis* abundance (cover and volume). Native vegetation diversity and abundance was also monitored prior to the biocontrol agent release and at 24 and 30-months post release to evaluate how native vegetation communities responded to changes in *T. fluminensis* abundance. *Kordyana brasiliensis* successfully established at all 50 release plots six-months post release and continued to survive over the 2.5-year monitoring period spreading within sites to nearby control plots. Disease incidence and severity peaked 18-months post release in the release plots with the highest levels of disease severity observed in the two most northern regions which have more humid and warmer climates. Disease severity was consistently lower during the monitoring study in in the most southern region which has a drier and cooler climate. Eighteen months post release *T. fluminensis* volume and cover significantly declined across the four regions with the largest magnitude of change occurring in the two most northern regions where *T. fluminensis* volume reduced by approximately 80 %. Disease severity at 6- and 18- months post-release was significantly negatively associated with the percentage change in cover of *T. fluminensis*.

Session 4 C: Diagnostics, Biosecurity

138

Small RNA sequencing (sRNAseq) for plant virus-viroid diagnostics at Post Entry Quarantine: Journey post-deployment

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Australia's Post Entry Quarantine facility (PEQ), in Victoria, conducts plant disease diagnostics on imported, high-risk nursery stock. In December 2022, sRNAseq was deployed as the primary screening method for detecting viruses and viroids in *Prunus*, *Rubus*, *Fragaria* and clonal grasses after ten years of test development and validation. Since sRNAseq enables detection of an entire viral repertoire in one test, biological indexing was phased out and PCR is only used as a secondary verification of the sRNAseq detections for these commodities. During the 2022/23 testing season, 201 plants were sampled during active growth for total RNA extraction in-house, whilst sRNA library preparation and Illumina sequencing were outsourced. Sample handling and extraction processes were modified to achieve RNA integrity suitable for sRNAseq. Double-sided bead clean up and ribosomal depletion on the sRNA library were evaluated for removal of host RNA. In addition, a stable internal control was introduced after comparing two commercial microRNA spike-in controls. Automated VirReport and GA-VirReport bioinformatics pipelines were used in data analysis and detections were categorised into confidence levels based on percent identity and the depth of coverage of the reference genome. A total of 137 viruses, and five viroids were detected from 20 plant-pathogenic species. These positive detections included quarantinable and non-quarantinable pathogens, putative novel viruses and host genome integrated virus detections. All detections except non-quarantinable were verified using specific PCR assays and Sanger sequencing. Most positive plants were asymptomatic and 32 were positive for multiple pathogens. Implementation of sRNAseq has resulted in thousands fewer PCR tests and the elimination of biological indexing for these commodities has increased potential for higher numbers of plant imports due to increased glasshouse space availability. The deployment and first season of plant virus-viroid testing using sRNAseq at PEQ will be discussed including triumphs, challenges and plans for future developments.

133

Border surveillance reveals new plant pathogen host records and geographic range extensions of Biosecurity concern for Australia

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Australia's biosecurity system protects the nation's agriculture, environment and export markets whilst enabling the benefits of international trade and travel (Maxwell et al. 2013). In Australia the Department of Agriculture Fisheries and Forestry (DAFF) manages biosecurity risk by imposing biosecurity controls on imported goods and conveyances including live plants that undergo inspection, and growth in post entry quarantine; authorising approved arrangement (AA) facilities for entities to undertake import related activities; and carrying out pest surveillance associated with AA's and ports. Here we report on new hosts, and range extensions of plant pathogens detected as part of DAFF's national border surveillance (NBS) program for 2017-2023.
More than 3500 AA facilities across Australia were risk assessed for pest entry and establishment potential. General and targeted surveillance for specific pests was undertaken at a frequency based upon that risk assessment. Surveillance targets included Australia's national priority plant pest (NPPP's) as agreed by plant health committee in August 2019 (DAFF 2023). Pathogens were isolated from diseased plants collected and identified using standard morphological and molecular techniques including multi-locus DNA sequencing.

Several new host and geographical range extensions were discovered: a potentially novel begomovirus; geographic range extensions for *Colletotrichum fructicola*, *C. karstii*, *C. liriope*, and *C. trichellum*; new host and geographic range extensions for *Cladosporium macrocarpum*, *Colletotrichum boninense*, *Harknessia globispora* and *Parapyrenochaeta acaciae*; new host records for *Didymocyrtis banksiae*, Passiflora Virus Y and for Turnip Mosaic Virus. New *Erysiphaceae* detections that were made are not included here.
These pathogens were not directly linked to the import activities of the AA's where these detections were made. The biosecurity implications of these detections are discussed.

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Maxwell A, Vettraino AM, Eschen R, Andjic V (2013) International Plant Trade and Biosecurity DOI: 10.1007/978-94-017-8560-0

24

Phenotyping tan spot (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*) of mung bean using specific quantitative PCR

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Curtobacterium flaccumfaciens pv. *flaccumfaciens* (Cff) is a pathogenic bacterium that causes tan spot and bacterial wilt of mung bean (*Vigna radiata*) and several members of the *Fabaceae*. Limited investigations of pathogenicity have been conducted despite the economic losses associated with tan spot in Australia. Early detection of tan spot is critical for effective management. Several studies have aimed to detect Cff by targeting a gene located on a virulence related linear plasmid, however, strains with no plasmid have been reported. This may result in an underestimation of Cff populations. This project presents a duplex quantitative PCR (qPCR) assay targeting a conserved region of the gyrB gene of Cff and the EF1α gene of mung bean. The assay was conducted to assess the relative virulence of six Cff strains on the mung bean variety Opal-AU in two glasshouse experiments. There was a positive correlation between the visual disease symptoms and qPCR results ($p < 0.001$, $R2 = 0.639$). In contrast, a negative association occurred between the trifoliate dry weight and Cff DNA biomass ($p = 0.003$, $R2 = 0.391$). Symptom development and pathogen biomass varied between strains ($p < 0.001$), demonstrating variability in virulence. Preliminary data suggest that linear plasmid copy number variation may impact the virulence of each strain, however, investigations are ongoing. Knowledge of the virulence potential within Cff populations and the qPCR phenotyping tool will assist mung bean breeding programs to address tan spot disease impacts.

145

Enhancing Plant Biosecurity Surveillance and Diagnostics capacity and capability through expertise Networks

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Effective diagnostic and surveillance capability and capacity are essential in achieving the Intergovernmental Agreement on Biosecurity objectives, including to 'prepare and allow for effective responses to, and management of, exotic and emerging pests and diseases that enter, establish or spread in Australia'.
Changes in agricultural practices, trade and people movement mean the Australian biosecurity system must continue to evolve to address new risks and pest pathways. People, infrastructure, standards and tools to deliver plant biosecurity surveillance and diagnostic services now and into the future are crucial to the system. This includes maintaining a high level of expertise in the diagnostic and surveillance areas, this is achieved through the professional development programs, national protocols, national plant health proficiency testing program and expertise Networks.
The National Plant Biosecurity Diagnostic Network (NPBDN) and Plant Surveillance Network Australasia-Pacific (PSNAP) were established in 2010 and 2017 respectively. These expertise Networks are to improve Australia's capability and capacity to detect, identify and respond to plant pests that impact plant industries, the environment and the community.
The NPBDN is an initiative under the Subcommittee on Plant Health Diagnostics (SPHD) and the PSNAP is an initiative under the Subcommittee on National Plant Health Surveillance (SNPHS). Funding for the two Networks been provided by the Department of Agriculture, Fisheries and Forestry (DAFF) and supports the management of professional development program, the national plant health proficiency testing program and national protocols.

14

The Diagnostic Frontier: Conquering *Fusarium oxysporum* with new tools

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Fusarium oxysporum (Fo) is a devastating fungal pathogen that infects a wide range of plant species, causing significant economic losses in agriculture. There are three putative species within the Fo species complex (FOSC), comprising both pathogenic isolates, usually defined as formae speciales (ff. sp.), and putatively non-pathogenic isolates ubiquitously present in soils.

Rapid and accurate detection of Fo is crucial for implementing timely management strategies and preventing the spread of the disease. Molecular characterisation of most Fo strains using conserved genes is impossible, as phylogenetic studies based on conserved genes have revealed considerable diversity between and within isolates of a forma specialis (f. sp.), placing them across several clades. Effector proteins secreted by Fo play a crucial role in host-pathogen interactions. These genes are expected to be similar between strains within a f. sp., as supported by the presence-absence effector phylogeny, which shows clustering of isolates based on strain, indicating high conservation in the pathogenicity mechanism of Fo. Strain-specific effectors in pathogenic Fo strains are responsible for host specificity.

Using strain-specific effectors as diagnostic markers for pathogenic strains offers several advantages, including high specificity, sensitivity, and potential for multiplex detection. Effector-based diagnostics present a promising approach for the rapid and accurate detection of Fo.

87

Current status of viruses infecting local garlic germplasms in Indonesia: old and new players

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Potyvirus and carlavirus are the two main economically important virus groups that have been reported infecting garlic in Indonesia. Asexual propagation of garlic increases the risk of virus infection, especially by clove-borne viruses. This is especially an important fact since the Indonesian government announce a national program for self-production of garlic. The objective of this study was to detect and identify main viruses infecting local garlic cultivars in Indonesia. Samples were obtained from field collection; germplasm collection; and imported bulbs. Specific RT-PCR test was done to detect onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), garlic common latent virus (GCLV), shallot latent virus (SLV), and garlic virus A–E, –X (GarVA–E, –X)). LYSV incidence reached up to 100% on both local (19/19) and imported garlics (16/16); while OYDV incidence reached 73.7% (14/19) on local garlic and 100% on imported garlic (16/16). Interestingly, we found 0% incidence of SLV on local garlic samples but 93.8% (14/16) on imported garlic. Both imported and local garlics have low incidence on GCLV i.e., 18.8% (3/16) and 31.6% (6/19), respectively. During the virus indexing, we detected GarVB on local garlic with 89.5% incidence (17/19). Sequencing analysis showed that GarVB from Indonesian garlic isolates shared 98.16% similarity with GarVB from China. For our knowledge, this is the first report of allexivirus infection in Indonesia. This finding is important as a basic information to develop a strategy to improve production of garlic in Indonesia.

Session 4 D: Plant Disease Management, Chemical Resistance

162

Analysis of *Phytophthora cinnamomi* in the Great Otway National Park

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The effect of the plant pathogen *Phytophthora cinnamomi* is extensive and devastating for many ecosystems and their component species throughout Australia. One such ecosystem is that of heathlands that support a multitude of Australia's rare and endemic species. Due to the relatively poor soil quality and high rainfall run off that are features of heathlands they are particularly susceptible to soil-borne pathogens, such as *P. cinnamomi*. High water run off spreads this pathogen rapidly through the landscape at rates from 4-7 metres per month (Weste and Taylor 1971). The Great Otway's National Park in South-Eastern Victoria is home to several heathland areas that contain around 25% of Victoria's native flora, many of which are endemic. The effects of *Phytophthora cinnamomi* within the park have been documented since its first discovery in 1972 (Hill et al. 2013) however management methods, such as the use of the chemical phosphite, are only just starting to build momentum for use on a larger scale. The studies being undertaken here include extensive disease monitoring where sites in the Central and Eastern Otways are being assessed over an extended period for any changes in the floristics of infested compared with uninfested sites. In addition, half of the sites have been treated with the chemical, phosphite. The results from these studies will demonstrate the variation in species diversity and density due to disease presence in a heathland ecosystem. The efficacy of phosphite application as a protectant of vulnerable Victorian vegetation communities and individual species will also be established. In addition to the floristic monitoring, trials of rehabilitating post disease sites by planting endemic and susceptible species, some with the addition of phosphite, in a cluster method has been undertaken. This mimics vegetation 'islands' of susceptible species observed surviving in known *P. cinnamomi* sites.

137

Adavelt™ active (Florylpicoxamid) – a novel fungicide for disease management in strawberry, lettuce, fruiting vegetables and cucurbits in Australia.

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AdaveltTM active (florylpicoxamid) is a novel naturally inspired solution from Corteva Agriscience for broad spectrum control of economically important fungal diseases in strawberry, lettuce, fruiting vegetables and cucurbits. Adavelt is a second generation picolinamide fungicide (FRAC group 21) that offers a new mode of action across a wide range of crops. Adavelt has shown excellent control of powdery mildews, *Alternaria sp*., *Sclerotinia* spp. and *Botrytis cinerea*. Adavelt is a second generation picolinamide fungicide, which inhibits fungal respiration by binding to the complex III in the mitochondrial electron transport chain at the Qi site (Quinone inside inhibitor - QiI). Adavelt shows no cross-resistance with other major fungicide modes of action and will be a valuable rotation partner for fungicide resistance management programs. This paper summarises registration trials for strawberry, lettuce, fruiting vegetables and cucurbits in Australia.

58

Comparative effect of bacterial cultures and cell-free supernatants on the development of *Sclerotinia sclerotiorum*

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Oilseed rape (*Brassica napus*), one of the main oil-producing crops worldwide is susceptible to white mold caused by the fungal pathogen *Sclerotinia sclerotiorum*. Control of this necrotrophic, polyphagous fungal pathogen still mainly relies on chemicals. Therefore, the development of new biocontrol agents appears to be a promising option for contributing to more sustainable oilseed rape production. Previous work carried out in our laboratories has identified two bacteria with particularly interesting activities under controlled conditions and in the field. An analysis of the entire genome of these bacteria shows that they have the genes to produce metabolites having antifungal activity. This result suggests that antibiosis is a possible mode of action explaining the protective efficacy of these bacteria against *S. sclerotiorum* on oilseed rape. The objective of this study is to validate this hypothesis through biological experiments.

To this end, dual culture plate assays were performed to evaluate the inhibitory effect on *S. sclerotiorum* of the bacterial strains and of their cell-free supernatants. In addition, tests on detached oilseed rape leaves were carried out to assess the protective efficacy of the cell-free supernatants compared with the living bacteria.

Results show that the bacterial strains exhibit antibiosis against *S. sclerotiorum*, reducing its mycelial growth and forming a zone of inhibition. This direct effect can be attributed to the synthesis by the bacteria of antifungal metabolites, present in the cell-free supernatant. Additional results showed that the cell-free supernatant could significantly enhance the efficacy of protection provided by both bacteria. Understanding the underlying mechanism should help guide the development of a future biocontrol product with stable efficacy.

141

Fungicide resistance in powdery mildew in Australian vineyards

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Grapevine powdery mildew, caused by *Erysiphe necator*, is a significant disease in Australia and worldwide. Fungicides are vital tools in controlling this disease, however the widespread and frequent use of fungicides has contributed to increasing resistance in the grapevine industry. To investigate resistance status, samples were collected from 150 vineyards from five states in Australia (NSW, Vic, SA, Tas and WA). A single spore colony was established for each sample using detached leaves in water agar plates. Isolates were phenotypically tested against seven fungicide groups; QoIs (11), DMIs (3), Amines (5), Aza-naphthalenes (13), SDHIs (7), Aryl-phenyl-ketones (50) and Phenyl-acetamides (U6), and genotypic analysis using next-generation sequencing (NGS) were also performed for known fungicide resistance mechanisms. *E. necator* showed varying levels of reduced sensitivity to most of the fungicide groups tested, with resistance only confirmed for fungicides from group 13. Two mutations, G143A and Y136F, were identified in *E. necator* populations. There were strong relationships between reduced sensitivity to QoIs and the presence of G143A mutation in CYTB gene, but not between reduced sensitivity to DMIs and Y136F mutation in CYP51 gene. The mutant H242R/Y (associated with group 7 resistance) was not detected, but reduced sensitivity was recorded for this group. Techniques have been refined for improved fungicide resistance monitoring by developing rotorod spore traps combined with NGS to detect mutations. The two mutants (G143A and Y136F) have been detected in the field using spore traps. A mini-greenhouse system was developed, and is an effective method for maintaining cultures of E. necator and evaluating the fitness of mutations. G143A and Y136F mutants were fit and stable for at least six months under laboratory and greenhouse conditions. Current research is developing In-field and high-throughput genotypic testing, which will enhance fungicide resistance monitoring in Australian viticulture.

34

Fungicide resistance monitoring with digital PCR – a case study in barley net blotch

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Fungicide resistance is a risk to sustainable crop production. DNA detection systems offer the ability to monitor for genotypes associated with fungicide resistance and estimate the frequency of specific genotypes. This knowledge is critical for informed fungicide and disease management strategies. Using droplet digital PCR of leaf lesion DNA and the net blotch pathosystem in barley, we demonstrate the ability to quantify both *Pyrenophora teres* f. *maculata* and *P. teres* f. *teres*, and 23 genotypes associated with different levels of sensitivity to DMI (demethylation inhibitor) or SDHI (succinate dehydrogenase inhibitor) fungicides. Across 20 fields sampled in Western Australia in 2021, *P. teres* genotypes associated with reduced sensitivity or resistance to DMIs were detected in every field (3 to 92%). Genotypes associated with reduced sensitivity or resistance to SDHIs were detected in 15 fields (1 to 89%). The most frequently detected pathogen was *P. teres* f. *maculata*. Using the same workflow in 2022 for 10 fields resulted in the first detection of DMI resistance genotypes in Queensland, with genotypes associated with reduced sensitivity or resistance to DMIs detected in eight fields (7 to 68%). Genotyping also suggested the presence of genotypes associated with reduced sensitivity to SDHI fungicides in six fields (1 to 13%). Both *P. teres* f. *maculata* and *P. teres* f. *teres* were present but varied in relative abundance among fields. This workflow will improve monitoring of fungicide resistance and disease management in barley crops.

Keynote Address 5

206

Emerging diseases of fruit and nut crops in California: the effect of climate change and intensive farming practices, and new control solutions

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California is experiencing a climate crisis that is increasingly affecting plant health. One major aspect of climate change is the increase of extreme weather events, including cold and heat waves, drought, heavy precipitations, floods and storms. In the current scenario of rapidly evolving climate change and high frequency of extreme weather events, California agricultural crops are more frequently subjected to stresses of both abiotic and biotic origin. Because most plant pathogens respond to weather, changes in weather events due to climate change are impacting the frequency and intensity of disease epidemics. The present review will summarize observations from the last decade of the effect of severe droughts, heat waves, cold snaps and unprecedented atmospheric rivers and flooding on fruit and nut tree health and disease emergence in California. These include the rise of canker diseases across multiple tree crops, including almond and sweet cherry, as well as recent outbreaks of Phytophthora in pistachio and almond. Emerging foliar and fruit diseases caused by fungal and bacterial pathogens affecting fruit and nut crops also will be presented. The intensification of agricultural practices, including mechanization, high and super high-density systems, intensive pruning also have played a role in disease emergence and outbreaks. Olive production, particularly has faced new challenges following the adoption of more intensive, higher-yielding and mechanized production practices. Adaptation strategies including chemical and biological control, as well as cultural practices to mitigate disease outbreaks will be discussed.

Thursday 23 November:

Conference Day Three

Keynote Address 6

217

Chasing spores: decision aids, fluid dynamics, GPUs, and 3D printing

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Agriculture producers are continuously faced with the conflicting goals of reducing their inputs and ecological footprint while meeting the ever-increasing demand for disease free products at reduced costs. This dilemma is further compounded by the low probabilities of current disease scouting approaches for detecting a problem early enough in the epidemic to avoid significant losses. This high risk makes growers favor approaches that minimize maximal regret and often results in less than efficient deployment of fungicide that can lead to development of fungicide resistance. Recent advances in molecular biology, computer sciences, and engineering can be leveraged to aid in monitoring pathogen presence, guide disease management decisions and assess risk of fungicide resistance. A 20+ year journey on developing inoculum monitoring of Erysiphe necator as a decision aid for timing fungicide applications will be presented. Along way, this goal led to new approaches to monitoring for fungicide resistance populations, improved understanding how air turbulence and canopies interact to disperse plant pathogens, and the biophysical modeling of pathogen dispersion and plant growth for the realistic simulation disease management practices impact disease development and pathogen evolution.

Keynote Address 7

207

Stakeholder engagement in research and extension programming: An essential process for managing recalcitrant plant diseases

Professor Lindsey du Toit[1]
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Despite decades of effort and resources, some diseases continue to plague plant production, much to the chagrin of plant pathologists. Effective engagement of stakeholders in research and extension efforts on plant diseases can be critical to gaining in-depth understanding of how the crops are grown and opportunities to enhance our capacity for practical management of the pathogens. This presentation reviews two case studies of recalcitrant vegetable diseases for which far more effective management has been facilitated by stakeholder engagement. The maritime Pacific Northwest is the only region of the USA with suitable climatic conditions for production of spinach seed, where ≤20% of the global supply of spinach seed is grown annually for western markets. However, the acid soils of this region have necessitated ≥15-year rotations between spinach seed crops to avoid major losses to Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *spinaciae*. By integrating a soil bioassay to quantify the risk of races 1 and 2 of the pathogen in growers' fields, screening spinach parent lines to quantify susceptibility to each race, optimizing soil tilth, and amending soils with agricultural limestone to increase pH, spinach rotation intervals can be reduced by ~50%, essentially doubling the carrying capacity for spinach seed production in that country. Onion bacterial rots are associated with a complex of bacteria that can be extremely difficult to manage. A multi-disciplinary research and extension project that engages stakeholders in examining the host, pathogens, environmental factors, and economics of bacterial diseases of onion is ensuring that disease management strategies developed are practical, viable, and sustainable. The mutual exchange of expertise by growers and other stakeholders with researchers is a cornerstone to the economic and logistical sustainability of agricultural production.

Session 5 A: New Technologies (Artificial intelligent (AI)) and Novel Methods in Plant Pathology and Disease Control

188

RNA interference (RNAi): dsRNA targeting pathogen-specific genes inhibits the physiological characteristics of *Botrytis cinerea* in chickpea

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Botrytis cinerea, the causal agent for Botrytis grey mould disease, is one of the devastating necrotrophic fungal pathogens responsible for significant yield losses in a wide range of crops, including chickpea. Repeated use of broad-spectrum fungicides may lead to loss of sensitivity through adaptation of the pathogen. Consequently, the use of double-stranded RNA (dsRNA) as RNAi-based bio-fungicides to target functional sequences triggering RNAi machinery are considered as a sustainable approach for managing fungal pathogens including *B. cinerea*. In this study, we demonstrated the impact of exogenous application of dsRNA targeting *B. cinerea* specific genes on the physiological characteristics of *B. cinerea*. For this, three-week-old chickpea plants were sprayed with BcDCL1/2 dsRNA or BcERG 13-1-11 (TripleStack) naked dsRNA and water, two days before inoculation (DBI) with *Botrytis cinerea* spores. Consequent physiological impacts of these treatments on *B. cinerea* growth indicators, were assessed 6-72 hours post inoculation (HPI). Of these, spore germination rate was significantly reduced, germ tubes were shorter and no appressoria formation was observed until 48 hours on leaves treated with DCL1/2 dsRNA or BcERG 13-1-11 (TripleStack) dsRNA. This indicated significant delays in pre-penetration events on the host tissues and subsequent reduced *In Planta* symptomology. Furthermore, using fluorescently labelled and exogenously applied dsRNA, the timing for dsRNA uptake from the leaf surface was determined to be 96-120 hours post application. Further investigations are required to assess potential for systemic movement of dsRNA and distal fungal protection. Together, these studies contribute to understanding the potential use of dsRNA target-specific sequences for systemic crop protection.

89

Double-stranded RNA for environmentally sustainable control of plant diseases

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We have been exploring the use of double-stranded RNA (dsRNA) as an environmentally sustainable control for plant diseases including Phytophthora root rot and rust myrtle rust, two aggressive diseases which are destroying natural plant populations across Australia and causing significant economic losses to horticulture, forestry and native plant industries. The approach, also known as spray-induced gene silencing (SIGS), involves exogenously applying pathogen-specific dsRNA to host plants, which is taken up by the invading pathogen to trigger RNA interference (RNAi) and silence essential pathogen genes. We have identified dsRNA molecules which have fungicidal effects against *Phytophthora cinnamomi* and *Austropuccinia psidii* in vitro and on planta. *A. psidii* appears to be particularly amenable to dsRNA-mediated control, with *A. psidii*-specific dsRNA significantly reducing urediniospore germination and the development of infection structures such as appressoria and infection pegs. dsRNA was similarly effective in planta, significantly reducing disease symptoms on detached leaves and in one-year old rose apple trees. We are now conducting RNA sequencing to investigate the mechanism of RNAi in *A. psidii*. We are also investigating different dsRNA delivery systems and whether dsRNA can provide plants with systemic protection. Our ultimate goal is to develop a clean green safe control strategy for these invasive diseases to safeguard Australian horticulture, forestry and native plant industries, with long-term benefits for conservation and biodiversity.

91

RNAi-based mechanisms of crop protection: uptake and translocation of dsRNAs targeting *Botrytis cinerea* in winegrapes.

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The fungal pathogen *Botrytis cinerea* causes botrytis bunch rot (BBR) in the common grapevine, *Vitis vinifera*. BBR epidemics in vineyards can cause substantial economic loss from a reduction in yield and a downgrade in fruit quality. Protection of grapes for winemaking requires integration of chemical and cultural control measures. Safe and effective chemical control options are limited, and the threat of fungicide resistance persists. Spray-induced gene silencing (SIGS) has potential to suppress BBR in wine grapes and be developed as new crop protection tool. We are investigating the efficacy and mechanisms of exogenously applied double-stranded RNA (dsRNA) targeting virulence-related pathogen genes in *B. cinerea*. Here we report results of dsRNA suppression of *B.cinerea* with in vitro and in planta assays using isolates collected from grapevines across Tasmania. Successful adoption of SIGS in grapevine depends on elucidating the mechanisms by which dsRNA enters and moves within grapevine tissues. We present results pertaining to the uptake of dsRNAs in leaves of *V. vinifera* and systemic movement of dsRNAs between tissues, including the inflorescence and developing berry. As part of a broader effort within the ARC Hub for Sustainable Crop Protection, the next stage of this work is to evaluate the efficacy and in planta movement of dsRNAs formulated as BioClay™ to aid persistence when applied under field conditions.

25

UMI-based high-accuracy full-length fungal rRNA sequencing using Oxford Nanopore Technologies platforms for accurate pathogen identification

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Accurate taxonomic identification is key to pathogen diagnostics and biosecurity. Sequencing of amplicons of marker genes, especially the ribosomal RNA (rRNA), has been widely used for identification of fungal plant pathogens. However, the read length of high-throughput sequencing platforms (e.g. 300 bp for Illumina sequencing) limited the taxonomic resolution of this method, leading to difficulties in differentiating closely related species. While long read sequencing technologies (e.g. Oxford Nanopore Technologies, ONT) are available, the interpretation of results was hindered by their high raw read error rates (~5%). The method developed by this study was able to generate full-length (~ 4000-8000 bp) fungal rRNA sequences using ONT platforms based on unique molecule identifiers (UMIs). UMI-tagged amplicons of the full-length rRNA were sequenced, and the consensus sequences of the original template molecules were generated based on UMIs, minimising the effects of sequencing errors. Assessment using a fungal mock community consisting of 34 isolates belonging to 20 species showed that this method was able to separate closely related species that short amplicons (ITS1/ITS2, LSU) failed to differentiate. Further assessment using over 300 visibly diseased plant tissues showed that this method was able to accurately identify disease-causing fungal pathogens that matched the symptoms. Overall, the results demonstrated that this UMI-based high-accuracy full-length fungal rRNA sequencing method could be used for accurate pathogen identification. Over 500,000 high quality full-length fungal rRNA sequences have been generated using this method, representing an invaluable resource for biosecurity, fungal pathogen diagnostics and fungal taxonomy due to the greatly improved taxonomic resolutions compared to the shorter amplicons.

211

A Novel and Rapid Method of Screening Sugarcane Variety for Red Rot Resistance

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Red rot is an important disease of sugarcane, caused by the fungus *Colletotrichum falcatum* worldwide. In the Sugar Research Australia's (SRA) variety-development program in Australia, breeding lines are screened for resistance to red rot before releasing for commercial production. Although, the existing method of screening for red resistance is effective, it takes 12 to 15 months to obtain the resistance rating. A rapid method of screening for resistance to red rot was developed, using six-months-old sugarcane stalks, inoculated with red rot fungus. In addition, two other methods, controlled condition testing (CCT), a method used in India, and a leaf midrib inoculation method were tested. In the new method, six sugarcane lines with known ratings were inoculated with red rot culture through holes made in the middle of the two-eye-setts and incubated at 30°C and 90% relative humidity for 2 weeks. Inoculated setts were split longitudinally and visually assessed for symptoms using standard disease indices and photographed. The images were analysed using the machine-learning algorithm Classification and Regression Tree to estimate the percentage of symptomatic pixels as image cover. Symptom expression was poor in the setts inoculated using the CCT method. The leaf midrib method showed no differences among the inoculated leaves. The new method produced excellent symptoms in all inoculated sugarcane setts, and visual indices of disease showed strong correlation (r=0.99) with the historical ratings of breeding lines. Image cover correlated strongly with disease indices (r=0.93) and historical red rot rating (r=0.88). The new method along with image analysis can substantially shorten the time required for screening for red rot s from over a year to about 3weeks. This approach will be implemented to screen for resistance to red rot in the SRA variety-development program.

50

Phenotyping for quantitative resistance to *Leptosphaeria maculans* in *Brassica napus* (rapeseed): A framework using machine learning and artificial intelligence (MLAI)

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Phenotyping of plant diseases has not kept pace with the rapid progress in genetic and genomic characterisation and is a bottleneck for breeding. Blackleg crown canker (caused by *L. maculans*) of *B. napus* causes large economic losses but breeding for quantitative resistance is challenging due to the large number of genes involved and the lack of an economically viable non-subjective phenotyping method deployable at the scale required for in-field screening in a breeding context. To overcome this issue, we developed machine learning and artificial intelligence (MLAI) techniques for automated assessment of crown canker severity in cross-sections of canola stems at plant maturity using RGB images. The MLAI algorithm was trained on a data set of 4000 images to extract the region of interest (ROI), resulting in an overall accuracy of 88%. Moreover, the disease was quantified based on the pixel values extracted from the images. To ensure the accuracy of our approach, we validated the results by comparing them with standard visual assessments. Overall, the MLAI framework provides an automated disease assessment approach which could form the basis to identify the genes underlying QR and help growers to have resistant varieties of canola to reduce Blackleg related yield loss.

Session 5 B: Epidemiology, Ecology, Modelling and Risk Analysis

60

Journey to the core: black core rot in citrus

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Black core rot in citrus is caused by *Alternaria alternata* and is a common disease in southern Australian citrus orchards. The disease is characterised by internal fruit rot and fruit drop. The incidence of black core rot impacts yields and if diseased fruit reaches the consumer, it may affect repeat purchases of citrus fruits. Its occurrence is erratic between varieties, regions and seasons, resulting in a varied impact on industry. The identity and diversity of the black core rot pathogen, as well as the disease occurrence and epidemiology, remain unclear. This has left the Australian citrus industry with little capacity to effectively manage the disease. The aim of this project is to improve our understanding of disease aetiology and epidemiology. Extensive sampling from citrus orchards across three Australian states has identified the pathogen as *A. alternata*. A field trial in a commercial orchard in the Riverina, New South Wales, was established to identify the main infection periods of black core rot. Ongoing sampling of citrus foliage and fruit during different physiological growth stages aims to pinpoint timing of infection as well as the source of inoculum. Studies investigating the relationships between infection, inoculum sources, disease incidence and impact are underway.

29

Genetic diversity of poleroviruses and luteoviruses in cereals and grasses in south-eastern Australia

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Poleroviruses and luteoviruses, including cereal yellow dwarf viruses (CYDV) and barley yellow dwarf viruses (BYDV), cause substantial yield and quality losses in cereals such as wheat, barley and oats, worldwide. Despite their importance and prevalence in Australia, little is known about the genetic diversity of these viruses in cereals and grasses in Australia, where the majority of studies have been conducted using serological techniques which are unable to differentiate between closely related species. In this study, tillers were collected from cereals and grasses from cereal fields across western Victoria during October 2020. Samples were tested for viruses using tissue blot immunoassay (TBIA), reverse-transcription polymerase chain reaction (RT-PCR) and high-throughput sequencing (HTS). Our results show that more polerovirus and luteovirus species are present in cereals and grasses in Australia than those that are currently described, and that although many of them might be detected by the current serological tests, they don't all fit neatly within the current species classifications. This information is particularly important for the more targeted development of virus-resistant cultivars and more effective diagnostic tests, which are being used to further assess the diversity and distribution of these important viruses.

46

Impact of sowing CMV-infected seed on growth and yield of lentil

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Lentil (*Lens culinaris*) is an important food legume crop and is considered a main source of protein. Lentils are susceptible to several viral diseases, but cucumber mosaic virus (CMV) can be considered the most important. It is transmitted through seeds and by aphid vectors. Virus surveys of pulses in Victoria in previous years have indicated that CMV is common in lentils in south-eastern Australia, but data is lacking on its impact on growth and yield of lentils. In this study, field trials were conducted at four locations in south-eastern Australia during 2022 to quantify the yield risk of planting CMV-infected lentil seed. In preparation for field trials, levels of CMV infection in 143 seed lots were assessed in the glasshouse, seedlings were tested for CMV using tissue blot immunoassay (TBIA) one month after sowing. CMV was detected in seeds of three lentil varieties SP1333, Eston and Indianhead with infection levels of 0.7%, 2% and 3% respectively. These varieties were sown in field trials in a randomised complete block design at four locations. Plant height was measured before maturity and the field plots were tested for CMV infection during the season to assess virus presence. The mean incidence of CMV across all the sites in SP1333, Eston and Indianhead was 24%, 13% and 8% respectively, while the mean incidence in control plots did not exceed 1%. The average plant height was lower in the virus-infected varieties compared to control ones. Plots were machine harvested and the grain yield from each plot was measured. Overall, mean yield across all trial sites was higher in the control plots than the virus-infected treatment plots. In general, in the virus infected SP1333 treatment plots, the higher the incidence of CMV, the lower the yield from that plot.

104

A century of banana disease epidemics

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Bananas are the world's favourite fruit with over 100 Billion consumed annually. Lately bananas have been in the news in Australia because of an incursion of freckle in the Northern Territory and an incursion of fusarium wilt TR4 in Tully as well as the global spread of TR4 to many banana producing countries. The early expansion of banana cultivation experienced very little disease problems which led Gäumann to state in 1922 "that in publications of tropical plant diseases until about 1910 banana diseases typically did not rate a mention". This statement, valid at the time is certainly no longer true today as the production of bananas is threatened by a range of major plant diseases spreading around the globe including Yellow Sigatoka, Black leaf streak, Eumusae leaf spot, Freckle, Fusarium wilt Tropical race 4, Banana bunchy top and the bacterial wilts Moko, Xanthomonas wilt and Banana blood disease. Data of the spread of these pathogens over the last century will be presented and the nature of spread over time of these diseases and the threat they pose to the large-scale monoculture plantations of the variety "Cavendish". The spread of these diseases and a global banana industry reliant on a single variety has resulted in an extreme level of genetic vulnerability of which the consequences will be discussed. The resistance to diversification in the Cavendish production chain and the lack of investment in genetics and plant breeding in the recent past means that currently very limited genetic solutions are available to replace the Cavendish banana with market acceptable resistant varieties utilising a range of different genetic backgrounds.

57

Understanding leaf wetness in Western Australian table grape production for disease modelling

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Leaf wetness duration is a key parameter for the infection and establishment of several economically important grapevine diseases. However, a combination of a lack of internationally recognised standards for the placement of leaf wetness sensors in canopies and sensor reliability, has meant accurately measuring leaf wetness is challenging. To understand the characteristics of leaf wetness in table grape vineyards in Western Australia (WA), leaf wetness sensors were placed in four vineyards in geographically different growing regions, Carnarvon, Swan Valley and Hamel, over three growing seasons.

Two brands of sensors were compared, the leaf wetness sensor (LWS) (Pessl Instruments) attached to iMetos 3.3 weather stations, and the PHYTOS 31 LWS (METER). The Pessl LWS records wetness via the conductivity on a filter paper between two stainless steel electrodes, while the PHYTOS 31 measures the dielectric constant on the upper surface of the sensor. The LWS's were placed at 1.5 metres high in the canopy on a 45° angle, facing south.

Over the three seasons of data collection, no significant difference was found between the type of LWS used for leaf wetness duration. A prediction model for leaf wetness is developed in each location, employing regression trees that incorporate specific constraints such as dew point, humidity, and temperature. A zero-inflated model was utilised to account for the inherent variability of rainfall. Each model undergoes testing and training through a ten-fold cross-validation approach with statistical error measurements of Mean Absolute and Mallow's Cp, among others.

The outcomes of this work will assist in the validation of disease models or decision support systems for endemic diseases, and risk profile of pathogens of biosecurity concern, for table grapes across multiple production regions in WA.

39

Grapevine trunk disease pathogen spore detection varies within and around vineyards

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Eutypa (ED) and Botryosphaeria dieback (BD) are grapevine trunk diseases caused by fungal spore infection via wounds. Burkard spore trapping in vineyards detected ED and BD pathogens throughout the year, predominantly from autumn to spring, in association with rainfall. Rotorod spore traps, analysed using qPCR assays, provide a research tool to understand the variability of spore detection in and around vineyards and the effect of management practices. In a 17-year-old Shiraz vineyard with substantial dieback symptoms located at the Waite Campus in Urrbrae, South Australia (SA), spores of both ED and BD pathogens were detected in five positions in and around the vineyard. ED pathogen spores were predominant, with an increasing gradient of spore numbers from the north-east corner to the southern edge of the vineyard, consistent with predominant wind recorded from the north. Spores were detected at heights from 40-250 cm, with greatest numbers at cordon height (100 cm). Lower spore numbers were detected at 40 cm, the height of the Burkard air intake orifice, suggesting that the Burkard spore trap may underestimate spore numbers. Rotorod spore traps were run for durations from 1 to 23 h and revealed that at least 23 h is required for optimal spore detection. To understand the impact of remedial surgery on spore detection, six rotorod spore traps were deployed in a 20-year-old Shiraz vineyard in the Barossa Valley, SA with substantial dieback symptoms, which had 15 rows remediated in the previous year. Spore detection was greater within control vines compared to remediated vines, due to the removal of infected wood. Although remedial surgery reduced infection risk, the presence of spores, presumably from sources outside of the vineyard, indicated wound protection is still necessary. Ongoing research is utilising rotorod spore traps to evaluate the detection of spores at distances from an infected vineyard.

Session 5 C: Diagnostics, Biosecurity

10

NEW METHOD FOR NUCLEIC ACID ISOLATION AND QUANTIFICATION FOR THE DETECTION OF RATOON STUNTING AND LEAF SCALD PATHOGENS OF SUGARCANE

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Australia is the third-largest raw sugar supplier in the world, generating 4-4.5 million tonnes of sugar from 30-35 million tonnes of cane annually. Major diseases including ratoon stunting, leaf scald, smut, chlorotic streak, and red rot, however, can significantly reduce sugarcane productivity if they are not managed properly. Accurate pathogen detection at an early stage of infection is thus critical for disease prevention and yield loss reduction. Significant progress has been made in the diagnosis of sugarcane pathogens during the last decade. The standard molecular methods currently employed by the Australian sugarcane industry to detect the causal agents of endemic and exotic diseases include next-generation sequencing (NGS), high-throughput metagenomic sequencing, and quantitative polymerase chain reaction (qPCR). Although robust, current diagnostic approaches include a multi-step sample processing stage starting with nucleic acid extraction and purification, which is time-consuming, laborious, costly, and needs skilled personnel and advanced laboratory facilities. This intensive sample preparation process also renders these methods inappropriate to use in resource-poor settings. Here, we are introducing a novel single-step method for in situ nucleic acid isolation and quantification from various sample types of sugarcane plants, such as leaves, meristematic tissues, and xylem sap. The method uses the benefits of traditional isothermal amplification-based quantification and naked eye detection, with positive results detected by a colour shift from pink to greenish yellow. To date, the method has sensitively detected *Leifsonia xyli* subsp. *xyli* and *Xanthomonas albilineans*, the causal pathogens of ratoon stunting and leaf scald diseases of sugarcane, respectively where the detection threshold was 1 cell/μL with good linearity (R^2 = 0.99) and reproducibility (%RSD <5%). This approach has the potential to be integrated into a handheld device and used by growers in the field to detect other sugarcane pathogens.

107

First report of *Neopestalotiopsis* spp associated with leaf spots of avocado and mango in Champasak Province, Lao Peoples Democratic Republic

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The authors initiated studies on the fungal species associated with leaf spots of horticultural tree crops
in Champasak Province, Lao Peoples Democratic Republic (Lao PDR), in 2019. *Calonectria pseudoreteaudi* was found associated with a distinctive leafspot on macadamia in 2019 (Phanthavong et al, 2020).
During February 2023 dark brown localised leaf spots were observed on a young avocado tree in the garden of the CPC Coffee Shop approx. 12 km west of Paksong on the Bolaven Plateau in Champasak province.
A brown leaf spot was also observed on a small mango tree at Wat Phou, near Champasak township in the
same province. Samples of both leafspots were collected and taken to the Diagnostic Laboratory at the
Champasak Provincial Agriculture and Forestry Office in the provincial capital, Pakse, where fungal cultures
were isolated from leaf spots of both hosts, purified and tentatively identified as *Pestalotiopsis* species.
A pure culture of the isolate from each host was sent to the International Centre for Microorganisms
From Plants (ICMP) in New Zealand for accession and identification. Duplicate cultures were also sent to the Department of Plant Pathology at the University of Sassari in Italy.
Preliminary identifications at ICMP based off ITS and 16S sequences, and morphology, indicated both isolates belong to the genus *Neopestalotiopsis*, and are possibly novel species. The results of molecular identification with ITS (regions ITS1-ITS2) with primers ITS1 and ITS4 at the University of Sassari supported the findings of the ICMP studies.
Additional genes will be sequenced and the findings reported at the APPS Conference.
Pathogenicity tests will be undertaken in Lao PDR.

96

National coordination of high-throughput sequencing (HTS) data for a connected diagnostics system

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Plant Health Australia was contracted by the Australian Government Department of Agriculture, Fisheries and Forestry to undertake the project: Phase 1. National coordination of high-throughput sequencing (HTS) data for a connected diagnostics system. This project developed an agreed foundation for the design and scope of a national high-throughput sequencing (HTS) database for plant pests, as well as developing data standards and governance arrangements for the database.
Growing numbers of travellers and increasing volumes of goods entering Australia place further pressure on Australia's biosecurity system for faster and accurate identification of plant pests and pathogens, especially where these may be exotic species, national priority plant pests, industry high priority pests and trade sensitive established pests.

HTS technologies offer a rapid, reliable and cost-efficient diagnostic platform to identify pests and pathogens in a single test, increasing Australia's diagnostic capacity, and delivering rapid, more accurate results to support both plant pest emergency responses and area freedom. However, the vast amount of data being generated by HTS technology has created a need for a secure, centralised storage database to support the uploading, sharing and analysis of trusted plant pest genomic data, with the potential to link to other diagnostic databases such as the Australian Plant Pest Database and the Pest and Disease Image Library.

Plant Health Australia will present the results of this project.

181

MALDI-ToF mass spectrometry is an effective tool for plant pathology

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Matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is a mature technology, deployed globally for bacterial identification in human and veterinary disease diagnostics, and food testing. MALDI-ToF MS measures the mass of molecules in a sample, with mass spectra from test specimens being compared against libraries of reference spectra ('fingerprints') to provide a list of nearest-matching species. As the mass spectra primarily feature ribosomal proteins, MALDI-ToF MS is a proteomic approach. The technique parallels genetic sequencing and can be performed at a fraction of the consumable costs and time. The value of MALDI-ToF MS for plant pathology is gaining global recognition, being included in recent EPPO diagnostic protocols for bacteria. Our biosecurity laboratory recently acquired a MALDI Biotyper® sirius (Bruker Pty Ltd). Our review of the 709 genera/4,274 species in the Bruker bacterial fingerprint library found more than 70 species of plant pathogens, from 28 genera. Similarly, their library of 65 genera/232 species of fungi includes over 60 species, across 31 genera, known to cause major plant diseases or post-harvest soft rots. MALDI-ToF MS improved our diagnostic workflow in plant pathology by providing identifications or indications at three decision points. First, directly from mycelium, including macro-fungi, or bacterial ooze on symptomatic material. Secondly, from primary culturing, enabling efficient selection of colonies to take through to pure isolates. Finally, from pure isolates, contributing confident identification at the level of species or genus. Detections and indications of non-pathogens have been equally valuable as detection of pathogens. MALDI-ToF MS provides rapid, cost-effective diagnostics, with minimal training required for basic, routine use. It is relatively easy to supplement the Bruker libraries by creating and sharing fingerprints from verified cultures. MALDI-ToF MS promises to be a valuable additional tool for diagnostics and research in plant pathology.

183

Modernising routine diagnostics for the management of viruses for the horticulture industry

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Viruses can cause significant losses to many vegetable crops, with effective vector control being the only limited option for control. Rapid, cost-effective diagnosis of these crops can inform effective management strategies. We have generated LAMP and multiplex qRT-PCR assays for vegetable infecting viruses for a more cost-effective solution to virus diagnostics. A potyvirus probe-based multiplex rt-qPCR was developed to detect four possibly occurring cucurbit infecting viruses in Queensland: cucumber green mild mottle virus, papaya ringspot virus, zucchini yellow mosaic virus and watermelon mosaic virus. The assay was optimised by testing different probe and primer ratios and validated with multiple locally occurring isolates. Rapid field-capable LAMP assays have been developed for typical viruses of solanaceous crops, such as capsicum chlorosis virus, tomato spotted wilt virus, tomato yellow leaf curl virus, tomato leaf curl virus, potato virus Y and potato leafroll virus. These assays have proven rapid, specific, sensitive, and more cost-effective than reverse transcriptase based assays. The newly developed assays are frequently used in our routine diagnostic survey samples for research projects and commercial clients, allowing rapid and sensitive diagnostics which contribute to management decisions affecting commercial success in the Horticulture industry.

Session 5 D: Integrated Disease Management/ Biological Control

88

Current Status of Banana Bunchy Top Disease in Indonesia and Its Alternative Control Strategy

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Various banana cultivars with different genome types (AA, AAA, AAB, ABB) are grown in Indonesia to meet domestic and export needs. Banana bunchy top disease (BBTD) caused by BBTV is an important disease that has the potential to affect banana production in Indonesia. Field surveys conducted in recent years indicated that BBTD has been found in almost all banana growing areas in Indonesia. Most commercial banana cultivars are known to be susceptible to BBTD. Transmission study of BBTV in the greenhouse showed that several wild banana species native to Indonesia, such as *M. acuminata* subsp. *malacensis*, *tomentosa*, *breviformis* and *macrocarpa* are moderately resistant to BBTD. One of the strategies attempted to manage BBTD is to increase the resistance of banana cultivars to BBTD through the provision of priming agents. Two separate experiments were conducted to evaluate the effectiveness of the priming agents, i.e. (1) application of liquid smoke at several concentration levels and time (since the shoot multiplication phase, since the rooting induction, and in the acclimatization phase; (2) application of *Pseudomonas fluorescens* and guano filtrate before and after BBTV infection. Liquid smoke treatment had a significant effect on the number of shoot multiplication and growth of banana plantlets; and it reduced disease incidence. *P. fluorescens* and guano filtrate was able to significantly reduce the intensity of the disease only when applied before BBTV infection occurred. This study proves that liquid smoke treatment on banana tissue culture and the use of beneficial microbes has the potential to increase plant growth and resistance to BBTD. Therefore, this treatment can be recommended as part of BBTD control strategy.

70

What we know and don't know about alternate host crops and Verticillium wilt of cotton.

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Verticillium wilt causes yield loss in many crops worldwide and is an important economic constraint to cotton production in Australia. The causal fungus, *Verticillium dahliae* Kleb, has a wide host range of over 300 plant species including weeds and economically important agricultural tree, vegetable and field crops. Recognised as a host-adapted pathogen, new plant species are being reported succumbing to the disease worldwide. The longevity of the pathogen in infected fields is aided by the ability of the pathogen to survive for up to 14 years or more as microsclerotia in soil and plant residues. Disease severity is influenced by many variables including the environment, host, plant tolerance, nutrition, the pathotype present and the virulence of the pathotype. More than one strategy is required to manage this disease and crop rotation is one tool recommended as part of an integrated disease management approach. Field trials in Northern NSW have highlighted that maize and sorghum are both good options to aid in reducing soil inoculum levels compared to continuous cotton. Glasshouse pathogenicity tests with two *Verticillium* pathotypes has revealed that some crops commonly rotated with cotton in NSW and Qld are in fact susceptible to infection, and seedborne in some. This may have implications for disease management with these crops having potential to aid pathogen survival between susceptible cotton crops.

156

Seed-coating with *Trichoderma atroviride* based generic bio-inoculant to control soil-borne and seed-borne diseases of some vegetable crops in New Zealand.

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A generic *Trichoderma atroviride* based bio-inoculant for disease control/plant growth promotion would benefit New Zealand's vegetable industry. The bio-inoculant must be capable of controlling both fungal/bacterial and soil-borne/seed-borne diseases in various hosts and under different production systems. Selected strains of *T.atroviride* isolated from New Zealand soils were screened for these attributes by testing them against the following disease/host systems:

Soil-borne diseases
Violet root rot (caused by *Rhizoctonia crocorum* / *Helicobasidium purpureum*) of carrot (*Daucus carota*)
White-rot (caused by *Sclerotinia sclerotiorum*) of dwarf bean (*Phaseolus vulgaris*)
Rhizoctonia root rot (*Rhizoctonia solani*) of red radish (*Raphanus sativus*)
Aphanomyces root rot (*Aphanomyces euteiches*) of garden pea (*Pisum sativum*)
Fusarium root rot (*Fusarium solani*) of tomato (*Solanum lycopersicum*)

Seed-borne diseases
Black-rot (caused by *Alternaria radicina*) of carrot (*D. carota*)
Black rot (caused by *Xanthomonas campestris* pv *campestris*) of cabbage (*Brassica oleracea*).

Depending on pathogen and host, a single strain of *T.atroviride* or a four strain mixture was applied as a seed treatment The growing medium was soil naturally infested by the pathogen (field and glasshouse experiments), potting-mix to which known amounts of the pathogen had been added, or a hydroponic growing system (both glasshouse experiments). The presence of *T. atroviride* enhanced plant growth, both in the absence and presence of the pathogen, significantly reduced disease incidence in all the systems assessed, and significantly increased yield in carrot, dwarf bean, radish and garden pea. The bio-inoculant was used successfully in a hydroponic system (tomato, cabbage). Work is now in progress with an industry partner to develop a commercial seed treatment product.

59

Simultaneous metabolomics and transcriptomics identifies key antifungal compounds and biosynthetic gene clusters during liquid-state fermentation of a *Streptomyces* biocontrol agent

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New, safe and sustainable fungicides are required to fill a growing gap in the crop protection market left by a decline in acceptance or increasing regulation of synthetic chemical controls. An opportunity exists to exploit bacteria such as *Streptomyces*, well known for their ability to produce antimicrobials but where up to 90% of their biochemical potential is yet to be discovered and characterised. From a unique terrestrial and plant-associated collection of *Streptomyces* isolated from south-west Western Australia, we identified through traditional in vitro bioactivity screens a *Streptomyces* strain linked to potent antifungal activity against a broad range of necrotrophic fungal pathogens of crops. Whole genome sequencing revealed this strain encoded a predicted repertoire of 50 biosynthetic gene clusters and capacity to produce up to 25 novel compounds. To facilitate identification of causal gene clusters and develop fermentation strategies for improved industrial scale antifungal compound production, we undertook a simultaneous targeted metabolomics and untargeted transcriptomics (RNAseq) approach to subsample *Streptomyces* cells, cell-free extracts and volatiles during a fermentation time-course and link differential gene expression and metabolite patterns to bioactivity. The process enabled rapid identification of candidate biosynthetic gene clusters underpinning bioactivity.

65

Interaction between the ginger soft rot pathogen *Pythium myriotylum* and the biocontrol agent *Pythium oligandrum*

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The use of natural antagonistic microorganisms, such as *Pythium oligandrum*, is a promising environmentally friendly approach to biologically control Pythium soft-rot disease of ginger (*Zingiber officinale*) caused by *Pythium myriotylum*. A critical part to improve the efficiency of ginger disease control is to understand both the mechanisms of action of *P. oligandrum*, and the mechanisms by which *P. myriotylum* responds. *P. oligandrum* antagonism involves hydrolytic enzymes such as cellulases and proteases, and recently we uncovered a role for *P. oligandrum*-produced volatile organic compounds in antagonism of *P. myriotylum*. The effects of these mechanisms are apparent from major morphological and cytological damage to *P. myriotylum*. The mechanisms by which *P. oligandrum* volatile compounds control ginger disease also involves ginger-mediated mechanisms such as growth promotion. Part of the response of *P. myriotylum* to *P. oligandrum* proteases appears to involve the upregulation of protease inhibitors. Also, in response to *P. oligandrum* volatile compounds, *P. myriotylum* showed a strong upregulation of putative detoxification-related genes. Overall, the responses of *P. myriotylum* to *P. oligandrum* suggest ways to reduce the ability of *P. myriotylum* to defend against and counter-antagonize *P. oligandrum* (e.g., by targeting *P. myriotylum* protease inhibitors or detoxification genes), and thus improve the efficiency of *P. oligandrum*-mediated ginger disease control. The interaction between *P. myriotylum* and *P. oligandrum* is doubly interesting because it is one of the few examples where the plant pathogen and biocontrol agent are from the same genus (i.e. the oomycete *Pythium* genus).

Sheikh, …, Wei and Daly (2023). Volatile Organic Compounds from Pythium oligandrum Play a Role in Its Parasitism on Plant-Pathogenic Pythium myriotylum. Appl Environ Microbiol 89(2): e0203622.
Daly, ..., Wei (2021). Dual-transcriptomic, microscopic, and biocontrol analyses of the interaction between the bioeffector Pythium oligandrum and the Pythium soft-rot of ginger pathogen Pythium myriotylum. Frontiers in Microbiology. 12(3461).

Session 6 A: Integrated Disease Management/ Biological Control

72

Potential for using rhizobacteria for biological control of barnyard grass (*Echinochloa crus-galli*) in rice fields

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Barnyard grass (*Echinochloa crus-galli* (L.) Beauv), a significant weed in paddy rice, is an annual species originally from Asia but has now spread globally. Currently, the main method of weed control is herbicide application. However, this may negatively impact on human health, the environment and food safety, and over-use can facilitate herbicide resistance in weeds. To address these concerns, the use of beneficial rhizobacteria presents an ecological alternative for weed management. In this study, we assessed the potential bioherbicidal effects of rhizobacterial strains isolated from the rhizosphere of barnyard grass. The root rhizosphere, which consists of microbes loosely attached to plant roots, was collected from three locations in the Northern Inland region of NSW. A total of 387 rhizobacterial strains were isolated in pure culture from 135 samples of barnyard grass rhizosphere. These strains were evaluated for their bioherbicidal potential against barnyard grass and rice in both in vitro and greenhouse experiments. Four soil microbial isolates, including one strain of *Pseudomonas fluorescens* and three strains of *Enterobacter ludwigii*, significantly reduced the growth of barnyard grass without inhibiting rice growth. These results highlight the possibility of developing these specific strains as bioherbicides for barnyard grass management, offering a promising alternative to conventional herbicide use.

203

Biological control of dieback disease of mango caused by *Lasiodiplodia theobromae* using endophytic actinobacteria in the United Arab Emirates

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Twenty-seven streptomycete and 11 non-streptomycete actinobacteria were isolated from surface-disinfested mango roots and evaluated for their potential to produce chitinase and to inhibit the growth of *Lasiodiplodia theobromae*, the causal agent of mango dieback in the United Arab Emirates. The most inhibitory isolate was identified as *Streptomyces griseus* which produced relatively high levels of chitinase and degraded the hyphae of *L. theobromae in vitro* causing extensive plasmolysis and cell wall lysis. A crude culture filtrate of *S. griseus* exhibited antifungal activity and significantly reduced ($P<0.05$) spore germination and germ-tube growth of the pathogen. The antagonist was recovered from inside the root at all samplings up to 8 weeks after inoculation indicating that the roots of healthy mango may be a habitat for the endophyte. *S. griseus* significantly reduced ($P<0.05$) the severity of the dieback disease under greenhouse conditions. An endophytic Streptomyces sp. isolate incapable of producing chitinase that did not produce detectable levels of chitinase, did not lyse hyphae of *L. theobromae* or reduce dieback in the greenhouse experiments, although colonization of the mango root by both these isolates was similar to that of the chitinase-producing wild-type isolate of *S. griseus*. This study is the first record of control of a soil-borne plant pathogen by a chitinolytic actinobacteria, endophytic in mango roots.

197

The focus on biocontrol in response to European constraints on fungicides

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With agricultural production largely reliant on crop protection products to achieve greatest yield potential, there is ever increasing pressure by consumers and the European Union (EU) to reduce the use of synthetic chemicals. Registration regulations may result in either outright banning or restricting their use by imposing low maximum residue limits (MRLs). Furthermore, resistance is a real threat to the viability and efficacy of many fungicides. These factors have a significant impact on what Australian producers can use to effectively grow and export produce.
For these reasons, the use and interest in biological control is rapidly increasing. Where once biocontrol was viewed as variable, unreliable and lacked data, biological products are now screened with more scientific rigor and offer good efficacy against many key diseases across a wide range of crops.

Biological agents offer a different mode of action, mitigate the risk of fungicide resistance and are perceived as safe to the environment. Additionally, biologicals provide an alternative option for acceptable disease control.

Bacillus species are well known for their role in biocontrol. Taegro fungicide (*Bacillus amyloliquefaciens* strain FZB24), is a recent development that is backed by robust scientific data and evaluation. It acts by competition for nutrients and space, releases anti-fungal metabolites and improves plant response to pathogen attack by triggering induced systemic resistance. Under greenhouse and laboratory conditions, Taegro showed consistent survival up to 10 days after application. Replicated trials conducted in vineyards showed Taegro improved control of powdery mildew (*Erysiphe necator*) and botrytis (*Botrytis cinerea*) by 10-30% when used to complement a conventional fungicide spray program. Results also showed Taegro improves disease control when used solo or when used close to harvest. The outcomes of this research provide the viticultural industry with a new option for biological control in Australian growing conditions.

45

Streptomyces strain improvement for crop protection applications

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Fungal pathogens account for 70-80% of all diseases associated with crops, affecting crop yield and quality. Development of resistance to fungicides in these pathogens is a growing concern. There is increasing interest in alternatives to chemical fungicides and solutions that are environmentally friendly, less toxic and biodegradable.

Streptomyces sp. are extensively studied gram-positive bacteria for their secondary metabolites which have antibiotic, antifungal, or anti-inflammatory properties. As such, microbial-derived crop-protection products like *Streptomyces* offer promise in crop protection. We are interested in identifying *Streptomyces* strains with strong antifungal activity that can be used to protect crops from fungal pathogens either applied as a bacterial spray or as cell-free products containing a biological cocktail. One roadblock to their uptake in-field is their relative efficacy and manipulative capacity.

In a strategy akin to production of pharmaceuticals, our approach to the problem is through domestication and improved efficacy of microbials for biocontrol. Using UV mutagenesis, we generated mutants for some of the *Streptomyces* strains from the CSIRO Actinobacteria collection. The mutants are screened using a standardised pipeline using solid-phase extraction followed by a cell-imaging based high-throughput bioactivity assay to measure the fluorescence from fungal pathogen spores such as *Verticillium dahliae* and *Fusarium oxysporum* tagged with reporter genes such as GFP inoculated with the concentrated metabolite cocktail from the Streptomyces mutants. If the *Streptomyces* mutants confer greater antifungal activity than the wild type, then no or reduced growth of the fungal spores can be observed as no fluorescence or reduction in fluorescence signal.
The screening pipeline is semi-throughput as it requires extraction and concentration of secondary metabolites from each of the mutants. Experiments are underway to develop a biosensor that could be used in a high-throughput screening platform minimizing the time and effort required for screening mutant libraries and *Streptomyces* strains from the culture collection.

77

Woodchip amendment can alter banana soil microbial abundance and disease-suppressive potential

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Long-term banana cultivation alters soil microbial communities compared with areas of native vegetation. Specifically, the fungal microbial communities in banana soils are less abundant and less diverse, allowing potentially pathogenic groups of fungi, such as *Fusarium oxysporum* complexes, to dominate. Woodchip amendments could potentially increase soil fungi, leading to greater disease suppression in banana soil. We tested three rates equivalent to 2, 20 and 100 t/ha of woodchips from leguminous trees, *Erythrina* sp., *Gliricidia* sp., and *Leucaena* sp., incorporated into the soil used for commercial banana production. One month after adding woodchips, Ducasse (Musa ABB) was planted into the soil. One month later, the plants were inoculated with a spore suspension of *Fusarium oxysporum* f. sp. *cubense* (Foc) Race 1 (VCG 0124). Results indicated that the incorporation of woodchips did not significantly impact the growth of banana plants except for the 100T/ha rate of *Gliricidia* sp. Woodchip amendment significantly increased the abundance of bacteria, archaebacteria and fungi in the soil compared to non-amended soil. Fungal biomass was significantly increased following the application of *Leucaena* sp., whereas bacterial biomass was enhanced following the incorporation of *Erythrina* sp. An increase in the rate of woodchip application increased the proportion of omnivorous nematodes and decreased the proportion of bacterivores. Woodchip amendments using *Erythrina* sp. and *Leucaena* sp. can increase the soil microbial biomass, which reduces FocR1 colonisation of the banana rhizome.

16

Stem colonisation by *Fusarium pseudograminearum* during cereal development and after harvest

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Fusarium crown rot (FCR) is one of the most important diseases affecting cereal crops in the northern grain growing region of Australia. *Fusarium pseudograminearum* (Fp) is the fungal species responsible for majority of infections in this region, surviving in infected cereal stubble for three years or longer. If adequate moisture is available, further colonisation of cereal stubble post-harvest (saprotrophic colonisation) may occur during intercropping and non-host periods, but this is not routinely considered in the integrated management of FCR. We therefore examined the growth of Fp within stems of cereal cultivars across both the pathogenic and saprotrophic phases. Ten cultivars including bread wheat, durum wheat, barley, and oat species, with a range of susceptibilities to FCR were tested. Both quantitative polymerase chain reaction (qPCR) and agar culturing was used to identify DNA levels and the extent of Fp colonisation. Four time points were assessed: stem elongation and anthesis (pathogenic phase), and maturity and two weeks post-harvest (pathogenic phase). During the pathogenic phase, some cultivars with higher FCR resistance ratings such as oat experienced lower FCR symptoms and pathogen DNA, and lower heights of Fp colonisation. Further colonisation by Fp after senescence (maturity and post-harvest) was observed in all cereal species tested, and largely resulted in an increase in fungal biomass and vertical progression of Fp within infected stems. Colonisation in the saprotrophic phase did not align with genetic resistance traits, with colonisation up to 23.5 cm higher in the highly resistant wheat germplasm LRC2012-122 compared with the most susceptible wheat cv. Kittyhawk. Growing cereal cultivars with improved FCR resistance therefore remains important for limiting disease and pathogen progression during the growing season but will not provide protection against saprotrophic colonisation of stubble by Fp after plant senescence.

Session 6 B: Microbiomes and Disease Complex and other

31

Pathogenicity and identification of a novel collar and root rot *Fusarium oxysporum* associated with yield decline of Australian processing tomatoes

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The Australian Processing Tomato Industry (APTI) in northern Victoria has experienced a yield decline over the last decade. Previous studies found that declining yield of processing tomatoes grown under sub-surface drip irrigation was in part due to diseases caused by soil-borne pathogens. Infected plants were characterised by stunting, collar and root lesion, poor root growth and ultimately yield loss. *Fusarium oxysporum* was identified as a major pathogen of processing tomatoes from previous trials. This study identified twenty *F. oxysporum* isolates using morphological and molecular based techniques, and several glasshouse bioassays were conducted to assess their pathogenicity, plant physiological parameters were measured. It found that most of the pathogenic isolates showed positive for *Fusarium oxysporum* f.sp. *lycopersici* (FORL) race 3 according to PCR-based differentiation, and various pathogenicity was hence determined from the glasshouse bioassays.

172

Amendment effects on soilborne pathogens and microbiome diversity in calcarosol soils

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Soilborne fungal pathogens are one of the major constraints to plant health and productivity in calcareous soils. Edaphic factors such as high pH and salt/carbonate toxicity in these soils can influence the diversity and function of organisms involved in C cycling, nutrient availability and disease incidence impacting crop production. Surface (0-10 cm) soils collected from four fields in South Australia (Minnipa, Poochera, PtKenny, Avon) were used in controlled environment reconstructed microcosm experiments. Soils were incubated with and without *Rhizoctonia solani* AG8 and amendments for 7 days at 15/20C in the dark prior to sowing wheat seeds. Microbial biomass, catabolic potential, composition and abundances of bacterial and fungal populations were measured using biochemical, functional gene qPCR and group specific sequencing methods in incubated bulk soils prior to sowing and 4-week-old rhizosphere soils. Results indicated that the composition and diversity of bacterial and fungal communities were significantly different in the four soils. Responses in microbial catabolic profiles were distinctly different between amendments and soil types. In both the bulk and rhizosphere soils, actinobacteria were most abundant phyla followed by Proteobacteria, Chloroflexi and Firmicutes but the responses varied with amendments and soil types. Alpha diversity indices for bacteria significantly reduced with sucrose, wheat and broccoli addition in Minnipa, Poochera and Pt Kenny soils but no significant change in Avon soils. In general, the effect of different amendments also varied for the growth of *R. solani* in soils with different chemical properties and suppression potential (eg., Avon vs. Poochera). Overall, these results suggest that understanding the intricacies of complex microbiome-pathogen interactions both in the bulk and rhizosphere soils is important to identify amendments that can promote disease suppression capacities of calcareous soils with chemical constraints both to plants and soil microbiomes.

53

Evolution of the fungal and bacterial fruit microbiomes of kiwifruit (*Actinidia chinensis* var. *deliciosa* and *A. chinensis* var. *chinensis*) from pre-harvest spray to storage in a cool store.

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Composition of the fruit microbiome affects the fruit health profile, including susceptibility to pathogens. Understanding the evolution of the kiwifruit microbiome can provide insights into how best to manage fruit after harvest. Therefore, a study to determine the evolution of the fungal and bacterial microbiomes present on the surface of kiwifruit was conducted in four 'Zesy002' (yellow-fleshed kiwifruit) orchards and three 'Hayward' (green-fleshed kiwifruit) orchards located in Bay of Plenty. We collected five fruit from each orchard at the following timepoints: before pre-harvest spray, after pre-harvest spray, when the fruit were in picking bins, when they were in trays after being sorted, and from the coolstore after about 4 weeks' storage. The fungal and bacterial microbiomes from each individual fruit were characterised by amplicon sequencing analysis.
This longitudinal study revealed that the microbiomes on 'Hayward' and on 'Zesy002' evolve differently. Major differences were found after the fruit had been stored for about a month in a coolstore. *Colletotrichum* became more prevalent or detectable in two 'Zesy002' orchards, only after the fruit had been picked. *Alternaria* was found on 100% of the 'Hayward' fruit that were sampled after 1 month in storage, but was found on only 37% of the fruit sampled earlier. Potential postharvest pathogens such as *Penicillium*, *Colletotrichum* and *Alternaria* species were found predominantly on 'Zesy002'. On that same cultivar, *Penicillium* was more prevalent when the fruit were sampled in the orchard. Finally, 93% of the 'Zesy002' fruit analysed carried *Alternaria*, while only 50% of the 'Hayward' fruit analysed carried this pathogen. This study highlighted possible sources of contamination by postharvest pathogens, and identified environments favourable to the establishment of those pathogens. It also highlighted that different cultivars need to be managed differently

112

Analysis of the effect of the chitin synthase inhibitor nikkomycin Z on *Phytophthora* species

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Chitin is a biopolymer that plays an essential role in the growth and development of fungi and multiple oomycete species. Paradoxically, however, oomycetes from the *Phytophthora* genus do not seem to contain chitin in their cell walls despite the presence of chitin synthase genes in their genomes. In addition, *Phytophthora* species show reduced mycelial growth in the presence of the chitin synthase inhibitor nikkomycin Z, which is surprising given the absence of chitin in their cell walls. In this work, we aimed to determine the mode of action of nikkomycin Z in *Phytophthora* species and shed light on the function of the chitin synthase genes in these micro-organisms. We first confirmed that nikkomycin Z inhibits the mycelial growth of *P. cinnamomi*, *P. cambivora*, *P. citricola*, and *P. nicotianae* and continued our studies by focusing on *P. cinnamomi* as this species is an important threat to agriculture and ecosystems worldwide. Nikkomycin Z treatment induced tip bursting and abnormal hyphal growth as well as a decreased level of cellulose in the hyphal cell wall. In addition, treatment with the inhibitor affected the level of expression of genes playing vital roles in the micro-organism, i.e., genes related to cell wall biosynthesis, the uridine diphosphate N-acetylglucosamine pathway, glycosylphosphatidylinositol anchors, effector proteins, and chitin deacetylases. Our data suggest that nikkomycin Z has a wide range of effects on *Phytophthora* species that might be useful in guiding the development of future anti-oomycete drugs.

44

INNOVATION IN TERTIARY AGRICULTURE TEACHING: TRAINING THE NEXT GENERATION OF PLANT PATHOLOGISTS

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The Australasian Plant Pathology Society has discussed, at various meetings, the need to ensure improved capacity in agricultural industries, particularly through quality tertiary training of plant pathologists. Nearly a decade ago now, a set of agriculture-specific national threshold learning outcomes (TLOs) for graduates and an associated best practice guide were co-created by industry, graduates and academics; and endorsed by the Australian Council of Deans of Agriculture (ACDA). The five TLOs reference: Knowledge; Understanding; Inquiry and Problem Solving; Communication; and Personal and Professional Responsibility. At that time, industry indicated that graduates required a broad knowledge of pathogens and associated basic diagnostic skills as well as a skill set that allowed them to make decisions when faced with a real world-problem that has economic and environmental implications. In 2023, employers consider intercultural and digital competency as well as employability skills (i.e. generic non-technical skills) as most important. The national Agriculture Learning and Teaching Academic Standards (including the TLOs) therefore need to be refreshed to remain relevant in guiding tertiary teaching practice in agriculture and related disciplines, including plant pathology.
This presentation by the ACDA Learning and Teaching Academy will explore opportunities to enhance the TLOs and requirements for the professional development of agricultural tertiary academics in innovative teaching practice. The Academy has been formed by the ACDA to inform innovation and quality in tertiary teaching practice in Agriculture and related disciplines (see https://www.acda.edu.au/learning-and-teaching-academy). The Academy aims to encourage the scholarship of learning and teaching (SoLT) for agricultural educators; provide reward and recognition for excellence and innovation in tertiary agricultural education; promote and sustain the implementation and a regular update of the national Threshold Learning Outcomes (TLOs) for Agriculture; and support interactions between tertiary and secondary agricultural educators and industry.

Session 6 C: Diagnostics, Biosecurity

210

Stop the rot: monitoring *Phytophthora* in nurseries

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Urban greening programs aim to improve tree canopy coverage, enhance biodiversity outcomes and mitigate the impacts of climate change, such as urban heat islands, in urban environments. However, many of these projects are negatively affected by a range of factors that result in high failure rates and early tree death during establishment. In numerous jurisdictions the effects of pest and disease negatively affect survival and establishment of plantings and also provide potential for pest and diseases to spread to already established urban forests and beyond into natural ecosystems. The genus *Phytophthora* presents a significant risk to the health of our Urban Forests and natural ecosystems. Following reports of tree failures as part of the New South Wales “million tree” initiative we initiated a project, “Stop the Rot”, to assess the frequency of recovery of *Phytophthora* species from nursery stock used to supply land management agencies. Soil samples were collected for *Phytophthora* testing from 1437 trees from six nurseries, which consisted of four commercial production nurseries and two community nurseries. A total of 62 host species were sampled cross 39 genera that were a mix of Australian native and exotic species. Soil samples were baited with germinated lupins, DNA extracted and tested identified through analysis of the cytochrome c oxidase subunit 1 (cox1) gene. A total of 329 of the 1437 sampled trees (23%) were positive for *Phytophthora*. The positive percentages by nursery ranged from 2.8% to 35% with a median positive rate of 26.5%. Nine described Phytophthora species were detected as well as several undescribed species. Given the high frequency of positive detections, the potential severity of the species recovered, we have engaged in a program of awareness raising and training with the nursery industry to improve standards and to minimise future impacts.

174

Testing statistics for diagnostics, biosecurity, and general surveillance in Victoria 2020 - 2023.

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Crop Health Services (CHS) is a business within the Department of Energy, Environment and Climate Action (DEECA) in Victoria, and conducts plant diagnostic and biosecurity testing in the fields of bacteriology, entomology, mycology, nematology, and virology. Staff members are using traditional methods such as culturing, microscopy, and immunological techniques as well as standard and advanced molecular technologies including PCR, LAMP and short and long read high throughput sequencing. Members of CHS belong to the National Plant Biosecurity Diagnostic Network and participate in proficiency testing, laboratory residentials and review and verification of National Diagnostic Protocols through Plant Health Australia. Within the years of 2020 – 2023, CHS has undertaken diagnostics on 8,623 submissions, equating to 82,206 samples and 265,084 individual assays (molecular/immunological/culturing). In 2021 alone, CHS undertook 92,136 individual assays of which 82,812 were conducted by the virology group. CHS and the wider diagnostic team in Victoria, provide support for biosecurity. Within the past four years, CHS has detected and responded to multiple pests that were new to Australia or are regulated, including tomato chlorotic dwarf viroid and potato spindle tuber viroid from ornamentals, *Alternaria gaisen* (Black spot of pear) and *Dickeya fangzhongdai* (Orchid soft rot), as well as multiple insect pests, including serpentine leaf miner (*Liriomyza huidobrensis*) and Braula fly (*Braula coeca*).

86

Improved diagnostics for *Trichoderma aggressivum* and *Cladobotryum* spp., two major fungal pathogens affecting commercial mushroom production in Australia

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Green Mould (*Trichoderma aggressivum*) and Cobweb (*Cladobotryum* spp.) are among the most serious fungal diseases threatening commercial mushroom production in Australia. These diseases lead to severe reduction in mushroom yield, harvest quality and shelf life. The current diagnostic methods employed at Crop Health Services (CHS), Agriculture Victoria for these fungal pathogens rely on culture-based approaches which are slow (4 - 6 weeks), laborious, unsuitable for fastidious species and need a high degree of technical expertise. Furthermore, control options are highly limited (i.e., fungicides) for fungal pathogens on a fungal host (mushroom), leaving industry reliant on rapid identification followed by physical removal of contaminated material as a control option. The Australian mushroom industry is increasingly requesting more rapid and accurate diagnostic testing of these pathogens and their endemic relatives from CHS, Agriculture Victoria. Hence, we aimed to design new rapid molecular tests for these two key fungal mushroom pathogens. For *T. aggressivum*, a published PCR assay has been validated *in vitro* against in house sourced VPRI positive controls. Further in silico validation was done using two high quality genome assemblies generated under this study, and a new qPCR assay has been developed and validated for high specificity and sensitivity. For *Cladobotryum* spp., a comprehensive search of published studies failed to identify candidate PCR/qPCR assays for validation, possibly due to its complex phylogeny. Whole genome assemblies have been generated for four *Cladobotryum* spp. to improve our understanding of the phylogeny of this species, and to identify potential marker genes for qPCR design. The diagnostic qPCR assay for *Cladobotryum* spp. is under development based on these sequence resources.

55

Aetiology of banana finger-tip rot (mokillo) in Australia

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Banana finger-top rot (mokillo) is a bacterial disease of banana fruit. Diseased fruit fingers have discoloured and necrotic pulp. The finger symptoms are indistinguishable from those caused by Moko disease or Blood disease of banana, and its superficial resemblance to Moko is why the disease was named 'mokillo' in Central America. Moko caused by *Ralstonia solanacearum*, and Blood disease caused by *Ralstonia syzygii* subsp. *celebesensis* are diseases of quarantine importance to many countries. The ability for laboratory diagnosticians to discriminate between these diseases of quarantine importance and banana finger-tip rot is vital. Therefore, this study aimed to elucidate the aetiology of banana finger-tip rot. Specifically, what is the identity of the causal agents of banana finger-tip rot in Australia, can they cause plant wilt, and how does infection occur? Experiments were conducted on banana fruit and potted banana plants. The completion of Koch's postulates demonstrates that *Burkholderia cenocepacia*, *Pantoea dispersa*, and *Ralstonia* sp. *nov*., are able to cause banana finger-tip rot. Whole genome sequencing, ANI and MLSA analysis of the *Ralstonia* sp. *nov*., confirmed it as a novel species within the genus *Ralstonia* - most closely related to *Ralstonia pickettii*. We also provide evidence to support the hypothesis that infection occurs through the open xylem of female banana flowers, and that the disease causal agents are opportunistic pathogens or plant commensals, and are unable to cause plant wilt. Clarification of the aetiology and identity of the causal agents of banana finger-tip rot assists with the laboratory discrimination of banana bacterial wilts of quarantine concern.

76

Development of Highly Sensitive RT-PCR Assays for Universal Detection of Orthotospoviruses and Internal Control in Thrips Testing

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Orthotospoviruses, economically devastating plant viruses transmitted by thrips, pose a significant threat to global crop production. Rapid and accurate detection of these viruses is critical for implementing effective biosecurity measures and preventing their spread.

In this study, two RT-PCR assays were developed. The first assay targets the orthotospovirus RdRp gene, enabling universal detection of all known orthotospoviruses. The second assay targets the elongation factor 1-alpha gene (EF1-α) and serves as an internal control for virus testing in thrips. The orthotospovirus RT-PCR assay was designed based on extensive analysis of all available orthotospovirus RdRp gene sequences. The internal control assay, on the other hand, was designed using EF1-α sequences from over 200 insect species.

Both assays demonstrated high sensitivity, being capable of amplifying their targets from as few as 10-6 fmol (approximately 600 copies) of artificially synthesised dsDNA templates. These templates were designed to include sequence patterns representing the theoretical weakest primer binding sites. The assays were further validated using real biological samples.

The developed assays have the potential to significantly enhance surveillance and early detection of orthotospoviruses. Additionally, the internal control assay enables more robust virus testing in thrips and other common insect vectors of plant viruses.

190

Automated air sampling for remote surveillance and high throughput processing of environmental samples for eDNA analyses

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A collaboration led by the National eDNA Reference Centre at the University of Canberra, with support from the Department of Agriculture Fisheries and Forestry (DAFF), has evaluated an end-to-end, at scale, eDNA based surveillance system using remote sampling units derived from the iMapPESTS Sentinel Surveillance for Agriculture project, coupled with high throughout pest/pathogen identification methods. In this study, the mobile surveillance platforms were satellite enabled to demonstrate end-to-end data connectivity and allow airborne pathogens monitoring anywhere in Australia. Using Myrtle Rust (*Austropuccinia psidii*) as a model pathogen, Sentinel units were deployed at strategic sites in Adelaide (recent but rare positive detections) and Sydney (established disease region) to demonstrate surveillance capacity using high-throughput qPCR diagnostics by SARDI Molecular Diagnostics and metabarcoding diagnostic by the Australian National University fungal genomics group. These placements also included state Botanic Gardens, to capitalise on flora diversity which included 'sentinel' plants. The dual diagnostic approach to the fully traceable air samples collected from sentinel devices combined target specific diagnostic for area-wide surveillance capability with genomic analysis of lineage defining loci for deeper investigation into species diversity within broader phylogenetic groups (e.g., rust) and potential detection of novel incursions. Future application of the system to well-studied pathogens will allow for adoption of population scale analysis including pathogen virulence profiles or biodiversity analysis of air biota applied strategically within Australia's Botanic Gardens. Digital output visualisation packages explored with the project's technology partner Data Effects, in collaboration with the University of Adelaide, extended the end-to-end surveillance pipeline of device-to-data delivery customised to stakeholder needs. Applications of the project outputs include deployments into risk pathways and border protection points of entry or trade protection underpinned by innovative and cutting-edge technology solutions for Australia's biosecurity programs.

Session 6 D: Plant Disease Management, Chemical Resistance

178

Characterization and detection of QoI resistance in *Colletotrichum* species associated with avocado in Australia

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Avocado anthracnose is an economically important disease caused by several species of the *Colletotrichum* genus. Formulations of copper and strobilurin (quinone outside inhibitors, QoI) fungicides are commonly used as sprays in the orchard for effective disease management. However, QoI fungicides are classified as high-risk for resistance development. More than 60 fungal pathogen species have been reported to have developed resistance to QoI fungicides. Preliminary *in vitro* fungicide sensitivity studies have evaluated the sensitivity of Colletotrichum isolates from avocado to two QoI fungicides used in Australia. Mycelial growth of two isolates, IN095 and IN096, on sPDA was not inhibited by azoxystrobin or fluopyram + trifloxystrobin fungicides at four different concentrations (1.0, 0.1, 0.01 and 0.001 mg a.i /ml). A different isolate, IN069 displayed 46.6% inhibition of mycelial growth *in vitro* when exposed to a high concentration (1.0 mg a.i/ml) of azoxystrobin fungicide. Additionally, a total of seventeen Colletotrichum isolates were screened for sensitivity to azoxystrobin using a molecular assay to detect the mutation in cytb gene. The site specific mutation was not detected in nine isolates obtained from orchards prior to registration of azoxystrobin, or in organic and minimally-sprayed orchards. The mutation was detected in six isolates, including IN095 and IN096, originating from orchards with history of azoxystrobin confirming their resistant status. Two isolates originating from azoxystrobin-sprayed orchards showed sensitivity. Further molecular and *in vitro* screening of isolates collected from organic and QoI-fungicide sprayed orchards across Australia is currently underway. The objective of the study is to gain a comprehensive understanding of QoI fungicide resistance patterns within populations of *Colletotrichum* spp. infecting avocado in Australia.

33

Exogenous double-stranded RNA as a biopesticide for sustainable control against soilborne pathogens

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Soilborne plant pathogens are one of the leading causes of plant diseases and contribute to significant annual crop production losses worldwide. Pesticides are the main control for plant disease, but can lack specificity, may be toxic to humans and non-target organisms, and can lead to the development of pathogen resistance. Hence, a new innovative strategy is key to environmentally friendly disease management of soilborne pathogens for greener and sustainable agriculture. An emerging non-genetically modified (GM), environmentally sustainable and target-specific crop protection platform is spray-induced gene silencing (SIGS), which uses exogenously applied double-stranded RNA (dsRNA) as a bio-fungicide. We have been examining the requirements for effective SIGS against plant pathogenic fungi and oomycetes including *Verticillium dahliae* and *Phytophthora cinnamomi*. We assessed dsRNA uptake efficiency by pathogens and different plant tissues, movement of dsRNA within host plants, and inhibitory effect of dsRNA targeting essential fungal/oomycete genes. We found that *V. dahliae* and *P. cinnamomi* could take up dsRNA from the environment but with varying degrees of efficiency. Interestingly, in model host crops, dsRNA applied to the shoots could be detected in root tissue and dsRNA applied to roots could be found in leaves. dsRNA targeting essential fungal genes significantly reduced pathogen growth *in vitro* and bioassays were developed to determine whether this translated to protection in planta. These findings will inform the development of sustainable exogenous RNAi-based crop protection approaches against soilborne pathogens.

81

Can secateurs spread Grapevine Trunk Disease pathogens?

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Grapevine trunk diseases (GTDs) such as Botryosphaeria dieback cause significant yield reduction in Australian vineyards. The causal pathogens infect primarily through pruning wounds resulting in dieback and eventually death of vines. The demand for organic and biodynamic wines has increased significantly in recent years. With no control strategies in place, the development of alternative plant protection strategies to manage GTDs for organic and/or biodynamic systems is essential. The contamination of pruning equipment, such as secateurs, has long been thought not to be a source of spread for GTD pathogens. In this research, the potential for contamination of secateurs with *Neofusicoccum luteum* and *Diplopia seriata* while pruning (cutting) inoculated canes was assessed. Preliminary results indicated that there is a potential for contamination of *N. luteum* between inoculated canes and secateurs, with the transfer of the pathogen by contaminated secateurs in 30% of the total samples cultured on sterile agar. A secondary experiment was conducted to validate the findings of the first preliminary experiment with a larger sampling size. In the secondary experiment *D. seriata*, 60% of samples were positive for the pathogen on agar plates. Whereas the contamination of the *N. luteum* only showed a 10% contamination rate on agar plates, with no contamination on control plates. Therefore, it is feasible for a level of transference to occur from contaminated vines to secateurs. Further research will be conducted to identify if inoculum can be spread to clean canes, and a more comprehensive study will be conducted to identify other possible pathogen transference, including multiple species.

176

Emergence of resistance to succinate dehydrogenase inhibitor fungicides in *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata* in Australia

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Net blotches are the most significant diseases of barley in Australia, causing AUD$88 million in losses annually. The two forms of the disease are Net-form net blotch (*Pyrenophora teres* f. *teres* [Ptt]) and Spot-form net blotch (*P. teres* f. *maculata* [Ptm]). Resistance to succinate dehydrogenase inhibitors (SDHIs) in net blotches in Australia was first identified in 2019 in Ptt on the Yorke Peninsula, South Australia (SA), followed by detection in 2020 in Ptm near Cunderdin, Western Australia (WA). On the Yorke Peninsula, the frequency of resistance to SDHIs (defined as growth at 10 µg mL-1 fluxapyroxad) was 10%, with a further 32% showing reduced sensitivity to SDHIs (defined as growth at 2 µg mL-1 fluxapyroxad) (n=425). In Cunderdin, the frequency of reduced sensitivity to SDHIs was 38%, with a further 2% resistant (n=333). In 2021, SDHI resistance and reduced sensitivity was detected in Ptt in both Victoria and the Great Southern region of WA. Eight mutations across three genes of the Sdh complex (SdhB, SdhC, SdhD) were found in Australian *P. teres*. The mutation H134R in SdhC was most common in Ptt in SA, and N75S in SdhC was most common in Ptm in WA. Two mutations were novel for *P. teres*, in SdhB (H277L) and SdhD (H134Y), and both mutations correlated with reduced SDHI sensitivity in microtiter assays. Homology modelling of the *P. teres* Sdh complex showed that the B-H277L and D-H134Y mutations result in altered binding modes and lower binding affinities of SDHI compounds compared to the wild-type. *In planta* studies indicated that seed dressing with 1× label rate of fluxapyroxad and inoculation with C-H134R SDHI-resistant isolates resulted in disease severity not significantly different from a zero-fungicide control. The effects on fitness of mutations found in Australian *P. teres* isolates were also assessed.

170

The impact of oat leaf (crown) rust on hay and grain yield and quality

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Oat leaf rust (*Puccinia coronata*) also known as crown rust, can cause significant yield and quality reduction in oaten hay and grain. Septoria is the most common oat disease in Western Australia (WA), however, historically leaf rust has been more damaging. While many current oat varieties have moderate levels of resistance to leaf rust, several commonly grown varieties (comprising >25% of area sown to oats in Western Australia) are susceptible to the disease.

To increase the understanding of how leaf rust impacts oaten hay and grain yield and quality, inoculated trials were conducted in Manjimup, WA (high rainfall zone) in 2020 and 2021. Trials contained three commonly grown oat varieties (with a range of resistance rankings for leaf rust) and three disease management strategies (untreated, single spray at flag leaf and multiple spray/ full disease control).

Oat leaf rust significantly impacted the yield and quality of both oaten hay and grain. These trials highlight the risk of growing susceptible varieties in locations or seasons favorable for leaf rust. In susceptible varieties, leaf rust develops rapidly, and the impact on yield and quality is significant. For growers this means multiple fungicide applications may be required to reduce disease severity to preserve yield and quality. Growing varieties partially resistant (MRMS) or better can significantly delay leaf rust development and limit impacts on yield and quality in both hay and grain. Where leaf rust pressure is high, these varieties may benefit from a single well-timed fungicide application (eg. when flag leaf fully emerged) to limit the possible impacts on hay and grain quality.

Keynote Address 8

215

Impacts and innovations of the ongoing environmental and industry threat of myrtle rust

Dr Louise Shuey[1], Alistair R McTaggart[2], Anne Sawyer[2], Rebecca Degnan[2], Flávia S Bonora[1], Rebekah Frampton[3], Tilly Davis[4], Dallas Anson[5], Michael Brand[5], Robert Beresford[3], Michael Bartlett[3], Geoff S Pegg[1], Grant R Smith[3]
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Myrtle rust, a disease caused by *Austropuccinia psidii*, is now a consistent presence in native environments and Myrtaceae-based industries since its invasion in Australia over a decade ago. Conservationists, custodians of native lands, and emerging native food and oil industries face challenges about how to manage disease and safeguard native flora from a pathogen that may potentially trigger a generational extinction event. The full impact of myrtle rust in Australia requires further understanding and may be difficult to quantify given the ecological importance of Myrtaceae and downstream ecological effects. We present some of the outcomes of collaborative research in efforts to better understand i) the impact of *A. psidii* in Australian ecosystems, ii) reproduction, its life cycle and pathways to evolutionary innovation, iii) how to prevent further threats from exotic genotypes, iv) whether natural resistance in Australian and New Zealand hosts can be integrated into disease management, and iv) new management tools, such as dsRNA sprays, to be implemented in native environments and plantations. Our ecological research has provided new knowledge on the effect of bushfire and management of myrtle rust in certain environments. We have changed long-held hypotheses on reproduction in *A. psidii* and whether industry faces a clone or a constantly changing pathogen, and new data will be presented on temporal changes in population dynamics. We share advances in how the community is tracking the global spread of *A. psidii* and how this can be implemented for biosecurity to prevent introduction of new genetic diversity. Our research has led to the discovery of potential host resistance mechanisms and pathogen effector genes. Our team has tested the application of double stranded RNA to prevent and cure infections of myrtle rust, and there is promise to protect culturally significant and near-threatened trees in native ecosystems.

Friday 24 November:

Post-Conference Workshops

Workshop 5: Xylella

Presenters: Dr Toni Chapman - DPI NSW, Menangle; Dr Monica Kehoe - DPIRD, South Perth WA

Summary

Xylella spp. our number one exotic plant pest. During this session we will share our knowledge and images on the disease, sampling of live and dormant plant material and the new National Diagnostic protocol and why the assays in the NDP were selected. We will show you what type of plant material to collect and how to process it. This session is aimed at anyone interested in *Xylella* from our biosecurity policy makers, emergency response teams, diagnosticians and industry personnel.

- Intro and Background on *Xylella* spp. (Toni Chapman)
- Intro and Background on vectors (Piotr Trebicki)
- *Xylella fastidiosa* learnings and perspectives from the National *Xylella* Coordinator (Craig Elliott)
- A New Zealand perspective, given the presence of a vector (Luciano Rigano)
- Importation of *Xylella* spp. and culture methods (Luciano Rigano)
- *Xylella* spp. a Plant Health Perspective, including the NDP verification process (Rachel Mann)
- The new National Diagnostic Protocol (Toni Chapman & Monica Kehoe)
- A guide to sampling in the field – field walk at WAITE
- Provide knowledge on *Xylella* spp. and our learnings from our recent field trips
    - Europe (Toni Chapman, Monica Kehoe & Luciano Rigano)
    - America's (Pragya Kant)
- Contingency Gaps & Future perspectives - discussion (Toni Chapman, Monica Kehoe, Luciano Rigano, Piotr Trebicki)

Poster Abstracts

Posters 1 - 9: Diagnostics, Biosecurity

18

Early disease detection = early farmer action

Ms Aylwen Cotter[1], Dr Peter Dracatos[1], Professor Travis Beddoe[1], Dr Kim Johnson[1]
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Pathogenic fungi can cause losses of up to 70% in susceptible crop varieties leading to millions of dollars each year in lost revenue to farmers, agriculture and food industries. The current methods for detecting fungal pathogens in the field are challenging, labor-intensive, and time-consuming. Typically, symptoms need to manifest before detection becomes possible. Early methods of detection before symptoms appear can mean early action to limit disease spread and crop damage. Highly sensitive molecular diagnostic assays that use environmental sampling, for example from soil, air and water have potential to act as early detection systems. We are developing field deployable molecular diagnostics assays that use loop mediated isothermal amplification (LAMP) and have potential for detection of fungal pathogens within 15 minutes. This study aims to develop LAMP assays for four fungal pathogens: *Botrytis cinerea, Golovinomyces cichoracearum, Zymoseptoria tritici* and *Puccinia striiformis* that affect a range of crops such as strawberries, cannabis and wheat. After optimisation, assays will be tested under field settings using both air and leaf sampling in associated horticulture and broadacre crops: strawberry, cannabis and wheat, respectively. LAMP assays will be investigated for their specificity to the targeted fungus and sensitivity measured as time to positive in the assay. A SKC Biosampler for air sampling is currently being tested in closed environment agriculture systems to determine what microorganisms are present. This project aims to fill a gap in knowledge of the effectiveness of molecular in-field detection assays in crops. The potential outcomes of the project are assisting farmers in the early detection of fungal outbreaks and to support local decision-making on treatment plans.

28

Achieving the Impossible in Biosecurity: The 5A's in Border Diagnostics

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The Australian Department of Agriculture, Fisheries and Forestry is committed to ensuring a resilient biosecurity system that safeguards against the introduction of exotic pests and diseases. Identifying potential biosecurity risks associated with imported goods is challenging and the diagnostic process at the border poses multifaceted constraints that demand innovative solutions and a comprehensive approach for effective management. Border diagnostics must be:

1. Accurate: Regulatory decisions based on accurate diagnostic data achieved by incorporating quality control measures, advanced molecular techniques, and expertise-driven interpretation ensures defendable results and enhances confidence in the diagnostic outcomes.
2. Automated: Diagnostics amenable to automation and leveraging on technological advancements allows streamlining of labour-intensive and time-consuming tasks and enables efficient processing of a higher volume of samples.
3. Affordable: Cost-effective diagnostic services achieved through streamlined processes and optimised resource allocation can alleviate financial burdens without compromising diagnostic quality.
4. Agile: The diagnostic system must be agile and able to identify organisms and emerging threats on any pathway to ensure comprehensive coverage of potential biosecurity risks.
5. Abrupt: Diagnostics must enable timely decision-making as delays result in economic losses to importers.

The Border Molecular Diagnostics Section had adopted innovative technologies such as Oxford Nanopore Technologies MinION™ sequencing and streamlined workflows to achieve the 5A's in border diagnostics. We developed a rapid DNA extraction, PCR amplification and sequencing workflow that allows identification of biosecurity risks within a day. This is a notably faster turnaround time compared to Sanger sequencing offered by external laboratories. MinION™ facilitated rapid detection of exotic fungi, bacteria, viruses and plants and allowed detection of pathogens directly on plant tissues without the need for isolating microorganisms into pure culture. These reforms facilitated faster release of goods under biosecurity control, resulting in efficient risk management and provision of effective risk mitigation options to the department's stakeholders.

40

Rapid on-site detection of *Phytophthora infestans* using real-time loop-mediated isothermal amplification (LAMP)

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Late blight of potato, caused by *Phytophthora infestans*, is a major constraint to potato production worldwide. Early detection, coupled with a knowledge of the genotype present, can ensure the timely implementation of the most optimal disease management strategies. Recently, loop-mediated isothermal amplification (LAMP) assays have become more widely used for the rapid on-site detection of *P. infestans*, but these assays have limitations. In this study, we developed a new LAMP assay using the ypt1 gene for *P. infestans* which can readily distinguish *P. infestans* from other oomycetes such as *P. erythroseptica, P. mirabilis, P. nicotianae,* and *Pythium ultimum* within 10 minutes on a Genie II or IIIC platform. In addition, six other published LAMP assays were compared with our assay on Genie IIIC using the same concentration of primers. Our assay was more reliable than other assays based on specificity and sensitivity on the Genie platform. Our LAMP assay based on the ypt1 gene did not cross-react with *P. mirabilis* or *P. phaseoli*. However, although our assay did cross-react with *P. andina* and *P. ipomoeae, P. ipomoeae* was easy to distinguish because it amplified very late in the reaction. The lower limit of detection (LOD) of our LAMP assay was determined to be 1 pg/µL (LAMP run for 25 min) for pure culture. LAMP and quick DNA extraction technology, coupled with a portable platform such as the Genie IIIC, enable the rapid on-site detection of *P. infestans*. Samples confirmed as *P. infestans* can be sent to the lab for further genotyping and molecular characterization.

51

Development and validation of diagnostic assays for detection of banana wilt associated phytoplasmas

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Phytoplasmas are pathogenic bacteria that live in the vascular system of a broad range of plants and are spread by sap feeding insects. Phytoplasmas can only survive inside their hosts and cannot be cultivated using standard laboratory growth media, which poses several challenges to the study of these pathogens. Banana wilt associated phytoplasmas (BWAP) causes leaf yellowing and leaf death in many varieties of banana. Internal discoloration and discontinuous brown vascular streaks and necrotic pockets of wet rot are visible in the vascular strands inside the pseudostem and leaf mid rib. BWAP has been reported in Papua New Guinea and Solomon Islands, posing a major threat to other banana producing countries in Southeast Asia and the Pacific. The most likely pathway into Australia of banana wilt associated phytoplasmas is by importation of infected plants. For that reason, imported banana germplasm must be screened for banana phytoplasmas as part of post-entry quarantine screening to ensure Australia remains free of these pathogens.

Although several published PCR-based methods used for detection of phytoplasmas in general are available, related endophytic bacteria are often detected in banana tissue and lead to false positives. We have developed and validated two novel PCR assays, based on BWAP draft genomes, and tested these against three published PCR diagnostic assays to identify the most reliable method to detect phytoplasmas in banana. Results showed that four of the assays tested generated less false positives than the currently used assay for phytoplasma indexing of imported germplasm in Australia and identified phytoplasma in samples previously identified as bacterium. These results provide support for a scientifically informed choice of assay for phytoplasma detection of imported planting material.

135

Optimisation of RNA extraction protocols for plant virus and viroid detection

Dr Pooja Sharma[1], Dr Dilani De Silva[1], Miss Stephanie Rosch Rosch[1], Dr Emma McFarlane[1], Dr. Ruvini Lelwala[1], Dr. Naima Tasnim[1], Mrs Cassie McMaster[1], Dr. Marie-Emilie Gauthier[2], Dr. Roberto Barrero[2], Mr Robin Eichner[1], Dr. Candace Elliott[1], Dr. Julie Pattemore[1]
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Exclusion of exotic plant pathogens is a pivotal strategy to safeguard plant industries. More than 300 plant lines are imported into Australia each year through the Commonwealth Post Entry Quarantine (PEQ) facility in Victoria, with screening for viruses and viroids of biosecurity significance. In 2022, high-throughput sequencing of small RNA (sRNAseq) was implemented as the primary method for screening Rubus, Fragaria and Prunus for viruses and viroids in PEQ. The quality and integrity of extracted RNA is critical to the success of sRNAseq and poor quality HTS samples can yield false negatives. At PEQ, plant total RNA is extracted using an automated platform Promega Maxwell RSC, with simplyRNA tissue protocol. While the quality of extracted total RNA is sufficient for PCR diagnosis, getting optimised quality for sRNAseq is a challenge. This study set out to optimise the routine RNA extraction protocol and explore other Promega RNA extraction protocols that may be fit for purpose.

Total RNA was extracted from 30 samples of Prunus and Rubus plants, infected with known viruses and viroids using three different extraction kits: RSC miRNA Tissue Kit, RSC Plant RNA Kit and RSC simplyRNA Tissue Kit. In a parallel experiment the effect of using different tissues on RNA quality was tested with Prunus, Fragaria, Rubus and Stenotaphrum, an ornamental grass, using routine RSC simplyRNA Tissue protocol. Preliminary observations indicated that the RSC Plant RNA Kit gave the best total RNA quality and integrity for both Rubus and Prunus samples. RNA integrity number (RIN) scores, varied between plant tissue type, with petioles and leaf mid-ribs giving higher scores. A comparison of the performance of the kits pre- and post-sequencing and PCR verification of the detections will be presented. The outcomes will benefit in providing better RNA quality required for sRNAseq to improve the viral diagnosis at PEQ.

143

Traditional pathology underpins modern diagnostic advances in Post Entry Quarantine

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Post entry quarantine (PEQ), Mickleham, Victoria is the exclusive entry point in Australia for most high-risk plant germplasm for imported food, agroforestry, turf, and ornamental plants. Imported plants undergo careful visual observation and other mandatory prescribed testing using both traditional and molecular diagnostic methods to detect exotic fungi, bacteria, phytoplasmas and viruses. Traditional methods including visual observations, ELISA, light microscopy, woody and herbaceous indexing, fungal/bacterial plating, pathogen baiting, and transmission electron microscopy (TEM), continue to play a critical role in detecting exotic plant pathogens at PEQ. As an example, TEM has been used in PEQ for approximately 30 years and remains a useful tool for detecting plant viruses, particularly those with filamentous and rod-shaped virions. Recently, TEM was instrumental in detecting a novel potyvirus and potexvirus in plants undergoing PEQ, which was confirmed by high throughput sequencing (HTS).

Prescribed testing for all plants undergoing PEQ calls for visual observation for all diseases and therefore, a traditional pathologist requires experience diagnosing the health of many different plant genera. If a plant has successfully completed prescribed testing but is presenting poorly, the pathologists rely on past plant health observations for decision making about further testing and ultimately the release of the plant from PEQ. There have been many innovations in prescribed testing including more sensitive qPCRs and HTS, however one limitation of these tests is that they are conducted only on a sample of the test plant could potentially miss pathogens that are unevenly distributed in plants. For molecular testing, samples of only 20-50 mg are tested whilst for traditional testing, although the sample size is larger it's still small in comparison to the entire plant. The role of traditional plant pathologists in conducting electron microscopy and visual plant health inspections remains key to identifying potential biosecurity risks in plants at PEQ.

152

Biosecurity bacterial LAMP assays: Residential training and surveillance

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The National Plant Biosecurity Diagnostic Network (NPBDN), delivered by Plant Health Australia (PHA) and funded by the Department of Agriculture, Fisheries and Forestry (DAFF), provides activities for diagnosticians to improve their capability to identify pests of biosecurity importance. A residential funded through NPBDN enabled capacity building for a SARDI diagnostician in the use of Loop-Mediated Isothermal Amplification (LAMP) assays for three exotic bacterial pathogens: *Xylella fastidiosa* (Pierces and leaf scorch diseases), *Xanthomonas citri* subsp. *citri* (citrus canker) and *Xanthomonas fragariae* (angular leaf spot in strawberries). These assays are available in new national diagnostic protocols (NDPs) but without positive controls it is not possible to effectively run a diagnostic assay. As a result of this residential, positive controls in the form of extracted DNA were supplied to SARDI by DPI NSW. These controls enabled the reproduction of the published assays using a portable Genie III apparatus in NSW, and again back in Adelaide at the Plant Research Centre. In the past, surveillance in SA for these pathogens had only been performed by visual symptom inspection. The residential has provided more sensitive LAMP assays to contribute to surveillance in SA. Following the residential, 39 samples were collected and found to be negative for these pathogens. They were collected from urban spaces such as community (24) and home gardens (15), from four hosts: olive (11), grapevine (9), strawberry (4) and citrus (15). This residential has contributed to building collaboration of the national diagnostic network and validated methods in National Diagnostic Protocols.

159

Biosecurity Queensland Plant Health Snapshot

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Biosecurity is critical to protect our economy, environment, human health and way of life. Biosecurity threats are on the rise with growing levels of trade, travel, urbanisation and climate change. In the past 10 years there has been a significant increase of new incursions each year which, coupled with active incursions from previous years, places a significant burden on our biosecurity agencies. Biosecurity Queensland Plant Health have numerous programs in place to prevent, contain, eradicate, and manage biosecurity threats either in the state or at our doorstep. These measures aim to minimise plant pest impacts on our $9.1bn crop and horticultural industries, our $253M forestry industry (2022 QLD primary industries data) and our invaluable natural resources. In addition, surveillance efforts also facilitate overseas and interstate trade by providing evidence of pest absence.

Here we present a snapshot of Biosecurity Queensland Plant Health current challenges, successes, and effort that contributes to a modern and robust biosecurity system.

160

Detection and Current Status of Guava Root-knot Nematode in Queensland

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Meloidogyne enterolobii (guava root-knot nematode, GRKN) is a highly pathogenic plant parasite, with a very broad host range and the ability to overcome root-knot resistance genes in a range of crops. As such, GRKN poses a significant threat to Australian plant industries.

The first Australian detection of GRKN was made in the Northern Territory in September 2022. Shortly after, in December 2022, the pest was detected in Far North Queensland. Following an awareness campaign, a second detection was made in January 2023, from a peri-urban property in the Wide Bay-Burnett region, approximately 1400km from the initial north Queensland site. Affected crops at the two Queensland properties included: cucumber, sweetpotato, tomato, zucchini, and watermelon. No linkage between the Northern Territory and Queensland properties, nor between the two Queensland properties has been found.

As part of the GRKN response, Queensland DAF have encouraged grower and public surveillance in all major horticultural production zones across the state and, at the time of writing, have received and processed around 160 samples of root-knot infested soil and roots. Extracted juvenile and female root-knot nematodes were tested using species-specific PCR assays and DNA sequence data to confirm the presence or absence of GRKN. Despite the wide geographic and host crop range surveyed, no further positive detections have been made in the state to date.

Pure populations of GRKN have been established from single egg masses on glasshouse grown tomatoes to produce inoculum for experiments. Research areas will include improved diagnostics, pathogenicity studies, host and rotation crop resistance and efficacy of disinfestation agents. There is a need to carefully monitor GRKN distribution to restrict movement to new areas and to develop management options to minimise inoculum build up, crop losses, and spread on infested properties.

Posters 10 - 11: Epidemiology, Ecology, Modelling and Risk Analysis

63

Effective and consistent soil application methods for basal stem infection by *Sclerotinia sclerotiorum*

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Sclerotinia sclerotiorum (Lib.) de Bary is an economically devastating soil-borne fungal pathogen known to cause Sclerotinia stem rot (SSR) in a broad range of plants. The most common pathway for infection by S. sclerotiorum is by production of airborne ascospores by carpogenic germination of sclerotia, that infect the upper parts of the plant. However, in conducive environments, sclerotia can also germinate myceliogenically forming hyphae that directly infect the plant stem base. The direct inoculation method of wrapping S. sclerotiorum mycelia agar plugs onto plant stems has been widely used, but effective methods mimicking basal stem infection are not currently available. Therefore, the objective of this study is to develop effective and consistent protocols that infect plant basal stems from the pathogen in the soil. We tested three host plant species at the cotyledon stage (canola, lupin, and lettuce) and found two effective methods that consistently produced basal stem infections. The first method used hyphal agar plugs placed just below the soil surface at a distance of 5 mm from each cotyledon, which led to 100% infection. The second method used pathogen-infested soil by mixing the soil with dry inoculum in the form of a powder prepared from mycelium-colonised organic substrates (10:1, w/w). Three types of substrates consistently led to the infection of all cotyledons tested, including whole wheat grains, a mixture of wheat bran and psyllium hulks, and a hulled millet and psyllium hulks mixture. In contrast, mycelia grown in chia seeds colonised the soil surface and cotyledons quickly but did not infect cotyledons. The two effective soil inoculation methods tested in this study will enhance future studies on the disease progression of SSR or suppression of SSR basal stem infection by soil microbiomes or chemical fungicides.

127

Temperature Effects of Botrytis Grey Mould Colonisation and Growth on Lens culinaris.

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Botrytis Grey Mould (BGM) caused by *Botrytis cinerea* (*B. cinerea*) and *Botrytis fabae* (*B. fabae*) is a major biotic stress constraint on lentil production in South-Eastern Australia. The region offers conducive conditions for the disease including warm temperatures (15-25°C), leaf wetness, and high relative humidity (>70%). However, there is little to no research on the actual impacts of climatic conditions (temperature in particular) on plant-pathogen interactions. With cropping areas continuously expanding into different climatic zones, there is a need to understand the effect of temperature on BGM infection to determine future risks and inform management strategies.
A detached leaf assay was conducted under controlled conditions to determine how the pathogen colonised and established during the early stages of infection under different temperatures (15°C, 20°C, and 25°C). Fourteen-day old lentil leaflets of two genotypes were detached and inoculated with the pathogen (two isolates of each *B. cinerea* and *B. fabae*). Leaflets were then microscopically assessed for spore germination percentage, germ tube length, and appressorium formation at 6, 12, 24 and 48 hours post-inoculation (hpi).
All isolates exhibited high germination percentages across the three temperatures but varied for average germ tube length and severity with the increase in temperature. Significantly longer germ tubes and increased disease severity were noticed when bioassays were exposed to 25 °C suggesting greater risk at warmer climates such as Wimmera and Mallee regions in the South-eastern Australia. *B. cinerea* showed greater capability to infect resistant variety CIPAL2022 compared to *B. fabae*. This indicated B. cinerea as the dominant form of both and needs appropriate management strategies for control. In summary, this research presented insights into the epidemiology of the BGM in lentils and showed environments where BGM can be a risk. Additionally, this information is valuable to develop predictive models and inform strategies to counter future epidemics.

Posters 12 - 14: Forest and Perennial Crop Diseases

54

Screening *Tasmannia lanceolata* for resistance against *Phytophthora cinnamomi* dieback

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Susceptibility of *Tasmannia lanceolata* (native pepper) to *Phytophthora cinnamomi* has been reported in wild stands and plantations, and surveys suggest that clones differ in susceptibility. The key objective of this study was to identify native pepper clones resistant to *P. cinnamomi*, and the defence mechanisms responsible. Plant material from 47 genotypes were successfully propagated as cuttings. Two disease screening experiments were conducted in "soil-free plant growth system" units, which enabled plant roots to be directly inoculated with droplets of a *P. cinnamomi* zoospore solution, or a sterile water control. Successful inoculation and infection were confirmed via observation of symptoms on simultaneously inoculated lupin roots and re-isolation of the pathogen from native pepper roots. Image analysis and machine learning was used to quantify root infection and discolouration. Based on pathogen re-isolation success and total root discolouration percentage, clones were categorised using a susceptibility rating method across multiple categories from highly resistant to highly susceptible. In the first experiment, all 47 propagated clones were challenged with one isolate of *P. cinnamomi* (Pc1), and pathogen re-isolation percentage and total root discolouration (brown and black) percentage differed significantly with clone ($P<0.001$). In the second experiment, three representative clones of experiment 1 were challenged with two isolates of *P. cinnamomi* (Pc1 and Pc2). The putative defence compounds callose, lignin, hydrogen peroxide and peroxidase were assessed visually and quantitatively in the clones used for experiment 2 and their abundance was found to be significantly ($P<0.05$) affected by both clone and inoculation treatment. The presence of polygodial, a known anti-fungal compound which gives native pepper it's characteristic flavour, was explored in roots to determine if clonal variability could contribute to root disease responses.

106

Ilyonectria and Dactylonectria species from *Pinus radiata* in New Zealand

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Ilyonectria and Dactylonectria species have been associated with diseases in agricultural, horticultural and forestry crops causing cankers, root rots and black foot disease (BFD). These genera are ubiquitous and are found in soil, plant roots or woody tissues, and can act at saprobes, endophytes, or weak and latent pathogens. They have Cylindrocarpon-like anamorphs and produce conidia that are spread through soil and water. Many species produce chlamydospores allowing them to survive in the soil for extended periods of time. Species in these genera have been associated with severely reduced yields and crop losses. Dactylonectria and Ilyonectria species have been identified from diseased Pinus radiata submitted to Scion's Forest Health Reference Laboratory (FHRL). These samples were showing symptoms of root rot and most often sampled from nurseries or from recently planted stands. A delimiting study was carried out to determine what Ilyonectria and Dactylonectria species are associated with symptomatic P. radiata. Using ITS 1/4, only four of the 19 isolates studied aligned phylogenetically with currently described species. The remaining 15 isolates could be separated into six distinct phylogenetic clades. Three further gene regions were used to determine how these isolates might be related to known Ilyonectria species. However, our analysis revealed that the isolates were phylogenetically distinct from the known Ilyonectria species we used in this study. Further validation and descriptions of new species is being undertaken to improve our understanding of distribution, host associations, and pathogenicity to *P. radiata* and other forestry and horticultural hosts.

147

Phomopsis Husk Rot in Macadamia: Endophytes as Potential Sources of Inoculum

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Initially limited to the subtropical regions of Australia's east coast, macadamia is now commercially cultivated worldwide. Australia is one of the major macadamia nut exporters, with a farm-gate value of over $200 Million. Despite a prolific production of approximately 25,000 flowers per macadamia tree, only 1% will develop into fruits with a hard shell and an outer husk. Over time, the nut matures within the husk until it reaches the desired size and flavour profile. Several pests and diseases significantly threaten the husk (fruit pericarp), resulting in substantial yield losses. Among the most important diseases of the fruit pericarp are husk spot, caused by *Pseudocercospora macadamiae*; anthracnose husk rot, caused by *Colletotrichum* species; and Phomopsis husk rot (PHR), caused by *Diaporthe* species. In Australia, *Diaporthe australiana* has been identified as the primary culprit of PHR, resulting in premature fruit abscission. *Diaporthe* species have been identified within macadamia trees as endophytes or saprophytes, in addition to their role as plant pathogens. This study examined whether endophytes are the primary inoculum sources during fruit development. Preliminary findings revealed the presence of two main *Diaporthe* species, namely *D. australiana* and *D. fraxini-angustifoliae*, in the rachis, petiole, and fruit pericarp at the "full size" stage (fruit growth stage four). Future studies will provide further insight into the association of *Diaporthe* species with different fruit stages and their role in disease epidemiology. This knowledge will help growers to implement more effective disease management strategies, thereby minimizing yield losses and optimizing productivity.

Posters 15 - 17: Host Resistance Breeding

4

Virulence spectrum of Western Australian *Parastagonospora nodorum* isolates on wheat.

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Nodorum blotch of wheat caused by *Parastagonospora nodorum* is an important necrotrophic fungal disease of wheat in Western Australia. The virulence of 12 Western Australian *P. nodorum* isolates selected on the basis of temporal and spatial diversity was assessed on 12 bread wheat varieties with known responses to nodorum blotch. Randomised trials were conducted in a controlled environment and seedlings were sprayed to run off at two and half leaf stage, using individual isolates, then kept under humid conditions for 48 hours. Responses of wheat varieties were evaluated 10 days post inoculation. Varieties displayed varying disease levels ranging from moderately resistant (6HRWSN125) to highly susceptible (Amery) as expected. The analysis of variance of data indicated that the interaction between isolates and wheat varieties was statistically significant but very low indicating no obvious pathotypes as also reported before. However, results indicated that there was a subtle difference in aggressiveness of the *P. nodorum* isolates. QTL profiling of these isolates is currently underway and may reveal their target loci associated with pathogenicity in wheat.

129

Selection for Phytophthora root rot resistance in chickpea crosses affects yield potential

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Phytophthora root rot of chickpea (Cicer arietinum) caused by Phytophthora medicaginis is an important disease. Resistance is based on chickpea x *Cicer echinospermum* crosses providing quantitative and partial resistance. We tested if selection for lines with low levels of foliage symptoms in two contrasting recombinant inbred (RIL) populations of chickpea x *C. echinospermum* crosses led to the selection of material that maximises yield. For the var. Yorker x *C. echinospermum* backcross RIL population (n = 196) with the highest level of resistance, in the absence of Phytophthora root rot, significant effects were observed for later flowering, later podding, lower grain yields and 100 seed weight between low and high foliage symptom RIL groups. These results were consistent with prior findings for an introgression segment from *C. echinospermum* being associated with reduced chickpea yields. For the second RIL population (n = 212) of var. Rupali crossed with same *C. echinospermum* backcross, with lower levels of resistance, the only significant difference between low and high foliage symptoms groups was for 100 seed weight. Across four *P. medicaginis* inoculated experiments and a natural inoculum experiment comparing low and high foliage symptom RIL groups, survival to maturity was the most consistently related parameter among experiments of four different foliage symptom parameters. Survival to maturity was more strongly related across experiments than grain yields. Comparison of foliage symptom parameters as grain yield predictors showed that for inoculated experiments the area under the disease progress stairs was the most consistent predictor of yield among five different foliage symptom parameters. Overall results showed foliage symptom based selection included some high yielding RIL in the most resistant population. Additional selection efforts in the absence of PRR disease will be required to identify the material with both the highest levels of PRR resistance and grain yield potential.

166

Johnson grass mosaic virus: resistance of Australian maize, sweet corn and grain sorghum hybrids and germplasm

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Johnson grass mosaic virus (JGMV) is widely distributed in the major perennial host Johnson grass (Sorghum halepense) in southern and central Queensland and north-west New South Wales.

Two strains of this aphid-transmitted potyvirus occur in Australia, the type strain (JGMV-JG) and the Krish -infecting strain (JGMV-K) which can infect grain sorghum hybrids/lines with the Krish resistance gene.

Although the economic impact of JGMV is currently low, the virus has caused sporadic severe losses in sweet corn, grain corn and grain sorghum over 30 years. Little work has been done on JGMV genetic diversity or resistance levels in commercial hybrids in the last two decades. Given the potential for economic impact, the resistance status of commercial sweet corn, grain corn and grain sorghum hybrids was assessed and current virus diversity determined.

We used glasshouse studies to determine resistance-breaking status of 38 JGMV isolates collected from 1992 to 2022, across three States. Of these, 19 JGMV-K strains were found from grain corn/sweet corn, sorghum and johnsongrass. One each of JGMV-K and JGMV-JG genomes have been characterised and complete genomes of a further 9 isolates of each strain are in progress.

All ten commercial grain corn hybrids tested by sap inoculation were resistant to both JGMV strains as were ten of 15 sweet corn hybrids. The remaining five hybrids were susceptible. The resistance status of sweet corn and grain corn hybrids to JGMV was well correlated with published resistance levels to the related exotic Maize dwarf mosaic virus. All 15 sorghum hybrids were susceptible to JGMV-K while 10 of these hybrids were resistant to JGMV-JG, suggesting the presence of the Krish resistance gene in their pedigree.

Posters 18 - 22: Integrated Disease Management/ Biological Control

94

An endophytic strain of *Pseudomonas poae*: Natural ally against Botryosphaeria dieback

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Botryosphaeria dieback (BD) presents a significant risk to global viticulture, causing substantial economic losses and decreased productivity. Traditional methods of controlling BD, such as chemical treatments and pruning, have limitations in terms of their effectiveness, and both a negative impact on environment, and safety for consumers. Biological control offers a promising alternative for combating BD. This research focused on exploring the potential of Pseudomonas spp. as biocontrol agents for BD by investigating 10 antagonistic strains for their ability to suppress the BD pathogen, *Neofusicoccum luteum*, in grapevine plants. The most promising strain, *Pseudomonas poae* BCA17, suppressed infection in potted grapevines by *N. luteum* by 80%. Investigations on the mode of action of BCA17 revealed that the bacterium was able to secrete bioactive diffusible compounds that significantly reduced the germination of spores and fungal biomass of *N. luteum*, as well as the other representative grapevine trunk disease (GTD) pathogens. MALDI-TOF analysis revealed the presence of an unknown cyclic lipopeptide in the crude extract, which was absent in a non-antagonistic strain, *P. poae* JMN13. This implies that this novel lipopeptide may play a role in the biocontrol activity of BCA17. This strain shows promise as a biocontrol agent that may be used as an alternative strategy for the management of BD. By harnessing its beneficial properties, BCA17 can effectively suppress BD pathogens and may promote vine health.

101

Fighting stubble-borne disease in Australian conservation agriculture farming systems

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Australian growers have universally adopted conservation agricultural (CA) practices, with retained crop residues and no-till. CA practices conserve soil moisture and protect from erosion with stubble forming the foundation to build soil C for soil health and provide carbon farming opportunities. However, CA also promotes the build-up and carryover of stubble-borne pathogens. The resultant disease epidemics and yield loss represent a rapidly emerging and increasingly wicked problem for farmers, threatening the sustainability of CA. Currently, growers are reliant on host genetics and fungicides, which are expensive and can be rendered useless due to pathogen evolution, or burning which creates pollution and soil-carbon losses. Clearly as CA practices are here to stay, and interest in broad acre regenerative agriculture grows, alternative approaches that are sustainable, resilient and socially acceptable are required.

Microbial decomposition of carbon-rich stubble is generally limited by N, P and S, and this likely underlies the surprising lack of soil-C increase in long-term CA systems. Specifically, the conversion of stubble-C to stable soil-C can be increased (7-29%) by nutrient supplementation of C-rich stubble with N, P and S to optimize microbial processing in a range of soils with rapid decomposition of stubble. This provides the opportunity to develop novel systems experimental approaches to identify innovative interventions to control stubble-borne disease by reducing stubble loads, while simultaneously increasing sequestration of soil C. The project investigates how to accelerate microbial and nutrient amendments to reduce the risk of pathogens including (i) strategic nutrient additions that balance stoichiometry and favour microbial stubble decomposition, (ii) introduced and naturally occurring microbes to speed stubble decomposition, (iii) introduced microbes with known competitive or antifungal properties to reduce pathogen growth on the stubble.

131

Using silicon to boost the cotton resistance against reniform nematodes

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The reniform nematode (*Rotylenchulus reniformis*) is a major plant-parasite of cotton worldwide and accounts for up to 11% of the total disease-related loss of cotton yield in the USA. In the Australian cotton industry, their presence is limited to the Central Queensland region while posing a biosecurity risk to other cotton-growing regions. Reniform can survive deep in the soil profile which makes it difficult to manage them once their population is established. There are currently no nematicides or resistant varieties against reniform nematodes. Silicon (Si) is known to play a significant role in plant defence against diseases and abiotic stress tolerance by providing physical resistance as well as by regulating the defence pathways. Glasshouse trials were conducted where cotton plants were primed using a readily available form of silicon (Plant Guard, 24% monisilicic acid) to test their effect against the reniform nematodes. The priming effect of silicon was tested in both direct seed-sown and transplantation settings. The silicon was applied on the day of sowing in both settings. The direct seed-sown pots were inoculated with reniform on the day of seed sowing, while the seedlings in the transplantation trial were grown in seedling trays for two weeks before being transplanted into the pots inoculated with reniform nematode. After 16 weeks, the reniform population in the soil was determined. It was found that silicon treatment significantly lowered the nematode population in transplanted cotton, while it had no effect on nematodes in direct seed-sown plants. These trials clearly showed that silicon provides resistance to cotton plants against reniform nematodes under controlled conditions; therefore, silicon needs more consideration from the scientific community. Future research should focus on understanding the mechanism of such resistance and optimising the use of silicon so that it can be adopted by growers in broadacre cropping systems.

153

Biocontrol of soilborne pathogens that cause poor root growth of Australian processing tomatoes

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Student Level: PhD student

Presentation Type: Oral Presentation

Abstract: Tomato is the second most cultivated vegetable crop both worldwide and in Australia. Processing tomatoes grown in the field in Victoria suffer a 10-15% yield loss due to soil-borne pathogens, with *Fusarium oxysporum* and *Pythium* spp. being the most aggressive. Biocontrol is a promising disease management practice with high sustainability, target specialization and cost-efficiency. This research focuses on identifying potential biocontrol microorganisms, examining their antagonism against *F. oxysporum* and *Pythium* spp., and evaluating their effectiveness in controlling root disease in processing tomatoes using both glasshouse pot trial and field test.

In glasshouse pot trials, the application of a local *Pythium oligandrum* strain both before and after inoculation with Fusarium and Pythium resulted in reduced level of root necrosis in tomato plants and increased shoot and root dry weight compared with the uninoculated controls. Further glasshouse trials showed that application of up to 10% v/v of *P. oligandrum* inoculum did not impact the growth or development of tomato plants. Therefore, this *P. oligandrum* strain was considered to have biocontrol potential and will undergo further field tests.

182

Suppression of Root-knot Nematode in Modified Commercial Sweetpotato Production Systems

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Meloidogyne spp. (Root-knot nematodes, RKN) are a major threat to the quality and quantity of marketable sweetpotato in Australia and world-wide. In relatively low numbers, RKN can affect the size, shape, appearance, and marketability of sweetpotato storage roots, and decrease both plant vigour and pest resistance.

Under commercial best practice, a field experiment comparing three different organic amendment treatments with a nematicide application, for the control of RKN, was conducted over four growing seasons. Resistant rotation crops were sown outside the growing season and incorporated into each plot. Plant-parasitic and free-living nematodes were quantified before planting and at harvest each season. Storage root quantity and quality were analysed at harvest, recording visual defects to assess any detrimental effects attributed to nematode damage or from the application of organic amendments.

Results to date indicate suppression of root-knot nematodes and promotion of free-living nematodes via the addition of either; banded organic matter, banded compost, or a V-furrow amendment of compost, to current best practice production. Initially, there were detrimental effects from the amendments on yield and quality of the harvested product. By the second harvest, there were no significant differences in total yield between treatments, however the organic matter treatment had less marketable yield than the nematicide treatment. By the third harvest, the organic matter treatment had significantly higher total and marketable yield when compared to other treatments.

Poster 23: Microbiomes and Disease Complex

126

Investigating microbial-driven formation of soil organic matter from crop residues to find novel strategies for promoting carbon sequestration and soil health

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It is estimated that global agricultural soils have lost between 50-60% of historical soil carbon. This is concerning in terms of both reducing global carbon emissions and maintaining healthy soil systems, as soil organic carbon helps retain soil moisture and positively influences crop productivity. Crop residues are the primary source material for formation of soil organic carbon, and consequently offer enormous potential for increasing soil carbon. The conversion of crop residues into soil carbon relies on the degradative activity of soil- and residue- associated microbial communities, a process that is yet to be comprehensively characterised.

Crop residues are managed in several ways, including burning, tillage, and no till management strategies. The practice of retaining crop residues offers potential for increasing soil organic carbon and helps prevent erosion and loss of soil moisture but can also promote the carryover of crop diseases. Finding ways to increase soil carbon via retention of crop residues, without increasing carryover of residue-borne disease is an area of great interest in agricultural research.

Our team at CSIRO are employing a combined metagenomic and metatranscriptomic approach to explore the impact that fungicide, fertilisers, and tillage have on the crop residue-associated microbiome responsible for conversion of crop residues to soil organic carbon. The knowledge gained from this project could help to improve crop residue management practices and contribute towards the development of microbial amendments that promote more efficient sequestration of carbon in soils and suppress residue-borne diseases.

Posters 24 - 28: Molecular Plant Disease Interactions

75

Unlocking the Secrets of Potato Plant Immunity: A Journey from Lab to Field

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The potato (*Solanum tuberosum* L.) is the world's third most commonly grown food crop with an exceptionally high yield potential. However, its production is hampered by various pathogens, leading to significant yield losses. Current control methods include frequent and costly fungicide applications and classical breeding is complicated in potatoes. A promising approach to developing long-term solutions involves improving our understanding of potato plant immunity and identifying factors that can provide broad-spectrum resistance. To facilitate such efforts, we applied comparative proteomic techniques to enhance our understanding of changes in protein abundance during immune responses in potato leaves. In the course of our study, we identified Parakletos, a potato protein that contributes to plant stress susceptibility. Knockout or silencing of Parakletos resulted in enhanced resistance to oomycetes, fungi, bacteria, salt, and drought, whereas overexpression of Parakletos decreased resistance. In response to biotic stimuli, plants overexpressing Parakletos exhibited a reduced amplitude of reactive oxygen species and Ca2+ signaling, while the silencing of Parakletos had the opposite effect. The function of Parakletos requires the calcium-sensing receptor, and Parakletos homologues have been identified in all major crops. After conducting two years of field trials, we found that the deletion of Parakletos improved resistance to *Phytophthora infestans* and increased yield. These findings could be exploited to enhance crop resilience towards both abiotic and biotic stress, potentially propelling progress towards low-input agriculture in a changing climate.

82

CHARACTERISATION OF GENES UNDERLYING A 3H QTL ASSOCIATED WITH NET FORM NET BLOTCH RESISTANCE IN BARLEY

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Net form net blotch (NFNB), caused by *Pyrenophora teres f. teres* (*Ptt*) is a destructive foliar disease of barley worldwide. The disease typically results in yield losses of 10-40%, however, extreme cases of infection may cause complete loss in highly susceptible crops. Breeding for genetic resistance to the pathogen is regarded as the most effective and environmentally friendly management method. Although many Quantitative Trait Loci (QTLs) which confer resistance to the pathogen have been identified in barley, little is known about the resistance mechanisms underlying those QTLs. Prior to this work, a Genome-Wide Association Study identified two significant QTLs on chromosomes 6H and 3H with resistance against *Ptt* in a Nested Association Mapping population developed from crosses between 25 barley wild accessions and the cultivar Barke (Pham and March, unpublished data). While the 6H QTL was likely the Rpt5/Spt1 locus previously described in other studies, little is known about the second most significant QTL detected on chromosome 3H. This research inspected the region spanning nearly 50 Mb and consisting of 700 genes between the two most significant markers linked to the resistant 3H QTL. Amongst these genes, candidate genes potentially associated with the plant defence response were identified for further investigation. These genes encode proteins including receptor kinases, ABC transporters, endochitinase, peroxidase and phosphatase. Differential gene expression of these candidate genes is also being analysed within the mapping population during the interaction with three *Ptt* isolates. Furthermore, bioassays using the proteinaceous extracts from the pathogen on intact barley leaves investigated if plant sensitivity response to toxins secreted by *Ptt* is the mechanism underlying the disease resistance. Once identified, the knowledge of the causal gene(s) and the mechanism of NFNB resistance conditioned by the QTL on chromosome 3H will be useful in future breeding efforts to develop novel resistant varieties.

118

The inhibitor of virus replication (IVR) is derived from anaphase-promoting complex 7 (APC7) and functions in resistance to infection by TMV in N gene tobacco

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The inhibitor of virus replication (IVR) is a well-known plant protein inhibitor shown to be effective against infection by several plant viruses. Recent work in our lab has shown that IVR is part of a novel resistance pathway mediated by the transcription factor (TF) SHE1 (Signaling Hub Effector 1), which is induced by TMV infection of N gene tobacco. SHE1 also interacts with the IVR protein, in vivo and in vitro. In addition, IVR interacts with the 1a protein encoded by RNA 1 of cucumber mosaic virus, suggesting that this replication-associated protein is a target for the action of IVR. IVR was shown to comprise the C-terminal 34% of anaphase-promoting complex 7 (APC7), part of the cellular cyclosome, a 13-subunit E3-ubiquitin ligase controlling the progression of mitotic division. APC7 contains six tetratricopeptide repeats (TPRs), each containing a helix-turn-helix structure, as well as three other non-TPR helices. The 199-amino acid IVR contains 10 helices (3.5 TPRs and three additional single helices), as well as an unstructured 28-amino acid C-terminus. The C-terminal half of IVR contains the SHE1 interaction site. However, structure modeling suggests that the number of IVR helices involved in interactions, rather than their particular position within IVR, may be more important for the various interactions IVR undergoes. The modeled structure IVR is identical to that of the C-terminal third of APC7, indicating a separate functional domain within APC7. The sequences of the tobacco APC7 gene, upstream of the IVR coding region, contain several putative promoter sites for various TFs including four GCC binding elements for SHE1, upstream of putative transcription start sites. We propose that these sequences located within the APC7 gene allowed the induction of expression of IVR by SHE1 to serve a novel function in plant defense.

125

CRISPR knock-out mutants of an S gene in potato leads to increased tolerance to both biotic and abiotic stress

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Potato is the third most important food crop, but potato production heavily relies on chemical fungicide application, which is regarded by many as an unsustainable control method. Susceptibility (S) genes often have a potential for increased broad-spectrum resistance when functionally knocked out. An S gene involved in hormonal regulation has been knocked out in potato in our lab. The S gene mutants show increased broad-spectrum resistance to biotic stress induced separately by the oomycete pathogen *Phytophthora infestans* and the fungal pathogen *Alternaria solani*. Furthermore, the mutant phenotype shows increased tolerance to abiotic stress caused by either salinity or drought. The tolerance is characterized by improved growth in controlled saline conditions and improved recovery from controlled drought conditions. The mutants show higher transcript abundance of stress-related genes after exposure to stress.
To our knowledge this is the first time an S gene mutant has led to improved tolerance to both biotic and abiotic stress. Additionally, field trials with the S gene mutants are currently going on for the fourth year in southern Sweden, where they will be evaluated in regard to biotic stress tolerance and yield.
These results can contribute to decreased fungicide use and improved yield stability, which is expected to become a more common challenge along with the changing global climate.

164

Isolation and physiological characterisation of *Ascochyta rabiei* exosomes

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Across kingdoms, exosomes are produced by pathogenic organisms that are associated with signalling for host recognition and invasion, including by fungi and when in contact with their plant hosts. However, very little is known about plant pathogenic fungal exosomes, with data limited to a few early studies on *Fusarium oxysporum* and *Penicillium digitatum*. Meanwhile, *Ascochyta rabiei* is an endemic and widespread necrotrophic ascomycete fungus that causes significant impact on chickpea production. Very few *A. rabiei* avirulence (avr) factors have been proposed and none have been functionally assessed for how they are delivered to the chickpea plant. Therefore, the current study focuses on determining if *A. rabiei* produces exosomes when in the presence of the chickpea host and their physiological characters. Isolation of *A. rabiei* exosomes will lead to their assessment as potential carriers of identified avr and putative effector sequences involved in the initial recognition and establishment. In this study, *A. rabiei* exosomes were isolated from liquid broth cultures through optimised ultrafiltration methods. They were then morphologically characterized under Transmission Electron Microscopy and Nanosight nanoparticle analyser, revealing typical cup-shaped structures with double membranes of 30-150 nm in size with greater frequencies produced in response to the presence of powdered and concentrated host tissues compared to whole detached leaves. This is the first time exosomal structures have been identified in an Ascochyta species and further work will determine cargo and predictive functionality.

149

Prevalence and biology of rachis tip dieback in macadamia

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Macadamia is susceptible to several flower diseases, including grey mould, green mould, dry flower disease, and wet blight, which are caused by species of Botrytis, Cladosporium, Pestalotiopsis, and Phytophthora, respectively. Necrotic dieback at the rachis distal end has been associated with all the symptoms of the four diseases affecting macadamia flowers. This study aims to investigate the prevalence and causal agents of rachis tip dieback in macadamia trees to better understand the cause of this issue. Surveys of commercial macadamia orchards in the three major production areas in Central and Southeast Queensland and the Northern Rivers will provide information on the occurrence of rachis tip dieback in the Australian macadamia industry. Preliminary results from the 2023 flowering season have revealed five different genera identified as Epicoccum, Cladosporium, Pestalotiopsis, Neopestalotiopsis, and Diaporthe associated with rachis tip dieback. Koch's postulates will provide crucial information on the causal agent(s). This study sheds light on how widespread rachis tip dieback is, the identity of the causal agents, and the putative role of rachis tip dieback in flower disease epidemics in macadamia. The insights gained from this study will facilitate the development of an efficient disease management strategy in macadamia cultivation practices.

Posters 30 - 31: New Technologies (Artificial intelligent (AI)) and Novel Methods in Plant Pathology and Disease Control

79

A split GAL4 RUBY assay visually shows association between the wheat Sr27 resistance protein and corresponding AvrSr27 stem rust effector proteins in planta

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The study of the interaction between plant resistance proteins and their cognate effectors is highly significant for understanding the mechanism of plant immunity activation. Moreover, it will provide guidance for engineering and strengthening immunity. The wheat stem rust resistance protein Sr27 provides resistance to *Puccina graminis* f. sp. *tritici* isolates that express cognate AvrSr27 effector proteins. In planta expression of Sr27 and AvrSr27 induces plant defense in the heterologous plant *N. benthamiana*, but interaction between these proteins is not detected in yeast-two-hybrid assays.
We have developed a novel, in planta protein-protein interaction (PPI) assay that confirms interaction between Sr27 and AvrSr27 proteins in *Nicotiana benthamina* leaves in real time. The assay uses a split GAL4 yeast transcription factor to induce transcriptional activation of a RUBY reporter gene upon PPI, leading to the production of the highly visual metabolite, betalain. Samples require no processing for in planta visual qualitative assessment, but with very simple processing steps the assay is quantitative and its accuracy is demonstrated using a series of known interacting protein partners and mutant derivatives. Using this method, we were also able to detect interactions between several pairs of R-Avr proteins, such as Sr50-AvrSr50, Sr35-AvrSr35, and so on. Using this PPI assay, interactions were observed between the Sr27 protein and each member of the AvrSr27 protein family, including the avrSr27-3 protein encoded by the corresponding virulence allele. However, differing interaction strengths were observed between Sr27 and these effector proteins with the avrSr27-3 virulence protein showing a weaker interaction, which coupled with its lower expression during stem rust infection, likely enables it to avoid Sr27 detection.

139

Cube Bioassays: A new high-throughput method for screening multiple fungal pathogens against multiple plant hosts

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When it comes to screening potential pathogens for use in biocontrol, the process can be arduous, time consuming and resource intensive. Due to the nature of the plant microbiome, one of the more confounding issues is contamination risk from other microorganisms when determining the cause of disease or symptoms.

Here, we present a novel bioassay method using a compact, closed, and sterile system, that includes a total nutrient agar that can support seedlings for months at a time. Our bioassay allows for multiple plant hosts (up to 4 or 5) to be grown together, and inoculated with with mycelial plugs or a spore solution from the pathogen(s) of interest. Once sealed, quantitative and qualitative observations of symptoms and mortality can be done without risking contamination, and pathogenicity can easily be determined upon completion, via re isolation, microscopy, and/or sequencing.

We created and optimized this bioassay in the context of biocontrol of weedy *Sporobolus* grasses. We screened over 100 fungal isolates to prioritize further testing of 15 candidate agents. However, we believe our Cube Bioassay method can be used for screening of any potential plant pathogen, as well as for the testing of fertilizers, herbicides and pesticides.

Posters 32 - 42: Plant Disease Management, Chemical Resistance

37

High throughput field screening protocols for Sclerotinia stem rot disease in canola and lupin

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Sclerotinia stem rot (SSR), caused by the widespread necrotrophic plant pathogen *Sclerotinia sclerotiorum* Lib. (de Bary), is a destructive disease of many important agronomic crops including *Brassica napus* L. (canola) and *Lupinus* spp. (narrow-leaf and albus lupin). Under favourable environmental conditions, outbreaks of SSR can lead to substantial economic losses in these crops through reduction in yield and grain quality. The utilisation of genetic resistance is an integral component of successful IPM strategies, however currently there is no complete SSR resistance available in Australia for either canola or lupins. Further, the development of a reliable, low-cost and rapid high throughput protocol for screening potential breeding lines in the field is required. We conducted detached leaf assays, whole plant and field plot inoculations (with and without misting) to determine the optimum carrier and technique for reliable and consistent SSR disease. Twelve plant-based inoculum carriers, colonized by mycelium from a single isolate (CU11.19) of *S. sclerotiorum*, were applied to fully expanded detached leaves of a semi-resistant canola, susceptible canola and susceptible lupin variety. Lesion area was measured using leaf imagery after 2 days. The three most consistent techniques causing SSR lesions were subsequently used for whole plant assays and field plot inoculations. Results are ongoing and will be finalized during the 2023 growing season.

99

In vitro fungicide efficacy testing against *Rhizopus stolonifer* the cause of hull rot of almond.

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In Australia, hull rot is a significant disease of almond fruit primarily caused by *Rhizopus stolonifer*. Symptoms include fungal growth, starting on the inside of the hull, and dieback of twigs and fruiting spurs due to accumulation of organic acids produced by the fungus. In vitro testing was carried out on a number of fungicides and biological agents, for their efficacy at controlling *R. stolonifer*. Isolates of *R. stolonifer* from each of the main almond growing regions, Riverina NSW, Sunraysia VIC, and Riverland SA, collected in 2019 were selected for testing. Products were assessed at their label rate by either incorporating into molten agar (fungicides) or spreading over the plates surface (biologicals). Mycelial plugs from the growing edge of 3-day old cultures were inoculated onto amended plates. Culture diameters were measured at 3 and 8 days to determine the effectiveness of each product at inhibiting fungal growth. Most of the fungicides and biologicals tested restricted the growth of *R. stolonifer* to a greater or lesser extent. There were some promising chemical and biological control agents identified in these preliminary laboratory screenings. The better performing products were then tested in a bioassay on detached almond fruit. When considering both the in vitro and detached nut assays the most effective chemical products, when applied prior to inoculation with the pathogen, were fluopyram + tebuconazole, isopyrazam, and copper hydroxide, restricting fungal growth by ≥ 99% and decreasing the number of infected nuts by up to 82%. None of the products had any curative effect except for fluopyram + trifloxystrobin which resulted in 45% fewer infected nuts in the detached nut assay. Our data suggests that the products containing *Bacillus amyloliquefaciens* were effective biological control agents performing comparably in the bioassay to the best protectant chemical products over the timeframe tested.

102

In vitro evaluation of fungicides for management of *Cryphonectria parasitica*

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Victoria accounts for 70% of Chestnut production in Australia generating approx. $6.3 M through domestic sales. Chestnut blight caused *Cryphonectria parasitica* (Cp) was discovered in Victoria in 2010 and due to active eradication and surveillance was confined to northeast Victoria. However, the continued new discoveries of infected premises led to the transition to management of the disease in 2019. This study aimed to identify fungicides that may provide preventative or curative treatments for chestnut blight. Nine chemical companies were contacted for potential fungicides. Twenty-one chemicals covering 10 activity groups and one combination were screened in vitro against Cp at 3 rates. At the recommended label rate, only 8 of the 22 had any inhibitory effect on spore germination and mycelial growth. The most effective, in order, were epiconazole (Soprano®, Group 3), captan (Captan 800 WG, M4) and carbendazim (Howzat®, Group 1), mefentrifluconazole (Belanty®, Group 3), propiconazole (Bumper 625®, Group 3) and prochloraz (Sportak®, Group 3). Tebuconazole (Orius® Group 3) and mancozeb (Dithane®, M3) showed a slight inhibitory effect, but it was minor in comparison with the previous six. There was considerable difference in activity among the Group 3 fungicides tested, indicating that active ingredient could be more important than fungicide group. Fungicides from Groups 7, 9, 11, 12, U6 and a combination of Groups 3 & 11 had no inhibitory effect. Disappointingly, the chemicals already registered for use on chestnuts in Australia – copper (M) and penthiopyrad (Group 7) – had no effect on Cp germination and growth. On this basis, epiconazole, captan, carbendazim, mefentrifluconazole and propiconazole are recommended for the next stage of trialling in planta.

111

Characterisation and management of leaf pot disease of cotton in New South Wales

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Leaf spot of cotton has been long considered a minor disease in New South Wales (NSW), especially when only Upland cotton (*Gossypium hirsutum*) are grown in commercial fields these days. However, outbreaks on cotton seedlings were reported in the 2017/18 season in southern NSW. In the 2021/22 season, an outbreak on mature cotton which was subsequently resulted in an estimated loss of 25% of a dry land crop, was recorded in northern NSW. Using multigene sequences, *Alternaria alternata* was identified as a predominant fungal species recovered from diseased cotyledons and leaves. In 2021/22 season, 15% of the fungal collection was identified as *Stemphylium solani*, which was for the first time fully characterised. We also recovered a small number of isolates belonged to unidentified *Alternaria* species. However, no *A. macrospora*, which was previously reported as a main pathogen on cotton, was recovered between 2017 and 2022 seasons. Both *A. alternata* and *S. solani* were highly aggressive on two-true-leaf seedlings. Similarly, historical *A. macrospora* isolates were able to incite severe infection on seedlings. We also found a comparative level of aggressiveness of *A. alternata* and *A. macrospora* on three most commonly commercial varieties (cv. 714B3F, 746B3F and 748B3F). Though growth best at 25 °C, *A. alternata* appeared more virulent on detached leaves at 20 °C. This could provide an insight into the disease occurrence in the field when growing seasons experienced temperature stress. Both Tebuconazole and Mancozeb, which are currently operated under permit, provided good preventative protection of cotton against *A. alternata* infection. Other alternative tested products were also highly effective against *A. alternata*. However, none was registered in cotton. This warrants further research to enhance preparedness for the Australian cotton industry.

119

In-field evaluation of products for the management of almond hull rot

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Hull rot disease of almonds is an ongoing concern in Australian orchards with management strategies historically centring around cultural practices. More recently, several fungicides have been registered for use in Australia for the suppression of hull rot. Field trials conducted in 2021 and 2022, as part of Integrated pest management project AL16005, compared both registered products and products purported to suppress hull rot disease development.
In both seasons, treatments were applied at 10% hull split (mid-January) within a Sunraysia orchard with industry standard plantings of Nonpareil on Nemaguard, with alternating rows of pollinisers, and spacings of 7.5m between rows and 5m between trees. Four replicated blocks of each treatment were applied in both years, with an experimental unit of 0.5 ha in 2021 and 0.2ha in 2022.
In 2021, treatments were: the standard orchard practice of Custodia® (200 g/L tebuconazole + 120 g/L azoxystrobin); Luna Sensation® (250 g/L fluopyram + 250 g/L trifloxystrobin); dipotassium phosphate; and Luna Sensation® + dipotassium phosphate. In 2022, treatments were: Custodia® (200 g/L tebuconazole + 120 g/L azoxystrobin (the current practice); Luna Sensation® (250 g/L fluopyram + 250 g/L trifloxystrobin); and diKaP™ ((P2O5) 31%, (K2O) 50%) which is a nutritional and stress tolerance product promoted by Dr Jim Adaskaveg (University California - Riverside) and imported by AVR from Redox Pty Ltd.
In 2021 dipotassium phosphate had no suppressive effect, while in 2022 diKaP™ showed similar efficacy to registered products Custodia® and Luna Sensation®. Across both seasons, current registered chemistries reduced hull rot but did not eliminate the disease. A timely application of a protectant product at hull split is beneficial in high-risk hull rot areas.

134

New country records of fungal pathogens of persimmon (*Diospyros* spp.) in Australia and in vitro evaluation of fungicides against *Neofusicoccum parvum* and *Neopestalotiopsis* species

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Persimmon (*Diospyros* spp.) is susceptible to attack by several plant pathogens. Samples from persimmon trees displaying both leaf lesions and dieback symptoms were collected from orchards in NSW. Diseased leaves exhibited black lesions starting at the tip of the leaf, whereas dieback-infected stems showed internal staining of vascular tissues. Fungal isolation, pathogenicity, and cross-inoculation between isolates derived from different plant materials, along with preliminary evaluation of several fungicides in vitro, were tested. A single isolate of *Neofusicoccum parvum* and *Neopestalotiopsis* spp. were used as representative isolates from symptomatic samples. The leaf isolate N. parvum 004-1b, and dieback isolate *Neopestalotiopsis* spp. 014-1a were identified using morpho-cultural methods and PCR assay using the internal transcribed spacer region. Pathogenicity of N. parvum 014-1a was confirmed on leaves of Jiro and Fuyu cultivars. *Neopestalotiopsis* spp. 004-1b was cross-inoculated to leaves and confirmed to be infective. Both pathogens were re-isolated from the inoculated areas. Recommended rates (RR) and ½ RR of tebuconazole + fluopyram (0.75 ml L-1 PDA and 0.38 ml L-1 PDA), fluazinam (1.00 ml L-1 PDA and 0.50 ml L-1 PDA), and fludioxonil (0.75 mg L-1 PDA and 1.50 mg L-1 PDA) amended in PDA plates exhibited 100% growth inhibition in both isolates regardless of the concentration. In pyraclostrobin, 100% growth inhibition was only observed for *Neopestalotiosis* spp. 004-1b while only 87.46% growth inhibition in *N. parvum* 014-1a. Isopyrazam (0.75 ml L-1 PDA and 0.38 ml L-1 PDA) and penthiopyrad (1.50 ml L-1 PDA and 0.75 ml L-1 PDA) also showed potential in inhibiting the growth of both isolates relative to the control plate. To our knowledge, this is the first report of *Neofusicoccum parvum* as a persimmon leaf pathogen and the cross-infectivity potential of *Neopestalotiopsis* spp. to persimmon leaves. Results from preliminary evaluation of different fungicides warrant further studies in glasshouse assays.

140

Validation of different collection methods to identify fungicide resistance in *Erysiphe necator* in South Australian vineyards

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Erysiphe necator, which causes powdery mildew in grapevine, is one of the most widespread pathogens in Australian vineyards and poses a significant challenge to the industry's sustainability worldwide. Detecting fungicide resistance of *E. necator* is a key element in resistance management strategies. The ubiquitous nature of this pathogen puts pressure on fungicides used for control, particularly the quinone outside inhibitors (QoIs), the DeMethylation Inhibitors (DMIs) and the Succinate dehydrogenase inhibitors (SDHIs). Intensive use of these fungicides has led to resistance in some areas worldwide. Different sample collecting methods for powdery mildew on grapes were evaluated in South Australian vineyards, including; rotorod spore trap, mini-vacuum cyclone separator, washing infected leaves, cotton bud swabs and swaps from working gloves. High-throughput sequencing (HTS) was used to identify three mutants, G143A, Y136F and H242R/Y, associated with resistance to QoIs, DMIs and SDHI fungicides, respectively. These methods were trialled in the laboratory, glasshouse and field. All collection methods were equally successful tools for detecting the genetic resistance of *E. necator*. HTS genetic analysis showed that two mutants, Y136F and G143A that are associated with DMI and QoI resistance, respectively, were identified from air samples and plant leaves. However, H242x (linked to SDHI resistance) has not been detected. The rotorod spore trap proved to be more sensitive and practical, especially early in the season when there were no visible symptoms of powdery mildew to be collected using other methods. Current research is developing sampling protocols for in-field and high throughput qPCR techniques to achieve large scale screening for mutant detections to monitor for fungicide resistance.

158

Sclerotinia is a real issue for WA lupin growers

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Sclerotinia is a polyphagous fungal disease with a wide host range mainly infecting canola and lupins in Western Australia's broadacre grain crops. Sclerotinia is seen as an emerging threat for lupin production in WA. A GRDC co-funded research project '' Sclerotinia management for narrow leaf lupin crops in Western Australian farming systems" commenced in 2021 to investigate the economic impact, epidemiology, pathogenesis, and development of strategies for managing sclerotinia disease in medium and high rainfall zones in WA. Surveillance data gathered in 2021 and 2022 revealed that disease incidence increased and spread in Western Australian commercial lupin crops. This is not only a risk for lupin cultivation, but also for other vulnerable rotation crops like canola and pulses which are important to retain within farming systems. Overall, 72% and 37% of paddocks were affected by canopy and basal sclerotinia respectively, across in the WA grain belt. In field trials incidence of plants with canopy infection was 29% and basal infection was 7%. Approximately 50% of field trials showed yield response to fungicide application. Sclerotinia is a disease highly responsive to environment and its patchy occurrence in paddock can make it challenging for growers to manage. This poster provides a brief overview of lupin sclerotinia disease, its progression and impact on narrow leaf lupin from a Western Australian perspective.

163

Unveiling the infection mechanisms of flower blight disease pathogens in macadamia

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The flower blight disease complex (FBDC) threatens macadamia orchards by destroying different stages of flower development, resulting in a poor nut set and significant yield losses if not managed properly. The FBDC comprises Botrytis blight caused by *Botrytis* spp., Cladosporium blight caused by *Cladosporium* spp., and dry flower disease caused by *Neopestalotiopsis* and *Pestalotiopsis* spp. Previous studies showed that the aggressiveness of *B. cinerea* and *B. macadamiae* are similar but *C. cladosporioides* is the most aggressive among the six *Cladosporium* species causing green mould. For dry flower disease, the most aggressive species is the *N. macadamiae* among the other eight pathogens. Although the etiology of the three flower diseases has been established in Australia, information on the infection process of the pathogens in macadamia is unknown. Therefore, this study aimed to elucidate the mechanisms used by the most aggressive species to penetrate, colonize, and proliferate in macadamia flowers. Using light and scanning electron microscopy, the infection process from inoculation to symptom expression in macadamia cultivar A268, at the four flower growth stages was monitored. The patterns of spore germination, host penetration, colonization, and sporulation on the floral tissues by each pathogen will be presented. This information would support management decisions for flower diseases, including the identification of the optimum timing of fungicide applications and the risk of spread in the Australian macadamia orchards.

180

Crown and peduncle mould of pineapples: industry implications and next steps

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Postharvest moulds of pineapple crowns and peduncles are a common yet often overlooked quality issue. The pathosystem comprises several mould fungi including *Penicillium*, *Fusarium*, *Aspergillus* and *Trichoderma* species, amongst others.

The incidence of pineapple crown and peduncle mould is influenced by several pre- and post-harvest factors. High-sugar cultivars are more susceptible, due to greater available energy for infecting microbes and increased translucency and leakage at the peduncle. The sugary crown/peduncle exudates promote post-harvest infection and mould growth.

There is mounting evidence to suggest potential human health effects caused by these moulds (i.e. through inhalation, ingestion), alongside quality/saleability issues. The mould fungi can quickly contaminate cold and storage facilities, setting up a persistent infection cycle. Contamination of such sites, particularly where there is poor ventilation, poses further risk to workers.

Aside from external mould, internal discolouration/browning of flesh has also been observed in trials. Incidence is dependent on variety, season and sugar content, and represents further quality implications to consider.

Current post-harvest fungicide regimes may be inadequate for controlling this disease. Furthermore, *Penicillium* moulds present a high fungicide resistance risk. As such, we are trialling novel approaches to manage this disease issue, including dual-action fungicide and sanitiser rinses, alongside cold room hygiene measures. We have identified several effective post-harvest treatments, which are undergoing further testing and confirmation.

We are also profiling the pineapple-peduncle mould fungi through molecular characterisation. We aim to determine the species commonly infecting pineapples after harvest, to better understand health and management implications. We have isolated over 20 different fungi (filamentous and yeasts) which will be sequenced, categorised and stored for subsequent pathogenicity testing.

199

Fungicide resistance screening of a *Botrytis cinerea* population from Australian vineyards and development of an in-field detection method.

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Botrytis cinerea, the causative agent of Botrytis bunch rot, is one of the most economically significant diseases of grapevines worldwide. The use of fungicides to control B. cinerea is an integral part of disease management in grapevines. Fungicide resistance in *B. cinerea* for all current single-site fungicides is geographically widespread. In this study, isolates (N = 456) were collected between 2017 – 2022 from 48 vineyards across Western Australia, South Australia, Victoria, and New South Wales. The isolates were screened on discriminatory concentrations of three chemical groups (9, 12 and 17). Resistance frequencies were 10.1, 5.9 and 5.9% for group 9, 12 and 17, respectively. All isolates resistant to group 9 and 17 were genotyped for the target genes pos5 or mdl1 (group 9) and erg27 (group 17). In pos5 and mdl1, the changes V273I, P293S, P319A or L142F/V and E407K or G408R were identified, respectively. In erg27, the changes F412I/S/V were found. These screening results have provided valuable information on the resistance status of *B. cinerea* in Australian vineyards and could assist in improving resistance management strategies. An in-field qPCR method to detect L412F and F412S mutations was developed and tested. This method was adapted from a previous report that detected G143A (group 11, cytB) in *Blumeria graminis* f. sp. *triciti* on barley leaves in situ (Dodhia et al. 2021). Within a vineyard setting, infected berries (N = 69) were sampled and hand ground in a buffer for the quick extraction of DNA using a micro pestle. The resulting slurry was diluted and then used as a template for qPCR in a lightweight qPCR instrument. The frequencies of L412F and F412S were 4.3 and 1.4%, respectively. This in-field qPCR method could provide a quick and simple early detection approach in vineyards with known control issues.

Posters 43 - 47: Taxonomy, Diversity and Evolution

35

Release and re-capture: tracking adaptation of clonal *Ascochyta rabiei* chickpea host genotype association

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Ascochyta rabiei is a necrotrophic fungus of chickpea (*Cicer arietnum*), which can lead to complete yield loss under favourable conditions. Meanwhile, the Australian *A. rabiei* population is clonally propagating with low genetic diversity; but is still capable of evolving and adapting to overcome host resistance. The effect of host resistance genetics on the *A. rabiei* adaptation is largely unstudied. Therefore, this study aims to uncover any potential host-mediated selection through release and recapture of known *A. rabiei* genotypes over three successive cropping seasons. For this, six *A. rabiei* isolate genotypes that were previously whole genome sequenced, were selected from the Northern NSW chickpea growing region, and unique SNP allele identifiers (haplotypes) were determined. A mixed, equal spore count solution numbers of each of the six isolates were inoculated under field conditions onto plots (6 m x 2 m rows) of six chickpea host genotypes and replicated four times in a randomised complete block design at latitude -31.157514 and longitude 150.982791. Each treatment plot was buffered with faba beans to minimise external infection. At early podding (165 DAS), 30 disease lesions per plot were sampled using a transect method resulting in the recovery of a total 477 *A. rabiei* isolates. All isolates were genotyped to assess for the presence of an applied haplotype with a partial sequencing method developed using the Allegro® Targeted Genotyping V2 platform (Tecan, 2023). Haplotype calling was done where alternate alleles to the *A. rabiei* Me14 reference genome assembly ASM401169v2 (GenBank GCA_004011695.2) were identified. The subsequent frequency and distributions of the haplotypes were mapped across the plots as an initial indication of selective host genotype association. Future analyses with multiple seasons data will uncover selective adaptation of the source population due to host genotype effects, including assessing for changes in avirulence sequences related to increased pathogen aggressiveness.

49

Novel Trichoderma species indigenous to New Zealand

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Endophytic Trichoderma fungi are widely used in agriculture and forestry to improve plant health and performance. The aim of this research was to identify novel Trichoderma strains and to test them for beneficial attributes. Endophytic Trichoderma were recovered from within roots of 167 out of 378 *Pinus radiata* trees sampled from Kaingaroa forest, New Zealand. Individual trees were selected using a novel method designed to identify superior growth after partitioning out microsite and inter-tree competition effects. Phylogeny of isolates was assigned based on tef1 gene sequencing which indicated that eight of the isolates are novel species indigenous to New Zealand. 58 representatives of these eight species were assayed for the production of antibiotics against common plant pathogens *Rhizoctonia solani, Sclerotinia sclerotiorum* and *Pythium sylvaticum*. 22 out of 58 isolates showed antagonism towards at least one of the pathogens, with clear trends within species observed. The production of siderophores by Trichoderma are an important mechanism for promoting plant growth. Siderophore production by the novel Trichoderma strains was investigated and two indigenous species consistently exhibited siderophore production. These two species were also antagonistic against Pythium, suggesting that competition for iron resources may be involved in the observed antagonism. Trichoderma are well-known produces of cellulases that are a valuable commodity in many industrial processes. Most of the novel isolates exhibited some cellulase activity, with seven strains scoring highly.

73

Discovery of Four Novel *Xanthomonas* Species and the First Report of *Xanthomonas cannabis* in Australia

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Xanthomonas is a genus of globally significant plant pathogens that cause disease in a wide range of crops, leading to significant economic losses. In this study, we report the discovery of four novel *Xanthomonas* species infecting strawberry (*Fragaria x ananassa*) and alfalfa (*Medicago sativa*). These species were isolated during routine biosecurity surveillance in NSW and QLD and were initially identified as *Xanthomonas* through biochemical assays. All four were classified as potential novel species as part of a larger project to sequence *Xanthomonas* within NSW Culture Collections. Phenotypic characterisation assays, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS), average nucleotide identity (ANI), digital DNA-DNA hybridization (dDDH), and extensive phylogenetic analysis confirmed these isolates as novel species of *Xanthomonas*. Preliminary results from pathogenesis assays in *Fragaria x ananassa* show angular leaf spots in multiple plant hosts, however, the causal agent has not yet been confirmed. Furthermore, we have identified multiple *Xanthomonas cannabis* isolates, a significant plant pathogen previously unreported in Australia. The pathogens were isolated from *Zinnia elegans* (common zinnia) and *Cucurbita pepo* (zucchini) in NSW and QLD. Phylogenetic analysis demonstrates that four of the five Australian *X. cannabis* isolates form a monophyletic clade separate from international isolates, indicating they are genetically distinct. Further analysis reveals Australian *X. cannabis* isolates contain a higher number of virulence factors compared to any other *X. cannabis* isolate documented in the NCBI database, suggesting higher pathogenicity. This study expands our understanding of the diversity and distribution of *Xanthomonas* species, highlights the potential risks they pose to economically significant crops, and underscores the importance of routine biosecurity surveillance.

74

Genotypic and phenotypic characterization of *Phytophthora infestans* populations in Bangladesh

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Late blight, caused by *Phytophthora infestans*, is an economically important disease of potato which causes significant yield losses, with severe outbreaks regularly occurring in Bangladesh. The objective of this study was to do a large-scale survey of potato fields in the main potato-growing divisions of Bangladesh examining genotypic diversity of *P. infestans* populations. A total of 160 samples were collected in 2018 from both potato (n = 140) and tomato (n = 20). Isolates were mainly collected on FTA cards (n = 143) but 17 were also collected and isolated into pure culture. Microsatellite analysis revealed high levels of subclonal diversity in *P. infestans* populations with 116 multilocus genotypes recorded from 160 samples. Comparisons with standards of European and US isolates showed that 74% of samples could be categorized as genotype EU_13_A2, 7% clustered near EU_6_A1 and EU_1_A1 and 19% were unique. Discriminant analysis of principal components showed that the *P. infestans* population clustered into four distinct groups, a main group that contained most of the samples from potato, two distinct tomato groups and one group of samples originating from the division of Mymensingh. Of 17 isolates from cultures, 15 were insensitive to metalaxyl-M, whilst 16 were EU_13_A2 and one was EU_6_A1. Out of 160 samples, 158 were categorized as mating type A2 and two as mating type A1. These results indicate that Bangladesh populations of *P. infestans* from potato, like those from neighbouring countries, are dominated by genotype EU_13_A2. However, populations from tomato were distinct and appear to be specific to tomato.

195

New species of *Colletotrichum* identified on Australian native plants

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The fungal genus *Colletotrichum* comprises around 340 species, many of which are significant plant pathogens of agricultural crops. While there is substantial knowledge of diversity and host range of species associated with agricultural crops, little is known about species infecting Australian native plants. This study aims to improve understanding of the taxonomic diversity of *Colletotrichum* spp. associated with Australian native plants. A collection of *Colletotrichum* cultures isolated from Australian native plants were acquired from state culture collections, VPRI (Victoria) and BRIP (Queensland), and new field collections in Victoria and Queensland. The isolates were identified using a polyphasic approach of multigene phylogenetics and morphological characterisation. Forty-two isolates were identified as members of the *gloeosporioides* species complex, nine isolates of the *acutatum* species complex, eight isolates of the *boninense* species complex and one isolate from each of the *dematium*, and *spaethium* species complexes. Of particular interest are two putative new species in the *gloeosporioides* species, which were isolated from a range of Australian native plants. These findings highlight the potential unexplored diversity of *Colletotrichum* spp. outside of agricultural systems and the importance of considering both agricultural and native plants when looking at diversity and host range. Pathogenicity bioassays will be conducted to understand the lifestyle and severity of these species and to ascertain the risk to Australian native ecosystems and agriculture.