BOOK OF ABSTRACTS

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ORAL PRESENTATIONS

1. Genomics

Genomic features associated with leaf and stem pathogenicity in *Teratosphaeria*

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Abstract

Teratosphaeria is a genus of fungi primarily associated with *Eucalyptus*. Several species are important pathogens of *Eucalyptus* trees planted in forestry settings outside of their natural habitat. While most are foliar pathogens causing leaf blight, two species cause stem cankers. The emergence of stem pathogens in a predominantly leaf-associated genus is intriguing and presents an opportunity to investigate the transition from leaf to stem specificity. We have recently established a *Teratosphaeria* genome project in which whole genome sequences have been determined for all major *Teratosphaeria* pathogens of *Eucalyptus*. These include the two stem pathogens *T. gauchensis* and *T. zuluensis*, the aggressive leaf pathogens *T. destructans*, *T. eucalypti* and *T. pseudoeucalypti* and the less aggressive *T. epicoccoides*. In this study, we characterised and compared all available *Teratosphaeria* genomes in search of features associated with organ-specificity traits. We hypothesised that genomic features and genes present in the stem pathogens, but absent from leaf-associated species, are associated with tissue specificity. Initially, single copy ortholog genes across all genomes were used to construct a phylogenomic tree. This phylogeny revealed that the stem pathogens share a common ancestor with the less aggressive *T. epicoccoides*, while the aggressive leaf pathogens reside in a discrete clade. The stem pathogens had few (<2 %) repeat sequences, in contrast to the highly repetitive (16-35 %) nature of the leaf pathogen genomes. Analysis of orthogroups revealed more than 200 gene duplications in the clade for the stem pathogens, in comparison to <40 duplications in other parts of the phylogeny. Numerous genes (142) unique to the stem pathogens were identified, rivalled only by 63 unique genes in *T. pseudoeucalypti.* Secondary metabolite biosynthesis capabilities were expanded in the stem pathogens but were similar among all aggressive leaf pathogens. Overall, stem pathogenicity found in *T. gauchensis* and *T. zuluensis* is associated with a genome architecture and metabolic profile that is distinct from that of the leaf pathogens. Further investigation of putative gene functions may reveal specific molecular mechanisms associated with these forms of tissue specificity.

Detection of novel mycovirus diversity in *Ceratocystis fimbriata*

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Abstract

Most research on viruses has focused on economically important plant and animal viruses, and mycoviruses (viruses that infect fungi) have largely been overlooked. The availability of fungal RNAseq datasets has dramatically changed this. As a consequence, the number of mycoviral genomes in public databases has increased exponentially. The availability of RNAseq datasets from *Ceratocystis fimbriata*, a notorious pathogen of sweet potato, has presented an opportunity for the identification and characterization of novel mycoviruses. In this study, analysis of deep sequencing data was used to detect viral sequences within RNAseq datasets from *C. fimbriata*. Contigs were assembled from these datasets using a de-novo-based assembly approach and were evaluated for the presence of an RNA dependent RNA polymerase (RdRp) domain. The RdRp containing contigs were further characterized using BLAST screening and phylogenetic tree construction. Several of these contigs were identified as containing novel mycovirus sequences, specifically belonging to the viral families *Totiviridae* and *Endornaviridae*. The mycoviral contigs associated with *Totiviridae* are presumed to belong to the genus *Victorivirus*, while those associated with *Endornaviridae* are either previously unclassified or belong to the genus *Alphaendornavirus*. As the contigs did not represent full viral genomes, species demarcation within these families was difficult, and future research will involve the generation of more complete genomes. This study demonstrates the suitability of using RNAseq data for the rapid discovery of mycoviruses and represents the identification of the first mycoviruses in *C. fimbriata*.

NRPS-dependent siderophore synthetase gene clusters and characteristics of NRPS siderophore synthetase genes in *Armillaria* and other species in the *Physalacriaceae*

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Abstract

Secondary metabolites are often pathogenicity or virulence factors in fungi. Genes involved in biosynthesis of these metabolites are usually contained in secondary metabolite gene clusters (SMGCs), such as nonribosomal polypeptide synthetase (NRPS) clusters. NRPSs contain domains, are either mono- or multi-modular, and produce peptides such as siderophores. Siderophores are high affinity ferric iron chelating compounds required for iron uptake under aerobic conditions. *Armillaria* spp. (*Basidiomycota*, *Physalacriaceae*) are mostly facultative necrotrophs of woody plants. Studies on mechanisms involved in their pathogenicity is thus warranted. NRPS-dependent siderophore synthetase clusters of *Armillaria* spp. and selected *Physalacriaceae* were therefore investigated using a comparative genomics approach. Siderophore biosynthesis by *Armillaria* spp. was also evaluated using CAS and split-CAS assays. Our results showed that the genomes studied generally contained at least one NRPS cluster and other clusters (NRPS-like, terpenes, type 1 polyketide synthetase, and some hybrid clusters). No correlation was observed between the number and types of SMGCs and reported pathogenicity of the species whose genomes were studied. The genomes contained one NRPS-dependent siderophore synthetase cluster each. All NRPSs were multimodular with the domain architecture (ATC)3(TC)2. NRPS clusters of the *Armillaria* spp. showed a high degree of microsynteny. Identified NRPS-dependent siderophore synthetase gene clusters in the genomes of *Desarmillaria* spp. and the closely related *Guyanagaster necrorhizus* were more syntenic to those of *Armillaria* spp. than to the other *Physalacriaceae* species studied. Three A-domain orthologous groups were identified, and atypical Stachelhaus codes were predicted for the A3 orthologous group of the NRPSs. We postulate that the siderophore biosynthesized by the identified NRPS-dependent siderophore synthetase clusters will be hydroxamates based on homology with characterized NRPSs, domain architecture, and predicted substrates of A-domains of the NRPS genes. Bioassays with strains of selected *Armillaria* spp. revealed in vitro biosynthesis of mainly hydroxamate siderophores and some catecholate siderophores. Hence, *Armillaria* spp. generally contains one highly conserved, functional NRPS-dependent siderophore synthetase gene cluster although some interspecific variations in the products of these clusters is expected. Results from this and future studies will elucidate the molecular biology of fungal phyto-pathogenicity.

The molecular changes that may have contributed to the adaptation of *Xanthomonas vasicola* pv. *vasculorum* to *Eucalyptus grandis*.

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Abstract

Xanthomonas vasicola is a Gram-negative bacterial species, and like other members of its genus, it is motile by a polar flagellum and is characterised by yellow mucoid colonies on artificial media. There are 27 described phytopathogenic species belonging to the genus, and they are known to cause disease in 124 monocotyledonous plants and 368 dicotyledonous plants. *Xanthomonas vasicola* has undergone recent host range modifications. In the early 2000s, it was suggested that *X. vasicola* pv. *vasculorum* jumped from sugarcane to *Eucalyptus grandis*, resulting in the emergence of bacterial blight and dieback. A comparative genomics study was thus undertaken to understand the molecular changes that may have contributed to this host jump and the adaptation of this bacterial pathogen to *E. grandis*. The genomes of five *X. vasicola* pv. *vasculorum* strains isolated from *E. grandis* were sequenced with Illumina HiSeq and were assembled and annotated. The core and accessory genomes of the five strains isolated from *E. grandis* and eighteen others *X. vasicola* strains isolated from hosts such as sugarcane maize and banana were determined, and their phylogenetic relationships were inferred. The complete repertoire of Type III, IV, and VI secreted effectors was predicted. These effectors are important in pathogenicity and interactions with other microbes. Furthermore, the signatures of adaptation drivers such as genomic islands, recombination and insertion sequences of the five strains were identified. The identified genomic islands are involved in virulence, metabolism, antibiotic resistance, and the symbiotic interactions between the strains, their hosts, and other microbes. These genomic island regions are also recombination regions, and many of the insertion sequences predicted are within these regions. In addition, the orthologous clusters were identified and annotated, and the phylogenetic inference of orthologous clusters was inferred. Orthologous clusters with unique evolutionary histories of the five *E. grandis* strains were identified. Unique genes from X. *vasicola* pv. *vasculorum* strains isolated from *E. grandis* were identified as being involved in chemotaxis, the breakdown of plant cell-wall proteins, suppression of reactive oxygen species and T4SS. Therefore, these genes may have contributed to the adaptation of *X. vasicola* pv. *vasculorum* to *E. grandis* from sugarcane.

Molecular basis of cycloheximide tolerance in the Ophiostomatales: A genome "needle in haystack" discovery

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Abstract

Most Eukaryotes are sensitive to cycloheximide but resistance to this powerful antibiotic is known in some fungal taxa. This phenomenon, for example, occurs in diverse and phylogenetically unrelated genera of the ascomycetous yeasts. Interestingly, most genera and species in the Ophiostomatales, an order that includes mostly symbionts of bark beetles and some of the most important tree pathogens, are highly resistant to this antibiotic. As early as the 1960's, it was shown that this resistance was associated with the 60S ribosomal subunit in the yeasts, but its molecular basis has never been considered for the Ophiostomatales. The recent availability of whole genome sequences for numerous members of the Ophiostomatales presented the opportunity to consider this question. We examined all the available genomes for the Ophiostomatales and discovered a transition mutation in the ribosomal protein eL42 gene. The result is the substitution of the amino acid Proline to Glutamine in position 56 of the predicted protein. The same substitution was also observed in many yeast species known to be resistant to cycloheximide and this had previously only been reported in *Kluyveromyces* lactis. This transition mutation in the ribosomal protein eL42 gene is most likely the cause of cycloheximide resistance across the Ophiostomatales. This change across all genera of an important and well-defined Order of the fungi suggests that the mutation arose early in their evolution. Furthermore, it most likely relates to their relationship with bark beetles and other environments where cycloheximide producing microbes occur enabling the Ophiostomatales to compete with other fungi.

Discerning the global phylogeographic distribution of *Phyllosticta citricarpa* by means of whole genome sequencing

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Abstract

Phyllosticta citricarpa is a fungal pathogen and causative agent of citrus black spot (CBS). As a regulated pest in some countries, the presence of the pathogen limits export of fruit and is therefore of agricultural and economic importance. In this study, we used high throughput sequencing data of 71 isolates from eight countries to infer the global phylogeographic distribution of this pathogen. Countries included Argentina, Australia, Brazil, China, Cuba, eSwatini, South Africa and the United States of America. Genomes were assembled and used in pairwise read mapping and enumeration of variants between isolates. SSR marker mining of the assembled genome with the best assembly statistics, yielded 1,987 markers, and was used for *in silico* genotyping of all isolates. Furthermore, 32,560 SNPs were identified relative to a reference sequence. The pairwise variant counts, SSR genotypes and SNP datasets were used in population genetic analysis, and all three analysis approaches gave the same overall results. The analyses revealed possible pathogen dispersal routes and evolutionary histories of populations. The Chinese population is the most diverse and is genetically the furthest removed from all other populations, and therefore it is assumed to be the origin of the pathogen. This study represents the largest whole genome sequencing survey of *P. citricarpa* to date and provides a more comprehensive assessment of the population genetic diversity and connectivity of *P. citricarpa* from different geographic origins. This information provides a better understanding of the epidemiology of the citrus black spot pathogen, its long-distance dispersal and dissemination pathways, and can be used to refine phytosanitary measures and management programmes.

Novel viral diversity associated with *Helianthus annuus* L. in Gauteng and Free State

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Abstract

Helianthis annuus L. (sunflower) is one of the world's most important oilseed crops, with South Africa contributing ~2 % of global production. Fungi, such as species of Sclerotinia, *Verticillium* and *Phoma* and various rusts, are the primary causes of pathogen-associated losses of sunflower in South Africa. A diverse range of viruses from the *Potyvirus, Begomovirus and Umbravirus* genera are known to infect sunflower, however little to no data is available regarding the sunflower-associated viral diversity in South Africa. In April 2021, sunflowers showing symptoms of either severe leaf mottle or mosaic, were collected from an experimental plot at the University of Pretoria experimental farm in Gauteng, as well as a farmer's fields in Free State. Total RNA from each sample was used to generate RNAtag-seq libraries, which were sequenced using an Illumina NextSeq 2K sequencer. De novo assembly of trimmed reads was performed using metaSPAdes 3.14.0 and blastn analysis of the resulting contigs, showed that samples were infected either with *Bidens mottle virus* (BiMoV) or a novel member of the *Umbravirus* genus. RT-PCR assays were optimized to confirm the presence of the respective viruses. This is the first time that BiMoV is being reported in South Africa and most of the genomes shared high nucleotide homology (~98 %) with other isolates from Taiwan. However, one population was associated with a divergent strain (<80 % nucleotide homology to other extant isolates) of BiMoV. All plants infected with BiMoV were associated with chlorotic mottle over the entire leaf blade. The novel member of the *Umbravirus* genus, associated with blotchy mosaic and ringspot symptoms, from plants from both locations. Tentatively named sunflower chlorotic ringspot virus, phylogenetic analyses suggest the virus is most closely related to Ixeridium yellow mottle-associated virus 2. Further research is required to determine the prevalence of the virus, as well as capacity the cause yield limiting disease.

Genome Wide Association approach to identify genes linked to growth in *Fusarium circinatum*.

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Abstract

Genome-wide association studies are invaluable for linking genetic variation with specific phenotypic traits. Several studies in fungi have successfully used this approach to identify genomic regions associated with phenotypes such as growth rate and virulence. Despite its economic importance in forestry globally, few studies have investigated the molecular basis of the pine pitch canker pathogen, *Fusarium circinatum*. To address this knowledge gap, we employed a genome-wide association study to identify genes and/or genomic regions associated growth rate of *F. circinatum* at different temperatures. For this purpose, we used a collection of 80 *F. circinatum* isolates representing the known diversity of the pathogen in South Africa. Genomes of these fungi were sequenced using the Ion TorrentTM sequencing technology. To determine the growth phenotype, all isolates were grown on half-strength potato dextrose agar at 20 °C, 25 °C, and 30 °C. Correlation between the genotypic and phenotypic data revealed twenty-one significant SNPs *($P < 0.05$)* associated with growth. Most of them were located in telomeric regions. Based on work from other fungi, these SNPs were associated with genes involved in diverse processes (e.g., heat stress, sugar transport, transcription regulation, and vegetative incompatibility), including virulence (e.g., the ral2 activator of Ras2, polyketide synthase, and thioredoxin). The result of this first genome-wide association study on *F. circinatum* has thus provided important clues regarding the molecular processes determining growth at different temperature. Our future research will seek to understand how the molecular functions encoded by these genes are potentially involved in the pathogenicity of this fungus to its pine host.

Identification of genes and gene clusters involved in the biosynthesis of mycotoxins produced by *Fusarium* species associated with maize in South Africa

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Abstract

Maize *(Zea mays* L.*)* is one of the most important cereal grains, but its production globally is constrained by the fungal genus *Fusarium*. This is especially true for several species in the *Fusarium fujikuroi* species complex (FFSC) that cause Fusarium ear rot (FER), an economically important disease that reduces yield and grain quality. Grain quality is further reduced by the deposition of mycotoxins that can cause diverse health problems in humans and animals when the contaminated grain is consumed. As most previous work on the FFSC-maize association focused on *Fusarium verticillioides*, little is known about other mycotoxigenic species in this complex that potentially affect maize in South Africa. Understanding the mycotoxigenic potential of each species is critical for assessing the toxicological risks associated with FER, because mycotoxin production differs between species and species complexes of *Fusarium*. The purpose of this study was to identify the functional gene or gene clusters underpinning mycotoxin biosynthesis in *Fusarium* species obtained from South African maize. DNA was extracted from two isolates each of *F. verticillioides, F. subglutinans, F. temperatum, and F. awaxy*. Genome sequences were generated by using both PacBio RSII and Illumina Novaseq 6000 sequencing technologies. The genomes were assembled and annotated and examined for the presence of mycotoxin biosynthesis gene clusters. The genome sequence of *F. awaxy* has not yet been reported in other studies and will therefore be the first available sequence. However, existing genome sequence data of *F. verticilliodes* NRRL 20956, *F. subglutinans* NRRL 66639 and *F. temperatum* CMWF 389 is currently available and will be compared with the genomes of the South African isolates. This will provide for a better understanding of the potential of these species to produce mycotoxins and contaminate maize in South Africa.

2.Molecular Biology

Disruption of a secondary mating-type gene de-regulates sex-associated pathways in *Huntiella omanensis*

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Abstract

The *Ceratocystidaceae (Ascomycota)* includes a wide variety of genera, each with unique ecological and morphological characteristics. These fungi also exhibit significant diversity in their sexual strategies, including heterothallism, mating type switching, and unisexuality. Despite this intriguing diversity, the genes that underly sexual reproduction are fairly well conserved, with *MAT1-1-1, MAT1-1-2, MAT1-2-1 and MAT1-2-7* being present at the mating-type *(MAT)* locus or loci of most studied species. Secondary MAT genes generally act to regulate the sexual cycle of filamentous ascomycete fungi and are typically dispensable for sexual reproduction. However, recent functional characterization of the *Huntiella omanensis* MAT1-2-7 showed that it is essential for the production of mature fruiting structures. We used a comparative RNA-seq experiment to show that the disruption of MAT1-2-7 in *H. omanensis* is associated with the differential expression of approximately 25 % of all the genes present in the genome, including the transcriptional regulators ste12, wc-2, sub1, *VeA*, and pro1. This suggests that MAT1-2-7 acts as a transcription factor and that *ΔMAT1-2-7* mutant sterility may be a result of layered de-regulation of a variety of signalling and developmental pathways. This study is one of only a few that details the functional characterization of a secondary MAT gene in a non-model species. Furthermore, it provides a molecular mechanism that underlies the inability of ΔMAT1-2-7 isolates to complete the sexual cycle. This gene occurs in other *Ceratocystidaceae* species and because there are diverse secondary *MAT* genes present throughout the *Ascomycota*, further studies considering these genes will provide a better understanding of sexual development in the fungi.

Molecular Diversity of Maize RNA and DNA Viruses in Ethiopia: Implication for Africa

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Abstract

Maize, a leading food security crop in many African countries, is affected by devastating virus diseases such as maize lethal necrosis (MLN) and maize streak. A study was conducted to determine the diversity of maize viruses in Ethiopia by ELISA followed by PCR-based tests, Rolling circle amplification (RCA), Sanger and NGS sequencing. Sequence analysis revealed that Ethiopian maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV) isolates causing MLN are genetically homogenous and very similar to those from Eastern Africa or elsewhere. On the other hand, mastreviruses causing streak disease are highly diverse. RCA and complete genome sequencing revealed the existence of three genetic groups of mastreviruses, each representing a distinct species. The first group represented the most common A-strain of maize streak virus (MSV) whereas the second group shared 96-98% identity with Maize streak reunion virus (MSRV), confirming its occurrence in East Africa for the first time. Sequence analysis of the genomes (each 2846 nt long) representing the third group indicated only a limited nucleotide identity of 70-71 % with MSRV. Isolates in this group are assigned to a new virus species tentatively named maize streak dwarfing virus (MSDV). PCR screening of 89 samples showing streak symptoms with mastrevirus-specific primers showed that MSV-A is the most prevalent (39.3 %) followed by MSRV (14.6 %) and MSDV (12.4 %). RT-PCR assessment for Maize yellow mosaic virus (MaYMV) using polerovirus-specific primer pairs revealed that 32 of the 47 samples (72 %) were infected. Direct sequencing of the RT-PCR products confirmed that all the Ethiopian sequences share 98 to 99 % identity to reference isolates (KU248489). Full-genome sequencing of three MaYMV isolates using Illumina MiSeq platform revealed nucleotide identities of 99.6 % to each other and 96.8 % with the reference MaYMV sequence respectively. The study provided useful information on molecular diversity of six viruses infecting maize in Ethiopia and suggested that the country is a hotspot for the diversity of maize mastreviruses. Since some of these viruses are either never or only recently reported from maize in Africa, coordinated continent-wide effort to further understand virus diversity can contribute to develop suitable management strategies for sustainable maize production.

Ceratocystis wilt in South Africa: An increasing threat to forestry and agriculture

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Abstract

Ceratocystis spp. are well known pathogens that cause vascular wilt and canker diseases on woody plants, and tuber rots on various root crops. These pathogens have caused significant economic losses to plantation forestry, and substantial ecological damage to native woody ecosystems in various parts of the world. The first report of a *Ceratocystis* sp. in South Africa was on ornamental *Protea gigantea* in 1977. Since then, eight of the 42 knowns *Ceratocystis* spp., have been found in South Africa, on native and non-native tree species. Of these, only two are significant pathogens. The most well-known is the native African pathogen, *Ceratocystis albifundus*, which causes a serious wilt and canker disease of non-native *Acacia mearnsii*, and *Protea cynaroides* cultivated for cut-flower production. More recently, we reported the first outbreak of a *Ceratocystis* disease on a *Eucalyptus* hybrid variety in KwaZulu-Natal, caused by *C. eucalypticola*. Population genetic studies suggest that *C. eucalypticola* in South Africa is represented by a moderate level of genetic diversity with no population structure, even for isolates collected 500 km apart. Strains of the pathogen are also similar to those collected 14 years previously from artificially induced wounds on trees, in the absence of disease symptoms. Our results provide good evidence that *C. eucalypticola* has been accidentally introduced into South Africa, from a presently unknown source. The pathogen is widely distributed in the country, including areas where it is not causing disease, and its origin remains unknown. Of particular concern is that we have recently identified *C. eucalypticola* causing a serious wilt disease on kiwi vines in South Africa and we have evidence that the *Eucalyptus* and kiwi isolates are genetically connected. Our current research includes questions relating to pathogenicity factors, host range, and the development of robust detection protocols which will lead to a better understanding of this important group of pathogens. This will facilitate efforts to manage disease problems and minimize local and intercontinental spread of *Ceratocystis* spp.

ARISE: An Infrastructure for Dutch Biodiversity – A Fungal Contribution

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Abstract

The Dutch project ARISE, which stands for "Authorative and Rapid Identification System for Essential Biodiversity Information", aims to build an infrastructure to identify all multicellular species, including the soil fungi, in the Netherlands. The project consists of five teams to achieve this goal*: Team Sampling* provides the samples from the field to the laboratories and into the collections. *Team Sequencing* is responsible for extracting and sequencing DNA for all the specimens collected by the sampling team as well as the samples obtained from the reference collections. Also, they process the bioinformatic analyses of the sequence data. *Team Digital Species Identification* builds tools and services to support development and deployment of artificial intelligence algorithms to detect, identify and track the animals in diverse environments. *Team Monitoring Demonstration* Sites establishes various digital sensors at selected sites in the Netherlands to be able to monitor biodiversity, biomass and movement of the species across space and time. Lastly, *Team Biocloud* is responsible to develop an infrastructure for the entire ARISE program for data management, storage, digital object identification and integration between the teams in ARISE and the outside world. As Westerdijk Fungal Biodiversity Institute, we are part of the Sampling, Sequencing and Biocloud teams in the ARISE project. We receive soil samples from the other collaborators in the project and process them to obtain pure fungal cultures to investigate the fungal biodiversity in the samples. These isolates, together with other soil isolates previously deposited in the CBS culture collection are included in the molecular analyses. We perform DNA extraction, amplification and sequencing using the molecular barcodes ITS and LSU and additional markers such as *TEF1, RPB1* and *RPB2* where necessary for species level identification. We discuss our first results pertaining fungal biodiversity in Dutch soils and provide more information about the other aspects of the ARISE project.

Plant Pathology in the era of modern breeding

Dr Cheusi Mutawila

Bayer

Abstract

Durable plant disease resistance has always been perceived as the ultimate goal in disease management as it requires the least intervention from the grower and minimal use of crop protection chemistries. Biotechnology-based approaches of gene-silencing, cis-genetic engineering and gene editing provide major opportunities to introduce disease resistance to cultivated crops. However, it is conventional breeding that has so far been pivotal to the discovery and introduction of new plant disease resistance genotypes without any regulatory hurdles. A paradigm shift is currently underway in plant breeding to at least double the rate of genetic improvements to meet the future demands of a growing population with changing dietary needs. This shift is driven by technologies such as advanced marker technologies (for rapid cycling), high throughput phenotyping, envirotypic-*assisted* selection and data science to accelerate the development of new high performing cultivars (so called speed breeding). Disease management's ongoing challenge is ensuring new cultivars retain adequate disease tolerance, while protecting genetic potential and still managing germplasm for protection against the unknown. This presentation will share how plant pathology is responding to the threat of global food security in an era of modern plant breeding from a Bayer perspective in our quest for breeding for all and hunger for none.

Neofusicoccum parvum: A global threat to plant health

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Abstract

Neofusicoccum parvum is an important pathogen with a wide distribution and capable of causing disease in a very large number of plant species. Despite its importance, *N. parvum* has only been recognized as a distinct species since 1985. *N*. *parvum* has a very large host range, reported from 225 plant species from 64 families. It is a major pathogen of many important cultivated plants such as grapevine, blueberries and eucalypts, but it also threatens trees in urban and natural ecosystems. The most common disease symptoms caused by *N. parvum* are die-back and branch or stem cankers. However, it is also very commonly encountered as an endophyte in healthy plant tissues that can become pathogenic once the host is subjected to biotic or abiotic stress. Before its formal description, *N*. *parvum* was often reported as *Botryosphaeria dothidea* or *Neofusicoccum ribis*. Misidentifications, however, continued to occur for the subsequent 15 years due to the constraints of morphological identifications and the fact that DNA sequence-based identification was in its infancy. Taxonomic difficulties arising from the existence of many closely related species and the low resolution provided by common phylogenetic markers continue to be a problem for this species. Haplotype analyses have shown that the global population is dominated by a few closely related haplotypes with little structure based on geography or host. *N*. *parvum* is spread over short distances by natural processes such as rain-splash and wind, however, long-distance and inter-continental dispersal are most likely through the movement of infected plant material. Prevention of long-distance dispersal is significantly hampered by the ability of the pathogen to persist in asymptomatic plant material. Application of state-of-the-art diagnostic technologies to screen symptomatic and asymptomatic plant material will greatly improve our ability to limit the global spread of *N. parvum*. In addition, recent genomic and transcriptomic studies have provided valuable insights into the molecular mechanisms that *N. parvum* utilises to cause plant disease. These will inform novel strategies to protect plants from infection or to mitigate losses caused by the pathogen.

Genetic diversity of esca and Petri disease pathogen, *Phaeoacremonium minimum*, in six vineyards and two nursery mother blocks in the Western Cape

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Abstract

Phaeoacremonium minimum is an important esca and Petri disease pathogen that causes decline and dieback of grapevines in South Africa. Little is known regarding the reproductive strategy of this pathogen. The study aimed to investigate the genotypic diversity and recombination potential of eight *P. minimum* populations in the Western Cape. This was achieved by developing and applying nine polymorphic microsatellites and mating-type-specific markers. Thirty-seven genotypes were identified of which six genotypes were shared among the populations. All populations were characterised by the same dominant genotype (65.43 %), low genotypic diversity that ranged from 4.18 % to 11.91 % and high levels of clonality (81.36 %). This indicates that asexual reproduction is dominant. Multiple introductions of different genotypes over time greatly accounted for the observed genotypic diversity. However, *P. minimum* populations where sexual fruiting bodies (perithecia) are known, had even ratios of mating types, several unique genotypes and a few closely related genotypes, which indicates that sexual reproduction is occurring infrequently in these populations. New genotypes introduced into a population either because of infected plant material or sexual recombination, will rapidly produce copious amounts of asexual spores to ensure the spread and establishment in its new environment. Individuals with shared genotypes were trapped in both older vineyards and neighbouring newly established vineyards. The two rootstock mother blocks had several shared and unique genotypes. Propagation material obtained from infected rootstock mother blocks could lead to the spread of shared and unique genotypes to newly established vineyards. Management strategies must focus on reducing aerial inoculum to prevent repeated infection and further spread of *P. minimum* genotypes. This can be achieved by removing pruning debris especially from older wood within vineyards and surrounding fruit orchards that host *Phaeoacremonium* species and applying pruning wound protectants. The health status of rootstock mother blocks needs to be determined to ensure that clean propagation material is used in nurseries.

A phylogenomic approach to elucidate lifestyles in *Dothideomycetes*

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Abstract

Dothideomycetes is the largest and most diverse class of ascomycete fungi with 23 orders, 110 families, 1300 genera and over 19000 known species, comprising an incredible diversity of lifestyles, many of which have evolved multiple times. Studying the evolution of *Dothideomycetes* has significant implications for our fundamental understanding of fungal evolution, and practical implications regarding the effects of climate change on these pathogens in agriculture. The availability of whole-genome data produced a high-confidence overall phylogeny of *Dothideomycetes* and provided a clearer picture of the relationships among the various families, especially those previously regarded as incertae sedis, and indicating that pathogenicity evolved multiple times within this class. Using machine-learning methods fungi were classified into lifestyle classes with >95 % accuracy, identifying a small number of gene families that positively correlated with these distinctions. Ancestral character state analyses support a terrestrial saprobic lifestyle as being ancestral within the class, as well as at ordinal and family levels. Several transitions have occurred to evolve lichenised, plant and human parasitic, ectophytic (sooty blotch and flyspeck) and more recently epiphytic (sooty mould) lifestyles. In addition, the classical definition of lifestyles for a given species are not always clear cut as a species can exhibit different lifestyles during its lifecycle as its interaction with its host changes. Whole genome analyses such as CATAstrophy predictions allow for the identification of overlap in lifestyle classifications and therefore a more accurate categorisation of the lifestyle(s) of a species.

Improved identification of fungi using high-throughput DNA barcode data from a curated culture collection

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Abstract

The accuracy and precision of fungal identification and classification based on DNA barcodes are challenging, particularly in environmental metabarcoding approaches as these often trade accuracy for efficiency given the large data volumes at hand. The main bottlenecks are the lack of reference sequences in many taxonomic groups and the uncertainty of the similarity thresholds used. At the Westerdijk Fungal Biodiversity Institute, we have generated more than 200,000 DNA barcodes for the strains preserved in the CBS collection. A large amount (~30,000) of fungal DNA barcodes were submitted to GenBank as reference sequences, representing an unprecedented data release event in global fungal barcoding efforts to date. Computational approaches have been developed to improve fungal identification. We assessed the deep learning approach as it is a successful paradigm for big data classification. Experimental results showed that the deep learning approach worked well on datasets that had many of the labels present in the training datasets. However, when classifying a dataset whose sequences were not present in the training dataset, BLAST performed the best in terms of taxonomic identification. In most ecological studies using BLAST, only a single similarity cut-off value is used for sequence identification. This is not sufficient since the most used DNA markers are known to vary widely in terms of inter- and intra-specific variability. We addressed this problem by presenting a new tool, dnabarcoder, to predict local similarity cut-offs for sequence identification for different clades of fungi. It was shown that the similarity cut-offs predicted for different fungal clades varied significantly. When classifying a large public fungal ITS dataset from UNITE against the WI-CBS barcode dataset, the local similarity cut-offs assigned fewer sequences than the traditional cut-offs used in metabarcoding studies. However, the obtained accuracy and precision levels improved significantly. Our study also showed that the resolving powers of full-length ITS, ITS1, and ITS2 sequences were similar for fungal species identification. Nevertheless, the complete ITS region had a better resolving power at higher taxonomic levels. Finally, it was found that the CBS collection clearly improved fungal identification in global soil samples, in particular for plant pathogenic fungi.

Defining species in *Fusarium* and the FUSARIOID-ID database

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Abstract

The genus *Fusarium* currently contains more than 400 species, many of which are plant pathogens or taxa that produce mycotoxins harmful to humans or domesticated animals. The use of the morphological, biological and phylogenetic species concepts, together with information on pathogenicity, host range and mycotoxin production has been essential in the delimitation of species. Until the 1970s, fungal species were defined and described based on morphological characters only. Several different species concepts are known, including the biological species concept, which has limited application in mycology, and the phylogenetic species concept. Although the latter is the most abstract concept, it has become prevalent in the study of fungi since the beginning of the 21st century. One form of this concept is the "Genealogical Concordance Phylogenetic Species Recognition – GCPSR" and its application has resulted in the identification of approximately 400 phylogenetic species in *Fusarium*. Three main challenges still remain, (i) to formally describe already known, distinct phylogenetic lineages, (ii) reveal cryptic or sibling species within collections labelled with names based on morphological characterisation only, and (iii) access still underexplored niches in different ecosystems or peculiar substrates. The FUSARIOID-ID database was developed to include all agricultural, environmental and clinically important fusarioid genera and species based on curated sequences of several DNA barcode genes. The main aim of the FUSARIOID-ID database is to provide a stable, regularly updated, and user-friendly platform for the identification of *Fusarium* and other species in allied genera through advanced BLAST-like queries of well-curated DNA sequences. In addition, information is provided for the included species, accepted species lists and protocols for species description, and for working with the genus *Fusarium* and other fusarioid sister genera. Presently, about 500 taxon names (of which 300 *Fusarium* species) and 1550 isolates have been included in FUSARIOID-ID and the database is continuously being expanded. We show how necessary information is generated and incorporated in the database and discuss a few examples of new species found on tropical grasses with agricultural importance.

From mutants to informants: Using infectious clones to study citrus tristeza virus-induced stem pitting

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Abstract

Citrus tristeza virus (CTV) has a long and complex history with citrus production worldwide and has rendered millions of trees dead or unproductive through the induction of severe disease epidemics. CTV remains the most important viral pathogen of citrus and causes several disease syndromes in different citrus hosts. CTV-induced stem pitting leads to substantial economic losses by negatively impacting on grapefruit and sweet orange vigour and yield. The exact mechanisms of stem pitting development in CTV-infected citrus remain unclear. This study utilised full-length CTV infectious clone mutants in a reverse genetics approach to study stem pitting induction. A panel of CTV mutants were generated by deleting or replacing the three open reading frames (ORFs) of CTV thought to be implicated in stem pitting induction, namely p33, p18 and p13. ORF deletion and replacement mutants were generated in order to investigate if severe pitting could be induced by exchanging the ORFs of a moderate-pitting infectious clone (genotype T36) with those of a severe-pitting isolate of CTV (T3-KB). Interestingly, the presence of ORF p18 from T3-KB yielded the most significant increase in stem pitting severity in Mexican lime plants among the three ORFs investigated. A range of stem pitting pressures was observed across the panel of clones investigated. Plant responses to different stem pitting pressures were further assessed by untargeted profiling of secondary metabolites as well as quantitation of the stress-responsive phytohormones, abscisic acid, jasmonic acid and salicylic acid. Stem pits were also anatomically characterised using nano-CT scanning, biological staining, fluorescence microscopy and various electron microscopy applications in order to better understand the nature of the xylem and phloem tissues impacted in cases of severe pitting. This study generated novel insights into the factors that influence stem pitting severity in Mexican lime and Duncan grapefruit plants. Viral open reading frames implicated in induction of severe stem pitting could be identified. The addition of biological plant data in response to differential stem pitting pressures provided a unique perspective into the changes that accompany stem pitting development and provides valuable opportunities to further characterise these pathways in future studies.

Evidence that a horizontally acquired laccase gene confers pathogenicity in the conifer pathogen, *Leptographium wageneri*

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Abstract

Leptographium wageneri is an ascomycete fungal pathogen that causes a vascular wilt disease of conifers known as black stain root disease (BSRD). There are three varieties of *L. wageneri*, each of which is host specific. Unlike other species of *Leptographium*, which are saprophytic or weakly pathogenic, *L. wageneri* is an aggressive primary pathogen that can infect and kill its hosts. Little is known regarding the genetic basis of pathogenicity of *L. wageneri.* A recent comparative genomic study (unpublished) has revealed a putative laccase (Lac-2) gene to be present only in the three varieties of *L. wageneri* and a closely related species*, L. douglasii* and absent in the other *Leptographium* species. In the present study, we considered the evolutionary origin of this Lac-2 gene and its possible role in pathogenicity. The molecular structure of the putative Lac-2 protein was investigated, and *in-vitro* and *in-planta* mRNA expression was analyzed and compared using qRT-PCR. Subsequently, Lac-2 mutants were generated using the CRISPR/Cas9 mediated gene knockout system followed by a pathogenicity assay under laboratory conditions using the wild type and mutants. The results revealed that Lac-2 is a functional secreted laccase, horizontally acquired by *L. wageneri* and *L. douglasii*. qRT-PCR analysis revealed that Lac-2 was upregulated *in-planta.* The Lac-2 deleted mutants were not pathogenic but the wild type isolates resulted in disease symptoms characteristic of the BSRD. Collectively, the results indicate that the horizontally acquired laccase (Lac-2) is an important virulence factor in *L. wageneri*.

Eucalyptus scab and shoot malformation: A threat to commercial forestry

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Abstract

In 2014, a new and serious leaf and shoot disease of unknown aetiology was discovered in *Eucalyptus* plantations of North Sumatra, Indonesia. The disease is characterized by black necrotic spots that first emerge on young leaves and petioles, which become scab-like as the lesions age. Infected trees respond to infection by producing shoots with small leaves that commonly appear feathered. Severely affected *Eucalyptus* clones usually die after a number of successive infection cycles, generally over a period of two to three years. Fruiting bodies, typical of known *Eucalyptus* pathogens, were not present and the symptoms were unlike any other foliar or shoot diseases known on these trees elsewhere in the world. The disease was recently shown to be caused by a novel species of *Elsinoë*, which we have described as *Elsinoë necatrix*. Pathogenicity tests showed unequivocally that *E. necatrix* is the cause of disease in Sumatra and the disease has been named as Eucalyptus scab and shoot malformation. A rapid screening technique is being established to effectively select disease tolerant planting stock, and to reduce the significant negative impact of this new and serious *Eucalyptus* disease. Research is also focused on determining the origin of the pathogen including attempts to understand the long-term threats to *Eucalyptus* plantation forestry in Asia and globally.

3.Host-Pathogen/Insect Interactions

Extracellular vesicles provide a window into the pathobiology of economically important South African fungal pathogens.

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Abstract

By ferrying molecular cargo, including proteins, nucleic acids, and secondary metabolites, from a source to a recipient cell, extracellular vesicles (EVs) occupy an integral role in cellular communication among microbes and also between microbes and their plant/animal hosts. As a result, EVs are frequently associated with a variety of virulence factors, including biofilms which represent architecturally complex microbial communities in which cells are protected from harsh environmental conditions. When it comes to plant-microbe interactions, EVs also play a role in pathogen defense and invasion. Advances in the field of EVs indicate that these nanoparticles and the bioactive compounds they contain have promising, yet untapped, agricultural potential, making EV research critical for many economically important fungal species. By characterizing vesicles derived from two economically important Fusarium pathogens, *F. circinatum*, and *F. verticillioides*, we aimed to reveal the role of EVs in mediating interactions between pathogenic fungi and their plant hosts. For this purpose, vesicles were isolated from biofilms and planktonic cells and characterized following the Minimal information for studies of extracellular vesicles (MISEV, 2018) guidelines, which included Transmission Electron Microscopy, Nanoparticle Tracking Analysis, and proteomic analyses. Additionally, we conducted vesicle-uptake assays to demonstrate the actual involvement of EVs during plant-microbe interaction. The latter included uptake between host derived EVs and fungal cells, as well as uptake between EVs derived from fungal biofilms and suspended (planktonic) cells. Our results showed that EVs are indeed exchanged bidirectionally between hosts and pathogens. In other words, our findings demonstrate the potential of EVs in mediating fungal interactions with their hosts, as well as the ability of these nanoparticles to act as vectors for a variety of beneficial compounds (e.g., antifungal compounds, immune modulators, etc.) between organisms. The ultimate goal of this research is, therefore, to introduce EVs as part of a novel and long-term control strategy for severe fungal diseases of key economic crops and trees, with importance to Southern Africa.

The intergenic region is a pathogenicity determinant of Tomato curly stunt virus in *Nicotiana benthamiana*

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Abstract

Begomovirus infection causes a significant decrease in yield production in economically important crops. This poses a problem to food security and to the livelihoods of farmers and employers in the farming industry. Tomato curly stunt virus (ToCSV) exists as two variants, V30 and V22, which negatively impact tomato production in South Africa. The intergenic region (IR), a non-coding region within the ToCSV genome, is of interest because of its involvement in binding host factors to initiate infection and transcription of viral proteins. The IR was investigated by agro-inoculating *Nicotiana benthamiana,* an experimental host, with wild-type infectious clone constructs of V30 and V22, and IR-swap mutants where IR sequences were swapped between the two wild type infectious clones. The IR-swap mutant with the V30 sequence containing the V22 IR segment induced upward leaf roll and resulted in an increase in viral load at 28 days post inoculation (dpi) compared to V30. The IR-swap mutant with the V22 sequence containing the V30 IR sequence resulted in a knockout of upward leaf roll with no changes to viral load compared to V22. Shorter IR-swaps on either side of the conserved TATA box also resulted in a change in the symptom phenotype induced by wildtype V30 and V22 suggesting that the cis-acting TATA-associated composite element (TACE) in the IR may play a role in the symptom phenotypes induced in *N. benthamiana*. Furthermore, the presence of IR transcripts in plants infected with V30 and V22 at 20 dpi was verified by RT-PCR using sequence specific primers. This suggests a potential source of IR-derived siRNAs which may be implicated in disease pathways. This study has identified a short nucleotide sequence within the 3'-region of the IR which plays a role in the upward leaf roll phenotype induced by V22 in *N. benthamiana*.

Two *Eucalyptus* pathogens, with different levels of aggressiveness, have similar infection biology

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Abstract

Teratosphaeria destructans is one of the most important and aggressive leaf pathogens of *Eucalyptus*. Very little is known regarding its infection biology, and this hinders efforts to develop measures to minimize disease. In this study, we compared the conditions required for spore germination in *T. destructans*, with the less aggressive *Eucalyptus* foliar pathogen, *Teratosphaeria epicoccoides*. The infection process and disease development for both pathogens were studied on a *Eucalyptus* hybrid clone using light and electron microscopy. The optimal temperature for spore germination in *T. destructans* ranged from 25 to 30 °C and 15 to 20 °C for *T. epicoccoides*. The germination of conidia in both *T. destructans* and *T. epicoccoides* was enhanced when they were exposed to light and high levels of relative humidity, close to 100 %. Both pathogens infected the host via stomata 48 h after inoculation and the hyphae grew intercellularly. No specialized infection structures such as haustoria were observed. Symptoms appeared three weeks post-inoculation and pycnidia developed below the sub-stomatal cavities after four weeks. Results of this study showed that *T. destructans* and *T. epicoccoides* have similar humidity requirements, but different temperature requirements for infection. This study provides a foundation for artificial inoculations to screen *Eucalyptus* genotypes for resistance to *T. destructans* and to study the interactions between this important pathogen and its hosts.

Functional characterization of *Phytophthora parasitica* PpRxLR1 and PpRxLR6 effector proteins in *Nicotiana benthamiana*

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Abstract

Phytophthora parasitica is one of the most destructive oomycetes. During its infection process, *P. parasitica* secretes hundreds of RxLR effector proteins (including the conserved RxLR effectors (CRE)) into the cytoplasmic region, as putative virulence factors. Remarkable progress has been made in understanding CRE from other *Phytophthora* spp. However, little is known about CRE from *P. parasitica*. The aim of this study was to assess the functions of two CRE from *P. parasitica*. Towards this end, in silico analyses revealed that *P. parasitica* INRA 310 secrete 71 conserved RxLR effectors. Among the 71 CRE, PpRxLR1 and PpRxLR6 effector proteins were selected for further functional characterization. Inoculation of *Nicotiana benthamiana* leaves with zoospores indicated that the life cycle of *P. parasitica* switches from asymptomatic to symptomatic phase. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analyses of the inoculated leaves showed that *P. parasitica* PpRXLR1 and PpRXLR6 are expressed during the biotrophic phase, suggesting their importance in virulence. Findings from *Agrobacterium tumefaciens*-mediated transient expression of PpRXLR1 and PpRXLR6 in *N. benthamiana* revealed potential mechanisms of *P. parasitica* PpRXLR1 and PpRXLR6 in promoting disease development, this includes inducing reactive oxygen species as well as callose deposition. In addition, RT-PCR analyses revealed that PpRXLR1 and PpRXLR6 induce phytohormones (SA, ET, JA) and MAPKs (MPK3 and MPK6). These data indicate that both PpRXLR1 and PpRLR6 are important virulence factors of *P. parasitica*. Therefore, functional characterization of CRE from *P. parasitica* is a promising route for the search of potential durable resistance breeding in plants.

Botryosphaeriaceae associated with the native tree species *Berchemia discolor* in agricultural and natural ecosystems in the Limpopo Province.

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Abstract

The *Botryosphaeriaceae* include various endophytic species, some of which are important latent pathogens that cause disease on various indigenous and exotic trees, usually when they are subjected to stress. Nothing is known regarding the diversity and occurrence of these fungi on indigenous *Berchemia discolor* (brown ivory) worldwide. This tree species plays a role in the socio-economic well-being of small-scale farmers and people living in rural communities. It is valued for its medicinal properties and nutritional values and used in the production of liquor. Our study aimed to explore the diversity of *Botryosphaeriaceae* on symptomatic and asymptomatic branches of *B. discolor* at sampling sites in agricultural and natural ecosystems in the Limpopo Province. Thirteen species in the *Botryosphaeriaceae* and one species in the *Pseudofusicoccumaceae*, a family that was previously part of the *Botryosphaeriaceae*, were identified based on analyses of DNA sequence data of the ITS rDNA region and portions of the β-tubulin, TEF-1α and Rpb2 genes. The fungi identified included three potentially new species, designated as *Alanphillipsia* sp. Group B, *Dothiorella* sp. Group F and *Oblongocollomyces* sp. Group A, which await morphological descriptions and formal naming. In addition, isolates of *Dothiorella diospyricola, Do. brevicollis, Lasiodiplodia crassispora, L. mahajangana, L. pseudotheobromae, L. margaritacea, Pseudofusicoccum stromaticum* and various isolates with uncertain identity, belonging to *Botryosphaeria* and *Dothiorella* were identified. Species in the genera *Dothiorella, Lasiodiplodia* and *Oblongocollomyces* were collected most frequently. Some species occurred on both symptomatic and asymptomatic branches of *B. discolor* trees in natural and agricultural ecosystems in Limpopo Province, and thus providing support for them being able to survive as both endophytes and pathogens. Overall, our results showed that *B. discolor* trees are rich reservoirs for the *Botryosphaeriaceae* in the sampling sites in the Limpopo Province.

Deciphering the role of the SlyA transcriptional regulator in cell metabolism of *Pectobacterium brasiliense* 1692 using LC-MS-MS based Molecular Networking.

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Abstract

Pectobacterium brasiliense (*Pbr1692*) is an economically important pathogen that infects potatoes and causes significant economic loss of crops worldwide. SlyA is a crucial transcriptional regulator in *Pbr1692* and plays an important role in regulating the virulence of *Pbr1692*. Moreover, SlyA has been shown to further regulate carbohydrate metabolism in *Pbr1692*, therefore the aim of this study was to employ untargeted metabolomics to identify potential metabolites that are under the control of SlyA. To determine the role of SlyA in Pbr1692 cell metabolism, bacterial cultures of *Pbr1692ΔslyA* and wildtype *Pbr1692* were grown in three biological replicates. Metabolites from the endo-metabolome and exo-metabolome were then extracted with methanol and subjected to untargeted liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS). The raw LC-MS data was converted into mzML files then analysed using Molecular Networking workflow in Global Natural Products Social Networking (GNPS) platform complemented with different in silico tools Network Annotation Propagation, DEREPLICATOR which were then combined in MolNetEnhancer within GNPS for the discovery of differential profiles between *Pbr1692ΔslyA* mutant strain and wildtype *Pbr1692.* The metabolome data indicated that various biological pathways of cell metabolism such as glycerophospholipid metabolism, starch and sugar metabolism, amino acid and nucleotide sugar metabolism, were affected in *Pbr1692ΔslyA* mutant strains. This study will assist in identifying the metabolites of Pbr1692 that are important for its growth phase and under the control of SlyA. Further studies need to be conducted for a better understanding of how metabolic pathways are affected by SlyA in relation to the virulence of *Pbr1692*.

"Short but Crucial": Role of Short Linear Motifs (SLiMs) in *Phytophthora parasitica* "Core" PpRxLR1 Effector

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Abstract

The relationship between microbial pathogens and their host plants is epitomized by an endless arms race in the battle for survival. Successful plant pathogens, including *Phytophthora* spp., are known to secrete specific effector proteins to manipulate host immune responses. Examples of such effectors are the RxLR effector proteins, named after conserved Arg-any amino acid-Leu-Arg (RxLR) motif at the N-terminus. Our understanding of specific functions of RxLR effectors is limited by lack of knowledge concerning the motifs that facilitate functioning of these effectors at the cellular and molecular level. In this study, a highly conserved PpRxLR1 effector from *P. parasitica* was shown to encode a 6 amino acid long (LWLKYQ) short linear motif (SLiM). Using both in *silico* and *in planta* analyses, we established that the SLiM mediates cell death inducing activity of PpRxLR1 effector that promotes the infection of *P. parasitica*. Similarly, the SLiM was shown to facilitate the interaction between PpRxLR1 effector and its host target protein. However, it was shown to be dispensable for effector subcellular targeting into the host cell. Together, our findings indicate that PpRxLR1 could be an important virulence RxLR effector of *P. parasitica*, promoting the pathogen's infection with the help of its SLiM. Uncovering the mechanisms of effector interference with targeted host functions is a critical step towards understanding host-pathogen interactions. Ultimately, this can be harnessed to breed for durable resistance in plants.

Population genetic diversity and structure of *Exserohilum turcicum* from smallholder and emerging commercial maize farms in the Eastern Cape province of South Africa

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Abstract

Exserohilum turcicum causes northern corn leaf blight (NCLB) of maize and is a major threat to South Africa's maize production. Previous population genetics studies in South Africa revealed a high genetic diversity of the pathogen, being driven in part by cryptic sexual recombination. The population genetic structure of *E. turcicum* in smallholder and emerging commercial farms from the Eastern Cape (EC) is, however, unknown, and we address that question in this study. We hypothesized that *E. turcicum* would be highly diverse and with no population differentiation across the province. To test this hypothesis, 203 isolates from three districts, namely Alfred Nzo, Chris Hani and OR Tambo, were genotyped using 12 microsatellite markers and two mating type markers. The results show that *E. turcicum* was highly diverse (Nei's gene diversity = 0.58; Shannon index = 1.29), but that no population differentiation was detected. Both mating types were recovered from all populations, with strains in OR Tambo segregating in accordance with the null hypothesis of random mating, while Alfred Nzo and Chris Hani populations were significantly skewed from the ratio of 1:1 in favour of an abundance of MAT1-2 alleles. Our study confirms the high levels of genetic diversity at local and regional scales in the Eastern Cape province of South Africa, underpinned by high gene flow and sexual recombination. These results are important to consider in future studies on resistance breeding in maize for NCLB.

Investigating the role of Tomato curly stunt virus V2 coding region on pathogenicity *in vivo* using site-directed mutagenesis.

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Abstract

Tomato curly stunt virus (ToCSV) is an indigenous tomato-infecting *Begomovirus* in the ssDNA virus family *Geminiviridae* which causes severe disease outbreaks in the tomato-growing regions of Southern Africa. Two variant group isolates were shown to induce differing symptom phenotypes in *Nicotiana benthamiana*: V30 induced severe leaf swollen vein and rugosity without recovery, whilst V22 induced moderate to severe upward leaf roll with a significant recovery phenotype being observed. We investigated the role of two V2 aa sequence differences on pathogenicity using V22→V30 substitution mutants. Plants infected with V2 V27S displayed significantly increased disease severity and reduced recovery levels at late infection timepoint (36 days post inoculation) with a corresponding increase in viral load. A significant delay in symptom development was observed in plants infected with V2 T58S although viral load was not significantly different. Deletion mutants were also generated to examine the role of newly identified putative C5 and C6 ORFs present in the V2 coding region on disease development. Deletion of the V22 C5 ORF, which encodes a protein 60 aa in length, did not affect disease symptom phenotype. Deletion of the 35 C-terminal residues of V30 C6, which are absent in V22, resulted in a slight change to swollen vein symptom severity but not viral load. Positive detection of RNA transcripts derived from the complementary-sense (cs) strand region spanning the C5/C6 ORFs using RT-PCR suggests that these ORFs are transcribed. Our findings indicate that ToCSV V2 aa sequence differences play a critical role in symptom severity and disease recovery in *N. benthamiana*. Further experiments are necessary to determine the specific function of V2 aa 27 and 58 in modulating the differing disease pathologies observed. Our study is the first to examine putative cs ORFs present in the V2 coding region of an indigenous begomovirus of tomato. Additional experiments aimed at characterising the function of these novel ORFs in begomovirus pathogenicity are warranted.

Postharvest mould of pome fruit stems and calyx sepals- an investigation into the incidence, cause and management.

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Abstract

Calyx and stem mould is a superficial mould restricted to the pome fruit pedicles and calyx sepals after coming out of CA conditions. Export destinations have reported these moulds seen on long-term storage of fruit after reaching destination consequently resulting in major losses in the South African export industry. The aim of this project was to provide the pome fruit industry with effective and appropriate management solutions to the problem of mouldy stems and calyx sepals of pome fruit. Isolates were collected from symptomatic apple fruit from various packhouses in the Western Cape and incidence recorded. Fungal isolates causing calyx and stem end mould were identified using species-specific PCR and various other molecular methods. Preliminary morphological data shows common species of *Alternaria* and *Cladosporium* as possible causal pathogens however molecular identification to be confirmed. A six-month storage trial at -0.5 °C RA conditions was completed on various apple cultivars 'Fuji', 'Cripps Pink',' Rosy Glow' and 'Sundowner' involving commercial packaging materials and chemical treatments with fungicides (fludioxonil and pyrimethanil). Results from this trial showed that packing fruit in bags exacerbates the conditions for mould development. Packaging material MAM sheets showed the best result in controlling these moulds (no significant incidence). Combining chemical and packaging treatments resulted in a reduction of incidence on both stem and calyx mould. In conclusion the preliminary data obtained shows a possibility that calyx sepals and stems are surface contaminated by latent pathogens before harvest and that favourable conditions and moisture build-up allow them to proliferate.

Population structure and diversity of *Dothistroma septosporum* in Spain suggests several pathogen introductions

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Abstract

Dothistroma needle blight (DNB), caused by *Dothistroma septosporum* and *D. pini*, is an important disease of *Pinus* species. DNB was first reported in Spain in 1975, however severe disease outbreaks have only been noticed in this country since 2009. In 2015, a needle blight disease was observed in stands of three sub-species of *Pinus nigra* planted in the Cantabria Province, and in 2016, in two locations in the Valencia Province on *P. nigra*. The aim of this study was to identify the pathogen present on the affected trees, and to compare the population diversity and structure of a collection of isolates using microsatellite markers and species-specific mating type markers. In total, 163 isolates were collected and all were identified as *D. septosporum*. DAPC and STRUCTURE analyses showed that there are two major genetic clusters in the three stands in Cantabria, one of which was also present at one of the locations in Valencia. An additional unique genetic cluster present in Valencia suggests an introduction from an unsampled source. The Cantabria populations had high levels of genetic diversity in contrast to the clonal populations in Valencia. The analyses provided no evidence of sexual recombination in any of the populations. This is despite the fact that both mating types of the pathogen were detected amongst the isolates from both provinces, and individuals of opposite mating type were commonly isolated from the same pine needles in Cantabria. This study represents the first report of *D. septosporum* in Cantabria. The results suggest that the pathogen was not recently introduced into Cantabria as is the case in Valencia, and that there have been multiple introductions of the pathogen into Spain.

Phytophthora cinnamomi CRN effectors and their roles in manipulating cell death to maintain their hemi-biotrophic lifestyle

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Abstract

Phytophthora cinnamomi is the causal agent of Phytophthora root rot in *Persea americana* (avocado). Our understanding of the mechanisms *P. cinnamomi* utilises to infect and successfully colonise avocado is currently lacking, especially pertaining to how the pathogen is able to maintain its biotrophic and necrotrophic lifestyles respectively during infection. Like fungi, oomycete pathogens also utilise effectors to aid in infection and colonisation. Crinkling and necrosis effectors (*CRN*/Crinklers) are a class of cytoplasmic effectors in oomycetes. Functional studies are severely lacking in *P. cinnamomi*. This research aimed to identify full length CRN genes within the *P. cinnamomi* genome. Using RNA sequence data obtained from a dual transcriptome experiment where avocado was inoculated with *P. cinnamomi*, candidate *CRN* effectors which may be involved in suppressing cell death during the biotrophic stage or inducing cell death during the necrotrophic stage were identified. A total of 25 full-length and 1 partial/*CRN*-like sequences were identified, of which seven are suspected to either induce or suppress cell death. The full-length coding sequences of five of the *CRNs* were confirmed to enable functional characterisation. Interestingly, *CRNs* JG11952 and JG9753 were both shown to have two different haplotypes which may perform different functions during infection. With the coding sequences of these *CRNs* confirmed, functional characterisation studies can be performed in order to confirm the roles these effectors play in manipulating cell death during infection. This will allow for a better understanding on how *P. cinnamomi* is able to maintain the different stages of their hemi-biotrophic lifestyle and successfully colonise the host plant. Following the characterisation of these effectors, further research can be done in the future to determine host target interactions, and possibly edit these targets to promote resistance in host plants.

A susceptible avocado rootstock is confused about the importance of NLR proteins during *Phytophthora cinnamomi* infection

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Abstract

Avocado (*Persea americana*) is an important crop plant, both agriculturally and economically in many countries worldwide. *Phytophthora cinnamomi*, a hemibiotrophic oomycete, remains one of the most destructive pathogens affecting avocado since it is near impossible to eradicate from orchards. One method of controlling Phytophthora root rot is the use of partially resistant rootstocks, which demonstrate stronger immune responses during *P. cinnamomi* infection. Plant Nucleotide binding- Leucine rich repeat (NLR) proteins play a significant role in pathogen recognition and the activation of Effector triggered immunity (ETI). To date, a comprehensive set of avocado NLR genes have not been identified, although their identification is crucial to unravelling molecular mechanisms behind rootstock immunity during *P. cinnamomi* infection. In this study, 161 *PaNLR* genes were identified and characterized using a West-Indian pure accession P. americana genome. *PaNLR* genes were grouped into 12 distinct chromosomal clusters, with high sequence similarity within these clusters identified using phylogenetic analysis. *PaNLR* expression analysis, using RNA-sequencing, revealed significant differences in the expression of 84 *PaNLRs* when compared between a partially resistant and susceptible rootstock, during infection. The partially resistant rootstock showed increased *PaNLR* expression, which was observed up to 24 hours post-inoculation (hpi). The susceptible rootstock however, only showed increased *PaNLR* expression during the first 6 hpi. Results indicate that *PaNLRs* in the partially resistant rootstock may contribute to a stronger, prolonged ETI response able to suppress *P. cinnamomi* growth and proliferation. The results of this research may be used for the development of molecular selection tools for *P. cinnamomi* resistant rootstocks, which can be employed to accelerate rootstock screening programs.

Effect of elevated CO_2 concentrations on maize susceptibility to grey leaf spot (GLS) disease

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Abstract

Atmospheric CO_2 concentrations have been rising considerably over the last century and are forecast to continue increasing from current levels of 415 ppm to 800-1000 ppm by 2100. These changing environmental conditions will undoubtedly have an impact on plant development and disease. Maize is an important C4 crop that is cultivated for multiple purposes around the world, however, its production is subjugated to pathogen attack. *Cercospora zeina*-induced grey leaf spot (GLS) disease is a prominent and devastating fungal infection proliferating across many continents, including Africa. The maize-C. *zeina* interaction in a high CO_2 environment remains unknown, and the aim of this study was to investigate the effect of elevated CO_2 levels on maize susceptibility to GLS. Maize inbred line B73 was grown at 415 and 700 ppm CO_2 in enclosed growth chambers and inoculated with C. *zeina*, while control plants in each chamber were mock-inoculated. Plant height was measured and there was no difference between the growth rate of the B73 maize in either CO_2 regimen. Disease development was recorded and significantly greater GLS symptoms were observed on plants grown at elevated CO_2 concentrations. Gas exchange parameters, namely the photosynthetic rate (A) and stomatal conductance (gsw), were assessed across different leaf ranks corresponding to the inoculated and mock-inoculated leaves. Increased photosynthetic rates were observed for all leaf ranks of inoculated plants grown at elevated CO_2 levels during the early chlorotic stage of lesion formation, while enhanced stomatal conductance was seen in all but the lowest rank of leaves. No significant difference in the gas exchange parameters were observed between the mock-inoculated plants in each chamber. These results demonstrate an adverse change in the dynamic between maize and a destructive pathogen at the predicted CO_2 concentrations of the future.

4.Applied Pathology

Biocontrol of *Fusarium* species utilising indigenous rooibos and honeybush aqueous extracts

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Abstract

Mycotoxins produced by several *Fusarium* species have a significant effect on the reduction of maize yield and grain quality and have led to food safety concerns. The antifungal activity of rooibos *(Aspalathus linearis*) and honeybush (*Cyclopia* spp.*)* tea extracts against the plant pathogen *Botrytis cinerea* was previously shown but their efficacy against *Fusarium* spp. is unknown. In this study, the effect of fermented and unfermented rooibos (*A. linearis*) and honeybush (*Cyclopia subternata*) aqueous extracts, as well as green tea (*Camellia sinensis*), were evaluated as potential antifungal agents against several *Fusarium* species. Conidia viability was determined using the BacTiter-GloTM assay by determining the percentage ATP. The mode of action was analysed by scanning electron microscope (SEM) and the number of polyphenols quantified utilising HPLC-DAD. When applied at the highest concentration of 20 mg/ml, the fermented rooibos and *C. subternata* aqueous tea extracts reduced spore viability of *F. verticillioides* MRC 826-E ($p < 0.0001$) to only 9.26 % and 8.40 % ATP production after 96 hrs. *F. subglutinans* MRC 8553 spore viability was inhibited to 9.53 % and 3.79 % viability with the fermented rooibos and honeybush, respectively. Tea treated conidia examined utilising SEM showed disruption of conidia hyphae with damaged cell walls. These effects could be linked directly to the presence of polyphenols in the tea extracts, suggesting that the extracts contain active compounds that should be further investigated for their potential as natural antifungal agents.

Fungal diversity and mycotoxin contamination of wheat grain produced under different crop rotation systems and tillage practices in the Western Cape

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Abstract

Wheat is South Africa's second most important staple crop, with roughly 50 % of local production occurring in the Western Cape. Over the past 25 years, production practices in this province have changed from wheat produced in monoculture on conventionally tilled fields, to crop rotation coupled with reduced tillage. The impact of these practices on fungal diversity and mycotoxin contamination of wheat grain remains unresolved. The aim of this study was, therefore, to determine the fungal composition and mycotoxin contamination of wheat grain produced under different crop rotation and tillage practices. The study was conducted in 2019 in long-term agronomical trials at Langgewens and Tygerhoek research farms in the Swartland and Overberg regions of the Western Cape, respectively. Identical trials comprised of a two-factorial randomised split-plot design with three replicates, with crop rotation as main factor and conventional, minimum, no and zero tillage as sub-factor. Crop rotations included wheat-canola-wheat-lupin, wheat-lupin-wheat-canola, wheat-medic-wheat-medic and a wheat monoculture. Fungal isolates were obtained from harvested grain from each subplot and representative isolates were identified by sequencing the elongation factor and RNA polymerase II 2nd largest subunit gene regions. Mycotoxins deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEA) from grain was quantified by liquid chromatography tandem mass spectrometry. Fungal diversity was relatively low at both locations and included species of *Alternaria*, *Fusarium* and *Stemphylium*. *Alternaria infectoria* was the most abundant fungal contaminant, followed by *Stemphylium vesicarium* and *Alternaria graminicola*. Fungal diversity was greatest in the wheat monoculture, while wheat grain produced, following rotation with canola and lupin, had the lowest fungal diversity. Tillage practices did not significantly influence fungal diversity. Mycotoxins were generally low and ranged from 0. 009 to 0. 066 mg kg^{-1} for ZEA and DON, with no NIV detected. Wheat rotated with canola and lupin had the lowest mycotoxin levels at both locations. Minimum and conventional tillage resulted in the lowest mycotoxin levels at Langgewens, while zero tillage gave the lowest levels at Tygerhoek. Some treatments at Tygerhoek, however, had ZEA levels close to EU-limits, highlighting the need to monitor and manage mycotoxigenic fungi and mycotoxins.

Does the severity of Phytophthora root-rot vary between genotypes of *Eucalyptus nitens*?

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Abstract

Eucalyptus nitens is an important cold-tolerant tree species commercially planted in South Africa. This eucalypt species is threatened by various native and invasive pests and pathogens, of which one is *Phytophthora*. Recently, a previously unknown root-rot disease has been affecting *E. nitens* plants in the first year of planting up to the age of four years. The severity of this disease resulted in a reduction of land planted to this species in South Africa. The causal agent of this disease could not be conclusively identified, but the symptoms observed in the field indicated *Phytophthora* as a plausible candidate. DNA fingerprinting of the affected trees showed that the selfed genotypes of *E. nitens* were highly susceptible to this root-rot disease. In this study, greenhouse trials were conducted to test the pathogenicity of two *Phytophthora* species (*P. alticola* and *P. cinnamomi*) on three genotypes of *E. nitens* (selfed, outcrossed, and hybrid-*E. nitens* x *E. grandis*). Data emerging from these trials showed that (1) selfed *E. nitens* seedlings were highly susceptible to the tested *Phytophthora* species, followed by the outcrossed genotype, (2) the severity of root-rot was highest among those seedlings inoculated with *P. cinnamomi*, and (3) the hybrid genotype showed tolerance towards both the tested *Phytophthora* species. This study reconfirmed the deleterious effects of inbreeding that can substantially reduce fitness among tree species, making them susceptible to an assortment of native and introduced plant pathogens such as various species of *Phytophthora*.

The continual emergence of new *Puccinia triticina* races on wheat in South Africa

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Abstract

Leaf rust, caused by *Puccinia triticina (Pt*), is an important disease of bread wheat in South Africa (SA) and is primarily controlled using resistant cultivars. Understanding the virulence and genetic diversity of *Pt* is essential for successful breeding and improved durability of resistant cultivars. Virulence analysis of the South African *Pt* population from 2017 to 2020 revealed the presence of 10 *Pt* races typed from 406 isolates, of which three, 3SA100, 3SA127, and 3SA170, represent first reports. Races 3SA10, 3SA38, 3SA146 and 3SA115 were most commonly found with average frequencies over the four seasons varying from 18 % (3SA146 and 3SA115) to 26 % (3SA10). The frequency of the new race 3SA170 was 41 % during the 2020 season, whereas the remaining four races were observed at less than 3 %. Race 3SA170 is virulent on key resistance genes *Lr24, Lr26, Lr13* and *L37.* Seedling infection type data for 113 South African wheat varieties revealed that 10 entries are susceptible to race 3SA144 followed by race 3SA115 (43 susceptible), 3SA145 and 3SA127 (52), 3SA146 (54), 3SA100 (69), 3SA38 and 3SA170 (72), 3SA10 (73) and 3SA248 (78). According to microsatellite analysis, the current South African *Pt* population consists of three distinct genetic lineages, where 3SA100 and 3SA127 were genetically similar to 3SA146, and 3SA170 to 3SA145, 3SA248, 3SA10, 3SA38 and 3SA115. This supports the local mutational development of 3SA127 from 3SA146, and 3SA170 from 3SA10, but indicates that 3SA100 could represent yet another introduction. With the detection of 3SA100, 3SA127, and 3SA170, the number of new *Pt* races reported over the past 10 years increased to 10. The results indicate continued variability of the *Pt* population in SA and stress the need for regular surveillance as an early warning system to report the effect of new and more virulent *Pt* races on cultivar response.

Sensitivity of *Alternaria* species from potato in South Africa to different fungicides

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Abstract

Foliar diseases of potato, caused by *Alternaria* species, are a serious constraint to potato production world-wide. These diseases are mainly controlled by the application of fungicides; however, the development of fungicide resistance is a growing problem in agriculture. *A. alternata* is classified by the Fungicide Resistance Action Committee (FRAC) as a high-risk pathogen for development of resistance, while *A. solani* is classified as medium risk. Both species have developed resistance to various classes of fungicides, including the QoIs (reports from USA, Europe and South Africa) and SDHIs (reports from USA and Europe). The objective of this study was to test selected *Alternaria* isolates obtained from symptomatic potato leaves against five classes of fungicides (FRAC codes 2, 3, 7, 11 and 30) registered for the control of early blight of potato in South Africa. Tests were conducted by inoculating spore suspensions in micro titre plates, with seven different fungicide concentrations, in order to calculate EC50 values. Results confirmed that *A. alternata* isolates with reduced sensitivity to QoI (FRAC 11) fungicides are widespread in all potato production areas in South Africa. In addition, *A. arborescens*, *A. solani* and *A. grandis* isolates also showed a reduction in sensitivity to QoI fungicides. Various isolates of the large-spored species *A. grandis*, that causes early blight symptoms on potato, also displayed a significant reduction in sensitivity to SDHI fungicides (FRAC 7). *A. arborescens* and *A. grandis* from several areas had a reduced sensitivity to dicarboximide fungicides (FRAC 2). Isolates from all four species had a reduction in sensitivity to DMI fungicides (FRAC 3). Isolates from 13 of the potato production regions were tested and different profiles of loss of sensitivity were recorded from the various areas. The result from this study emphasises the need for proper management of spraying programs in order to extend the useful lifespan of the fungicides that are available for the control of foliar diseases of potato.

First report of black spot disease and seedling wilt of pecans (*Carya illinoinensis*) caused by *Alternaria alternata* in South Africa

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Abstract

The pecan (*Carya illinoinensis*) industry in South Africa is growing rapidly, and it is crucial to mitigate the challenges by understanding the risk of fungal pathogens poses to the industry. During disease surveys, black spot symptoms were observed on leaves, twigs, and nuts. Healthy and diseased samples were collected from various pecan orchards from major production regions. In this study, *Alternaria* isolates were identified based on molecular identification. Multi-locus DNA sequencing analysis of glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), RNA polymerase II 2nd largest subunit (rpb2), translation elongation factor 1-α (tef1), and *Alternaria* major allergen (Alt a 1) gene regions revealed that the isolates were *A. alternata sensu stricto*, forming part of the *Alternaria* species complex. The virulence of *A*. *alternata* isolates were tested on detached nut cultivars of Wichita and Ukulinga, detached leaves (Wichita), and seedling blights. The results differed significantly between resistant and susceptible cultivars of the detached nuts (wounded and unwounded) for all the tested isolates. Similarly, the wounded detached leaves were significantly different from the unwounded leaves. The bioassay on the pecan seedlings confirmed that the *A. alternata* isolates are pathogenic, causing overall decline (wildered) on the plants and fulfilling Koch's postulates. These results confirm that *A. alternata* causes black spot disease and seedling blight of pecans in South Africa. The study further developed and evaluated Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP) assay to screen a large collection of *A. alternata* isolates (222) retrieved from different pecan localities. *Alternaria* major allergen (Alt a-1) gene region was used, followed by digestion of the amplicons with *HaeIII* and *HinfI* endonucleases. The approach was useful in differentiating novel isolates into haplotypes, allowing an indication of variation. It allowed rapid and early detection of novel haplotypes which may also represent other *Alternaria* species. The data was then used to compare whether there were any significant differences between healthy and diseased pecan tissues. This study highlights the importance of *A. alternata* as a pathogen of pecans in the various production areas of South Africa.

Fungicide deposition on wheat leaves as influenced by seeding density, spray volume and tractor forward speed

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Abstract

Chemical control of foliar diseases of wheat is an expensive, but inescapable part of wheat production in the Western Cape. Fungicides are applied at rates of 50 – 200 L ha^{-1}, at a tractor forward speed of 6 – 20 km h^{-1}. Little information is currently available on how factors such as plant biomass (seeding density per m^2), spray water volume and tractor forward speed influence spray deposition parameters achieved on wheat leaves following fungicide application. Previous studies have used artificial targets, such as water sensitive paper strips, to quantify deposition parameters. However, artificial targets do not accurately represent leaf characteristics. The aim of this study was, therefore, to determine how the mentioned factors influence fungicide deposition (deposition - quantity, uniformity, and quality) using wheat leaves as deposition targets over two seasons. The effect of three seeding densities (160, 200 and 240 plants m^{-2}), three spray volumes (150, 200 and 300 L ha^{-1}) and two application times (advanced tillering and flag leaf) was determined in the first year. In the second-year lower spray volumes (50, 100 and 150 L ha^{-1}), tractor forward speed (6.3, 9.8, 15 and 20.7 km h^{-1} at 150 L ha^{-1}) and application under calm or windy conditions was investigated. The spray liquid consisted of water and yellow fluorescent pigment. Lower, middle and upper leaves of 12 randomly sampled plants / treatment / replicate / year was taken and deposition was determined with fluorometry macrophotography and digital image analyses. Results for year 1 showed that seeding density and spray volume did not significantly influence deposition at either growth stage. Significantly lower deposition was achieved on all leaves at the flag leaf stage when compared to the advanced tillering stage, due to the horizontal orientation of leaves at the earlier stage. Results from year 2 showed that significantly lower deposition was achieved on all leaves at 50 L ha^{-1} compared to 150 L ha^{-1} volume. An increase in tractor speed did not significantly influence deposition. Similar results was obtained under windy than calm conditions. Establishment of spray deposition benchmarks for disease control will be investigated in coming years.

The dream of boosting African crop productivity vs the reality of crop loss to pathogens

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Abstract

Agriculture is a main driver in the Southern African economy, a sector in which more than 60 % of the population is active. Plant diseases are a major obstacle in sustainable agricultural production, yet the effective transfer of knowledge in the better management of plant diseases in the industry is often lacking. This paper aims to highlight the challenges in "on the ground" plant disease management in sub-Saharan Africa in various crops from extensive knowledge gained over 20 years of service to the agricultural industry as a specialist industry researcher and consultant. An overview of the basic challenges encountered and explored in more than 13 crops in the field, orchard or logistics chain will be discussed. Other than the major known challenges of availability of agricultural inputs, reliable infrastructure, and access to markets this paper will focus on crop production challenges relevant to crop protection and crop health. These include the lack of knowledge farmers face in terms of pro-actively promoting plant health and the discord between academic research output and real-world farming needs. There is a crucial gap, brought on by a lack of support in plant disease management, between Southern African crop production realities and the dream for improved agricultural productivity in the region. This paper therefore also seeks to communicate what the specific needs of the farmers of this region are in terms of sustaining crop production from the perspective of crop health. Amongst others, the importance of building capacity, training crop health specialists, making reliable, rapid and affordable diagnostic services available and building collaborative networks in sub-Saharan South Africa, to promote sustainable agriculture, will be discussed.

Assessment of *Sclerotinia sclerotiorum* sclerotia recovered from soybean and sunflower silos across South African production regions

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Abstract

South African legislation prohibits sclerotia of *Sclerotinia sclerotiorum* > 4 % in graded soybean and sunflower according to the Agricultural Product Standards Act No. 119 of 1990. The South African National Seed Organisation (SANSOR) apply a stricter threshold of 0.2 % sclerotia in certified seed. The aim of this study was to assess sclerotia recovered from South Africa soybean and sunflower silos, between 2012/2013 and 2019, for each crop respectively. Publicly available historic data collected from South African Grain Laboratories, who were also responsible for the sampling and sclerotia percentage determination were utilised in this study. A total of 169 soybean silos were sampled between 2012 and 2019, 3-kg samples ranged between 100 and 150 per annum during this period. The prevalence of sclerotia recovered ranged between 33 % (2015) and 70 % (2017) in soybean production regions. A total of 143 sunflower silos were sampled between 2013 and 2019, with 152 and 176, 3-kg samples per annum during this period. The prevalence of sclerotia recovered ranged between 9.1 % (2013) and 89.5 % (2018) in sunflower production regions. Significant differences, determined by one way analysis of variance, between the years were reported for both crops. No sclerotia recovered exceeded the 4 % legislation threshold, only two soybean production regions had samples above the 0.2 % SANSOR threshold, both from prominent soybean production regions. In sunflower production regions, one sample from the North-West province, in 2014, was above the legislative threshold. Although, multiple production regions were above the SANSOR limit in 2014 and 2017 to 2019, there were also associated with the greatest area planted to sunflower. Sclerotia recovered from the silos may provide an indication of sclerotia retained in soybean used for farm-saved seed, as well as the persistence of sclerotia associated with previously infected fields. South Africa is one of the few countries who have National and industry legislation to monitor and restrict sclerotia in seed. This practice should continue, however, cognisance should be taken of ability for *S. sclerotiorum* to remain seedborne, as mycelium in the cotyledon, embryo and testa, and cause infections in the field.

Bibliographical analysis of *Sclerotinia sclerotiorum* disease management on oilseed crops.

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Abstract

Sclerotinia sclerotiorum associated diseases have been extensively studied due to the pathogens' ability to cause tremendous yield losses. Disease associated with this complex pathogen remains a challenge, probably due to ineffective and inefficient management. Six disease management strategies (i) avoidance; (ii) eradication; (iii) exclusion; (iv) protection; (v) therapy; and (vi) resistance, have been outlined as key tools. Each of these strategies includes different practices that help to suppress and ultimately manage *Sclerotinia* diseases. This review aimed to identify the leading *Sclerotinia* disease management practices, using bibliographic information yielded from abstracts of research articles published in English and accessible to our institution. In total, 2 597 relevant articles from Scopus, Science Direct and Web of Science were analysed. The results indicated that the two leading countries with *Sclerotinia* disease research outputs were China and Brazil, with the majority focusing on canola in the former and soybean in the latter. The preferred author keywords were "*Sclerotinia sclerotiorum*", “antifungal activity” and “biological control”, which were supported by the top management practices recorded. Eradication and protection were the leading management strategies, while the preferred management practice for each was biological and chemical control, respectively. The number of studies on these two practices were significantly higher than any other management practice. *Coniothyrium minitans* was the biological control agent most studied, while carbendazim and boscalid yielded the most articles in the fungicide category. More research is needed on several other management practices, especially the role of disease-free seed and weed control since both can prevent inoculum introduction and build-up.

Understanding fungal nut diseases of Macadamia fruit in South Africa

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Abstract

Husk rot, a fungal disease of macadamia fruit, is an important and complex problem for growers, as it often results in premature drop of nuts impacting overall yield. Different fungi have been implicated as the causal agents of this disease including a number of species of *Colletotrichum*, *Diaporthe*, and more recently *Calonectria*. The diversity of the causal agents of husk rot in South Africa is uncertain. The aim of this study was to accurately identify the species associated with macadamia fruit with husk rot symptoms in the three main macadamia production regions in South Africa (Mpumalanga, Limpopo and KwaZulu Natal), and to understand putative biological differences between the causal agents that may impact the management of the disease. Based on analyses of DNA sequence data of selected gene regions, those informative to each genus, three species of *Colletotrichum*, a number of *Diaporthe* spp. and three species of *Calonectria* were identified, including at least one novel species. Pathogenicity trials on detached macadamia fruits revealed isolates of *Co*. *siamense*, *Co*. *fructicola*, and two *Diaporthe* spp. most closely related to *D*. *macadamiae* are the most aggressive. Cultivar screening including 695, Nelmak 2 and A4 also showed a comparative tolerance in A4 to the husk rot pathogens. Growth studies revealed a greater affinity for temperatures between 30 °C – 35 °C in the *Colletotrichum* species, than the *Diaporthe* spp. which grew optimally between 20 °C – 25 °C. As both *Colletotrichum* and *Diaporthe* consist of known endophytes, various stages of the macadamia fruit were sampled and both genera detected from the asymptomatic pea-sized fruit stage, suggesting the possibility of a latency stage for some species. Calonectria husk rot, an emerging disease in South Africa previously detected in Limpopo only was identified in Mpumalanga suggesting further monitoring is necessary to determine its potential impact on the industry. This study has provided new insight into the diversity and biology of husk rot revealing potential for latency, variations in optimal temperatures and pathogenicity within known genera, and monitoring a new genera of husk rot pathogens to South Africa, *Calonectria*.

IPM: The only option for managing *Spongospora subterranea* f. sp. *subterranea* on potatoes

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Abstract

Once believed to not pose a threat to the South African potato industry, *Spongospora subterranea* f. sp. *subterranea* (Sss) is now one of the most important pathogens affecting this crop. This soil- and seed-borne Plasmodiophorid causes root galling and powdery scab, which results in severe economic losses to the international potato industry. Despite years of research by numerous scientists and industry partners globally, this pathogen remains notoriously difficult to control, due to the longevity of its resilient resting spores in soil, scarcity of effective chemical controls, and lack of fully resistant cultivars. No single measure will control powdery scab, and accordingly growers must apply various practices to manage the disease. For this reason, research projects in the Potato Pathology Programme @ UP have focused on elucidating the epidemiology and biology of the pathogen in South Africa, to develop a sustainable integrated management plan for Sss. Such a strategy begins with field selection and preparation. If planting in fields with a history of powdery scab, it is wise to apply soil- and / or seed treatments. Our research has demonstrated the potential of fluazinam, metam sodium, zinc compounds, calcium cyanamide and biological control agents containing *Bacillus subtilis* and / or *Trichoderma* spp. to reduce the pathogen in the soil and the roots of the host plant. Our research has shown a range of susceptibility in the commercial cultivars in South Africa, allowing growers to make an informed decision regarding selection thereof. Results from long-term survival studies of Sss in three contrasting argo-ecological potato growing regions in SA indicated that land use had the most noticeable effect on Sss inoculum concentration in the soil. Soybeans, black oats / rye mixture and fallow periods resulted in a decrease in inoculum concentration; while potatoes, maize and sorghum increased the concentration of Sss in the soil. Rotation with non-hosts or trap crops identified in recent studies done in the Potato Pathology Programme @ UP will aid in a gradual decrease in soil inoculum levels. Implementation of these and other agronomical practices will contribute to management of this pathogen and sustainable potato production.

The heteroecious life cycle of *Puccinia digitariae* on *Digitaria eriantha* and *Solanum* species, the first to be elucidated in South Africa in a century.

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Abstract

Rust fungi are important plant pathogens and have been extensively studied on crops, but much less so on wild host plants worldwide. Recent studies have confirmed the heteroecious life cycle of a rust fungus on *Digitaria eriantha* (finger grass) and the *Solanum* species, *S*. *lichtensteinii* (large yellow bitter apple), *S*. *campylacanthum* (bitter apple) and *S. melongena* (eggplant) in South Africa. Following field observations, inoculation studies involving telial isolates collected from *Digitaria* plants produced spermogonia and aecia on *S*. *lichtensteinii*, *S*. *campylacanthum* and *S*. *melongena*. Likewise, inoculation of finger grass with aeciospores collected from the aforementioned *Solanum* species produced uredinia on *D. eriantha*. *Pennisetum glaucum* (pearl millet varieties Milkstar and Okashana, as well as 17 experimental lines) and *S. elaeagnifolium* (silverleaf nightshade or bitter apple) were resistant to the rust isolates. Morphological characteristics and molecular phylogenetic data confirmed the identity of the rust on *Digitaria* as *Puccinia digitariae*, herein reinstated as a species and closely related to *P*. *penicillariae* the pearl millet rust, also reinstated. *P. digitariae* has a macrocyclic, heteroecious life cycle in which teliospores overwinter on dormant *D*. *eriantha* plants. Aecia sporulate on species of *Solanum* during spring and early summer to provide inocula that infect new growth of *Digitaria*. This is the first life cycle of an indigenous heteroecious rust to have been fully elucidated in a century in South Africa, the last having been published in 1923.

Exploring the diversity of mycotoxigenic fungi in South African food and feed

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Abstract

Mycotoxins are one of the biggest threats to African food security with associated problems expected to increase due to climate change and the unpredictable weather conditions that brings. Knowledge of what mycotoxigenic species occur on food and feed, and understanding their biology is important to establish effective management practices. Unfortunately, basic diversity knowledge is lacking in South Africa. Our project thus aimed to expand, document, and disseminate our understanding of the diversity of mycotoxigenic fungi and mycotoxins in food and feed across South Africa. Maize and sunflower seeds and animal feed were collected from across South Africa. Mycotoxin levels in these samples were determined using liquid chromatography-mass spectrometry (LC-MS). Fungal isolations were made by plating seed and feed onto dichloran 18 % glycerol agar, potato dextrose agar and water agar, with the latter two supplemented with chloramphenicol (100 ppm) and streptomycin (50 ppm). Plates were incubated at 21 ºC and subsequent purifications were made after 5–7 days. Strains and DNA were accessioned into a working collection housed at FABI. Strains were initially identified to genus level based on morphology, but species identifications were based on DNA sequences. This survey resulted in 843, 115 and 735 strains isolated from maize, sunflower, and animal feed. Strains were identified to 49 genera and 255 species. Across all samples, *Fusarium* (n=409), *Aspergillus* (n=381), *Penicillium* (n=324), *Cladosporium* (n=167) and *Talaromyces* (n=108) were the most commonly isolated. Several known mycotoxigenic species were recovered in high numbers (e.g., *F. verticillioides*, *A. flavus* etc.) but this did not necessarily correlate with high mycotoxin levels. This survey was crucial to expand our culture collections and build baseline knowledge needed to start addressing pressing questions about the biology and ecology of mycotoxigenic fungi in South Africa.

Validation of high-throughput sequencing for routine plant virus diagnostics

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Abstract

High-throughput sequencing (HTS) was applied successfully to discover novel viruses and viroids in many agricultural crops and the logical next step is to validate the application of this technology for routine pathogen detection adhering to basic validation principles. Citrus plants were established with a known cocktail of viruses and viroids. Four plants (one healthy plant and three infected) were sampled in triplicate and total RNA was extracted using two different methods and sent for Illumina HTS. One replicate sample of each plant for each RNA extraction method was also sent for HTS on an Ion Torrent platform. One year later the same plants were sampled in triplicate and sent for Illumina HTS. The data were evaluated for biological and technical variation. The sensitivity of the HTS assay was also compared to routine RT-PCR assays in a time course experiment. The study evaluated the influence of different HTS protocols on the sensitivity, specificity, reproducibility and repeatability of HTS as a detection tool. Extraction method and sequencing platform contributed the most to variation observed between data sets. Using a de novo assembly approach, complemented with read mapping, the Illumina data allowed a greater proportion of the expected pathogen scaffolds to be inferred, and an accurate virome profile was constructed. The complete virome profile was also constructed using the Ion Torrent data but analyses showed that more sequencing depth is required to be comparative to the Illumina protocol and produce consistent results. The CTAB extraction protocol lowered the proportion of viroid sequences recovered with HTS, and the Zymo Research kit resulted in more variation in the read counts obtained per pathogen sequence between replicates. The use of a LiCl-free CTAB extraction protocol significantly improved the sensitivity for viroid detection. This study highlights the need to measure the level of variation that can arise from the different variables of an HTS protocol. HTS is more comprehensive than any assay previously used, and with the necessary validations and standard operating procedures, the implementation of HTS as part of routine pathogen screening practices is possible.

Rust diseases of food and forage crops in South Africa: new threats and research to mitigate their impact

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Abstract

Rust pathogens of food and forage crops are known as production constraints, often resulting in additional disease management costs. The rusts of wheat have been comprehensively reported on in South Africa (SA), but rust diseases of barley, oat, maize, sunflower, soybeans and beans have not been systematically studied. Outbreaks of wheat stem rust on spring wheat in the Western Cape during 2020 were associated with the new race BFGSF, virulent to Sr38, and in the Free State with another new variant, race PTKSK, virulent to a previously effective adult plant resistance gene present in several winter wheat cultivars. Increased incidence of the wheat leaf rust pathogen on wheat in the Western Cape is predicted following the first report of isolates virulent to *Lr9* in 2021. In a recent study the dominant race of the barley leaf rust pathogen in the Western Cape showed virulence to *Rph3* postulated to be present in several of the current cultivars. On oat, the detection of crown rust race LLBB during the 2019 season impacts on the susceptibility of black oat cultivars planted as cover crops or as animal feed. Similarly, a new race of the bean rust pathogen was detected in 2021 on green beans planted in KwaZulu-Natal. Bean varieties postulated to carry the *Ur-5* resistance gene are now susceptible. Rust of spring onion and leek, visible as unappealing brown, sporulating pustules on the foliage of fresh products sold in supermarkets, is a major challenge for vegetable producers in continuous production cycles. Rust of teff is expected to become a problem along with expansion in production of this cereal grass for grain. From an epidemiological point of view, *Thinopyrum distichum*, the common coastal wheat grass, can host cereal rust pathogens and thus contribute to survival and early season inoculum in SA. Research on pathogen identity, race composition, resistance genetics and breeding, cultivar response, the impact of disease on yield and quality and other management methods is required for sustained control of rust diseases in SA.

The plant disease pyramid: Reflecting on a century of plant pathology development

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Abstract

During 2020 I had the opportunity to contribute a commentary to the South African Journal of Science as part of a special issue commemorating the International Year of Plant Health. The commentary reflected on a talk by Professor Paul A. van der Bijl in 1926 to the South Africa Association for the Advancement of Science, entitled 'Landmarks in the development of the science of plant pathology and of disease control'. Professor Van der Bijl had been appointed five years earlier as the first Professor of Plant Pathology and Mycology in South Africa at Stellenbosch University. The 1926 address serves as an interesting 'lens of history' on Plant Pathology today. It is clear from this comparison that more knowledge and more powerful tools do not automatically reduce the threat of plant diseases to food security and environmental health, as is true for human diseases and pandemics. The impact of the knowledge and tools rather lie in their implementation in a complex context. This is well illustrated by the changing perspectives on the disease triangle. Prof Van der Bijl did not use the term 'disease triangle', but reflected on the interactions between environment, host and pathogen in the development of diseases on local scales and how it might be manipulated to manage those diseases. Recently, researchers have argued that this picture is incomplete without a fourth dimension, namely the influence of biotic factors such as the microbiome on disease development. I added a fifth dimension to this model, namely the critical role that human social systems play in the emergence, evolution and severity of disease developments. The influence of all these elements of the system also needs to be considered in the context of interrelated local, regional and global scales. It is essential that Plant Pathologists consider how to develop more holistic, integrated and globally connected systems to optimize the use of the powerful tools at our disposal to reduce the impact of plant pathogens. This is an urgent task in the context of a growing human population and an environment under increasing pressure that will require foresight and dedicated leadership.

Timing of infection drives fungal growth and fumonisin production by *Fusarium verticillioides* in maize grain

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Abstract

Maize is commonly subjected to infection by fungal pathogens such as *Fusarium verticillioides* which causes Fusarium ear rot (FER). Moreover, fumonisins, the most important mycotoxins produced by *F. verticillioides* is associated with nocuous effects in humans and animals. In this study, maize lines with known response to FER/fumonisin contamination was utilised to investigate the role of timing of kernel infection by *F. verticillioides* on infection indicators (FER disease severity, *F. verticillioides* target DNA and fumonisins). Maize was inoculated 7 days after pollination (dap) (R2 blister kernel stage) while an independent set of maize plants were inoculated 35 dap (R5 early dent kernel stage) and subsequently harvested at 7, 28, 42 and 52 days after inoculation (dai). The infection indicators were evaluated at every sampling time for each inoculation event. *Fusarium verticillioides* growth and fumonisins increased progressively over time following inoculation, reaching a maximum at 52 dai for both inoculation events with significant differences between inoculated and control maize grain. Inoculated grain of resistant lines CML 444 and CB 222 accumulated lower levels of *F. verticillioides* target DNA (0.002 – 0.656 ng μL^{-1}) and fumonisins (0.1 - 12.8 mg kg^{-1}) when inoculated 7 dap. However, when inoculated at 35 dap these lines showed an increase in fumonisin contamination (0.1 – 19.0 mg kg^{-1}). The susceptible R2565Y accumulated high levels of fungal DNA (<0.209 ng μL^{-1}) at 7 dap and up to 1.600 ng μL^{-1} at 35 dap while fumonisins of 52.2 mg kg^{-1} (7 dap) and 4.8 mg kg^{-1} (35 dap) were quantified. Fumonisins showed a strong significant correlation with *F. verticillioides* target DNA (r= 0.42; P = 0.003) 7 dap while fungal DNA had a strong significant correlation with FER severity (r= 0.469; P = 0.001) 35 dap. *F. verticillioides* growth and fumonisin accumulation in maize grain is dependent on the timing of infection rather than a specific kernel stage with maximum fumonisin contamination occurring in matured grain. The response of maize lines should also be assessed by artificially inoculating maize ears at the early stages of kernel maturation to determine accurate plant response in matured grain.

Identification of new *Puccinia helianthi* race variants in South Africa and their impact on sunflower hybrids

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Abstract

Sunflower rust, caused by *Puccinia helianthi* Schw. (Phe), is a major disease of sunflower (*Helianthus annuus* L.) and when left uncontrolled, can result in significant yield and quality losses. The pathogen is known to regularly evolve into more virulent races by overcoming deployed resistance genes. In a previous study, more phenotypic variation amongst current Phe race isolates was expected than depicted by the standard set of nine differentials and two additional lines. In the current study, ninety South African Phe isolates, representing eight of the nine described Phe races in South Africa (SA), were used to screen twelve additional sunflower differentials. Nine of these differentials contains uncharacterised resistance sources, while the remaining three possess R13b, R14 and R15, respectively. Phe3000 was the only race with isolates avirulent to all twelve differentials, while race Phe7721 isolates were the most virulent. Four differentials produced low infection types to all Phe isolates. Differentials Ph2 and Ph7, TMAD01A and TOF09A, as well as RHA 461 and RHA 479, produced similar low and high infection types (ITs), indicating that they share the same source(s) of resistance. Sixteen unique avirulence/virulence profiles were observed among the ninety Phe race isolates. Thus, isolates of the eight original Phe races could be grouped into 23 unique sub-races. No variation was recorded for isolates that typed to races Phe3621 and Phe7331, Phe3320 could be sub-divided into two sub-races, Phe3000 and Phe3001 into three each, Phe3401 into four, and Phe7221 and Phe7320 into five sub-races, respectively. Low and high IT patterns recorded for the differentials TMAD01A and TOF09A, as well as for PHRR3 (R3), were commonly observed for several Agricultural Research Council national sunflower trial hybrids. Four of the hybrids were resistant to all Phe isolates. This study represents the most comprehensive report on Phe race analysis and sunflower hybrid responses in SA, with both breeders and producers benefitting from these results.

Influence of crop rotation and different tillage practices on Fusarium crown rot and agronomical performance of wheat in the Western Cape.

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Abstract

Fusarium crown rot (FCR), caused by *Fusarium pseudograminearum*, is an economically important stubble-borne disease of wheat. The disease is favoured by warm, dry conditions after anthesis and agronomical practices like wheat monoculture. Disease management relies on integrating practices like crop rotation and tillage with host tolerance and seed treatments. The aim of this study was to determine the effect of crop rotation and tillage practices on specific FCR and agronomical parameters of wheat. The study was conducted during 2020 and 2021 in long-term trials at Langgewens and Tygerhoek research farms, located in the Swartland and Overberg regions of the Western Cape, respectively. Four cropping systems consisted of a wheat monoculture, wheat rotated with medics and two rotations where wheat followed canola or lupin in a wheat-canola-wheat-lupin system. Each rotation was sub-divided into four tillage treatments, namely conventional, minimum, no, and zero tillage. Stubble decomposition rate and chlorophyll-free length 60 days after planting was measured in all treatments. Wheat samples were taken from each treatment at the soft dough stage to assess disease incidence, tillers diseased (%) and average lesion length. Agronomical parameters including yield, biomass and grain protein was measured post-harvest. At Langgewens, stubble decomposition rates were higher under zero- and no tillage than minimum and conventional tillage. Crop rotation and tillage significantly ($P \leq 0.05$) affected certain disease parameters at both locations. At Langgewens, zero tillage reduced FCR incidence compared to more disruptive treatments, while crop rotation reduced diseased tillers compared to wheat monoculture. At Tygerhoek, zero tillage reduced diseased tillers and average lesion length better than more disruptive treatments, while crop rotation lowered diseased tillers compared to wheat monoculture. In 2020 at Langgewens, grain, straw and biomass yield, ear bearing tillers m^{-2}, and grain protein were greater for wheat planted in rotation. At Tygerhoek, zero tillage promoted straw and biomass yield of wheat. The chlorophyl-free length of seedlings significantly decreased as soil disturbance increased in 2021, indicating seeds were planted deeper as tillage increases. The reduced length of sub-crown internodes under zero-tillage may contribute to the lower disease levels obtained. Conservation agriculture practices can, therefore, help reduce FCR.

A standardised assay to assess risks of *Fusarium circinatum* (pine pitch canker) infection

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Abstract

Fusarium circinatum is a globally regulated pathogen that causes a serious canker disease of *Pinus* species known as Pine Pitch Canker (PPC). Since its first discovery in the United States, the pathogen has been reported as an invasive alien in many parts of the world. It is particularly damaging to non-native *Pinus* species established in plantations. In this study, we compared and quantified the aggressiveness of *F. circinatum* strains from diverse locations, including Chile, Colombia, Guatemala, Mexico, Nicaragua, South Africa, Spain, and the United States. This was achieved by inoculating 6-mo-old *Pinus patula* seedlings in a greenhouse setting. The results showed that strains differed markedly in their relative aggressiveness. Most strains were highly aggressive (n=41), with only a few producing small lesions. Prior to this study, the aggressiveness of some of these isolates were unknown. Without this large, standardised assay we could not have reliably compared aggressiveness between isolates from different populations. This emphasises the need for standardised assays to compare independent studies and understand the risk from different *F. circinatum* populations. *F. circinatum* will likely continue to spread and standardised studies such as this will be key to protecting countries that rely on *Pinus* species.

Assessment of apple trees to be remove due to dieback pathogens and the detection of *Diplodia seriata* on chipped apple wood

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Abstract

Apple orchards need to be replaced over time to maintain production volumes. Once removed, the old apple trees are often chipped and used as mulch in younger orchards. The presence of dieback pathogens in old apple plantations are often not considered when the trees are used for mulch. Therefore, the extent of dieback pathogens presents in apple trees to be removed and the presence of *Diplodia seriata*, were investigated from chipped apple tree wood pieces. Trees showing symptoms of dieback were sampled from 14 orchards in the Grabouw, Vyeboom and Kouebokkeveld apple-producing areas. For the detection of *D. seriata* from mulched wood pieces, samples were collected in October 2020 and February to April 2021 from orchards in the Grabouw (two orchards) and Vyeboom (one orchard) production regions. Visual inspections for the presence and viability of *D. seriata* were done on samples from four collection events, whereas quantitative real-time PCR analyses (qPCR) were done on samples from the first and last sampling events. Canker and wood rot pathogens were isolated from 118 of the 144 trees sampled. *Eutypa lata* was most often isolated (from 18.6 % of trees) followed by *D. seriata* (9.3 %). The predominant wood rot pathogen associated with dieback of apple trees was *Trametes versicolor* (from 33.1 % of trees). Visual inspections of mulch wood pieces indicated the presence of *D. seriata* pycnidia and or conidia from all material assessed between October 2020 and April 2021. Viable *D. seriata* cultures were obtained from 87 % of mulch wood pieces investigated. *Diplodia seriata* remained present and viable for a period of 20 months on the wood chips. DNA was extracted from water washes of 120 wood piece samples of which 84 % tested positive for *D. seriata* with qPCR. The mean DNA concentrations of *D. seriata* increased for the two F1 orchards from October 2020 to April 2021, however a decrease was observed for the F2 orchard. This study showed that apple trees that are chipped and used for mulch harbour important canker and wood rot pathogens. The presence of viable *D. seriata* inoculum on apple tree wood chips used for mulch in younger orchards illustrates that there is a risk involved in using wood chips made from old fruit trees. Infected tree material should thus be removed prior to chipping of older trees.

Back to the future: using herbarium specimens to reconstruct the genetic development of two wheat rusts in South Africa

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Abstract

Puccinia triticina (Pt) and *P. graminis* f. sp*. tritici (Pgt),* the causal agents of wheat leaf and stem rust respectively, are postulated to have entered South Africa (SA) during early European settlement. While studies on virulence evolution within these two species are impossible due to the lack of viable isolates older than 40 years, herbarium specimens provided an opportunity to study their genetic adaptation. A microsatellite study combining both current and herbarium *Pt* specimens revealed the existence of five genetic lineages over the last 110 years. Three of these, putatively representing genetic remnants of the original races, have all disappeared with one lineage surviving at least 82 years in the field. The remaining two lineages most probably arose from foreign introductions into SA. The dominant of these two lineages appeared to consist of at least three sub-lineages that independently developed from separate introductions over the last 35 years. A similar study revealed at least three genetic *Pgt* lineages in SA over the last 115 years. The oldest lineage, now extinct, was discovered in collections from 1906 to 1938. Records of the current non-Ug99 lineage dates back to 1930. With no herbarium specimens clustering with the Ug99 race group members, it is concluded that this lineage first appeared around 2000 in SA as an introduction. Despite drawbacks such as DNA quality, and limited numbers of suitable microsatellite markers and available specimens, herbarium material allowed us to partially reconstruct the genetic development of two wheat rusts in SA. The results emphasise the role of incursion events and the consequent dominance and further adaptation of certain races. This study acknowledges the vision of early scientists who contributed wheat rust specimens to herbaria.

Resistance of *Ralstonia* biofilms to disinfectants

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Abstract

Ralstonia solanacearum, the causative agent of bacterial wilt, is probably the most destructive phytopathogenic bacterium worldwide. Bacterial wilt is causing losses of $ 950 million each year worldwide with yield losses recorded of up to 100 %. Broad spectrum disinfectants have showed to control *R. solanacearum* 100 % after only 30 seconds of exposure on planktonic forms (bacterial cells floating in solution). Biofilm growth increases microbial resistance to disinfection that can lead to substantial economic losses. There was an urgent need to determine the effect of disinfectants on *R*. *solanacearum* biofilms to reduce the spread of this pathogen. During the study, *R*. *solanacearum* biofilms were successfully formed on steel (nails), polyethylene or polycarbonate (plastic) and rubber. The biofilms were exposed to broad-spectrum disinfectants for periods of 1, 5 or 10 min. After exposure to the disinfectants, the cells present in the biofilm were released into nutrient medium with the help of an ultrasonic bath. The number of viable bacterial cells was determined by plating onto nutrient medium to determine the effectiveness of the disinfectants. *R. solanacearum* biofilms formed on steel after treatment with the disinfectant for 10 min decreased between 25-99 % and there were 37 million cells (10 min, 0.5 X recommended dose), 23 million cells (10 min, 1 X recommended dose) and 45 thousand cells (10 min, 2 X recommended dose) still present. In the case of rubber, there was a decrease between 81-89 %, with 18 million (10 min, 0.5 X recommended dose), 10 million (10 min, 1 X recommended dose) and 1.8 million (10 min, 2 X recommended dose) live cells present. Although there were fewer *Ralstonia* cells after the 1-, 5- and 10-min treatments with the different disinfectants, the number of bacteria that remained alive was significantly higher. *R. solanacearum* biofilms which were formed on plastic, steel and rubber were protected in the biofilm against the disinfectant treatments. Thus, increasing the concentration of the disinfectant will not eliminate the biofilm and it is extremely important to control or prevent the formation of biofilms in the management strategy against *R*. *solanacearum*.

Members of the genus, *Cladosporium,* and their role in pecan scab in South Africa

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Abstract

The pecan (*Carya illinoensis)* is native to Mexico and North America, and one of the more important nut crops in the world. Annual pecan production in South Africa is more than 20 000 tons. Pecan scab is the most widespread and destructive disease in the USA, and is caused by the fungus, *Venturia effusa*. In South Africa, however, *Cladosporium* spp. is constantly isolated from scab symptoms, although no evidence exists that these fungi cause scab. This study determines whether members of *Cladosporium* can cause pecan scab and investigates the diversity and distribution of the genus in South Africa. Samples were taken from nuts, leaves and surrounding air in pecan orchards where symptoms of scab were observed. Air samples were also taken from orchards where scab symptoms appeared to be absent. Fungal isolates resembling *Cladosporium* were identified based on the elongation factor (EF) 1-α gene region. Only members of the *Cladosporium cladosporioides* species complex were found. Observations revealed that scab was only found in the northern and eastern parts of South Africa, including the provinces of Limpopo, Mpumalanga, KwaZulu-Natal, Eastern Cape, and North-West. An undescribed species, *Cladosporium* sp. 5 was most frequently isolated in all areas and pathogenicity trials confirmed that it can cause scab on nuts and leaves. Twelve species of the *C. cladosporioides* species complex were identified, of which seven appear to be novel. Five known species, including *C. angulosum*, *C. anthropophilum*, *C. asperulatum*, *C. perangustum*, and *C. tenuissimum*, were isolated, of which none have been associated with pecans before. Only *C. perangustum* was previously isolated from South Africa. Thus, all 12 species of *Cladosporium* are new reports on pecans, and 11 of the 12 species are newly reported from South Africa. Results indicated that the pecan cultivar, Ukulinga, is significantly more resistant to scab development than Wichita, with nuts to be more prone to show symptoms than leaves. This study contributes to the overall understanding of the diversity of *Cladosporium* spp. in South Africa, and more specifically on pecans.

Pewenomyces kutranfy, causal agent of an important canker disease on *Araucaria araucana* in Chile

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Abstract

The monkey puzzle tree or pewen (*Araucaria araucana*), is an ancient conifer endemic to the Chilean and Argentinian mountain ranges. These trees live up to a thousand years, are slow growing and have both a sacred and economic relevance to indigenous communities in both countries. Between 2015 and 2016, an unrecorded disease-causing severe crown dieback was noticed on trees of all ages throughout most of their natural distribution. Four areas in the Chilean mountain ranges were surveyed, and the most important symptom of the disease were cankers on branches and stems resulting in copious resin exudation. Trees were monitored for a period of two years showing that the disease typically began on the leaves or at the leaf bases and progressed downwards to initiate cankers that could girdle branches or stems within 24 months. Samples were collected from symptoms and isolations were made from the margins of freshly formed cankers and directly from black ascomata that were found emerging from older cankers. These ascomata resembled those of *Caliciopsis* species previously described from *A. araucana*. However, morphological studies showed that they did not belong to any of those species. Phylogenetic analyses of the ITS, nucSSU, and nucLSU gene regions showed that the fungus resides in the *Coryneliaceae* but is distinct from other genera in that Family. The fungus has been described as a new genus and species, now known as *Pewenomyces kutranfy*. Inoculations conducted on naturally regenerated trees in the field (trees up to 3 metres high), and on young plants in the nursery, caused discolouration and girdling symptoms similar to those observed under natural conditions. The factors triggering the emergence of this canker disease are unknown, however, we believe that climate change is, at least in part, involved.

Fig rust: an unexplored disease in South Africa

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Abstract

Rust of cultivated fig *(Ficus carica)* was first recorded in South Africa (SA) as *Cerotelium fici* in 1927. Recently, a high incidence of rust on fig trees in residential gardens and commercial orchards was observed in the Overberg. Rust severity, often associated with leaf necrosis and untimely defoliation of trees, prompted an investigation into the identity of the pathogen, the infection process and cultivar response. Uredinia were scattered over the abaxial leaf surface, singly but frequently becoming crowded alongside leaf veins. Telia were visible as dark brown crusts surrounding uredinia. Uredinia were sub-epidermal, erumpent and telia sub-epidermal and non-erumpent. Paraphyses were also present. BLASTn analysis using the partial ITS2-28S rRNA locus revealed that the local fig rust isolate (PREM63073) shared 99 % similarity to a *C. fici* isolate (most likely incorrectly identified), and accessions of *Phakopsora nishidana* and *P. myrtacearum.* Five other *C. fici* accessions shared between 91 and 93% identity with PREM63073. Phylogenetic analysis distinguished PREM63073 and three *P. nishidana* accessions, all collected from *F. carica* trees in SA, the United States of America and Mexico, first from *P. myrtacearum,* as well as the five *C. fici* accessions. Microscopy of inoculated leaves of the Cape White and Kadota cultivars showed germinated urediniospores, germ tubes and appressoria forming over stomata on the lower leaf surface. Remarkably long germ tubes, often extending from one trichrome to another without apparent leaf surface contact, were observed. Mulberry, a previously recorded host for fig rust, was highly resistant to isolate PREM63073. Although rust developed on all fifteen fig cultivars tested for host response, White Genoa, Black Genoa, Adamsvy, Cape Brown and Deanne showed reduced receptivity to infection. This study confirmed *P. nishidana* for the first time in SA and on the African continent, and the second species attacking fig in SA.

An integrated control strategy for managing Fusarium head blight of wheat using epiphytic yeasts and a plant defence inducer

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Abstract

Fusarium head blight (FHB) is an important disease of wheat *(Triticum aestivum* L*.)* and other small grain crops worldwide, resulting severe yield losses. The aim of this study was to control FHB and associated mycotoxins using epiphytic yeasts and acibenzolar-S-methyl (ASM) as single and combined treatments. A total of 344 yeast epiphytes were isolated from various plants and screened against *Fusarium graminearum in vitro.* The best performing isolates were selected and tested for their efficacy against FHB on wheat plants under shade house conditions. Thereafter, different concentrations of ASM were tested against FHB *in vivo* as well as the efficacy of combining the best treatments from the biocontrol and ASM trials. The best performing yeast isolates provided up to 64.3 % reduction in mycelial growth of *F. graminearum* compared to the pathogen control. In all *in vivo* trials, an effective reduction in disease severity, an increase in the hundred seed weight (HSW) and a reduction in percentage seed infection (PSI) were observed. A reduction of up to 13.4 %, 29.0 % and 41.8 % in disease severity was observed in the biocontrol, ASM and integration trials, respectively. The reduction in deoxynivalenol (DON) concentrations was up to 28.96 %, 18.79 % and 19.35 % in the biocontrol, ASM and integration trials, respectively. Two of the best three performing yeast isolates were isolated from symptomless wheat plants and the third from a weed plant, *Ophiopogon japonicus* ((L.f.) Ker-Gawl) (dwarf lilyturf). The treatment that provided the best integrated control of FHB was $0.075g^{-1}$ ASM applied at anthesis with isolate WL6 (identified as *Papiliotrema flavescens).* This study presents the first report of *P. flavescens* providing *in vitro* inhibition of *F. graminearum* and in *vivo* reduction of FHB severity, respectively.

Impact of crop rotation and tillage practices on the incidence of Fusarium crown rot of wheat in the Western Cape

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Abstract

Fusarium crown rot (FCR), caused by *Fusarium pseudograminearum,* is an economically important disease of wheat globally and in the Western Cape province, where roughly 50 % of SA's wheat is produced. FCR is managed by integrating agronomic practices (tillage and crop rotation with non-host crops) with the use of tolerant wheat cultivars. Over the past 25 years, production practices changed from wheat produced in monoculture on conventionally tilled fields to crop rotation and reduced tillage. This raises concern since an increased incidence of FCR has been reported under reduced tillage. The aim of this study was, therefore, to determine the effect of crop rotation combined with different tillage practices to manage FCR. The study was conducted in long-term field trials at Langgewens and Tygerhoek research farms in the Swartland and Overberg regions of the Western Cape, respectively. Identical field trials comprised a split-plot design with three replicates. Crop rotation was the main plot factor and tillage practice (conventional-, minimum-, no- and zero till) served as sub-plot factor. Crop rotations consisted of three different 4-year rotations (wheat-canola-wheat-lupin, wheat-lupin-wheat-canola, wheat-medic-wheat-medic) and a wheat monoculture (control). Sampling of wheat plants was conducted at the grain filling stage during the 2018 and 2019 seasons. Disease incidence (percentage infected plants) and disease severity (number of infected tillers per plant and the length of visible lesions on tillers) was determined. Disease assessments were correlated with agronomical data, including grain yield, hectolitre mass and thousand kernel weight. Crop rotations all significantly ($P < 0.05$) reduced disease parameters at both localities, compared to the wheat monoculture, although disease suppression was more pronounced at Langgewens than Tygerhoek. Zero till significantly ($P<0.05$) reduced all disease parameters at Langgewens, compared to the other tillage practices. Although the same trend was evident at Tygerhoek, results were not always significant. Disease incidence and percentage infected tillers was significantly ($P<0.05$) negatively correlated with yield (r=-0.620 and -0.612, respectively) and hectolitre mass (r= -0.498 and -0.455, respectively), confirming the impact of FCR on agronomical parameters. Crop rotation and reduced tillage practices can, therefore, contribute to effectively manage FCR.

Facial Eczema: An important yet poorly understood disease of dairy cattle in South Africa

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Abstract

Facial eczema (FE) is a descriptive term for hepatogenous photosensitivity in sheep in cattle, caused by the mycotoxin sporidesmin. This toxin is produced by a saprophytic fungus, *Pseudopithomyces chartarum.* When cattle or sheep graze on contaminated pastures, sporidesmin damages the liver and bile ducts resulting in the accumulation of phylloerythrin in the blood and tissue. This photodynamic compound remains in circulation and causes the typical lesions of photosensitivity associated with FE, noticeable as sunburn-like lesions on non-pigmented areas of skin. Facial eczema was first reported in New Zealand in 1894 and occurs frequently there, but is also known in Argentina, Australia, France, the Netherlands, Portugal, South Africa, Spain, Turkey, the United States, and Uruguay. The clinical manifestations of the disease described above are obvious, but the subclinical effects are more subtle and easily overlooked. These include a loss in milk solids, reduction in milk production, frequently accompanied by a loss in body condition. These subclinical effects can contribute to significant economic losses to livestock-dependant industries. In South Africa, FE is increasingly recognized as a problem in the dairy industry, especially in the Eastern Cape region. After a recent outbreak in and around Humansdorp, mixed-grass samples were collected from these areas. These were surface disinfested and plated onto Potato Dextrose Agar (PDA) supplemented with chloramphenicol (50 ppm) and streptomycin (100 ppm). Fungal communities from pastures were diverse with 738 strains isolated. These represented 57 genera, but only 12 of them included more than ten isolates. Three different species of *Pseudopithomyces* were isolated from the pasture samples together with many other mycotoxigenic genera including *Fusarium* and *Aspergillus.* The results illustrate a paucity of knowledge regarding the identification of *Pseudopithomyces* spp. and the role that they, as well as other fungi, play in the health of cattle in South Africa. These questions will be considered in future studies.

Evaluation of fungicide seed treatments for control of *Pythium* and *Rhizoctonia* on soybean under glasshouse conditions

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Abstract

Pythium species and *Rhizoctonia solani* anastomosis groups (Ags) cause economically important soybean seedling diseases worldwide. Species and Ags, and isolates within these two groups of pathogens, can vary in aggressiveness. *Pythium ultimum* var. *ultimum* isolates were shown to be the most aggressive towards soybean based on damping-off (9-26 % survival). *Pythium ultimum* var. *sporangiiferum* (14-35 % survival) and *P. aphanidermatum* (22-33 % survival) caused significantly higher damping-off than *P. irregulare* (38-78 % survival). However, based on seedling length reductions the four species had more or less comparable aggressiveness. *R. solani* AG-4 HGI was more aggressive than AG-4 HGIII, with all the AG-4 HGI isolates causing significant damping-off (29-61 % survival) and seedling length reductions and only 43 % of the AG-4 HGIII isolates causing length reductions and/or damping-off (84-87 % survival). The three seed treatments evaluated, ST1 (penflufen, prothioconazole and metalaxyl), ST2 (mefenoxam and fludioxonil) and ST3 (thiabendazole, azoxystrobin, fludioxonil and metalaxyl-M), significantly improved seedling survival and -length reductions incited by four *Pythium* spp. and two *R. solani* Ags relative to the untreated inoculated seed. With a few exceptions, the treatments were all highly effective since it prevented seedling survival and -lengths reductions so that it did not differ significantly from the uninoculated seed control. ST1 prevented *Pythium* damping-off but could not be evaluated reliably for its effect against seedling length reductions since in the *Pythium* trials the uninoculated ST1 seed resulted in significant length reductions. ST2 prevented *Pythium* damping-off and seedling length reductions, except for damping-off caused by two isolates (*P. ultimum* var. *ultimum* and *P. irregulare).* ST3 prevented *Pythium* damping-off but not seedling length reductions caused by *P. irregulare* and *P. aphanidermatum* isolates and most of the *P. ultimum* variety isolates. Against *R. solani* AG-4 HGI, ST3 prevented damping-off and length reductions caused by all the isolates, whereas the ST1 and ST2 treatments did not prevent damping-off caused by 40 % of the *R. solani* AG-4 HGI isolates. In conclusion, there were no clear differences in the efficacy of the three seed treatments due to the treatments being highly effective at preventing damping-off versus seedling length reductions for all the investigated isolates and pathogens.

Plum decay control using gum arabic edible coating with salicylic acid or thymol

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Abstract

Stone fruit pathogens *Monilinia laxa* and *Botrytis cinerea,* causing brown rot and grey mould, respectively, result in major postharvest losses of plums world-wide. The alternative bioactives thymol and salicylic acid are antifungal compounds for brown rot and grey mould control of stone fruit. This study aimed to verify efficacy of salicylic acid or thymol *in vitro* as well as in fruit inoculation trials on 'Angelino' plums treated curatively with edible coating. Media amended with thymol at concentrations ranging from 0.005 to 2.5 mM effectively inhibited growth of both pathogens at 0.25 mM *(M. laxa)* or 1.00 mM *(B. cinerea)* The tested salicylic acid concentrations ranging from 0.005 to 4.70 mM did not show complete fungal inhibition *in vitro.* In fruit trials, 'Angeleno' plums were either wound inoculated wit *B. cinerea* (1x 10^5 spores /ml) or *M. laxa* (100 spores/ml) and curative treatment of one of the two bioactives was applied after an initial 24 h incubation at 25 °C in a moisture chamber. After treatment and further incubation fruit was evaluated for decay severity after 4, 6, 8 and 10 days. Both applications of salicylic acid (2.00 mM) and thymol (4.00 mM), reduced decay by more than 50 % during the 10-day shelf-life storage for both pathogens. The alternative bioactives thymol and salicylic acid are therefore potential candidates for commercial plum postharvest application. Although further formulation, and tests of combined treatments could possibly enhance efficacy.

The influence of storage practices and facilities on seed quality of on-farm saved soybean seeds produced by smallholder farmers in the Lesedi Local Municipality, Gauteng

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Abstract

Over the recent years, the area of soybean (*Glycine max* (L.) Merrill) production has expanded due to the activities of emerging smallholder farmers in various parts of South Africa. The farmers use basic practices to store their seed under varying unregulated storage conditions without knowing the implications of such practices. This study aimed to investigate the influence of storage practices and facilities on seed quality of on-farm saved soybean seed produced by smallholder farmers. The objectives of this study were to i) evaluate and compare viability and vigour of stored soybean seed, and ii) determine and compare the diversity of fungi and mycotoxins associated with on-farm saved soybean seeds. On-farm saved seeds stored under different storage conditions were sourced from twelve smallholder farmers in the Lesedi Local Municipality, Gauteng. The samples were analysed for parameters such as seed moisture content (MC), viability, vigour: accelerated aging (24-, 48- and 72 hr); controlled deterioration (12-, 14-, 16-, 18- and 20 % MC) and conductivity, as well as the health status. The results revealed that the seed sample FS10, which was stored under high temperature (27.7 °C) and placed directly on the floor had the lowest MC (9.1 %) and germination (30.8 %). In accelerated aged samples, germination ranged between 0 to 78.3 %. In terms of the controlled deterioration, the percentage germination reduced with the increasing MC and the aging period. Conductivity leachate values ranged from 26.6 to 112.6 µS.cm^{-1}.g^{-1}. The results further showed that there were significant differences in the incidence of fungi associated with the seed. The highest fungal incidence of 51.3 % was from sample FS4, which was stored in polypropylene bag at 21.2 °C and 61.1 % relative humidity. Moreover, the sample was found to be contaminated with the mycotoxin Deoxynivalenol (DON), which was detected to be above the LOD (9.5 µg/kg). The most common fungal genera isolated from the soybean seeds included *Aspergillus*, *Alternaria*, *Penicillium*, *Cladosporium*, *Chaetomium*, *Trichoderma*, and *Fusarium*. The results indicated that the quality of on-farm saved soybean seeds stored under different unregulated storage facilities was significantly affected. Recommendations can be made regarding the most suitable storage conditions, practices and facilities used by the smallholder farmers.

Invasion frameworks: a forest pathogen perspective

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Abstract

In the discipline of invasion science, researchers studying different taxonomic groups have developed separate ways of investigating the phenomenon of biological invasions. While there have been efforts to reconcile these differences, a lack of knowledge of microbial diversity, biogeography and ecology hampers researchers seeking to understand invasive microorganisms, including invasive forest pathogens. However, advances in molecular technologies such as gene and genome sequencing and metagenomics studies have increased the "visibility" of microorganisms, providing opportunities to better integrate forest pathology and invasion science. The two fields have much to gain from closer collaboration. We propose a modified version of the Unified Framework for Biological Invasions of Blackburn et al. (2011; Trends in Ecology and Evolution 26: 333-339) to accommodate invasive forest pathogens, recognizing the challenges and limitations, and suggest options for tackling these issues. With a clearer understanding of the stage's microorganisms pass through as they become invasive, we hope that forest pathologists will better understand how and why invasions occur, and importantly, where when and how invasions can be stopped or mitigated. We call for a broader incorporation of ecological and evolutionary concepts to underpin the studies of invasive forest pathogens, so as to address the complex challenges of identifying and managing forest pathogen invasions.

5. Climate Change and Digital Technology in Plant Pathology

Real-time PCR high-resolution melting curve analysis to detect *Ceratocystis* species in the Latin American clade

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Abstract

Sixteen species of *Ceratocystis* are accommodated in the Latin American Clade (LAC) of the genus. These species are important plant pathogens that infect a wide range of hosts in many different parts of the world. Despite efforts to reduce the spread of these pathogens, new disease outbreaks have continued to occur. To respond to these outbreaks more rapidly, and to mitigate future spread, there has been a growing need to detect the presence of *Ceratocystis* propagules in different substrates without making cultures. The aim of this study was to develop a quantitative real-time PCR high-resolution melting curve analysis (qPCR-HRM) to detect, differentiate and quantify several pathogenic *Ceratocystis* species in the LAC from different substrates. Primers targeting the cerato-platanin (CP) gene were designed to amplify species in the LAC, targeting a 172 bp region. These primers successfully detected all 14 species in the LAC screened in this study, and further differentiated between eight of these species using HRM. To determine the range of applications for this tool, various substrates including infected wood samples, sawdust, frass, and insect vectors were screened. Based on the results, the qPCR-HRM diagnostic method provides an automated, rapid, and sensitive tool to detect and differentiate between several economically important *Ceratocystis* species in the LAC from various substrates. Further work includes generating standard curves which can then be used to also quantify the amount of detected LAC *Ceratocystis* species in these various substrates. This tool will be useful to screen for pathogen propagules in substrates shipped across global borders and should aid in minimizing the emergence of new disease outbreaks.

A non-destructive technique for early detection of Phytophthora root rot using hyperspectral leaf reflectance

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Abstract

Climate change is increasing the prevalence of *Phytophthora* diseases globally. In this scenario, early detection and mitigation of Phytophthora root rot is increasingly important for protecting both natural and planted forests. Currently, a majority of the approaches used for detecting Phytophthora root rot involves destructive sampling. Thus, in this study, we used *Phytophthora alticola* and *Eucalyptus benthamii* as model systems to see if leaf reflectance data can be used as a non-invasive approach for early detection of root rot. For this, we used a sand-infestation pot trail to inoculate 19 families of *E. benthamii* with an isolate of *P. alticola* (CMW48711). Each family included ten infected and five control plants. Four months after inoculation, hyperspectral data from both control and infected plants were collected in duplicate using a leaf clip connected to an ASD FieldSpec 4 spectroradiometer. The final dataset had around three million data points that were analysed using a multi-step machine learning pipeline developed in this study. This multi-step pipeline comprised data normalisation, data visualisation, model optimization, model training, and determining the relevant wavelength for disease detection. Using this pipeline, we successfully trained a model capable of differentiating between infected and healthy plants and identified a hyperspectral index specific to *P. alticola* infection. This study demonstrates the future potential for using hyperspectral sensing as a tool for detection of Phytophthora root rot. Additionally, our research establishes the usefulness of a machine learning pipeline as a platform for efficiently managing large datasets such as these.

POSTER PRESENTATIONS

1. Molecular Biology

MB1

Development of a LAMP assay for on-site diagnosis of the novel *Eucalyptus* pathogen, *Elsinoë necatrix* using the elsinochrome toxin cluster as a marker.

Miss Alishia Van Heerden[1], Mr Nam Q. Pham[2], Prof Brenda D. Wingfield[1], Prof Michael J. Wingfield[1], Dr Tuan A. Duong[1], Dr Markus Wilken[1]

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Abstract

Eucalyptus scab and shoot malformation caused by *Elsinoë necatrix* is an emerging disease and a serious threat to the global commercial forestry industry. The disease was first discovered in North Sumatra, Indonesia in 2014. An early, simple and effective method is required for early detection of *E. necatrix* in infected material. In this study, a rapid and sensitive Loop-mediated isothermal amplification assay (LAMP) was developed for the on-site detection of *E. necatrix*. The assay targeted an intron region of the Efhp1 gene that is unique to *E. necatrix*. This gene is a main component of the elsinochrome toxin cluster that plays a key role in the pathogenicity of all *Elsinoë* species. To test the robustness of the assay, 28 strains of *E. necatrix* were tested, with positive amplification in all strains. A specificity test against 23 closely related *Elsinoë* species and three fungal species frequently found in *Eucalyptus* showed that the LAMP assay exclusively amplified the *E. necatrix* isolates. This assay also showed a high level of sensitivity, being able to detect the presence of *E. necatrix* in amounts as low as 0.1 ng of genomic DNA. Furthermore, the LAMP assay was able to detect *E. necatrix* from infected *Eucalyptus* leaves using simple DNA extraction methods, proving the potential of this system for field applications.

MB2

The *MAT1-1-2* gene in *Ceratocystis albifundus* plays a role in gene deletion during unidirectional mating-type switching

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Abstract

Ceratocystis albifundus (Ascomycota, Ceratocystidaceae) is an economically important pathogen of *Acacia mearnsii* and *Protea cynaroides* in South Africa. The fungus is self-fertile due to unidirectional mating-type switching, an unusual form of sexual reproduction known only in a small number of fungi. The switching process results in two types of progeny that differ in their sexual phenotypes. Self-sterile isolates lack the ability to produce sexual structures (ascomata) or sexual spores (ascospores) in the absences of a compatible mating partner. Self-fertile isolates can complete the sexual cycle in isolation, due to a switch in the mating type of some nuclei that result in a compatible "mating partner." Self-fertile and self-sterile isolates also differ in the gene content at their mating-type (*MAT1*) locus, the master regulator of sexual reproduction. The self-fertile version of the locus contains four genes known as mating-type (*MAT*) genes. During unidirectional mating-type switching, two of these genes are lost, producing the self-sterile *MAT1* locus with only two *MAT* genes. Two direct repeats flank the region lost during switching, and likely facilitate a recombination event that results in the deletion of the target region. These direct repeats have been studied in *Chromocrea spinulosa*, a non-pathogenic species unrelated to the *Ceratocystidaceae*. However, no study has considered the role of the *MAT* genes in a species that utilizes unidirectional mating-type switching. The work presented here addressed this question using a CRISPR/Cas9 system to delete mating-type genes from the *MAT1* locus in *C. albifundus*. Unexpectedly, the deletion of the secondary mating-type gene *MAT1-1-2* removed the ability of the fugus to undergo unidirectional mating-type switching. This was despite the fact that both direct repeats remained present and unaltered. This deletion also rendered the transformed isolate self-sterile. Furthermore, the deletion of the *MAT1-1-2* gene also had further pleiotropic effects, although these require further study. This is the first report of targeted gene editing in a *Ceratocystis* species, paving the way for future functional studies in a group of globally important plant pathogens.

MB3

Calonectria species associated with *Eucalyptus* plantation soils in Colombia

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Abstract

Plantation forestry is expanding rapidly in South America in order to supply an increasing demand for wood and wood products. As these plantation resources expand, the threat from pests and diseases is also growing. *Calonectria* species represent an important group of pathogenic fungi that cause serious disease problems in *Eucalyptus* plantations and nurseries in the region. During 2016, extensive surveys were conducted across *Eucalyptus* plantations in four forestry regions of Colombia. This resulted in a large number of isolates from soil samples collected under symptomatic trees. The aim of this study was to identify and resolve the phylogenetic relationships among the resulting isolates using DNA sequence comparisons for six gene regions. From a collection of 108 isolates, seven *Calonectria* species residing in three species complexes were identified. Of these, two represented undescribed species. *Calonectria parvispora* and *Calonectria spathulata* were the most commonly isolated fungi, each of which accounted for approximately 30 % of the isolates. This is the most extensive study yet to consider the diversity of *Calonectria* species in Colombia. The results suggest that new species of these important fungi will most likely emerge as surveys are extended to crop plants beyond *Eucalyptus*.

MB4

Calonectria species isolated from diseased leaves and soils in *Eucalyptus* plantations in the GuangDong province of southern China

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Abstract

Many *Calonectria* species cause an important disease of *Eucalyptus* known as *Calonectria* leaf blight. In this study, *Calonectria* spp. were isolated from diseased leaves and soils in Eucalyptus plantations in GuangDong Province, China. The aims of this study were to identify these isolates, to ascertain the distribution of *Calonectria* species between diseased leaves and soils, and to assess their pathogenicity to *Eucalyptus* hybrids widely planted in the area. A total of 606 *Calonectria* isolates were obtained, of which 399 were from diseased *Eucalyptus* leaves and 207 were from soils beneath infected trees in *Eucalyptus* plantations. Based on sequences of the elongation factor-1α (tef1), β-tubulin (tub2), calmodulin (cmdA), histone H3 (his3), DNA-directed RNA polymerase II subunit (rpb2) and actin (act) gene regions as well as morphological characteristics, 303 isolates were identified to reside in the *C. kyotensis* species complex, and the remaining 303 isolates were in the *C. reteaudii* species complex. Nine previously known *Calonectria* species and a new species were identified. These species include *C. aconidialis* (12.0 % of the total isolates), *C. curvispora* (0.3 %), *C. hongkongensis* (24.8 %), *C. ilicicola* (0.9 %) and *C. kyotensis* (12.0 %) residing in the *C. kyotensis* species complex, and C. *crousiana* (1.0 %), *C. pseudoreteaudii* (40.7 %), *C. queenslandica* (7.3 %) and *C. reteaudii* (0.7 %) as well as the undescribed species (0.3 %) residing in the *C. reteaudii* species complex. Most (97.6 %) of the isolates obtained from soil resided in the *C. kyotensis* species complex, and most (98.3 %) of the isolates in the *C. reteaudii* species complex were isolated from diseased leaves. Pathogenicity tests showed that all ten species of *Calonectria* identified in this study were pathogenic to the two tested *Eucalyptus* genotypes, and that in some cases, the pathogenicity of isolates of the same species was significantly different.

MB5

Grapevine infecting viroid diversity in South Africa.

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Abstract

Six viroid species have been reported to infect grapevine, namely, hop stunt viroid (HSVd), grapevine yellow speckle viroid 1 (GYSVd-1), grapevine yellow speckle viroid 2 (GYSVd-2), Australian grapevine viroid (AGVd), Japanese grapevine viroid (JGVd), and citrus exocortis viroid (CEVd). In this study, Next Generation RNA sequence data (NGS), from 229 *Vitis* accessions from the field maintained South African *Vitis* germplasm collection vineyard, were analyzed to determine the diversity of the viroids present. Five of the six known grapevine infecting viroids were identified in 214 of the 229 samples, with each positive sample containing at least one viroid species. HSVd, GYSVd-1, GYSVd-2, AGVd, and JGVd were identified in the NGS data of the samples and confirmed with RT-PCR detection and Sanger sequencing. The HSVd sequences indicated the presence of two variants, with one showing multiple nucleotide insertions. AGVd and GYSVd-2 did not display high levels of sequence diversity, thus confirming past international studies. GYSVd-1 occurs as four major variants worldwide and representatives of each were identified in this vineyard. This is the first report on the diversity of viroids infecting grapevines in South Africa and the first report of JGVd outside of Japan. Further studies are needed to fully assess the population and to identify new potential viroid species.

MB6

Pathogenicity and microsatellite characterization of *Puccinia hordei* in South Africa

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Abstract

Barley is an important winter cereal in South Africa (SA) with the Western Cape as the main production area. This region, with its Mediterranean climate, is prone to foliar diseases such as barley leaf rust caused by *Puccinia hordei* (Ph). Isolates of Ph collected from trial plots and commercial fields were pathotyped using differential cultivars and lines with 27 designated Rph-resistance genes. Single pustule isolates derived from field isolates, collected between 2015 and 2020, typed as Ph race UVPh7235 with increased virulence to Rph3 when compared with the two previously described races from SA, UVPh3231 and UVPh7231. Results for barley cultivars with designated sources of adult plant resistance unexpectedly, revealed low seedling infection types (ITs) for Baronesse (Rph20+Rph24) to all Ph isolates and for Lenka (Rph20+Rph23+Rph24) to isolates of Ph races UVPh3231 and UVPh7231. The barley cultivars Agulhas and Cristalia also showed low seedling ITs and moderate levels of adult plant resistance under field and greenhouse conditions. Genotyping of 48 Ph isolates with 20 microsatellite markers revealed low gene diversity and allelic richness levels between individuals. While STRUCTURE analysis suggested three genetic clusters, no clear division of the isolates into the clusters was evident with all isolates being strongly admixed for all three clusters. Linkage disequilibrium analysis, as well as higher HO versus HE values, supported the hypothesis that the South African Ph population is clonal. Thus, single-step mutational events occurring within this single genetic lineage may explain the increased virulence observed for the recently deployed Rph3 resistance gene. Results from this study will assist barley breeders in their efforts to develop and release new cultivars resistant to leaf rust. Furthermore, the cultivar response data should assist barley producers in managing the disease through cultivar choice and optimising timing of chemical application on susceptible cultivars.

MB7

Fungal diversity in Namibian *Stipagrostis* 'fairy circles' including descriptions of four new *Curvularia* species

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Abstract

The Namib desert is home to an unexplained phenomenon known as 'fairy circles'. These are barren, almost-circular, patches of land typically surrounded by flourishing *Stipagrostis* species (*Poaceae*) at their margins. Fairy circles have attracted considerable attention for more than 50 years, without any clear conclusions as to their cause. One of the more recent hypotheses has been that microbial phytopathogens could be involved. In this study, we considered the culturable fungal diversity associated with *Stipagrostis* species associated with fairy circles. Two sampling regions were selected where one circle was sampled at the first site, and two circles at the second. For each circle, five *Stipagrostis* plant samples were collected from the lush margin, five from the vegetation between circles, and five from the barren centre. Roots and shoots were surface disinfested and plated on Fusarium Selective Media (FSM; containing PCNB), Malt Extract Agar (MEA) and Dichloran-Glycerol (DG18), supplemented with chloramphenicol and streptomycin. A total of 553 strains, 58 genera and 118 species were identified based on DNA sequencing results for the BenA (*Penicillium*), CaM (*Aspergillus*), GAPDH (*Bipolaris*, *Curvularia*, and *Exserohilum*), ITS (unidentified genera), RPB2 (*Phoma*-like genera), and TEF (*Trichoderma* and *Fusarium*) gene regions. The most prevalent genera identified included *Curvularia* and *Fusarium* with the former genus including the largest number (20) of species. Four *Curvularia* species were recognized as novel taxa and have been described. The overall results of this study show that there remains a wealth of fungal diversity yet to be explored in the Namib desert fairy circles and that could be involved in the biology of this intriguing phenomenon.

MB8

Population genetic analysis of the pitch canker pathogen *Fusarium circinatum* suggests a limited introduction into Colombia with little subsequent change.

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Abstract

The global distribution and incidence of plant pathogens has increased dramatically in the 21st century. This is strongly associated with human activities and has allowed the pitch canker pathogen, *Fusarium circinatum*, to become one of the most important pathogens of *Pinus* spp. The pathogen now occurs in plantations of many countries that propagate non-native *Pinus* spp. including Colombia. *F. circinatum* first emerged in Colombia in 2005 and disease has gradually increased in plantations, especially as basal stem cankers, with infections, probably originating in nurseries. The aim of this study was to consider the mode of reproduction and population structure of *F. circinatum* in Colombia. This was achieved using 10 microsatellite markers to analyse 111 isolates obtained from multiple geographical regions and hosts including *Pinus patula*, *Pinus kesiya*, *Pinus maximinoi* and *Pinus tecunumanii* (HE) in the country. The diversity and distribution of multilocus haplotypes were investigated based on host species, geographical distribution, and temporal variation. This population was found to be predominantly clonal including 7 genotypes. Isolates were dominated by the MAT 1-1 mating type, and there was no evidence of sexual recombination ($P < 0.001$) having occurred. Overall, the results support the view that *F. circinatum* was introduced into Colombia, probably via a limited genetic base that has not changed greatly over time. This suggests that resistance to pitch canker should be relatively durable for the future.

MB9

Molecular detection of *Ramularia collo-cygni* and *Pyrenophora teres* from barley in South Africa

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Abstract

Net form of net blotch (NFNB) caused by *Pyrenophora teres* f. *teres* and spot form of net blotch (SFNB) (*P. teres* f. *maculata*) are important diseases of barley (*Hordeum vulgare* L.) in South Africa (SA). Ramularia leaf spot (RLS) caused by the fungus *Ramularia collo-cygni* (*Rcc*) was identified for the first time in SA in 2015. Identification and management of these diseases has been challenged by their overlapping symptoms. Therefore, the objective of this study was to use molecular techniques in the detection and differentiation of these diseases, with a major emphasis on RLS. Surveys were conducted from 2018 to 2020 and barley leaves with small brown spots suspected of infection with the aforementioned diseases were collected. Two methods were used to detect *Rcc*. The first method, developed by CenGen (Pty) Ltd., involved Loop-mediated isothermal amplification (LAMP) which used two sets of primers, that were designed based on the *Rcc* translation elongation factor gene sequence. The second method used a gel-based marker and has higher sensitivity than LAMP. Relevant PCR-based assays were used to identify net blotch as well. In 2018, brown leaf spots were observed in 21 of 52 barley fields surveyed, and the DNA of *Rcc* was found in 28 % of 18 samples collected in the Western Cape. In 2019, symptoms were noted in 39 of 52 fields but no *Rcc* DNA was detected in any of 37 samples tested. However, from 35 samples analysed in 2019, 49 % were positive for SFNB and 20 % for NFNB. About 14 % were positive for SFNB and NFNB. In 2020, brown spots were observed in 31 of 48 fields surveyed, and *Rcc* was found in 17 % of 42 samples tested. Overall, the results confirmed presence of RLS at different localities in the Western Cape. However, its incidence and severity appear insignificant suggesting that RLS may not pose an immediate threat to local barley production in SA. Moreover, molecular methods have been successfully used in this study to detect RLS, NFNB and SFNB which are not easily distinguishable based on their symptoms. Application of these methods in SA will greatly assist in accurate identification and effective control of these diseases.

MB10

Diversity and structure of secreted LysM domain-containing peptides in the *Botryosphaeriaceae*

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Abstract

LysM domains are non-catalytic protein domains with a high binding affinity to N-acetylglucosamine (GlcNAc)-containing glycans. These domains are widespread among prokaryotic and eukaryotic organisms. In plants, proteins containing these domains play an important signalling role in pathogen-triggered immunity as they recognize chitin oligomers resulting from fungal cell wall degradation. However, plant pathogenic fungi themselves make use of these domains to sequester chitin oligomers and thereby circumvent host detection. Secreted LysM-containing peptides are thus an important class of fungal effectors but have been poorly characterized in the *Botryosphaeriaceae*. In this study, we structurally and phylogenetically characterised the secreted and non-secreted LysM domain-containing proteins among prominent taxa of *Botryosphaeriaceae*. Initial analyses of predicted proteomes using Pfam domain Hidden Markov Models (HMMs) were not well suited to identify LysM domains in the *Botryosphaeriaceae*. Using structural domain predictions, we were able to annotate additional LysM domains compared to the existing Pfam LysM domain model. Newly created HMMs improved our ability to identify these domains. Species of *Botryosphaeriaceae* had between two and ten genes encoding LysM containing peptides. The LysM domain occurred multiple times per gene and between two and seven repeats were observed. Predicted secreted genes were classified as effectors. Effector LysM domains were phylogenetically distinct from those of non-effector genes and had between two and three LysM domains per gene, compared to the five to seven repeats of non-effector genes. Additional structural analyses indicated that many of the effector LysM genes are capable of forming homo- and heterodimers. The precise function of these proteins has yet to be determined but this remains an important goal for future research aimed at better understanding the molecular basis of pathogenicity in the *Botryosphaeriaceae*.

MB11

Characterization of the mating-type locus and associated genes from five *Sclerotinia* species

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Abstract

Sclerotinia belongs to the fungal family *Sclerotiniaceae* that includes many plant pathogens. The genus has approximately twenty species, although the taxonomy of the group is poorly defined. Together, *Sclerotinia* species infect over 400 plant species globally. Infection is driven by sexual ascospores released from mushroom-like fruiting bodies called apothecia. In *Sclerotinia*, sexual reproduction is controlled by the mating-type (MAT) genes present at the mating-type locus (*MAT1*). The *MAT1* locus of *S. minor* and *S. sclerotiorum* consists of four genes: *MAT1-1-1, MAT1-1-5, MAT1-2-1 and MAT1-2-10*. Both species are self-fertile (homothallic) but can produce two versions of the MAT1 locus through inversion. Most isolates of *S. trifoliorum* are also homothallic, but two versions of the *MAT1* locus are known. These differ in gene content and are produced following uni-directional mating-type switching. This study aimed to characterize the mating-type loci of five previously unstudied *Sclerotinia* species using whole genome assemblies. To achieve this, the genomes for representative isolates of these species were sequenced and assembled. These genome sequences were used to identify the *MAT1* locus and associated *MAT* genes. The *MAT1* locus was present in all the genomes at a conserved position, although differences in gene content and gene arrangement were apparent. *S. sativa* and *S. matthiolae* had a *MAT1* locus structure like that of the homothallic *S. sclerotiorum* and *S. minor*, with four *MAT* genes in an inversion positive arrangement. This inversion truncated the *MAT1-1-1* gene, while inverting the orientation of *MAT1-2-10* and *MAT1-2-1* relative to *MAT1-1-5* and the flanking genes. The presence of repeat sequences makes it likely that these species can invert the *MAT1* locus during meiosis. *S. spermophila*, *S. bulborum* and *S. sulcata* had three mating-type genes, lacking *MAT1-1-5.* The presence of both *MAT1-1-1* and *MAT1-2-1* points to homothallism, while the presence of direct repeats in *S. sulcata* could indicate that this species might be capable of unidirectional mating-type switching. This study is the first to provide both genome sequences and *MAT* gene information for these species and will provide a better understanding of sexual reproduction in the genus *Sclerotinia*.

MB12

A comparison of the population structure of *Teratosphaeria pseudoeucalypti* from *Eucalyptus* plantations in Australia and Uruguay

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Abstract

Teratosphaeria pseudoeucalypti (*Dothideomycetes*: *Teratosphaeriaceae*) causes severe leaf blight of planted *Eucalyptus* trees. It occurs in and is presumed to be native to Australia but is also well established and presents important constraints to plantation forestry in Uruguay. In this study, microsatellite markers were used to compare the genetic diversity of *T. pseudoeucalypti* outbreaks occurring in plantations in New South Wales (NSW; Australia), where *Eucalyptus* is native, and in Uruguay where the trees have been introduced for plantation forestry. Sixteen polymorphic microsatellite loci were developed using *T. pseudoeucalypti* genomes that we previously sequenced and these markers were used to genotype the isolates. Only five genotypes were detected in the 36 individuals from the NSW plantation and one of these occurred in >80 % of individuals. Genetic diversity was, therefore, low, despite the expectation that *T. pseudoeucalypti* is native to that region. Both mating types occurred in NSW and, even though *MAT1-2* isolates far outnumbered *MAT1-1* and clonal reproduction appeared dominant, the hypothesis of random recombination was not rejected. The population structure of *T. pseudoeucalypti* was consistent with that of an introduced pathogen, either from the surrounding native forests or recently dispersed from northern Queensland. The 21 individuals collected from *Eucalyptus* trees across Uruguay were of a single genotype not found in Australia and all had the *MAT1-1* type. This clonal nature of the Uruguayan *T. pseudoeucalypti* population suggests a single accidental introduction of the pathogen and subsequent dissemination from that source.

MB13

Soilborne *Fusarium* and *Phytophthora* species diversity on avocado and macadamia in South Africa.

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Abstract

Avocado and macadamia are crops of economic importance in South Africa. These crops form part of the major exported crops with 89 433 and 56 550 tons of avocado and macadamia exported in the 2018/2019 growing season. Soilborne pathogens such as *Fusarium* and *Phytophthora* pose a serious threat to the production of these crops in South Africa and across the world with yield losses of up to 60 % caused by *Phytophthora cinnamomi*. A study was conducted during the 2016/2017 and 2017/2018 growing seasons with the aim to investigate species diversity, morphological and molecular characteristics as well as phylogenetic relationships of *Fusarium* and *Phytophthora* species isolated from the Limpopo and Mpumalanga provinces in South Africa. The TEF-1α and COX II genes were sequenced for *Fusarium* and *Phytophthora* species, respectively. *Fusarium* species accounted for 78.95 % (493 isolates) of the total (624) isolates obtained throughout the study whereas *Phytophthora* species accounted for 3.62 % (25 isolates) of the total isolates. Both morphological and molecular characteristics of *Fusarium* and *Phytophthora* matched identification characteristics from the previous studies. Phylogeny revealed that *Fusarium* was divided into three species complexes (FOSC, FSSC and FIESC). *P. cinnamomi*, *P. macrochlamydospora* and *P. kelmania* were obtained for the *Phytophthora* genus. Geographic location, crop and growing season had an impact on species diversity. Findings from this study provided us with valuable insights into the species diversity and phylogeny of soilborne pathogens of avocado and macadamia in South Africa. Agricultural practices and farming systems play a role on the occurrence of soilborne pathogens. More studies are required to determine the virulence, pathogenicity, and effective control strategies of soilborne pathogens isolated in this study.

MB14

Identification and pathogenicity of *Rahnella* species isolated from diseased onion bulbs from the United States of America and South Africa

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Abstract

In the family *Yersiniaceae*, the genus *Rahnella* includes Gram-negative, facultative anaerobic bacteria that have a ubiquitous distribution. The species of *Rahnella* have been isolated from water, human wounds, oak trees, beetle guts, and recently, from symptomatic onion bulbs and leaves. Onion (*Allium* cepa L.) is one of the most consumed vegetables worldwide. Ten states of the USA produce an average of 33 million tons of bulbs each year, whereas, in South Africa, approximately 600 000 tons are produced, mainly from the Western Cape province. Onion production is greatly hampered by pre-and post-harvest diseases caused by bacterial pathogens, resulting in up to 100 % yield loss. Amongst bacterial strains isolated from diseased onion bulbs and leaves in the USA and South Africa, 43 strains of *Rahnella* were identified based on their 16S rRNA gene sequencing. As the 16S rRNA gene lacks the phylogenetic resolution to delineate *Rahnella* strains at a species level, in the current work, a multilocus sequence analysis (MLSA) based on the four housekeeping genes: rpoB, gyrB, atpD, and infB, were conducted. Furthermore, the onion pathogenicity of *Rahnella* strains was determined using the red onion scale, onion bulb, and foliar assays. A concatenated phylogenetic tree of the four housekeeping genes showed that the 43 *Rahnella* strains primarily belonged to five *Rahnella* species, namely, *Rahnella perminowiae*, *R. aceris*, *R. aquatilis, R.variigena* and *R. victoriana*. Most onion *Rahnella* strains clustered with either *R. perminowiae* or *R. aceris* and were found to be non-pathogenic using the red onion scale and foliage assays. However, the onion bulb necrosis assay revealed that some isolates were pathogenic, non-pathogenic, and weakly pathogenic. The study provides valuable insight into the diversity of *Rahnella* species associated with diseased onion bulbs in the USA and South Africa and their pathogenicity in onion bulbs and foliage. This study is also a new host report of *Rahnella* species on onion in South Africa.

MB15

A CRISPR-Cas9 mediated gene knockout system to functionally characterize virulence genes in *Fusarium circinatum*

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Abstract

Fusarium circinatum is the causal agent of pine pitch canker disease and is one of the most destructive pathogens of *Pinus* species worldwide. Infection by this fungus causes severe mortality of nursery seedlings and in plantations it is associated with substantial loss in quality and yield. However, there is a paucity of mechanistic understanding of its virulence mechanisms and pathogenicity factors. Various putative virulence or pathogenicity associated genes have been identified in previous work, but none have been functionally characterized so far. The aim of this study was to develop a method based on CRISPR-Cas9 for disrupting particular gene targets. For this purpose, we used a gene encoding a putative virulence protein, which was identified in a previous genome-wide association study. A single guide RNA was designed to target the gene in a number of *F. circinatum* wildtype strains. The deletion mutants were then generated through a protoplast-based transformation approach and a CRISPR-Cas9-mediated genome editing system. The transformation reaction was completed by replacing the gene with a donor DNA carrying a hygromycin B resistance cassette. Gene knockouts were confirmed by hygromycin B-assisted selection and PCR amplification of the integrated antibiotic cassette. This study is the first to report the use of CRISPR-Cas9 in *F. circinatum* and provides a protocol for which routine gene characterization can be conducted. Future research will seek to further characterize this gene's role with regards to virulence in *F. circinatum* and this knowledge may contribute to the development of novel strategies to mitigate this devastating pathogen.

MB16

Characterisation of the *Fusarium oxysporum* species complex associated with sweet potato in South Africa

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Abstract

Sweet potato (*Ipomoea batatas*) is a popular food security crop in South Africa and has considerable commercial value. Fusarium wilt (FW) caused by the fungal pathogen *Fusarium oxysporum* has been reported worldwide and is widespread in sweet potato production areas in South Africa. *F. oxysporum* formae specialis (f. sp.) *batatas* was reported to cause FW worldwide and to date the only formae specialis associated with FW in South Africa. Preliminary molecular identification of South African isolates from diseased sweet potato plants indicated that there are other formae speciales besides *F. oxysporum* f. sp. *batatas* associated with Fusarium wilt. The objectives of the study were to conduct a field survey and to characterise the isolates of the *F. oxysporum* species complex using phylogenetic analysis and morphological characterisation. Phylogenetic relationships amongst isolates from diseased sweet potato material and soil collected in South Africa were characterised based on sequence data for the translation elongation factor-1 alpha *(tef-1α*), RNA polymerase II 2nd largest subunit *(rpb2),* β-tubulin and internal transcribed spacer (ITS) gene regions. Morphological characterisation was done to support the phylogenetic analysis. Phylogenetic analysis revealed two other f. sp., namely *F. oxysporum* f. sp. *tuberosi* and *F. oxysporum* f. sp. *vanillae* associated with FW and the results were supported by a significant bootstrap value. This study has contributed to a new body of knowledge and understanding of *F. oxysporum* species complex associated with FW of sweet potato in South Africa.

MB17

Characterisation of the association of apple stem pitting virus with pear stony pit disease

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Abstract

Pear Stony Pit Disease (PSPD) is a disease on pears which has been found in orchards since 1919, but only described in 1939. Graft transmission of the disease was demonstrated in 1938, when healthy trees produced pears with PSPD symptoms after being inoculated with buds from diseased trees, implicating an unknown virus as the likely cause of this disease. In 1999 it was theorized that apple stem pitting virus (ASPV), a foveavirus that infects pome fruit trees as well as their closely related species, may be associated with the disease. In recent years it was hypothesised that the observed fruit symptom could also be Antestiopsis stink bug feeding damage. The increased observation of PSPD symptoms in the last few seasons, was reason for concern and warranted urgent attention. High-throughput sequencing (HTS) was employed and identified the presence of ASPV in samples that tested negative, indicating a potential failure of the RT-PCR detection assay used. To prevent the distribution of virus infected material, detection assays need to be regularly validated to ensure that they are robust and accurate. In this study, pear orchards were surveyed for PSPD-like symptoms to investigate the association between PSPD and ASPV. The genetic diversity of ASPV was determined through a combination of HTS and amplicon sequencing, mainly to develop a sensitive detection assay for all known variants of ASPV, and to evaluate its impact on disease aetiology. This study developed a new RT-PCR detection assay that can detect all known ASPV variants. The use of HTS allowed for the construction of complete virome profiles of several samples and led to the discovery of two viruses not previously detected in pears in South Africa. The presence of stink bugs was observed in some orchards and therefore their contribution to the symptom development cannot be excluded. Determining the cause of PSPD, whether it is due to the virus or if it is stink bug feeding damage, will help to mitigate the large economic losses recently incurred by the South African pear industry.

MB18

Population studies indicate multiple introductory events of *D. pini* into France

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Abstract

Dothistroma septosporum and *D. pini,* the causal agents of Dothistroma needle blight (DNB), are important pathogens of *Pinus* species worldwide. The emergence of DNB outbreaks in France in the 1990s have led to increased investigations of the *Dothistroma* species. Previous population studies conducted on *D*. *pini in* central France revealed significant levels of genetic diversity and the presence of both mating types, indicating a possible native area of the pathogen. Outbreaks of DNB have also occurred in southern France, but these populations have not been investigated. Due to the fact that *D*. *pini* has been present in central France since 1907 and is well-established in these areas, we hypothesised that *D*. *pini* was introduced to southern France from central France. The aim of this study was, therefore, to compare *D. pini* populations in these two regions to determine whether they are genetically related. The pathogen species obtained from outbreaks in southern France was confirmed as *D*. *pini* by sequencing the internal transcribed spacer (ITS) region, while the population diversity and structure was determined using 16 microsatellite markers. The resulting data was compared to datasets obtained from *D*. *pini* populations from central France. Central France contained 25 % more unique multilocus haplotypes than southern France and no multilocus haplotypes were shared between populations in these two regions. Populations from central France displayed high levels of genetic diversity and admixture, which is typical of well-established populations. On the other hand, populations in southern France displayed lower levels of genetic diversity and there was little to no evidence of gene flow, even between these populations. These findings suggest multiple introductory events into southern France. The lack of genetic similarity between the populations from central- and southern France refutes our hypothesis. An alternative hypothesis is that *D*. *pini* was introduced to southern France from the neighbouring country, Spain, which could be investigated in future studies.

MB19

Revisiting *Clonostachys*

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Abstract

Clonostachys (*Bionectriaceae*, *Hypocreales*) species are common soilborne fungi that also occur as mycoparasites and in plants as endophytes, epiphytes, and saprotrophs. The sexual morphs of *Clonostachys* are members of *Bionectria*, which was subdivided into six subgenera, *Astromata*, *Bionectria*, *Epiphloea*, *Myronectria*, *Uniparietina* and *Zebrinella*. However, with the introduction of the One Fungus-One Name concept the genus *Clonostachys* was recommended for conservation over *Bionectria*. Species of *Clonostachys* are typically characterised by penicillate, frequently sporodochial, and, in many cases, dimorphic conidiophores (primary and secondary conidiophores). Primary conidiophores are mononematous, either verticillium-like or narrowly penicillate, and form heads of slimy conidial masses. Secondary conidiophores can be mononematous, loosely aggregated, and generally form imbricate conidia that can collapse to slimy masses that vary from white, to pale orange, yellowish or green. In the present study we investigate the species diversity within a collection of 458 strains of *Clonostachys* deposited in the CBS culture collection based on morphological and DNA sequence analyses of the nuclear ribosomal internal transcribed spacer (ITS), partial sequences for the 28S large subunit (LSU) nrDNA, partial RNA polymerase II second largest subunit (*rpb2),* partial translation elongation factor 1-alpha (*tef-1α*) gene and partial beta-tubulin (*tub2*) regions. Based on these results, the subgenera *Astromata*, *Bionectria*, *Myronectria* and *Zebrinella* are supported within *Clonostachys*. The genus *Sesquicillium* is resurrected for the subgenera *Epiphloea* and *Uniparietina*. One new genus, 30 new species and several new combinations are proposed based on results of this study.

MB20

Identification of putative NLP effectors in *Phytophthora cinnamomi* and determination of their expression profiles in *Persea americana*

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Abstract

Effectors are small protein molecules secreted by pathogens like *Phytophthora cinnamomi* to aid in the infection process. A fairly understudied but important group of effectors are the Necrosis-and-ethylene-inducing protein-like proteins (NLPs). These effectors have been well studied in fungi and bacteria, but little has been done in oomycetes. The main function of these proteins is to induce the hypersensitive response and necrotic cell death in the host plant, but they may hold other functions such as inducing the change in oomycete lifestyle from biotrophy to necrotrophy as NLPs have been identified that do not have a toxic function. Putative NLP proteins were identified from the *P. cinnamomi* GKB4 genome using a Hidden-Markov model (HMM) built from protein sequences obtained from NCBI as well as functional searches. Sixty-one potential NLPs were identified and an additional one was identified from functional analyses using BLAST2GO. These putative NLPs were then manually curated based on RNA seq data and verified to be NLPs based on the presence of the NLP motif GHRHDWE, a signal peptide and the number of C residues upstream of the motif. The expression patterns of these NLPs were investigated in a susceptible and resistant *Persea americana* rootstock at various timepoints after infection. The results indicated that their expression patterns varied over the course of infection suggesting their roles in the infection process.

2. Genomics

G1

Transcriptomic responses to the banana bunchy top virus in banana

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Abstract

Banana bunchy top disease (BBTD) is the most important viral disease of banana and plantain. This disease is caused by banana bunchy top virus (BBTV) to which there is no natural resistance in any *Musa* species. Towards identifying potential susceptibility factors that facilitate BBTV infection, the current study profiled transcriptomic responses to BBTV in banana using RNASeq. Using a time course approach, 563, 1052 and 818 genes were differentially expressed between BBTV-infected banana plants and uninfected controls at 7-, 30- and 45-days post-inoculation, respectively. Enrichment analysis revealed that genes involved in regulation of the cell cycle, DNA replication, far-red light signalling, stomatal movement and chromatin-associated regulation of transcription, were among those associated with BBTV infection. Upregulated differentially expressed genes (DEGs) included the proliferating cell nuclear antigen (a key factor in the cell cycle), DNA replication licensing factors mcm2-6 that are central to initiation of replication, histone subunits involved in epigenetic regulation of transcription, and HY5 and MYB transcription factors. DEGs involved in defence, including a thaumatin-like protein, ethylene-responsive transcription factor ERF024, a germin-like protein and chalcone synthase, were downregulated in BBTV-infected plants. The observed BBTV-induced upregulation of the cell cycle and DNA replication confirms similar infection strategies between the ssDNA babuviruses and geminiviruses. Currently, CRISPR/Cas9-mediated knock-out of candidate susceptibility factors identified among DEGs in this study and implicated in facilitating virus infection in other studies, is being pursued towards characterising their roles in BBTV infection and determining their potential utility in engineering resistance to BBTV in banana.

G2

RNA interference-related genes in *Ascomycota*

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Abstract

RNA interference (RNAi) is a post-transcriptional mechanism that inhibits translation or degrades messenger RNA by using small RNA molecules. Transposon suppression, protection against viruses and DNA damage, transgene silencing, gene regulation, and heterochromatin formation are all functions of RNAi in fungi. Here, we identified and inferred the evolutionary history of seven known RNAi genes in the pine pathogen, *Fusarium circinatum*, and a select number of fungal species. A search of 33 publicly available genomes revealed the presence of homologs for all seven genes in each species, with three copies of the Argonaute gene found in all species except *Neurospora crassa*, *Fusarium oxysporum* f. sp. *lycopersici*, *Fusarium mundagurra*, *Beauveria bassiana* and *Melanomma pulvis-pyrius*. Moreover, most species possessed two genes encoding Dicer, four encoding RdRP, two encoding RPA, one gene each for RecQ helicase, QIP and MRPL3. Phylogenetic analyses of identified homologs indicated that the respective RNAi genes are highly conserved and likely evolved along with the species harbouring them. Also, protein domain analysis with InterProScan showed that most of the genes contained the expected domains. The exceptions were the Argonaute, Dicer and QIP genes of some species that lacked particular domains or had novel protein domains. In all species except *N*. *crassa* we also found for the first time an extra copy of the Argonaute gene, with some having more than three copies. In most cases, the extra Argonaute gene(s) appears to be most closely related to one in *N*. *crassa*, although half contained a PAZ domain that is absent in the *N*. *crassa* Argonaute. This study is the first to interrogate the essential genes required for RNAi in the pine pathogen *F*. *circinatum* and its close relatives and our findings provide a valuable framework for further studies on the potential for RNAi-based technologies in those species.

G3

Whole genome sequencing of *Exserohilum turcicum* races present in maize growing fields of South Africa

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Abstract

Exserohilum turcicum is the causal agent of northern corn leaf blight (NCLB). The fungus can cause yield losses of up to 70 % in maize fields with high humidity and when temperatures reach 25–30 °C during the day. NCLB symptoms are recognised by grey-green cigar shaped necrotic lesions from where the fungus can sporulate and disperse. It is difficult to control NCLB, but to some degree can be managed by fungicide applications or the use of resistant maize cultivars. For breeding, four widely used resistance genes are used, namely Ht1, Ht2, Ht3 and HtN. However, some *E. turcicum* strains can overcome these resistance genes and are thus classified into different race types (e.g., a strain that can overcome Ht1, Ht2 and HtN is known as race 12N). Knowing which races are present in a region will allow breeders to utilise resistance genes more effectively. Race typing is a tedious process, needing inoculation trials with specific maize lines that contain the panel of Ht genes. The aim of this study is to do genome comparisons to identify potential molecular markers that can be used to identify races in a more time efficient manner. South African strains were obtained for race 1 and 23N from local culture collections and pilot studies. From these collections, a race 23N strain was selected and sequenced using PacBio, and several race 1 and race 23N strains was sequenced using the Illumina platform. Here we report the genome analysis and comparisons of the isolates of these different race types.

G4

Gene editing to understand RNAi pathways in the maize pathogen *Cercospora zeina*

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Abstract

Grey leaf spot (GLS) disease of maize caused by the pathogenic fungi *Cercospora zeina* is a threat to food security in sub-Saharan Africa. The aim of this project is to evaluate the gene editing efficiency of the CRISPR-Cas9 system to knockout genes in *C. zeina*. CRISPR gene editing is a highly versatile tool allowing precise genetic manipulation in crops, animals and microorganisms. We have transformed a wild type *C. zeina* with a *gfp* reporter gene and Southern blot analysis revealed the transformed *C. zeina* contains three copies of *gfp*. A single-guide RNA (sgRNA) upstream of the PAM motif targeting *gfp* has been designed for the CRISPR system. The gene editing experiment is currently underway in which the sgRNA and Cas9 protein is delivered as a ribonucleoprotein complex. A homologous direct repair of the Cas9-induced cleavage is carried out using a donor DNA (dDNA) containing geneticin (g418) selectable marker flanked by arms that are homologous to the region being targeted by the sgRNA. Upon successful optimisation of the CRISPR gene editing system using the reporter gene, we will target RNAi pathway genes to determine their importance in dsRNA-based gene silencing in *C. zeina.* We have identified *C. zeina* target genes such as *Dicer-like* and *Argonaute* through phylogenetic analysis. In previous work, we have also demonstrated dsRNA knockdown of *gfp* in the gfp -transgenic *C. zeina* line. Gene editing of RNAi pathway genes will also be done in the *gfp* -transgenic *C. zeina* background. This will enable testing the effect of knockout of RNAi pathway genes on dsRNA silencing of *gfp in-vitro*. The research will provide fundamental information about RNAi in this important maize pathogen. It will also inform ongoing efforts in the lab to implement RNAi based fungicides against grey leaf spot disease of maize.

G5

QTL identification of *Ceratocystis albifundus* genomic regions associated with laccase activity using GWAS.

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Abstract

Ceratocystis albifundus is an economically important pathogen of non-native *Acacia mearnsii* in South Africa. *C. albifundus* differ in their ability to cause disease on *A*. *mearnsii*. Also, the fungus does not appear to cause disease symptoms in native hosts. Laccases, known to catalyze a wide range of substrates from phenols to non-phenolic compounds, are known to influence the outcome of plant-pathogen interactions. Given the value of genome-wide association studies (GWAS) for identifying genes associated with important phenotypic traits, we used this tool to find genes or genomic regions associated with laccase expression in *C*. *albifundus*. For this purpose, a set of *C*. *albifundus* isolates, originating from a wide geographic range, was genotyped using low-coverage genome sequencing technologies. Correlation between the single nucleotide polymorphism data for the isolates and their corresponding phenotype information allowed the identification of a collection of genomic regions that were significantly (P-value < 0.05) associated with laccase expression. For example, one of the regions contained a gene that codes for a protein in the multicopper oxidase superfamily, of which laccase is a member. Results of this study demonstrated that laccase expression represents a quantitative trait in this fungus. Laccase expression also differed based on the host. Our future research will thus use GWAS to further explore the possible link between laccase expression and virulence in *C*. *albifundus*, with the ultimate goal, of improving our knowledge regarding pathogenesis in this economically important fungus.

G6

Bacterial and viral inhabitants of storage onion bulbs in the USA

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Abstract

Microbes occur in complex communities within plants and establish a network of interactions that may influence their health either positively or negatively. They can be pathogenic, exhibit synergistic interactions or confer abiotic and biotic stress tolerance. Some bacterial pathogens can switch their lifestyle from being non-pathogenic to pathogenic or vica versa. The aim of this study was to identify bacteria and viruses present in storage onion bulbs collected from Georgia and Washington State in the USA and try to determine their role in this environment. DNA was extracted from nine healthy bulbs and nine bulbs displaying symptoms of bacterial rot from a commercial storage facility from each State. The Illumina platform was used to sequence the hypervariable region of the 16S rRNA gene (V3-V4). DNA was then pooled for the healthy bulbs and for the diseased bulbs and metagenome assembled genomes (MAGs) assembled and analysed. The 16S rRNA profiling revealed the presence of numerous bacteria including *Panteoa* and *Burkholderia*. Once the MAGs were assembled, *P*. *agglomerans*. *P*. *ananatis* and *B*. *cepacia*, known bulb rot pathogens, were identified. In addition, 89 unique viral genomes were identified of which 67 could be classified. The bacterial and viral genomes were different in healthy vs. symptomatic bulbs from each of the two sampled locations. Functional metagenomics revealed that the bacterial species possessed genes linked to fitness and had both Type II and III secretion systems. Viral populations were screened for possible auxiliary metabolic genes (AMGs) and genes involved in fitness and pathogenicity of bacterial hosts were found. Onion bulbs were thus shown to host endophytic bacteria and viruses that were potentially beneficial and pathogenic to the host.

G7

Improving specificity and throughput of diagnostic PCRs aimed at maize pathogen detection

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Abstract

With a global population of over 7.9 billion people and over 800 million people estimated to fall into the category of undernourished according to a 2020 study carried out by the World health organization. This number is unacceptable and shows how food security is a major issue that needs to be addressed. Conventional diagnostic methods rely on disease identification based on symptoms, followed by the confirmation of the causal species that relies on the laborious and skills specific approach of isolating and identifying the organism. To increase efficiency of disease detection, it would be ideal to have a single multiplex assay that can detect all important pathogens accurately and robustly. This project is aimed at discovering unique genes associated with a range of maize pathogens, which includes 11 fungal, one bacterial and one viral pathogen, with the aim of aiding in molecular diagnostic identifications. This will be done by generating a multiplexed primer panel that can be used to identify multiple different pathogens in a single PCR run. Publicly available genomic resources of these pathogens and associated closely related species, were utilized to identify unique genes for each maize pathogen using the OrthoFinder software. On average 4 unique genes were identified for each pathogen, with 56 identified in total. Genomic location of these unique genes was determined for those species having assemblies in chromosomal format to investigate if these genes are associated with the variable regions or core components of the genomes. Characterization of these unique genes was done using Blast2GO, with the majority not having a known function. Those with functions were often components of the cell membrane. The number of unique genes for each pathogen was dependent on the availability of genomic data and the variability of closely related species. If this multiplexed panel is successful in amplifying the pathogen's sequences of interest, then it will set up the groundwork for a larger primer panel that can include more pathogens, or the framework can be used to generate a panel for different crops and related pathogens.

G8

Detection of mating allele diversity in South African populations of the multi-host pathogen, *Sclerotinia sclerotiorum*

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Abstract

Sclerotinia sclerotiorum is a fungal pathogen capable of infecting hundreds of plants, including several agriculturally important crops such as canola, sunflower and soybean. In this species, sexual reproduction leads to the formation of apothecia and ascospores, producing large amounts of inoculum that can drive new infections. Sexual reproduction in ascomycetes is controlled by mating-type (*MAT*) genes at the mating-type (*MAT1*) locus. The *MAT1* locus of *S. sclerotiorum* contains four *MAT* genes and the fungus is considered self-fertile, as it is capable of completing the sexual cycle without a mating partner. Interestingly, an inversion event at the mating-type locus creates two variants of the *MAT1* locus every meiotic generation. It has been suggested that these variants (known as Inv+ and Inv-) might act as mating-type alleles that could facilitate outcrossing in this self-fertile fungus. The aim of this study was to determine if both the Inv+ and Inv- versions of the MAT1 locus are present in South African isolates of *S. sclerotiorum*. To do this, PCR primers were designed to identify the Inv- and Inv+ alleles and used to test a population of South African isolates. The primers were able to successfully distinguish between the Inv+ and Inv- alleles in a set of test isolates. Our results also showed that both these alleles were present among the isolates tested, although the exact ratio of Inv+ and Inv- individuals remains to be determined. These primers provide a reliable tool to screen *S. sclerotiorum* for *MAT1* locus structural variation, and the presence of both *MAT1* locus variants provide preliminary evidence of genetic diversity among South African isolates of this agricultural pathogen. This work lays the foundation for future studies aimed at understanding the genetic diversity of *S. sclerotiorum* in South Africa.

G9

Identification and characterization of a growth QTL of *Fusarium circinatum* on pine-based medium.

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Abstract

Fusarium circinatum, commonly referred to as pine pitch canker is an economically important pathogen of *Pinus* species globally. This fungus is a member of the *Fusarium* fujikuroi species complex and can hybridize with another member of the complex, *Fusarium temperatum* that is commonly isolated from maize and its wild progenitor. Genetic and genomic analyses of the hybrid progeny from a laboratory cross between them has greatly aided our understanding of the molecular processes underpinning differences in their growth at particular temperatures. In this study, we aimed to compare the growth of *F. circinatum* and *F. temperatum* and their F1 progeny on pine tissue-derived medium. Using a combination of the growth data, a previously determined genetic linkage map, and the genomic sequences of the parental species, we were able to identify and characterize a significant Quantitative Trait Locus (QTL) associated with fungal growth on this medium. Genome analyses revealed that the QTL is located in a region containing sequences that are non-homologous between the two parents. The *F. circinatum* parent had a 7 Kb region lacking any detectable genes, while the *F. temperatum* parent encodes two genes in this region, which is also present in and other sequenced strains of the *F. circinatum* and other members of the F. *fujikuroi* species complex. The 7 Kb region in the *F. circinatum* parent were also characterized by low GC content, retrotransposable elements and repeat-induced point (RIP). These findings thus demonstrated a possible role for transposable element activity in gene deletion, and this could lead to alterations in the phenotype of a fungus.

G10

The mating-type locus in Ambrosial fungi from the *Ceratocystidaceae*

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Abstract

Mating in filamentous ascomycetes is governed by a small genomic region called the mating-type (*MAT1*) locus. The genes present at this locus define two mating-types termed *MAT1-1* and *MAT1-2*. Homothallic species are self-fertile and carry both mating types in a single genome. In comparison, heterothallism is defined by a mating interaction in which each individual possesses either the *MAT1-1* or *MAT1-2* mating type. In the past many filamentous ascomycete species were regarded as asexual solely based on the lack of an observable sexual structures. The *Ceratocystidaceae* (order *Microascales*) is a family of fungi best known for the pathogenic genera *Ceratocystis* and *Endoconidiophora*. Although the *MAT* region of these and other *Ceratocystidaceae* species has been studied, little is known regarding the mating strategies in genera that has a close relationship with Ambrosial beetles. These ambrosial genera include *Meredithiella*, *Toshionella*, *Phialophoropsis*, and *Wolfgangiella*, all considered asexual. The aim of this study was therefore to determine the mating strategies of species from these genera. To identify the MAT1 locus and associated mating-type genes, the genomes of *Meredithiella fracta*, *M*. *norrisii*, *Toshionella taiwanensis*, *Wolfgangiella franznegeri* and an unknown species of *Phialophoropsis* were sequenced and assembled. The assembled sequences were used in a tBLASTx search using the *MAT* genes from closely related species (such as *Ceratosytis fimbriata*) as query. The results were used to guide the de novo annotations to screen for the presence of mating-type genes. Genes coding the *MAT1-1-1, MAT1-2-1, MAT 1-1-2*, and the *Microascales*-specific *MAT1-2-7* proteins were present in all species investigated. The presence of all four genes in a single *MAT1* locus indicates that these fungi are likely homothallic. In some of these species, two copies of a direct repeat (≤192 bp) were identified flanking the *MAT1*-2 region. In other *Ceratocystidaceae* species, these repeats are responsible for the deletion of the *MAT1-2* region through unidirectional mating-type switching, and this might also be present in these fungi. The findings presented here is another step towards a better understanding of the genomic basis of mating and reproductive biology in the *Ceratocystidaceae*.

G11

Pathogenicity-related gene expression in the aggressive *Eucalyptus* foliar pathogen, *Teratosphaeria destructans*, under nitrogen starvation

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Abstract

Teratosphaeria destructans is one of the most aggressive foliar pathogens of planted *Eucalyptus* trees. It causes severe shoot and leaf blight on young trees, resulting in losses to the forestry sector. The biological factors underpinning *T. destructans* infections have not been interrogated and it is not known how the pathogen overcomes host defences. In the absence of an inoculation protocol for *T. destructans*, identification of genes upregulated in a nitrogen-starved environment could provide insights into the process. In this study, we used transcriptome sequencing to compare gene expression in a South African isolate of *T. destructans* grown on nitrogen-deficient and complete media. The results support the hypothesis that nitrogen starvation in *T. destructans* likely mirrors an in planta genetic response. This is because 45 % of the genes that were highly upregulated under nitrogen starvation have previously been associated with infection in other pathogen systems. These included several CAZymes, fungal effector proteins, peptidases, kinases, toxins, lipases and proteins associated with detoxification of toxic compounds. The 25 secondary metabolite biosynthetic clusters predicted from the genome were expressed in both nitrogen-deficient and complete conditions. Additionally, the most highly expressed genes in both growth conditions had pathogenicity-related functions. This study is the first to consider gene expression in this important pathogen and highlights the large number of expressed genes associated with pathogenicity and overcoming plant defences.

G12

Filling in the gaps: expanding our knowledge of the South African plant virome

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Abstract

The advent of non-targeting, high-throughput sequencing has represented a paradigm shift in the way in which novel plant virus diversity is uncovered. Comprehensive knowledge of viruses of agricultural crops is essential for effective certification schemes, as well as the development of diagnostic capacity. Most plant-associated viruses have RNA genomes but the large-scale use of RNAseq is still prohibitively expensive. Technologies such as RNAtag-seq allow for the parallel generation of multiple RNAseq libraries in a single reaction and reduces the per sample sequencing costs by an order of magnitude. The Plant Virology Group at FABI is making use of multiplexed RNAtag-seq sequencing to evaluate the metaviromes of previously understudied crops in South Africa. Focus crops include *Malus domestica (*apple)*, Prunus avium* (sweet cherry)*, Olea europaea* (olive*), Glycine max* (soybean)*, Helianthus annuus* (sunflower)*, Brassica rapa* (canola)*, Triticum aestivum* (wheat)*, Hordeum vulgare* (barley)*, Fragaria × ananassa* (strawberry)*,* and *Humulus lupulus* (hops). The metaviromes of 480 samples are in various stages of preparation and is expected to contribute to uncovering an unprecedented level of plant viral diversity. Previous applications of this workflow to crops and ornamental plant species in South Africa, have led to the discovery of more than ten novel viral species and the generation of thousands of complete genomes of known viruses. The availability of such resources should ultimately assist with the improved management of these pathogens, with a concomitant improvement in crop yields.

G13

The latent pine pathogen *Diplodia sapinea* contains two dispensable chromosomes with distinct genomic characteristics

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Abstract

Diplodia sapinea (*Dothideomycetes*) is a fungal pathogen of conifers that predominantly affects Pinus spp. This pathogen typically infects healthy trees and remains in a latent phase until the onset of stress when disease symptoms such as shoot blight, stem cankers, root disease and die-back develop. Isolates of *D. sapinea* have been shown to display varying degrees of aggressiveness, but little is known regarding the genetic basis for this variation. Through a hybrid approach of using Nanopore and Illumina sequencing technologies, we produced complete genome sequences for three *D. sapinea* isolates which were assembled into 14, 15 and 16 pseudo-chromosomes. Comparison of these genomes revealed the existence of two dispensable chromosomes (DCs) of 0.46 Mb and 0.64 Mb that encode for 80 and 152 proteins, respectively. Low-coverage Illumina sequencing of seven additional isolates from various countries revealed that one DC was prevalent and shared by 8 of the 10 total isolates, whereas the other DC was present only in a single isolate that harboured both DCs. These DCs had distinct genomic features compared to that of the core chromosomes in that they had lower gene density, lower GC content and higher proportions of transposable elements. Sequence analysis showed that genes on the DCs are rapidly evolving suggesting that the DCs act as evolutionary hotspots in *D. sapinea*. Sequence homology searches indicated that the DCs were likely horizontally acquired. Gene ontology enrichment analysis showed that the DCs have enriched GO terms associated with transposable elements and pathogenicity. Pathogenicity trials conducted on *Pinus patula* seedlings showed no obvious association between the DCs and virulence, and their biological roles remain to be identified.

G14

Comparative genomics of the mitochondrial genomes of fungal species in the *Ceratocystidaceae*

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Abstract

The small, relatively conserved, and uniparentally inherited mitochondrial genomes are useful determining evolutionary relationship, resolving phylogenetic incongruences and other evolutionary studies due to their maternal inheritance property, high copy number and small genome size. *Ceratocystidaceae* is an ascomycete fungal family that contains many important plant pathogens of great economic concern. Despite a large number of whole genome sequences available for species in the *Ceratocystidaceae*, fully assembled and annotated mitochondrial genomes are available for approximately 11 species from only 2 genera in this family. Knowledge on the conservation, organization and evolution of mitochondrial genomes in this family is thus limited. The aims of this study were to characterise and compare the mitochondrial genomes of species of the *Ceratocystidaceae*. Using the available genomics data, we assembled, annotated and compared mitochondrial genomes of 18 species residing in 10 genera in the family. The results show that their mitochrondial genomes were highly variable in terms of size, which ranged from 98 kb in *Ceratocystis manginecans* to 225 kb in *Endoconidiophora laricicola*. Despite the large variation in genome sizes, gene orders and coding sequences were highly conserved. The size variation observed in these mitochondrial genomes were mainly due to the variability in the number and size of introns in the protein coding genes. Phylogenies obtained from mitochondrial genes and nuclear genes were largely similar except for one node which had very low support in the mitochondrial gene phylogeny. This study provided a set of complete and fully characterized mitochondrial genomes for a large number of species and genera in the *Ceratocystidaceae* which will be an important resource in understanding the evolution of these important fungi.

G15

Evaluation of pines important to South Africa forestry for susceptibility to *Dothistroma septosporum* in a Colombian sentinel planting

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Abstract

Dothistroma needle blight (DNB) is one of the most important needle diseases of conifers. The disease is caused by two fungal species of *Dothistroma* of which *D*. *septosporum* is best known. During the course of last decade, *D*. *septosporum* has caused serious disease outbreaks on tropical pine species in Colombia. Preliminary analyses indicated that a unique, clonal and aggressive Northern population represented by a single mating type was resulting in this report. A separate Southern population, also clonal and having the opposite mating type was also found. The aim of this study was to determine the susceptibility of five tropical pine species and two hybrids currently utilised by the South African forestry industry to the two populations of *D*. *septosporum* present in Colombia. In addition, the haplotypes of *D*. *septosporum* recovered from infected material in Colombia were characterised using microsatellite and mating type data to determine which population of isolates was causing the disease. To achieve these objectives, a cooperative sentinel tree screening project was established between Colombia and South Africa. The forestry farm of Cedral (Colombia) was selected for the trial due to the high natural inoculum load of *D*. *septosporum* in the area. Initial symptoms in the field were observed on pines six months and older with clear differences among species. The controlled crossing of *P*. *patula* x *P*. *tecunumanii* Low Elevation, and the species of *P*. *tecunumanii* LE, *P*. *patula* and *P*. *maximinoi* were tolerant while *P. oocarpa* was highly susceptible. Surprisingly, the microsatellite markers revealed a higher level of diversity, new alleles and evidence for sexual recombination. On further inspection, the sexual stage of the pathogen was discovered as the first record for the Southern Hemisphere. These data indicate that new introductions of the pathogen have occurred in Colombia and that the population has changed over a ten-year period to include sexual reproduction and high levels of genetic diversity. This emphasizes the risk associated with of the global movement of pathogens and the need for continual pathogen screening in pine breeding programs.

G16

Variation in azole resistance within South African population of *Fusarium circinatum.*

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Abstract

Pine pitch canker is one of the most devastating forest diseases worldwide and is caused by *Fusarium circinatum*. This fungus causes growth reduction, wilting, and root disease in pine seedlings and the development of cankers in pine forests and plantations are associated with significant quality and yield losses. In the seedling nursery setting, management of the fungus often involves the use of fungicides, but the pathogen's resistance to most of these chemicals has not been widely investigated. This is an important knowledge gap, given the rapid and widespread evolution of fungicide resistance in many agricultural ecosystems. The objective of this study was to examine the sensitivity of South African populations of *F. circinatum* to tebuconazole and imazalil, which are both sterol biosynthesis inhibitors. A set of 86 strains obtained from diverse geographic origins and various pine species were grown in media amended with increasing fungicide dosages (0.1, 1, 10 and 100 mg/l). The effective concentration reducing growth *in vitro* by 50 % was calculated for each strain. The highest and lowest EC50 -values were considered as less sensitive (LS) and sensitive (S), respectively. Our results showed that tebuconazole was a more effective in terms of mycelial growth inhibition, compared to imazalil. All strains had EC50 -values of 0.04-6 mg/l in the presence of tebuconazole, while those for imazalil ranged between 1-34 mg/l. The results allow a better understanding of isolates of *F. circinatum*, aiming at more effective management of pine pitch canker in South Africa.

3. Applied Pathology

AP1

Colletotrichum theobromicola causes a shoot and leaf disease on *Eucalyptus* in South Africa

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Abstract

Anthracnose disease caused by *Colletotrichum* spp. occurs in tropical, sub-tropical, and temperate regions and it has a relatively wide host range, including economically important crops. Recently, one-year-old nursery plants of *Eucalyptus grandis × Eucalyptus urophylla* displayed symptoms of leaf and shoot anthracnose disease in a South African nursery. Samples were collected from symptomatic tissues and isolations were made. Isolates were identified based on their morphological characteristics and DNA sequence data based on eight gene regions. Phylogenetic analyses led to the isolates being identified as *Colletotrichum theobromicola*. Pathogenicity trials with *C. theobromicola* isolates were conducted on *E. grandis* and hybrids of *E. grandis x E. urophylla* and *E. grandis × E. camaldulensis*. The typical circular anthracnose-like leaf spots symptoms became visible on *E. grandis x E. urophylla* plants from two days after inoculation and the fungus was easily re-isolated from the infections. No symptoms were found on *E. grandis* and *E. grandis × E. camaldulensis* plants. As far as we are aware, this is the first report of *C. theobromicola* on *Eucalyptus* in South Africa. Importantly, this pathogen is able to cause severe defoliation and death of plants, and this can occur rapidly when conditions are conducive to disease development. However, there is also clear evidence of tolerance to infection in some *Eucalyptus* varieties, reducing the concern that the pathogen will emerge as an important constraint to large-scale *Eucalyptus* propagation.

AP2

Development of a protocol to screen *Eucalyptus* genotypes for resistance to the aggressive foliar pathogen *Teratosphaeria destructans*

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Abstract

Leaf blight caused by *Teratosphaeria destructans* is one of the most important diseases of *Eucalyptus* planted in tropical and sub-tropical areas of the world. This pathogen was first described from diseased trees in Indonesia in 1996 and has subsequently spread widely in Southeast Asia. Its first appearance outside that region was in 2015 when it was recorded in South Africa. This has resulted in a need to screen *Eucalyptus* planting stock for resistance to *T. destructans*. A first step was to determine the optimal inoculum concentration for inoculations using a *Eucalyptus grandis* (G1) clone known to be highly susceptible to infection. Subsequently, the relative aggressiveness of the pathogen was tested on six *E. grandis x E. urophylla* (GU) genotypes (GU1, GU2, GU3, GU4, GU5 and GU6) with the susceptible *E. grandis* genotype as a control. Susceptibility was calculated using two measures i.e. percentage of leaf area covered by lesions (PLACL) and a novel disease susceptibility index (SI) to categorize host reaction as Highly Resistant (SI= 0), Resistant (SI= 0.01–0.2), Moderately Resistant (SI= 0.21-0.6), Moderately Susceptible (SI= 0.61-1), Susceptible (SI= 1.01-1.5) and Highly Susceptible (SI= > 1.5). The genotypes GU2 (SI=0.54; PLACL= 9 %) and GU3 (SI=0.49; PLACL=6.5 %), were classified as moderately resistant compared with the most susceptible genotype GU4 (SI= 1.52, PLACL= 48 %). The PLACL values were positively correlated with the percentage of stomata infected. The latter was determined by microscopy and indicates more points of infection in more susceptible hosts. However, sporulation was not strongly correlated with the PLACL values. Results of the study provide a robust basis to select resistant *Eucalyptus* against one of the most aggressive foliar pathogens of *Eucalyptus* and to study the mechanisms involved in the *T. destructans* - *Eucalyptus* interaction.

AP3

A new mating-type gene discovered in the chestnut blight pathogen *Cryphonectria parasitica*

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Abstract

Cryphonectria parasitica (*Sordariomycetes, Ascomycota, Cryphonectriaceae*) is amongst the most important pathogens of trees. The fungus was accidentally introduced into Europe and North America where it has devastated natural chestnut (*Castanea*) forests. Given its notoriety, *C. parasitica* is one of the most intensively researched tree pathogens. Yet intriguingly, its mating system remains poorly understood. Broadly, the fungus has a heterothallic mating system where two individuals of opposite mating types are required for sexual reproduction. There is, however, evidence that *C. parasitica* is occasionally homothallic where a single individual of a single mating type can produce sexual fruiting bodies. The hypothesis is that the fungus is preferentially heterothallic but occasionally mates unisexually. In this situation, two individuals of the same mating type physically interact, thereby allowing sexual reproduction to take place. To test this hypothesis, *C. parasitica* isolates of the same mating type were co-inoculated on a single plate in the presence of an individual of the opposite mating type. Isolates of opposite mating types were crossed to serve as a positive control. The experiment, which took place over six months, showed no evidence of sexual crosses between isolates of the same mating type. But a few of the control crosses produced sexual structures suggesting that the conditions were conducive for sexual reproduction. The second element of this study was to consider variation in the *MAT1* idiomorph of a large collection of *C. parasitica* isolates. The sequences were analysed and shown to have high levels of interspecies variation but extremely low intraspecies variation in *MAT1* genes. This study led to the discovery of a novel *MAT1-2* gene that we have designated *MAT1-2-16*, as it is the 16th gene identified from the ascomycete *MAT1-2* idiomorph. This *MAT1-2-16* gene lacked any known conserved domains, and its function remains unknown. Overall, these studies have revealed new and interesting insights into mating in *C. parasitica* that deserve further study.

AP4

Grey mould control of strawberries using orange oil

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Abstract

Grey mould caused by *Botrytis cinerea* Pers. is one of the major destructive diseases of strawberries worldwide. It can result to yield losses of up to 25 % for untreated strawberries. This study aims to establish whether orange oil can be incorporated in the postharvest management of grey mould during cold storage of strawberries. Orange oil is generally regarded as safe and has been shown to effectively inhibit fungal growth *in vitro* for several postharvest pathogens (*Aspergillus* spp*., B. cinerea, Penicillium* spp*., and Rhizopus* spp.). D-limonene, one of Citrus oils' active compounds, is a known antimicrobial compound and likely to be the key contributor to the fungistatic effects of orange oil. The efficacy of orange oil as aqueous dip application (0.05 %, 0.5 %, 1.0 % v/v for 30 s) was tested on strawberries ('Albion' and 'Sabrina') applied curatively on fruit that was wound-inoculated with *B. cinerea* conidiospores. The efficacy of decay control of two formulated orange oil products (OR79 and OR007) was compared to pure essential oil. The fruit was stored at 4 °C for two weeks after treatment. Incidence of grey mould decay was evaluated during storage on days 14 and 17 after treatment. On day 14, the incidence of grey mould was reduced from 82 % of the untreated control to 55 %, 21 %, and 44 % on the OR79, OR007, and orange oil only treatments, respectively. When the fruit was left at ambient temperature for further 3 days, it became infected with grey mould. In this study, strawberry trial data showed the potential of orange oil only and orange oil-based products in prolonging the quality of strawberries when evaluated for grey mould control in cold storage. These treatments could be applied immediately after harvest through dip application and allow the fruit to dry before cooling.

AP5

Survey of potato soft rot- and blackleg-causing bacteria in South Africa

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Abstract

The blackleg-soft rot disease complex is an increasing problem for the South African potato industry. Recent years have seen an upward trend in its incidence and severity, mostly attributed to *Pectobacterium brasiliense*, the predominant species in the country, although other species of the *Pectobacterium* and *Dickeya* genera have also been implicated. This disease is mainly managed through the use of certified, disease-free seed tubers. Certification includes visual inspection of plants in season and seed at harvest, and testing for the causal agents. The introduction of new species into the country and the taxonomic reclassification of existing ones warrants a revision of the current species composition. Changes in species composition has implications for certification which needs to test for the correct species. Hence, a survey was conducted to determine the predominant blackleg-soft rot causal agents in South Africa. Symptomatic tubers and haulms were collected from across the country, and isolations were done on Crystal Violet Pectate agar. The isolates were identified by species-specific PCR assays followed by a gapA PCR-sequencing assay. A multilocus sequence phylogeny of the concatenated atpD, dnaX, rpoS, infB, and leuS gene sequences were also constructed for newly identified species. The overwhelming majority of isolates were identified as *P. brasiliense*, indicating that it remains the predominant species in South Africa. Three new species in the country were also identified, namely *Pectobacterium versatile, Pectobacterium actinidae,* and *Dickeya chrysanthemi*. Greenhouse pathogenicity trials confirmed Koch's postulates, resulting in the first reports of these three species as causal agents of potato blackleg in South Africa. The pathogenicity trials further showed a lower incidence of blackleg for these species compared to *P. brasiliense*, suggesting them to be less pathogenic and hence, to have a lesser impact on potato production in the country.

AP6

Alternaria species causing leaf spot on potato in South Africa

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Abstract

Alternaria solani and *A. alternata* are the causal agents of early blight and brown spot of potato, respectively. However, several other *Alternaria* species have been associated with leaf spot of potato in other parts of the world. These include *A. arborescens, A. cantlous, A. grandis, A. protenta* and *A. infectoria*. Foliar diseases can be a major limiting factor in potato production and correct identification of the pathogens is essential for the effective management of the disease. The South African potato industry is spread over 16 production regions, covering various climatic regions. All of these regions regularly experience outbreaks of leaf spots associated with *Alternaria* species. The aim of this study was to do a survey of the various regions in order to isolate and identify the *Alternaria* species associated with foliar diseases of potato in each region. Sampling was conducted from 2018 to 2021 in 13 of the 16 production areas. Isolates were identified based on culture morphology, as well as DNA sequencing and phylogenetic analyses. Gene regions sequenced included *Alt a 1, gapdh* and *rpb2*, depending on the morphological group the isolates were placed into. Isolates obtained were screened with a detached leaf assay to select virulent isolates. These isolates were inoculated onto plants in the glasshouse. Pathogens were reisolated and identified to fulfil Koch's postulates. Results showed that in addition *to A. alternata and A. solani, A. arborescens* and *A. grandis* are also present in all growing regions in South Africa and can cause disease on potatoes. This study revealed that the diversity of *Alternaria* species causing disease on potato is larger than previously thought. This may have implications for control of the disease and further studies are needed on the interaction of the different species in disease development.

AP7

Survey of *Alternaria alternata* populations from different crops in Mpumalanga, Gauteng and Limpopo Provinces in South Africa

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Abstract

Alternaria alternata can cause diseases in over 100 host plants. A variety of diseases are caused by *A. alternata* including brown spot-on potatoes and tangerines, leaf spot on rough lemon, black rot on several citrus fruits and black mould on tomato. The pathogen can also cause postharvest diseases (e.g. Alternaria fruit rot of tomato and Alternaria black rot of citrus). The objectives of the study were to isolate *A. alternata* occurring on potato, tomato and citrus crops in the Limpopo, Gauteng and Mpumalanga Provinces and conduct cross-infection studies with the isolates, in order to determine the ability of selected isolates to infect other hosts. Symptomatic plant material of citrus, tomato and potato were collected from 15 different farms in Gauteng, Mpumalanga and Limpopo. A total of 242 isolates were obtained and screened for virulence using a detached leaf assay, infecting the same crop it was obtained from. The virulent isolates selected were characterised based on morphology, including sporulation patterns and conidial morphology, as well as phylogenetic analyses. The three most virulent isolates from each crop were also used in a cross-infection study in the glasshouse. The glasshouse trial showed that all the *A. alternata* isolates evaluated were able to cause disease symptoms on the tested crops during cross infection. The findings of the study will serve to guide future disease control strategy development, since the ability of a single population to infect multiple hosts will have a significant impact on the success of the control measures.

AP8

Postharvest application of SO_2 volatiles on tomato to prevent Alternaria rot

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Abstract

Tomatoes are prone to postharvest decay resulting in a short shelf-life of less than 2 weeks. Alternaria rot was identified as a major cause of postharvest decay of tomatoes. Sulphur dioxide packaging potentially provides protection against postharvest decay causing pathogens whilst maintaining fruit quality. Two tomato varieties 'Round' and 'Roma' were exposed to a SO_2 sheet applied as a top sheet or bottom sheet in standard 5 kg tomato cartons. The fruit was stored at 10 °C over a period of 14 and 21 days, each with a respective shelf-life storage at 18°C. The results indicated that SO_2 application successfully reduced natural decay development on 'Roma' tomatoes during the 14 d - storage period reducing decay by more than 50 %, even after shelf-life. For long term (21 d) cold storage of 'Roma' tomatoes decay was reduced 60 % with a bottom sheet application. Only marginal decay control (22 %) was observed on the 'Round' tomatoes. SO_2 damage occurred on tomatoes with the application of a bottom sheet. The damage on 'Roma' tomatoes was marginal (5.4 %) but more on 'Round' tomatoes at about 16 %. Overall, the SO_2 damage observed could be easily sorted and removed after cold storage as the damage does not develop further after shelf life. The application of SO_2 did not have any negative effects on other quality parameters such as firmness, brix, and shrivelling.

AP9

Potential sources of *Phytophthora* spp. infestation in South African citrus nurseries

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Abstract

Phytophthora root rot is one of the most devastating diseases in citrus production. Sporadic outbreaks of Phytophthora root rot are often reported in citrus nurseries. The occurrence of outbreaks can be attributed to several factors including the presence of *Phytophthora* propagules in growth media and/or irrigation water, over-seasoning inoculum present in asymptomatic nursery stock and wind-blown particles carrying inoculum. *Phytophthora nicotianae* is commonly found in nurseries of potted ornamental fruit trees and is widespread in South Africa. *Phytophthora citrophthora* is frequently isolated together with *P. nicotianae* and typically occurs in the Western Cape. These pathogens cause severe damage to citrus production. They can also produce large amounts of secondary inoculum in a single growing season. The frequent association of these pathogens with asymptomatic plants make their detection difficult. The main objective of this study was to identify additional sources of *Phytophthora* contamination in citrus nurseries. Surveys were conducted in 16 citrus nurseries and three orchards located in the Limpopo, Mpumalanga, North West, Eastern- and Western Cape Provinces. Samples from water, soil, and potting media were collected for isolation of *Phytophthora* species. DNA was extracted from putative *Phytophthora* isolates using the CTAB DNA extraction protocol for species identification by ITS sequencing using oomycete specific ITS primers, namely, ITS4 and ITS6. A total of 955 soil samples, inclusive of potting media, and 64 water samples were collected from the nurseries and orchards, collectively. From the processed samples, 61 putative *Phytophthora* isolates have been recovered and identified microscopically, and of the 28 putative isolates whose ITS was sequenced, three isolates were confirmed as *Phytophthora* spp.. The other fungal genera recovered were *Fusarium* (12), *Phytopythium* (5), *Pythium* (6), *Neocosmospora* (1) and *Aspergillus* (1). To date, most putative *Phytophthora* isolates were recovered from potting media collected from asymptomatic potted citrus seedlings, indicating a potential contamination source in nurseries, although it is too early to state definitively. The picture may change as more samples are processed and putative *Phytophthora* isolates are identified molecularly.

AP10

Neofusicoccum parvum and other members of the *Botryosphaeriaceae* associated with dieback in pecans in South Africa

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Abstract

Members of the *Botryospheariaceae* are distributed globally and are known to cause dieback and cankers on a wide variety of economically important woody plants. Recently, pecan producers have experienced dieback in pecan trees, which is a growing concern for the industry in South Africa. Previous studies indicated that fungi in the *Botryosphaeriaceae* are regularly isolated from diseased pecan twigs and leaves with symptoms of dieback. In this study, healthy and diseased pecan material were collected from all the major production areas of South Africa. In total, 53 isolates were obtained and characterised using sequence data of the ITS rDNA, EF1-α, and RPB2 gene regions. The data indicated that at least nine clusters or potential species in the *Botryosphaeriaceae* are associated with pecan dieback, including species in *Neofusicoccum*, *Lasiodiplodia*, *Botryosphaeria* and *Dothiorella*. The majority of isolates grouped in *Neofusicoccum parvum*, and these were isolated from both diseased and healthy plant material. Three isolates of *N. parvum* that were respectively isolated from a symptomatic branch, leaf and nut were selected for pathogenicity tests. The pathogenicity trials produced significant lesions on nuts and stems, and only small lesions on leaves. Up to 100 % surface damage was noted in the nut assay within 14 days with all three isolates. Results also indicated that light exposure and high humidity favour disease development in nuts. Side branches of 2-year-old pecan trees produced lesions of up to 276 mm within 1 month after inoculation. In conclusion, *N. parvum* was the most frequent species of the *Botryosphaeriaceae* isolated from pecan in South Africa and was associated with a variety of disease symptoms.

AP11

Evaluation of fungi for dry retting of kenaf

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Abstract

Kenaf (*Hibiscus cannabinus)* is a warm season, annual herbaceous bast fibre plant in the *Malvaceae* family. It is traditionally used as a cordage crop (rope, twine, and sackcloth), but can also be developed into absorbents, paper products, building materials, and animal feed. Efforts over the last 20 years were made to produce kenaf in South Africa. One of the challenges, however, is that the retting process of loosening the cellulose fibres entails the use of bacteria and excessive river water. In South Africa, water is, however, a limited resource and alternative ways need to be found, such as a dry retting process. Under such conditions, fungi that need less water than bacteria, could be used to enzymatically break down the pectin to loosen the cellulose fibres. The present study investigated the use of fungi isolated from kenaf stems in the Winterton area of KwaZulu-Natal for their potential to be used in the retting process. Fungal cultures from the CGJM culture collection of the Department of Plant Sciences (University of the Free State) were also tested. Fifteen fungal isolates from the genera, *Aspergillus*, *Mucor*, *Rhizopus*, *Penicillium*, *Trichoderma*, and an isolate of *Trichothecium roseum* were selected for investigation. Enzyme assays were performed to determine which isolates produced the enzymes required for retting, namely cellulase, pectinase, and lingninases. Nearly all isolates tested, except for *Penicillium expansum* and *Rhizopus oryzae*, displayed cellulase activity. Fungi with the greatest potential to digest cellulose included *Penicillium chrysogenum* 1*, Trichothecium roseum, Mucor* sp. 2*, Aspergillus ochraceus, and Penicillium crustosum*. All tested isolates that displayed pectinase activity are regarded as plant pathogens, except for *Trichoderma viride*. The present study confirmed that a randomly selected isolate of *Trichoderma viride* (CGJM 3511), displayed the greatest and most consistent potential to produce enzymes necessary for retting kenaf at 25 and 30 ^{o}C. The isolate produced pectinase, cellulase, and ligninase correlating to a water activity of 0.887 at 25 ^{o}C and 0.906 at 30 ^{o}C, well within the range required for fungal growth. This isolate was the top producer of pectinase (12.0397 U/mg) and acceptable levels of cellulase (183.6133 U/mg).

AP12

Lophodermium species diversity and distribution on commercial *Pinus* spp. across the Southern Hemisphere

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Abstract

Lophodermium is a large genus with over 100 recognized species on *Pinus* spp., these include the important pathogen *L. seditiosum* and approximately 37 species that are recognized as common endophytes. Isolates of the well-known endophytic species, *Lophodermium pinastri*, collected from infected needles of both native and planted *Pinus* spp. in the Northern Hemisphere have been shown to include cryptic taxa. This, and a few other species, have been reported on non-native *Pinus* spp. in the Southern Hemisphere, but the identifications have mainly relied on morphological characterisation. The aim of this study was to obtain and identify isolates of *Lophodermium* spp. from *Pinus* plantations across the Southern Hemisphere, including Australia, Chile, Colombia, New Zealand, and South Africa. Over 100 isolates were sourced from culture collections or isolations made directly from symptomatic needles bearing characteristic *Lophodermium* apothecia. The morphology of these fungi was studied and a multi-locus (ITS, ACT and TEF1) phylogenetic approach was used to delineate the species. The results confirmed the presence of five *Lophodermium* spp. previously reported in the Southern Hemisphere, although the species boundaries were not clear. A species in the *L. conigenum-australe* complex was present in all countries other than Chile and is recorded for the first time on *Pinus maximinoi*. *Lophodermium indianum* was identified for the first time on *Pinus tecunumanii* and this species was confined to Colombian collections. *Lophodermium molitoris* was found only in the New Zealand collections. Based on phylogenetic analyses, three distinct lineages of *L. pinastri* were present in Australia, Chile, and New Zealand, respectively, and morphological analyses showed several overlapping features. The results support the fact that various cryptic *Lophodermium* species occur on *Pinus* spp. Resolving these boundaries will require larger collections of *Lophodermium* from native *Pinus* spp. in the Northern Hemisphere to establish a robust database for this group of fungi. Such studies are needed in order to evaluate the potential threats of *Lophodermium* spp. to commercial plantation forestry in the Southern Hemisphere.

AP13

Quantifying susceptibility to *Fusarium euwallaceae* in popular avocado cultivars

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Abstract

An ambrosia beetle, commonly known as the Polyphagous Shot Hole Borer (PSHB) *Euwallacea fornicatus*, and its fungal symbiont *Fusarium euwallaceae*, have emerged as an invasive pest in Israel and California (USA), causing severe damage and significant economic losses to agricultural, fruit, forest and ornamental trees, specifically to *Persea americana* (avocado). This fungal symbiont invades and inhibits transport functions of the xylem leading to Fusarium dieback and the eventual death of the host tree. Recently, this pest-pathogen complex was detected on avocado, *Carya illinoinensis* and *Macadamia integrifoli* trees in South Africa. Control management strategies are limited by inefficiencies in trapping, biocontrol and fungicides. However, the use of resistant/tolerant crop trees could serve as a potential control strategy. Previously, the interaction between popular international avocado cultivars and the PSHB or the fungal symbiont have been studied, but there is currently no information available on the susceptibility of important popular cultivars planted in South Africa. The aim of this study was to conduct pathogenicity trials on commercially important and popular avocado cultivars towards *F. euwallaceae* using qualitative and quantitative assessments to assign susceptibility, tolerance or resistance status. Two pathogenicity trials were conducted. The first trial used *F. euwallaceae* inoculated detached avocado branches from 10 different cultivars and measured the discoloured lesions under the bark (upper, lower, left and right directions from the inoculation site). The second trial used inoculated whole plantlets and measured the discoloured lesions under the bark, as well as, quantify the amount of *F. euwallaceae* DNA within each cultivar using q-PCR. The detached branch trial lesion lengths indicated that the majority of the screened cultivars were susceptible to *F. euwallaceae*, with 'Hass' as the most severely affected cultivar. The plantlet trial revealed statistically significant differences in lesion lengths (lower) between infected and control for the 'Fuerte' and 'Hass' cultivars. Whereas, the right lesion length was statistically significant between cultivars, particularly for 'Hass'. No statistically significant differences were observed for quantified levels of *F. euwallaceae* DNA between the cultivars. Our results revealed that popular and commercially important cultivars including 'Hass', 'Maluma®' and 'Fuerte' were susceptible to the pathogen causing Fusarium dieback.

AP14

Three new *Fusarium* species isolated from Eastern Cape dairy farm pastures

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Abstract

During a survey of the fungal diversity associated with potentially 'toxic pastures' in Eastern Cape dairy farms, one of the dominant genera observed was *Fusarium*. These pastures are thought to contain high amounts of sporidesmin produced by *Pseudopithomyces chartarum*. This is an economic important toxin as it causes facial eczema and decreased milk production in dairy cattle. Because *Fusarium* species are well-known for producing many harmful secondary metabolites, we thus questioned whether sporidesmin was the only mycotoxin present in the pastures. The aim of this study was to identify these pasture-inhabiting fusaria to species level, and to provide descriptions for new species. A total of 138 *Fusarium* strains isolated from pastures collected from the Eastern Cape were used in this study. A multi-locus dataset encompassing the translation elongation factor (*TEF*), calmodulin (*CaM*), partial RNA polymerase largest subunit (RPB1), and partial RNA polymerase second largest subunit (RPB2), was generated for these strains. Analysis of these data showed that the vast majority of strains (110) belonged to the *Fusarium incarnatum-equiseti* species complex (FIESC). Here they represented *F. croceum* (n = 41), *F. clavum* (n = 18), *F. coffeatum* (n = 5), *F. brevicaudatum* (n = 3) and *Fusarium* sp. FIESC 27 (n = 1), while the rest were new to science. The latter included three new species, consisting of 16, 23 and 3 strains, respectively, and were compared to close relatives based on growth rates and morphology. Potato dextrose agar and oatmeal agar were used to study macromorphological characters (e.g., colony colour and texture), carnation leaf agar was used to evaluate sporodochia and conidial features (e.g., macroconidia and microconidia, by taking into account their size, shape, and number of septa), and Spezieller Nährstoffarmer agar was used to study conidiophores and chlamydospores. Our findings provide new information on species distribution within the FIESC and the potential of mycotoxin production by pasture infecting FIESC in the Eastern Cape. Our study also forms a baseline for further investigation to understand the role fungal communities play in dairy pastures.

AP15

Isolation and characterisation of phages for potential biocontrol of *Pantoea ananatis* and *P. agglomerans*

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Abstract

With more than 700 000 tonnes produced in 2018-2019 at a gross value of around R 2.3 billion, onions (*Allium cepa* L.) are the second most popular vegetable in South Africa. In 2020 China and the United States, the top onion producers, collectively produced more than 29 million tonnes of onion. Besides being of economic importance, onions are also culturally significant due to their use in cuisine. Onions are susceptible to multiple diseases, one of which is bulb rot. Bulb rot of onion is characterised by foliar blight which progresses to rotting of seed stalks and inner bulb scales. Under favourable conditions yield loss of 100 % is possible. The disease is caused by a complex of bacterial pathogens that include *Pantoea ananatis* and *P. agglomerans.* Both are ubiquitous and often isolated as epiphytes, yet a variety of plants and even humans in some instances can suffer from disease caused by these two species. Considering their role in onion bulb rot, both pathogens are seed-borne and transmitted by insect vectors such as onion thrips. In addition, the emergence of copper-resistant strains and limited management strategies make this a difficult disease to control. Bacteriophages are promising biocontrol agents against plant pathogenic bacteria. However, there have been few reports of phages infecting *P. ananatis* and *P. agglomerans*. This study aimed to isolate and characterise phages infecting these two bacterial pathogens and to assess their efficacy as biocontrol agents. To achieve this, *P. ananatis* and *P. agglomerans* were used as hosts to isolate phages from different sources, including onion bulbs that displayed symptoms of rot, using soft agar overlays. Phages were purified using CsCl density ultracentrifugation and morphologically characterised with electron microscopy. Phage DNA was extracted, subjected to whole-genome Illumina sequencing and gene annotation was performed to ensure the absence of lysogeny and antibiotic resistance genes. One of the phages isolated belongs to an unclassified genus in *Studiervirinae*. Genomic analysis revealed an absence of lysogeny and antibiotic resistance genes, indicating that this phage is a potential biocontrol agent against *P. ananatis*.

AP16

The fungal diversity and mycotoxins associated with South African stored soybeans

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Abstract

Soybeans *(Glycine max)* are a significant source of oil and protein and are a growing component of Africa's agricultural economy. Various fungi can affect yields by causing diseases or their presence may result in elevated mycotoxin levels in seeds. Understanding the fungal diversity and mycotoxin levels in stored seed is considered important to understand its potential impact on soybean production in South Africa. Currently, this knowledge is lacking. Our study thus aimed to isolate, identify and preserve fungi associated with stored soybean seeds collected across South Africa. Surface–sterilised soybean seeds were plated onto potato dextrose agar (PDA), Dichloran 18 % glycerol agar (DG18), and water agar (WA), and incubated for 5–7 days at ±21 ºC. Strains were isolated into pure culture and preserved in vials containing 10 % glycerol and kept at -80 ºC, while DNA was extracted and kept at -20 ºC. Subsequent identifications to genus level were made using morphology. DNA sequences were generated as follows to identify strains to species level: the nuclear ribosomal internal transcribed spacers (ITS; for unidentified strains), partial sequences of beta-tubulin (*BenA*: for *Penicillium*), calmodulin (*CaM*: for *Aspergillus*), translation elongation factor 1-a (*EF1*-a: for *Cladosporium*, *Fusarium* and *Trichoderma*), glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*: for *Alternaria* and other *Pleosporaceae*), and DNA-directed RNA polymerase II subunit *(RPB2*: for *Didymellaceae*). A multi-mycotoxin analysis of soybean seeds was performed at The South African Grain Laboratory (SAGL) using liquid chromatography-mass spectrometry (LC/MS). Isolations and preservations resulted in 553 fungal strains that belonged to 25 genera and 109 species. Most notably, strains belonged to *Aspergillus* (n = 164), *Cladosporium* (n = 88), *Penicillium* (n = 66), *Fusarium* (n = 39), *Didymella* (n = 31), *Alternaria* (n = 31), and *Talaromyces* (n = 26). No mycotoxins were detected in any samples analysed during this study. This survey provides important baseline knowledge on the fungal diversity and mycotoxins of stored soybean seeds, with strains that will be made available for future studies. The DNA sequences generated will also serve as reference data to make future identification faster and easier.

AP17

Carry-over effect of the long-term application of organic amendments on apple replant disease

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Abstract

Apple replant disease (ARD) is a phenomenon where the growth of young apple trees is reduced when trees are replanted into soil previously cultivated with apple or related crop species. In South Africa, ARD is caused by a complex of organisms including *Phytophthora cactorum*, *Pythium* spp., *Pratylenchus* spp., bi-nucleate *Rhizoctonia* AGs, and '*Cylindrocarpon'*-like spp. ARD is managed through pre-plant soil fumigation with chloropicrin and 1,3-dichloropropene in South Africa. Long-term applied organic amendments may have the potential to reduce ARD in subsequent crops. The study investigated this by utilizing five orchards where mulch, compost plus mulch or no amendments have been applied for 6-years on a semi-commercial scale. A glasshouse trial, replicated over time, with the soils showed that mulch combined with compost was the only treatment that resulted in the suppression of ARD, but only in one trial repeat for one of the orchards. Since environmental conditions in the glasshouse are very conducive towards ARD development, tree roots have also been sampled from the five orchards to determine whether the organic amendments were able to reduce the ARD causative agents in apple tree roots under orchard conditions.

AP18

Biodiversity of fungi on maize agricultural soils in the Free state and North-West.

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Abstract

Maize is a staple food in South Africa and our biggest produced crop with a production of 16 211 265 tons recorded in 2021. The Free State and North-West provinces, together with Mpumalanga and KwaZulu Natal are the major maize producing regions. Soil health plays an important role in producing healthy maize. Studying microbial communities associated with maize rhizosphere soil is thus essential for understanding its contribution to healthy soil. The aim of our study was therefore to complete a survey and identify and characterize fungi from rhizosphere soils collected from maize farms in the Free State and North-West provinces. Fungi were isolated by preparing dilution series and plating samples onto potato dextrose agar, Fusarium Selective Media, and water agar, all supplemented with streptomycin and chloramphenicol. Strains were identified using DNA sequences from gene regions appropriate for the genus they belong to (e.g., translation elongation factor 1-alpha (TEF) for *Fusarium* and *Trichoderma* and beta-tubulin for *Penicillium*). Isolations and identifications resulted in 716 strains classified into 29 genera and 84 species. *Fusarium, Neocosmospora, Penicillium* and *Trichoderma* were found to dominate rhizosphere communities. *Fusarium oxysporum, Neocosmospora solani, Penicillium raperi, and Trichoderma afroharzianum* were the most common species in soils across surveyed farms. Other identified genera included *Aspergillus, Chaetomium, Cladosporium, Metarhizium, Talaromyces,* and *Umbelopsis*. Notably, *Fusarium chlamydosporum, F. oxysporum, F. subglutinans, F. temperatum, Neocosmospora solani* and *Setophoma terrestris* were isolated from soils and have previously been reported to cause stalk, root, and /or crown rots of maize plants. Potentially beneficial species commonly used as biological control agents (such as *Beauveria bassiana*, *Clonostachys rosea*, *Metarhizium pinghaense*, *Trichoderma hamatum*, and *Trichoderma gamsii*) were also isolated. The findings presented here provide baseline knowledge needed to start understanding fungal community compositions of maize rhizosphere soils and give us an insight on what soilborne pathogens are associated with maize on these farms.

AP19

Pathogenicity of diverse African isolates of *Cercospora zeina* on maize

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Abstract

Cercospora zeina is the main causal agent for grey leaf spot (GLS) in Africa causing up to 80 % yield loss. High genetic diversity was reported among the *C. zeina* population from South Africa, Zimbabwe, Kenya, Uganda, and Zambia with the latter being the most distant population. In this study, five diverse *C. zeina* isolates were selected with the aim to assess aggressiveness in a glasshouse trial on a susceptible maize hybrid. Five quantitative traits of aggressiveness – disease severity index (DSI), lesion size, infection efficiency, latent period, and sporulation capacity were used to rank the selected isolates in levels of aggressiveness. AUDPC values for the five *C. zeina* isolates differed significantly ($P<0.0001$) with the isolate from Kenya having the highest DSI value (139) followed by South Africa (107), Uganda (52), Zimbabwe (22), and Zambia (15). The same trend was observed in the latent period data where the isolate from Kenya was at 28 days post-inoculation (dpi) followed by RSA (29 dpi), Uganda (31 dpi), Zimbabwe (45 dpi) and Zambia (55 dpi). The results prove that the genetic diversity reported in the African population of *C. zeina* has a profound effect on the aggressiveness level. This knowledge can be used to identify mechanisms and genes involved in the aggressiveness of *C. zeina* which is essential for developing resistant maize cultivars. Additionally, it also helps in maximizing selection gain when relying on artificial inoculation as a way to evaluate the resistance of maize plants to GLS disease.

AP20

Three new species of *Pewenomyces (Coryneliaceae)* from *Araucaria araucana* in Chile

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Abstract

The genus *Pewenomyces* was recently described based on *Pewenomyces kutranfy*, a fungal pathogen that causes cankers on branches and stems of *Araucaria araucana* in Chile. This genus resides in the *Coryneliaceae*, a relatively small Family, and its morphology resembles those of species in the closely related genera *Caliciopsis* and *Hypsotheca*. During the study in which *Pewenomyces* was described, three additional novel taxa were identified from *A*. *araucana*. Cultures of these three putative species were obtained from making isolations from infected plant tissues and in only one case could cultures be made directly from sexual fruiting structures present on the samples. Although preliminary phylogenetic analyses showed that these three putative species were distinct from *P. kutranfy*, their novelty was uncertain given that two species of *Caliciopsis* (*C*. *brevipes* and *C*. *cochlearis*) had previously been described from the same host and location, but for which DNA sequence data were not available. Therefore, the herbarium specimens for these two *Caliciopsis* species were obtained, and their morphology was compared with all known and putative *Pewenomyces* spp. These comparisons showed that none of the *Pewenomyces* corresponded to the previously described *Caliciopsis* species. The results of this study, based on phylogeny using seven gene regions and morphology, consequently, confirmed the presence of three novel species of *Pewenomyces* on *A*. *araucana* and these add to the large diversity of *Coryneliaceae* found in Chilean native forests.

AP21

Fungi and their mycotoxins associated with maize ear rots from emerging farms in the Eastern Cape, South Africa

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Abstract

Maize is an important commodity produced across the world. In South Africa, much of its production is focused on the central provinces. The Eastern Cape contributes less than 1 % of the gross total but is steadily increasing its production and has been identified as a priority region for future growth. During its production, maize is prone to several ear, foliar and root diseases. These are of economic importance as they result in reduced yields, while ear rots may result in mycotoxin contamination of kernels. In this study, we surveyed ear rots from emerging maize farms in the Eastern Cape and tested mycotoxin levels in the maize. Fungal isolations were made directly from moldy cobs and identified using morphology and DNA sequencing. A total of 93 fungal strains were isolated belonging to *Cladosporium* (7), *Fusarium* (68), and *Stenocarpella* (18). The translation elongation factor *1-α (TEF-1-α*) gene region was used to identify *Cladosporium* (2 species) and *Fusarium* (9 species) strains, while the internal transcribed spacer region (ITS) was used for the identification of *Stenocarpella* (2 species). LC-MS/MS multi-mycotoxin analysis of ears collected at each farm was conducted, with 58 % of samples containing deoxynivalenol (DON) at levels well above South African regulatory limits, 58 % of samples containing extremely high levels of diplodiatoxin, while 25 % of samples contained zearalenone (ZEA) but with concentrations below South Africa regulatory limits. Also, 42 % of samples contained low levels of 15-acetyl-deoxynivalenol (15-ADON). A strong correlation was found between samples containing high DON concentrations and the presence of 15-ADON. No fumonisins (FUM) were detected in any samples even though the producing species were present. The contamination of maize with multiple mycotoxigenic fungal species as well as the subsequent mycotoxin cocktails is a concern for food security and consumer health. This study has highlighted the importance of the identification of ear rot causal agents and obtaining a better understanding under which conditions these species may produce mycotoxins.

AP22

Diversity of the *Botryosphaeriaceae* on Macadamia leaves, nuts, and racemes and their association with disease

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Abstract

Species in the *Botryosphaeriaceae* are common in the endophytic microbiome of woody plants and many have the ability to cause disease under conditions of stress. The *Botryosphaeriaceae* are well-known to cause branch die-back and tree declines on Macadamia trees but have also been associated with diseases of nuts and leaves. Little is, however, known about the diversity or the impact of *Botryosphaeriaceae* species on Macadamia plant tissues other than branches. The aim of this study was to characterize species of *Botryosphaeriaceae* from healthy and diseased Macadamia nuts, leaves, and racemes. Symptoms on diseased tissues included die-back of the racemes, husk rot on nuts, and brown leaf blight. Thirteen species were identified based on the analyses of DNA sequence data of the ITS, rDNA, β-tubulin, TEF-1α, and RPB2 gene regions. These included *Diplodia* sp*., D. allocellula, Lasiodiplodia* sp. 1*., Lasiodiplodia* sp. 2*., L. gonubiensis, L. iraniensis, L. pseudotheobromae, L. theobromae, N. algeriense, N. kwambonambiense, N. luteum, N. parvum and N. ribis*. The number of isolates obtained from both diseased and healthy tissues was relatively low for nuts and racemes in comparison to leaves. *Lasiodiplodia theobromae*, *L*. *pseudotheobromae* and *N*. *parvum* were found in high numbers, occurring on both diseased and healthy tissues and on all plant tissues sampled. A greater diversity of species was observed on nuts and leaves in comparison to racemes. This study provided new insights into the diversity of *Botryosphaeriaceae* species on an important nut tree crop in South Africa and provides a foundation to study the role of different species causing disease on these trees. Pathogenicity tests will now be conducted to better understand their association with husk rot and brown leaf blight.

AP23

Mycoflora and mycotoxins associated with cowpea seeds in South Africa

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Abstract

Cowpea *(Vigna unguiculata* (L.) Walp) is an important legume crop for many people living in less developed countries of the tropics and serves as a relatively inexpensive source of proteins for humans. Although it is not considered a commercial crop in South Africa, cowpea plays an important part in subsistence and smallholder farming systems. Cowpea seeds when stored under suboptimal conditions are susceptible to fungal contamination and the subsequent production of harmful mycotoxins. This study was conducted to determine which fungi and mycotoxins are naturally present in cowpea seed, and by so doing establish whether there is a potential health risk for consumers. Forty-eight seed samples were collected from South African subsistence and smallholder farmers from various localities in Gauteng, Limpopo and Mpumalanga provinces and analysed for the presence of mycoflora and mycotoxins. Seeds were plated onto potato dextrose agar, isolated and grouped morphologically and identified using molecular techniques. In total, 16 different fungal genera were identified at various incidence levels. Mycotoxigenic fungal genera such as *Fusarium brachygibbosum, Fusarium chlamydosporum, Fusarium graminearum, Fusarium verticillioides, Aspergillus niger, Aspergillus flavus, Penicillium citrinum* and *Penicillium chrysogenum* were also isolated. Mycotoxins were analyzed using ultra performance liquid chromatography (UPLC) – electrospray ionization (ESI) tandem mass spectrometry (MS/MS). No mycotoxins were found from the seed samples. However, some of the fungi produced mycotoxins such as fumonisin B1 (0.014 – 1092.402 mg/kg), B2 (0. 030-247.906 mg/kg) and B3 (0.026 – 54. 010 mg/kg), ochratoxin A (0.053 - 0.042 mg/kg), Zearalenone (0,036 - 0,277 mg/kg), Deoxynivalenol (0.190 - 0.658 mg/kg) and Nivalenol (7. 550 – 25. 099 mg/kg) when grown on maize patty media. This study confirmed the presence of potentially harmful mycotoxigenic fungi isolated from stored cowpea seed samples across the cowpea-producing areas of South Africa.

AP24

Characterization of potential plant growth promoting rhizobacteria isolated from sweet potatoes (*Ipomoea batatas* (L.) Lam) in South Africa

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Abstract

Sweet potatoes (*Ipomoea batatas* (L.) Lam) are nutritious, drought tolerant crops with a high yield potential. The average yield of sweet potatoes produced in South Africa (SA) is not enough to meet the demands of a growing population as a source of nutritious food and for income generation. The application of plant growth promoting rhizobacteria (PGPRs) has large potential to improve crop production. This study was aimed at characterizing PGPRs associated with sweet potato plants in SA. Potential PGPRs (64 rhizobacteria and 194 endophytic bacteria) associated with sweet potatoes were isolated from sweet potato fields located at the Roodeplaat campus of the Agricultural Research Council (ARC), SA. The isolates were morphologically characterized and tested for their ability to solubilize phosphate, produce siderophores, antagonize soil-borne pathogens, produce indole 3 acetic acid (IAA) and increase the growth rate of sweet potato seedlings. *In vitro* results showed that 17 % of the rhizobacterial isolates produced siderophores and 2 % solubilized phosphate. For endophytic bacteria, 27 % produced siderophores, and 1 % solubilized phosphate. More than 70 % of the total isolates produced IAA at levels of 50 µg/ml and above. None of the isolates (both rhizobacteria and endophytic bacteria) inhibited the growth of *Pythium ultimum* and *Sclerotinia rolfsii* in a dual culture assay. However, 24 % of endophytic bacteria and 16 % of rhizobacteria showed some level of inhibition towards the growth of *Fusarium oxysporum* in a dual culture assay. *In vivo* trials showed that 29 isolates significantly increased the growth of sweet potato seedlings, compared to the control plants. The improvement in the growth of sweet potatoes will enable farmers to produce higher yields, thus leading to improved food security.

AP26

Fungicide sensitivity among South African *Puccinia graminis* f. sp. *tritici* isolates

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Abstract

Insensitivity or resistance to fungicides within fungal populations, resulting in poor disease control, can occur following their widespread, continued and often incorrect application. Systemic fungicides with site-specific mode of action are at higher risk for resistance development, a widespread problem in global agriculture. Genetic variants of the CYP51 gene, encoding the target sterol 14α-demethylase protein of Demethylation Inhibitor (DMI) fungicides, have been associated with varying levels of triazole insensitivity for many plant pathogenic fungi. This study aimed to confirm a possible correlation between CYP51 sequence variants and fungicide insensitivity among 45 South African *Puccinia graminis* f. sp. *tritici* (*Pgt*) isolates collected between 1981 and 2020. Urediniospore germination percentage was measured for each isolate in replicated experiments against the triazole-based fungicides propiconazole and tebuconazole at four concentrations of their active ingredients (0.0313 g/l; 0.0417 g/l; 0.0833 g/l; 0.1667 g/l and 0.0069 g/l; 0.0103 g/l; 0.0138 g/l; 0.0206 g/l, respectively). After determining the EC50 values, significant differences in germination were detected among isolates. The EC50 values for tebuconazole ranged from 0.004 (isolate 22) to 0.018 (isolate 28) with a mean EC50 of 0.013. *Pgt* isolates 13 and 33 (EC50 estimates 0.009), 30 (0.014), 22 (0.004), 16 (0.01) and 11 (0.008) were the most sensitive to tebuconazole. The EC50 values for propiconazole showed a larger range with a minimum of 0.0002 (isolate 44) and a maximum of 0.09 (isolate 41). The mean EC50 was 0.057, with isolates 44 (0.0002), 37 (0.0098), 21 (0.0038) and 11 (0.0006) being the most sensitive. Overall, the *Pgt* isolates were more sensitive to increased active ingredient concentrations of tebuconazole compared to propiconazole with the full recommended commercial dose achieving 100 % germination inhibition for both fungicides. Twenty-three *Pgt* isolates selected for CYP51 sequence analysis are currently being analysed. The results from the study showed that although both fungicides remain effective in the inhibition of urediniospore germination of *Pgt* in South Africa, significant differences in sensitivity do exist among isolates.

AP27

Functional analysis of the AvrSr50 avirulence gene in South African *Puccinia graminis* f. sp. *tritici* isolates

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Abstract

In a gene-for-gene manner, resistance in plants depends on the recognition and binding of an effector protein encoded by the *Avr* gene of the pathogen, by a disease resistance receptor protein encoded by the corresponding plant *R* gene. Mutational variants of the *Avr* gene could evolve rapidly, resulting in virulence acquisition by the pathogen against the deployed *R* gene, making the plant susceptible. The Sr50-AvrSr50 gene-for-gene interaction between wheat and *Puccinia graminis* f. sp*. tritici (Pgt*) was recently confirmed with the cloning of the AvrSr50 gene. The aim of the study was to use allelic sequencing of the AvrSr50 gene to identify mutational variants within South African *Pgt* race isolates. Infection types produced by 45 field and historic isolates of known South African *Pgt* races on seedlings of wheat line Fed*3/Gabo*51BL.1RS-1-1 (+Sr50) included a combination of small (2- to 2=) to medium (2) uredinia with chlorosis. Similar symptoms were observed for variety Gabo (+Sr50), although medium sized (2) uredinia were absent with a higher incidence of necrosis being evident. Sequence analysis revealed that the encoded AvrSr50 protein sequence was highly conserved between most isolates. However, a single base pair deletion occurring in the heterozygous state in four Ug99 race group members, was detected. This deletion resulted in a severely truncated protein, which in a homozygous individual, could lead to Sr50 virulence. However, in its current heterozygous state, the wild type allele is still recognized by the plant, resulting in resistance. These mutational variants will improve our understanding of the potential future evolution of Sr50 virulence within South African *Pgt* race isolates. Wheat breeders should take note of this finding when considering the deployment of Sr50 in local wheat cultivars.

AP28

Population genetic analysis of *Fusarium pseudograminearum* associated with Fusarium crown rot and Fusarium head blight of wheat in different production regions of the Western Cape

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Abstract

Fusarium pseudograminearum is responsible for Fusarium crown rot (FCR) of wheat globally, including South Africa and infects the lower stem (crown) region, resulting in yield losses. The pathogen has also been associated with Fusarium head blight (FHB) of wheat in the Western Cape. Epidemiological differences between FHB and FCR raises the question whether *F. pseudograminearum* populations responsible for these diseases are the same or genetically distinct. The genetic diversity of populations associated with FCR and FHB in the Western Cape was investigated with 210 isolates from six localities using published simple sequence repeat (SSR) markers. Isolates were associated with FCR at five localities (Pools and Langgewens in the Swartland, and Tygerhoek, Napier and Riversdale in the southern Cape) and with FHB at two localities (Vissershok and Napier in the Swartland and southern Cape, respectively). The mating type of the isolates was also determined by PCR. Nine SSR markers were polymorphic, yielding 2-10 alleles per locus. Vissershok population was least diverse (Hs = 0.435) while Langgewens was the most diverse (Hs = 0.544) population. The Pools population could be significantly distinguished from the remaining populations ($P < 0.05$). The population from Langgewens (Fst = 0.260, P = 0.001) was genetically closest to the Pools population while the population obtained from Napier was genetically most distant (Fst = 0.347, P = 0.001). No significant genetic differentiation was found between populations from Langgewens and Tygerhoek (Fst = 0.000, P = 0.702) nor Riversdale and Napier (Fst = 0.003, P = 0.339) populations. Pools is the only locality where the two mating types could be significantly differentiated (Fst = 0.103, P = 0.001). Isolates associated with FHB and FCR could only be weakly distinguished from each other on a genetic basis (Fst = 0.310, P = 0.001). Considering both locality and symptom, no significant genetic differentiation was found (Fst = 0.022, P = 0.056) between populations obtained from different diseases. The results of this study highlight the need to limit the spread of the pathogen to other areas and will lead to a better understanding of the epidemiology of *F. pseudograminearum*.

AP29

Characterising plant health promoting microorganisms (PGPM's) and potential biological control agents (BCA's) from the soybean microbiome.

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Abstract

There is a critical need for new biological products to promote plant health and control highly aggressive plant pathogens, as there is a movement to alter our approach to conventional agricultural practices. In this study, the soybean microbiome was investigated to elucidate whether it can be a source of plant growth promoting microorganisms (PGPM's) and biological control agents (BCA's) for controlling Sclerotinia stem rot caused by *Sclerotinia sclerotiorum*. In 2021, phyllosphere, rhizosphere and endosphere samples of soybean plants from a field site in Delmas, Mpumalanga were collected. A total of 112 morphologically distinct microorganisms were isolated from this material. The study yielded bacteria and yeast that have plant growth promoting properties including, phosphate solubilisation, IAA production and nitrogen fixation. More than 20 isolates were shown to significantly ($P<0.05$) increase germination of soybean seed as well as shoot and root length of seedlings. Furthermore, *in vitro* trials conducted revealed that 30 of the 112 isolates showed varying degrees of growth inhibition of *S. sclerotiorum* mycelium, with three isolates showing great potential. Selected isolates showing potential to be utilised as PGPM's or BCA's are currently being studied in glasshouse and field trials. These results provide potential for the use of indigenous microorganisms associated with crops to be developed into commercial biological products to promote plant health and offer sustainable crop production strategies.

AP30

Survey of fungal and oomycete diversity from maize field soils in the Eastern Cape

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Abstract

Maize is one of the most important grain crops in South Africa. Most of our maize is produced in the Free State, North West, and Mpumalanga provinces, with the Eastern Cape, contributing about 1 % of the total maize produced. However, the province is expected to play a larger role in the future. Grain research in the Eastern Cape has long been neglected and very little is known about the fungal diversity or potential soil-borne plant pathogens occurring in these soils. The aim of this study was thus to complete a survey of fungal and oomycete diversity at maize farms across the Eastern Cape province. Rhizosphere soil samples were collected at 22 farms across four districts in the Eastern Cape. Fungal isolations were made by preparing serial dilutions and plating these onto potato dextrose agar, water agar and Fusarium Selective Media, all supplemented with chloramphenicol (50 ppm) and streptomycin (100 ppm). For oomycetes, the soil was baited using rose petals and leaves, and plated onto the selective media NAR (Nystatin Ampicillin Rifampicin) for *Pythium* and NARPH (Nystatin Ampicillin Rifampicin Pentachloronitrobenzene) for *Phytophthora*. Isolation plates were incubated at room temperature for 7 days, after which colonies of interest were transferred into pure culture. In total, 768 fungal strains were isolated. These were identified using both morphology and DNA sequencing. *Penicillium* (n=182), *Fusarium* (n=172), and *Trichoderma* (n=130) were the most abundant genera found, but *Chaetomium, Cladosporium, Epicoccum, Neocosmospora, Phoma* and *Talaromyces* were also identified. Thirty-four oomycetes were isolated and identified as *Pythium irregulare* and *P. torulosum*. This survey will create much-needed baseline knowledge on the fungi and potential plant pathogens present in Eastern Cape maize farms.

AP31

Hypovirulence associated with Diaporthe stem canker pathogens for biocontrol

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Abstract

Mycoviruses are widespread in fungi, oomycetes, and yeast, with the majority having double-stranded RNA (dsRNA) or positive sense (+) single-stranded RNA (ssRNA) genomes. They are generally transferred vertically (via spores) to progeny or horizontally (via hyphal anastomosis) to other isolates. Some mycoviruses from plant pathogens can induce hypovirulence (reduced virulence) in their fungal hosts. This trait has made mycoviruses attractive as candidates for the development of potential biocontrol tools against major fungal diseases caused by their hosts. Canker and dieback diseases of pome and stone fruit decrease the longevity of trees and can lead to serious losses if not adequately managed. *Diaporthe* species, responsible for major dieback diseases of apple, peach and plum, were tested for hypovirulence and screened for their potential as biocontrol agents on apple. *Diaporthe* isolates displaying reduced growth on PDA medium indicative of potential hypovirulence were used in horizontal mycovirus transmission tests (hyphal anastomosis) with faster-growing vegetatively compatible isolates. The reduced-growth phenotype was demonstrated to be transmitted to several isolates and confirmed on Czapek Dox Agar medium. Hypovirulence of isolates was tested by conducting pathogenicity tests on apple shoots. Mycelial agar plugs from the putative hypovirulent (V+), virulent (V-; faster-growing) and converted (CV; mycovirus-transmitted) isolates were inoculated in triplicate on apple shoots and all isolates were re-isolated after 3 months to fulfil Koch's postulates. Three months post-inoculation, the mean lesion lengths of the converted isolates were comparable to those caused by the hypovirulent isolates whilst being 43 % shorter than those caused by the virulent isolates. The aforementioned 3-month-old lesions were subsequently treated with the corresponding hypovirulent isolate and examined after 3 months. Results from this experiment showed that there was no difference in lesion lengths before and after treatment of converted isolate-inoculated shoots. However, lesions originally caused by virulent isolates decreased in length by up to 69 % and were comparable to those caused by converted (treated or untreated) isolates. These pathogenicity results confirmed the hypovirulence of isolates tested, that this trait was horizontally transmissible, and indicate that with further investigation these isolates have potential as biocontrol tools.

AP32

Exploring soybean and sunflower microbiomes for beneficial bacterial microorganisms.

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Abstract

An array of bacterial and fungal species are harboured by plants in various microbiomes, above- and below-ground, that display beneficial effects on plant growth and yield. Beneficial microbes can, for example, solubilize essential nutrients such as phosphorus and can also produce ammonia and indole-acetic acid (IAA), that improve plant growth, while others produce toxins that inhibit plant pathogens. These organisms can be used in commercial formulations as biofertilisers or for biological control of pathogens. The aim of this study was to isolate and identify beneficial microbes associated with microbiomes of soybean (*Glycine max*) and sunflower (*Helianthus annuus*) that promote plant growth and control the pathogen, *Sclerotinia sclerotiorum*. In 2021, roots, pods (for soybean), stems and leaves of soybean and sunflower plants at different growth stages were collected in Delmas, Mpumalanga. A total of 40 bacteria and yeast were isolated and stored as pure cultures on nutrient agar. Although most bacterial isolates displayed plant growth promoting properties and inhibited four isolates of *S. sclerotiorum*, only 11 bacterial isolates were selected for identification. Selected isolates were sent to INQABA Biotech for sequencing and identification, using 16S-27F and 16S-1492R primers. Identified genera included, *Bacillus* spp*., Lysinibacillus* spp*., Pantoea* spp*., Pseudomonas* spp*.,* and *Sternophomonas* spp. Root length and biocontrol effect experiments were done *in vivo* with water agar media and malt extract agar media, respectively, and incubated at 25°C for a period of 7 days. *In vitro* seedlings trials were conducted in pots with vermiculite and incubated for a 7-day period, thereafter, roots and shoots were measured (mm). Two *Bacillus velezensis* isolates promoted soybean growth and inhibit pathogen colonization by more than 50 %, as well as an unidentified bacterial isolate and one *Bacillus* sp. not distinguished to species level. Further studies are necessary to determine whether these beneficial microbes could be combined with other disease management strategies for soybean and sunflower.

AP33

Organic amendments transformed the apple (*Malus domestica L.)* rhizosphere fungal, but not bacterial, community in an orchard replant soil.

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Abstract

Apple replant disease (ARD) is a direct negative plant–soil feedback that exists worldwide. It represses plant growth and crop yields of apple trees that are replanted into orchard soils previously cultivated to similar plant species. A complex group of organisms (fungi, oomycetes, and nematodes) are among the causative agents of ARD. Organic soil amendments have potential as a sustainable disease management strategy when applied long-term by influencing specific soil microbes and soil quality parameters. Our study investigated changes in the composition of the apple rhizosphere microbiome under glasshouse conditions in response to the long-term application of organic soil amendments (mulch, mulch + compost, or untreated) in an ARD apple orchard. High-throughput amplicon sequencing and amplicon sequence variant (ASV)-based bioinformatics analysis were conducted to elucidate the bacterial and fungal community composition. Proteobacteria comprised the highest relative abundance among the bacterial phyla in all treatments (32- 33 %), with the genus *Conexibacter* being the most abundant (7 %). All treatments possessed a highly similar assemblage in the bacterial communities. The *Ascomycota* dominated the fungal phyla detected in the apple rhizosphere with the untreated (control) having a lower relative abundance (67 %) than the mulch (84 %) and mulch + compost (79 %) treatments. Differential abundance was observed among treatments for the phylum *Chytridiomycota* which had the highest relative abundance in the control (14 %), followed by the mulch + compost (4 %) and mulch (3 %). The genera *Coniochaeta* and *Zopfiella* occurred at higher abundance in the mulch (8 % and 5 %) and compost + mulch (5 % and 3 %) than in the control (1 % and 0.3 %). These genera are known to possess cellulolytic activity. The genus *Entoloma* had a higher relative abundance in the untreated control (2.2 %) than in the mulch and mulch + compost (0.16 % and 0.13 % respectively) treatments. The composition of the fungal communities in the two organic amendment treatments were similar based on a beta diversity analysis and were significantly different (R^2 = 0.16, PPERMANOVA = 0.001) from the control. The study has increased our knowledge on transformation of the apple rhizosphere microbiome induced by long-term application of organic amendments.

AP34

Exploring the cannabis (*Cannabis sativa*) microbiome for beneficial microorganisms

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Abstract

Plant growth promoting rhizobacteria (PGPR's) can greatly impact the health and development of their hosts by increasing nutrient availability, producing plant growth hormones and antibiotics, and priming plant defenses. Endophytic bacteria are also major role-players of a plant's microbiome and can confer similar benefits to their hosts. The cannabis (*Cannabis sativa*) industry is developing fast in South Africa for medicinal purposes and there is a demand for chemical-free end use products. There is therefore a need to develop alternative methods of boosting crop production by means of using biofertilizers such as bacteria that are capable of phosphate solubilization or ammonia (NH3) production. Similarly, bacteria that produce hormones such as indole-3-acetic acid (IAA), which function in regulatory and growth processes, can also be valuable to the industry. The objectives of this study were to isolate endophytic and rhizosphere bacteria from cannabis plants that display the potential ability to improve growth rate or suppress common plant pathogens such as *Botrytis cinerea* Went & Prins.Geerl. Various *in vitro* tests were conducted to determine the most superior bacteria with regards to these specific traits. The tests yielded numerous bacterial isolates that had the ability to produce IAA, solubilize phosphate (with the highest index value being above 7), produce NH3 and inhibit the growth of *B. cinerea* by up to 60 % *in vitro*. These superior bacterial isolates included known beneficial species such as *Bacillus velezensis, Pseudomonas putida* and *Kliebsella oxytoca*. The isolates were subsequently applied to potted plants via soil inoculation to determine their ability to suppress the pathogen *in vivo*. The results confirmed that bacterial isolates that were highly antagonistic to *B. cinerea in vitro* were also able to significantly ($P<0.05$) reduce infection of young cannabis plantlets via soil inoculation.

AP35

And then there were three: A new rust incursion in the South African sugar industry

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Abstract

Puccinia kuehnii E. J. Butler, causal organism of orange rust, was identified on sugarcane in South Africa for the first time in February 2022, after being detected periodically on spore traps located in Mpumalanga since 2016. This is the third rust pathogen to infect sugarcane in South Africa, joining *P. melanocephala* H. & P. Sydow (brown rust) and *Macruropyxis fulva* L.A. Martin, S.A. McFarl. and L.A. Castl. (tawny rust). Orange rust resulted in substantial economic losses in the Australian sugar industry in the early 2000s and has since spread through the Americas and much of Africa, including Mauritius and Reunion. An Emergency Response Plan, developed in anticipation of an orange rust incursion in South Africa, guided the industry's initial response. This included confirmation of pathogen identity through direct sequencing using primers Pk2-F and Pk2-R targeting the internal transcribed spacer (ITS1-5.8S-ITS2) region, the application of a fungicide (a.i. azoxystrobin / cyproconazole) pre-emptively registered for orange rust on sugarcane in South Africa in infected fields, notification of key contacts in southern Africa, and industry-wide surveys to determine prevalence and varietal susceptibility. Long term management will include screening for varietal resistance using molecular and traditional methods and the registration of additional fungicides against the disease. Research on the conditions favouring infection will allow the inclusion of orange rust in the local sugarcane rust forecasting model, while trials to assess the effect on yield will provide an indication of the impact of the disease on production.

AP36

Detection and assessing Potato Virus Y strains infecting tobacco (*Nicotiana tabacum*) in South Africa.

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Abstract

Tobacco (*Nicotiana tabacum* L.) contributes to the country's economy through annual tax contributions and job opportunities. Potato virus Y (PVY) continues to threaten the tobacco industry with infections that results in poor production and low-quality produce. The aim of this study was to identify the different PVY strains infecting tobacco in the major tobacco production areas of South Africa. During the 2016 growing season, the major flue-cured tobacco producing areas (Brits, Rustenburg, Groblersdal, Nelspruit and Vaalwater) in South Africa were surveyed and samples were analysed for the presence for PVY using double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) and nucleic acid-based techniques. Biological assays were conducted by mechanically infecting *N. tabacum* cv. Samsun plants with five selected PVY-positive isolates from the field samples and assessed for symptom development over a 4-week period. From the 116 samples tested, 64.7 % tested positive for PVY, with 8.6 % in Brits, 15.5 % in Rustenburg, 22.4 % in Groblersdal, 10.3 % in Nelspruit and 7.8 % in Vaalwater. Nucleic acid-based techniques confirmed the presence of PVY, and the N strain of PVY, amongst others, was identified as the most common strain infecting tobacco plants. Inoculated healthy *N. tabacum* cv. Samsun plants also expressed virus like symptoms, and these were confirmed to be infected with PVY. The work highlights the importance of continuous investigation of viruses/strains infecting solanaceous crops, to enable the development of management strategies against plant viruses.

AP37

The effect of phosphite treatment against *Phytophthora cinnamomi* on *Leucadendron argenteum* (L.) R.Br.

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Abstract

Phytophthora cinnamomi is recognised as a damaging and important invasive plant pathogen, with over 5000 susceptible host species recorded globally. Previous studies in South Africa have identified *P. cinnamomi* as the cause of Protea root rot. In recent years, high rates of mortality of silver trees (*Leucadendron argenteum*) have been observed in Kirstenbosch National Botanical Gardens (KNBG), with *P. cinnamomi* readily isolated from roots and collar lesions of dying trees. A systemic fungicide, phosphite (Phi) or phosphorous acid (HP03-), is the main form of chemical control against *Phytophthora* diseases. It acts by slowing the growth of the pathogen and inducing defence responses in *Phytophthora* challenged plants. The current study aims to determine the efficacy of phosphite applications in controlling *P. cinnamomi* infection in silver trees. Field and glasshouse treatment trails have been established to determine the optimum phosphite application rates and treatment regimes to confer protection against *P. cinnamomi* in cultivated silver trees. Two hundred silver trees of at least 50 mm diameter were identified at KNBG. Half were trunk injected with two rates of Phi and the other half were left as untreated controls. Seedlings planted into beds known to be infested with *P. cinnamomi* were treated by applying a randomised block approach, with an equal number of treated versus untreated seedlings. All treated and control plants will be monitored to quantify the effect of phosphite on silver tree growth and survival. Another group of seedlings will be grown in a glasshouse to conduct phosphite treatment trials under controlled conditions. This is the first time phosphite treatment has been used on indigenous South African flora. Silver trees are currently classified as rare and vulnerable. It is therefore essential that practical management options be developed to reduce the rate of die-back and mortality caused by *P. cinnamomi*. Preliminary trials show promising results, with 87 % of phosphite-sprayed seedlings surviving 6 months after spraying, compared with 60 % survival of the untreated controls. It is anticipated that the data generated from this study can be used to support restoration projects for this iconic tree species.

AP38

Isolation and identification of three *Bacillus* spp. causing soft rot of spineless cactus pear

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Abstract

Soft rot is a disease of cactus pear that has in recent years become very prevalent on cactus pear orchards in South Africa. Spineless cactus pear (*Opuntia ficus-indica* L. – var. Monterey) cladodes with soft-rot symptoms were collected in a cactus pear orchard established on the campus of the University of the Free State, Bloemfontein. The diseased cladodes were covered with brownish, circular lesions on the surface with rotten tissue inside the cladode. Isolations from the diseased cladodes yielded 15 yeast and bacterial isolates. After following Koch's postulates, only three species of bacteria produced the symptoms originally observed in the orchard. The I6S RNA region of each isolate was sequenced and was subsequently identified as: *Bacillus amyloliquefaciens* (Fukomoto) Priest, *Bacillus methylotrophicus* Madhaiyan. and *Bacillus proteolyticus* Liu. The isolate of *B*. *amyloliquefaciens* was the fastest-growing isolate and fully colonised a 90 mm Petri dish containing Luria-Bertani (LB) agar in 4 days. Artificial inoculation of 'Monterey' cladodes with *B*. *amyloliquefaciens* resulted in necrotic lesions of 850 mm^2, 20 days post-inoculation. After the same period, artificial inoculation with *B*. *methylotrophicus* and *B*. *proteolyticus* resulted in necrotic lesions of 650 and 400 mm^2, respectively. Thirty days after inoculation, lesions produced by *B*. *amyloliquefaciens* had a rotten internal tissue mass of 16.29 g per lesion, significantly more than 8.47 and 7.62 g for *B*. *methylotrophicus* and *B*. *proteolyticus*, respectively. All three bacterial isolates tested positive for cellulose and pectin hydrolysis. The three *Bacillus* species identified in this study threaten the cactus pear industry in South Africa and *O. ficus-indica* varieties must therefore urgently be screened for resistance to prevent the disease from spreading.

AP39

Biocontrol of Rhizopus rot on spineless cactus pear (*Opuntia ficus-indica L.)*

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Abstract

Post-harvest diseases of fruit are controlled mainly by the application of fungicides. There is, however, considerable public concern regarding the safety of chemicals such as fungicides used on produce in agriculture. Biological control of post-harvest diseases using cultures of bacteria and yeasts can be used as an alternative. In the present study, an isolate of the yeast, *Aureobasidium pullulans* de Bary (CBS584.75), was used as a preventative treatment to suppress fruit decay of spineless cactus pear (*Opuntia ficus-indica* L.) caused by *Rhizopus oryzae* Went & Prins.Geerl. Inhibition of *Rhizopus* mycelium *in vitro* by *A. pullulans* was demonstrated in dual cultures on potato dextrose agar (PDA) in Petri plates. Results demonstrated 40 % inhibition of *Rhizopus* mycelial growth in dual culture trials and no inhibition in the control treatment. Studies performed *in vivo* showed that portions of cactus pear fruit dipped in cell suspensions of *A. pullulans* (1 x 10^8 cfu/mL) developed significantly less mould infestation (20 %) than 100 % infestation in the control treatment after 5 days. On the 8th day, mould infestation was 100 % for both treatments. In a separate trial, cactus pear fruit showed a significantly lower mould infestation of 40 % when fruit was first dipped in a 1 x 10^8 cfu/mL yeast cell suspension and then inoculated with *R*. *oryzae* after 24 hours compared to 100 % mould infestation of fruit dipped in distilled water and then inoculated with *R*. *oryzae*, after 8 days of storage. The study demonstrated that an isolate of *A. pullulans* has potential to protect cactus pear fruit against post-harvest decay caused by R. *oryzae*.

AP40

Root growth promotion of spineless cactus pear cladodes using *Debaryomyces fabryi* and *Aureobasidum* pullulans

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Abstract

Spineless cactus pear (*Opuntia ficus-indica* L.) is propagated vegetatively using mature cladodes. Adventitious roots develop from areoles that are in contact with the soil. Root growth is crucial for the growth and development of the newly propagated plant as it grows into a mature plant. Exogenous application of synthetic plant growth regulators such as indole butyric acid (IBA) and naphthalene acetic acid (NAA) can be used to improve the rooting of plant cuttings. However, this is not a common practice in cactus pear propagation due to its ease of forming roots. The current study's objective was to evaluate the root growth promotion ability of two yeast species, *Debaryomyces fabryi* (CBS789) and *Aureobasidium pullulans* (CBS584.75)*, in vivo.* A watery mixture of the yeasts was applied to cladodes at a concentration of $1x10^8$ cfu/mL in three separate trials; the control treatment was similarly treated with sterile water. In the first two trials, mature cladodes of the variety Monterey were harvested in spring, and the trials were conducted in growth chambers. In the third trial, cladodes of one-year-old tissue cultured plantlets of the variety Mexicana were used in pot trials in the glasshouse. In the first trial, 12 areoles displayed roots after 3 weeks of yeast application compared to 6 rooting areoles for the control treatment. Treated cladodes sprouted roots 5 days earlier than untreated cladodes. In the second trial, the root length of treated cladodes was 20 mm compared to 8 mm of the roots in the control treatment. Root length (70 vs. 30 mm), root dry mass (0.25 vs. 0.05 g), and plant height (15 vs. 9 cm) of the plantlets propagated from the treated one-year-old 'Mexicana' cladodes were significantly increased compared to the non-treated control plantlets. Treated plantlets also produced new miniature cladodes. This study demonstrated that a mixture of *A. pullulans* and *D. fabryi* isolates significantly shortened the period of root formation as well as the number of roots produced by cladodes. The yeast mixture also significantly influenced the growth and development of the newly propagated plantlets and therefore has the potential to improve the rooting of cactus pear cladodes under controlled environments.

AP41

Identification and occurrences of fungi and mycotoxin contamination in sorghum grain fractions

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Abstract

Sorghum is the third most significant grain crop in South Africa, after wheat and maize. This study aimed to identify the fungi associated with superficial and deep-seated sorghum grain colonisation. Furthermore, a portion of the grain samples (N = 24) were used to quantify concomitant mycotoxins, i.e., aflatoxin (AF), deoxynivalenol (DON), fumonisins (FB), and zearalenone (ZEA). Harvested grain, of three commercial cultivars, obtained from previous National Cultivar Trials in Kwa-Zulu Natal, planted across seven planting dates was used. Each cultivar-environment replicate was dehulled at 1-, 2-, 4- and 6-minutes (T0 = whole grain; decorticated fractions = T1, T2, T4, T6). The grain was surface sterilised and cultured for fungal isolation and identification. Identification was based on morphological features and by PCR complemented with DNA sequencing with ITS and Ef primers. Higher frequencies of *Bipolaris* spp. and *Epicoccum* spp. were found to colonize superficial layers of the grain (T0 and T1), although these genera were also found in lower frequencies in deep-seated tissues, i.e., endosperm of grain T6. *Colletotrichum* spp. and *Exserohilum* spp. were only identified in superficial layers (T1). Mycotoxigenic fungi associated with the *Alternaria*, *Curvularia*, *Fusarium* and *Phoma* genera were found superficially and deep-seated in grain layers (T1 and T6). One of the *Fusarium* spp. was identified (*Fusarium graminearum*). These results suggest fungi associated with grain surface layers, i.e., hilum and pericarp, are easily removed at the recommended dehulling rates. However, deep-seated mycotoxigenic fungi may constitute a concern to food safety since no amount of economic dehulling would effectively remove it. Mycotoxins were detected in only nine samples, where only one sample exhibited ZEA concentration of 1250 µg/kg, which is higher than the maximum legislative limit. All the recorded DON concentrations were lower than the maximum legislative level but were detected in seven samples. Aflatoxins and fumonisins were not detected. To avoid fungal growth and mycotoxin contamination entering the food chain, it is necessary to establish suitable fungicides to use in the fields and the proper environmental conditions to apply in storage.

AP42

The distribution of White Root Rot caused by *Rosellinia necatrix* on avocado in South Africa

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Abstract

Rosellinia necatrix Berl. ex Prill. is an ascomycete plant pathogen that targets a broad host range and is the causal agent of white root rot (WRR), which has caused significant economic losses within the agricultural and forestry industries in Spain, Israel and Japan. In 2016, the first report of WRR was confirmed in a commercial avocado orchard in South Africa. Therefore, the principal aim of this study was focused on monitoring the spread and distribution *of R. necatrix* in South African avocado orchards. Additionally, a semi-selective medium, containing Rose Bengal, nystatin, cycloheximide, chlorothalonil and 2-phenylphenol, was developed to improve isolation of *R. necatrix*. Previous studies have confirmed that this pathogen is present in the Western Cape on apple, grape and pear. Morphological and molecular results confirmed that *R*. *necatrix* is also present on avocado in the Limpopo, KwaZulu-Natal and Mpumalanga provinces. The semi-selective medium allowed for an average recovery rate of 73.5 %, while acidified PDA (APDA) allowed for an average recovery rate of 43.5 %. Therefore, the semi-selective medium allowed for improved recovery of *R. necatrix* with less contaminants overtaking the growth of the target pathogen. The significance of this study is shown by the fact that *R. necatrix* is a formidable plant pathogen and, once established in an area, control and eradication of WRR has proven very difficult. Therefore, it is crucial to detect and determine the distribution of this pathogen to prevent further spread to different hosts.

AP43

First report of post-harvest decay caused by *Penicillium, Botrytis* and *Rhizopus* spp., in Cape Gooseberry (*Physalis peruviana*) in the Western Cape, South Africa

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Abstract

Cape gooseberries (*Physalis peruviana*) is native to South America and is grown in South Africa as an underutilised crop. Despite the industry being small, South Africa is the second-largest exporter of Cape gooseberries in the world after Colombia. The fruit is consumed fresh, and parts of the plants are used in the medicinal and cosmetic industries. Although Cape gooseberries are hardy and can grow well in various climates, they are susceptible to diseases, including pre-and post-harvest diseases. Yet, in South Africa, these diseases have not been studied. Mature Gooseberry fruit was harvested from an experiment that investigates Cape Gooseberry production on reclaimed soils in tunnels in the Western Cape. Two weeks after harvest, when post-harvest decay became apparent, fungi were isolated from the diseased fruit by plating 2 x 2 mm parts of the disease lesions on fruit onto Petri dishes containing potato dextrose agar. The culture plates were incubated at room temperature and pure cultures were sub-cultured. *Botrytis, Penicillium* and *Rhizopus* spp. were identified. Freshly harvested gooseberries of two cultivars, "Supper Size" and "Buffelsvlei", were sterilised and inoculated with the spore suspensions of the pathogens respectively and incubated at 20˚C for 10 days. In all instances, post-harvest decay was induced in the fruit of both cultivars. The pathogens were successfully re-isolated from the diseased fruit to satisfy Koch's postulates. Hence, this is the first report of these fungi causing post-harvest decay in Cape gooseberries in South Africa.

AP44

An overview of oat diseases in the Western Cape, South Africa

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Abstract

Oat (*Avena sativa* L.) is the sixth-largest cereal crop in the world following wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.) and sorghum [*Sorghum bicolor*,(L.) Moench.]. In South Africa, oat is cultivated under irrigation in the Eastern Cape, Free State and Northern Cape provinces. In the Western Cape, it is mainly grown under dryland conditions. Oat is consumed as a breakfast porridge, but also as an ingredient in health snack bars, biscuits and other products. Oat is also valued as an animal feed as it is a high yielding, fast-growing crop. Although high-quality oats may be produced in large quantities in most areas, oat diseases significantly hamper production. During the 2021 and 2022 oat growing seasons, two oat cultivar field trials were planted under dryland conditions near Caledon and Moorreesburg and two field trials were planted under irrigation near Caledon and Graaffwater in the Western Cape. In both seasons oat diseases were observed and studied. This included crown rust caused by *Puccinia coronata*, which is well known in South Africa, but also oat leaf spot diseases caused by *Alternaria alternata* and *Bipolaris* sp., and leaf blotch caused by *Pyrenophora* sp. These leaf spot and blotch diseases of oat have not been reported in South Africa before. This paper gives an overview of these diseases of oat under dryland and irrigated conditions and gives insight into their potential impact on oat cultivation in South Africa.

AP45

Surveying maize foliar diseases in the Eastern Cape.

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Abstract

The Eastern Cape is fast developing as an important maize producing region in South Africa. Research in the province has, however, long been neglected and very little is known about the diseases that affect maize in the region even though farmers report that diseases are greatly affecting their production. A maize foliar disease survey was thus conducted during the 2020/21 and 2021/22 growing season. The survey focused on four growing districts in the Eastern Cape province for which we determined disease incidence and severity. The most prevalent foliar diseases during the 2020/21 growing season were Northern corn leaf blight (NCLB), eyespot, Phaeosphaeria leaf spot (PLS), common rust and Grey Leaf Spot (GLS). The most severe foliar diseases during the same growing season were NCLB and GLS. During the 2021/22 growing season, the most prevalent foliar diseases were NCLB, common rust, eyespot, Diplodia Leaf Streak (DLS) and GLS, with varied severity observed between districts. The most severe diseases were NCLB and common rust. The survey shows that NCLB is a prevalent and severe disease and is of concern in the Eastern Cape. Fungal strains related with South African grains, especially pathogens, is not widely accessible to the research community. It is thus our goal to isolate fungi associated with foliar diseases, identify them based on modern taxonomic approaches, and expand biological resources available to researchers. To date, close to 1000 strains have been isolated that were classified to 28 genera, including important maize foliar pathogens like *Aureobasidium zeae* (Eyespot), *Cercospora zeina* (GLS), *Exserohilum turcicum* (NCLB), *Stenocarpella macrospora* (DLS), and *Puccinia sorghi* (common rust). We envision this survey to continue for many years to come. This will be important to help understand how diseases affect production and anticipate emerging diseases in the Eastern Cape.

AP46

Seed-borne fungi associated with Pinus seeds in South Africa: Effect on seed germination and pathogenicity studies

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Abstract

Natural occurring and introduced pathogens have been recorded in South African pine plantations. In South African nurseries, it has been noted that *Pinus* seeds often exhibit a low germination percentage, which can be as low as 25 %. This can be attributed to various factors, with seed-borne fungi reported as a potential cause. Seed lots of eight different *Pinus* spp. harvested in South Africa were screened for fungal presence using nutrient rich (potato dextrose agar) and nutrient deprived (malachite green agar) growth media. Numerous fungi, including *Alternaria, Diplodia pinea, Lasiodiplodia theobromae, Pestalotiopsis funerea and Penicillium* spp., were amongst the most frequently isolated. Isolated fungi were purified, grouped morphologically and selected isolates of known and unknown pathogens of pine were subjected to molecular identification. Selected fungi were further evaluated for their impact on seed germination and subsequent seedling development. Certain fungi, such as *Diplodia pinea* and *Sydowia polyspora,* had a negative impact on seed germination and seedling development. Among the mycoflora isolated, *S. polyspora* was isolated from seeds of *P. elliottii, P. maximinoi*, and *P. patula*, which represents the first report of this fungus within South Africa in pine seeds. Pathogenicity tests were carried out by artificial spray inoculation with *S. polyspora* on one year-old *Pinus* seedlings. Typical chlorosis and necrosis disease symptoms were observed on the needles after two weeks post inoculation with a disease incidence of 100 % and disease severity ranging from 5 to 25 % while the uninoculated, control seedlings remained symptomless.

AP47

In vitro fungicide evaluation and antifungal activities of pecan leaf and husk extracts against *Alternaria alternata*: HPLC analysis of phenolic compounds

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Abstract

Alternaria black spot (ABS) of pecans (*Carya illinoinensis*) in South Africa is caused by *Alternaria alternata*. This pathogen impedes the development of pecan trees and leads to low yield in nut production. The study investigated the *in vitro* effect of six fungicides against mycelial growth of *A. alternaria* isolates from ABS symptoms. Fungicides tested include, Ortivia (Azoxystrobin), Ag-Tin (Fentin hydroxide), Bellis (Boscalid + pyraclostrobin), Coptin (Fentin hydroxide), Tilt (Propiconazole), and Bumper 250 EC (Propiconazole). All fungicides were applied in 3 concentrations (0.2, 1 and 5 mg/mL). The assays suggested that propiconazole fungicides have a higher inhibitory effect on the *A. alternata* isolates even at a concentration of 0.2 mg/mL but were significantly effective at 1-5 mg/mL. Future approach would be to conduct field trials to determine if the lowest concentration of propiconazole would still effectively inhibit the pathogen and thus, avoid unnecessary excessive use of fungicides. This study also investigated the antifungal activities of acetone and ethanolic leaves and husk extracts of pecan cultivars Wichita and Ukulinga. Contrary to ethanolic crude extracts assay, the acetone crude extracts with concentration levels at 60-90 mg/mL was shown to have more antifungal efficacy against all the tested *A. alternata* isolates, with inhibition zones ranging from 11 - 39 mm. Acetone crude extracts of cv. Wichita and cv. Ukulinga showed more efficacy against the isolates compared to that of the ethanolic crude extracts and were significantly different ($p = 6.9e^{-4}$). SEM imaging showed major morphological damages on the conidia from assayed cultures. HPLC was used to investigate individual phenolic constituents and total phenolic content in the leaves and husks of both cultivars. Twelve individual phenols were detected in the respective extracts. The total phenolic content was higher in the leaf extracts of Wichita (102.19 mg GAE/g) and Ukulinga (110.13 mg GAE/g) relative to the husks extracts of Wichita (62.03 mg GAE/g) and Ukulinga (85.07 mg GAE/g). This study showed that propiconazole based fungicides are superior in controlling *A. alternata* and should be promoted. In addition, this study also highlights the potential utilisation of pecan bioactive compounds as antifungal agents in future.

AP48

Random amplified microsatellites (RAMS) analysis showed no link to geographical location of *Alternaria alternata* populations causing black spot of pecans (*Carya illinoinensis*) in South Africa

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Abstract

Limited information regarding the occurrence of black spot disease of pecans (*Carya illinoinensis*) associated with *Alternaria* species (spp.) in South Africa is known. The pecan industry is growing rapidly, so it is essential to understand the risk of the fungal pathogen. In this study, the genetic variation of 364 *Alternaria alternata* isolates was investigated by two RAMS primers (CCA5 and CGA5). In total, 6525 alleles were produced, with a minimum of 3182 alleles on the CGA5 primer and maximum of 3343 alleles for CCA5 primer. Further analysis of the primers showed relatively low genetic diversity *of A. alternata* isolate populations, with mean values; (H = 0.12) and Shannon's information index (I = 0.20). The analysis of molecular variance (ANOVA) revealed significant differences between populations, with 88 % of the genetic variation found within populations (*Nm* = 3.59, PhiPT = 0.12), and were not significantly different ($p > 0.001$). However, 12 % variation was observed among populations (*Nm* = 2.89, PhiPT = 0.08) and the estimates were statistically significant ($p < 0.001$). STRUCTURE HARVESTER output showed that K value is K = 8, where ΔK cannot find the true number of populations because of less variation. The dendrogram cluster tree generated by Ward's analysis unveiled two main distinct clades and 10 sub-clades, revealing similar findings as those of PCoA analysis clusters. Therefore, it was evident that these analyses depicted no distinct relationship between the *A. alternata* isolates and their geographic locations or the prevalence of distribution among the populations.

AP49

Zorvec Encantia®, a new tool to manage late blight in potatoes.

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Abstract

Zorvec Encantia® is based on oxathiapiprolin (Zorvec active), the first member of a novel class of fungicides controlling diseases caused by oomycete pathogens. Zorvec Encantia® is a suspo-emulsion (SE) preparation containing 30 g/L oxathiapiprolin (Group 49) and 300 g/L famoxadone (Group 11). The two actives belong to different classes of fungicides. Both offer late blight control with different modes of action. The primary rationale for the combination of the two active substances is the management of the fungicide resistance risk. FRAC has determined that oxathiapiprolin has a medium-to-high risk of developing resistance. A robust resistance management strategy is therefore required. Zorvec Encantia® applied at 0.5 L/ha at 7–10-day spray intervals show a high level of control of late blight. Key attributes and benefits of Zorvec Encantia® are longer-lasting control, giving growers the opportunity to optimize the protection program; rain fastness, ensuring the plant is secured and protected just 20 minutes after application; and protection of new and emerging leaves, an essential attribute at rapid vegetative growth of the crop. In efficacy field tests conducted under conditions of severe disease pressure, Zorvec Encantia® achieved high levels of disease control and outperformed reference products. Zorvec Encantia® demonstrated outstanding protection of established and new growth, contributing to cleaner foliage and healthier plants. Zorvec Encantia® is registered for the control of late blight on potatoes in South Africa. Corteva Agriscience™ plans to develop the product on other crops such as tomatoes and onions for late blight and downy mildew control, respectively.

AP50

Evaluation of *Mangifera indica, Azadiratha indica* and *Hyptis suaveolens* for the management of seedling blight pathogen, *Lasiodiplodia theobromae* of Cashew (*Anacardium occidentale*)

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Abstract

Cashew (*Anacardium occidentale*) is a significant economic crop in Nigeria, whose sustainable production begins in the nursery. Cashew seedlings are susceptible to blight disease causing more than 65 % economic loss. High toxicity of synthetic fungicide necessitates an alternative measure at management. Extracts of *Azadirachta indica*, *Mangifera indica*, and *Hyptis suaveolens* were evaluated in management of *Lasiodiplodia theobromae* and compared with mancozeb. Infected seedlings were obtained from cashew nursery of Cocoa Research Institute of Nigeria (CRIN), assayed and pathogenicity of the isolates determined. Aqueous extracts of *M. indica*, *A. indica* and *H. suaveolens* prepared from the dried milled leaves (1:4 w/v) soaked for 24 hours, 25 % and 50 % concentrations obtained. Fifteen-day old spore suspensions of *L. theobromae* inoculated on seedlings in triplicates 2 weeks after planting (WAP). The infected seedlings were sprayed with the plant extracts singly and in combinations with mancozeb and water as control 2 weeks after infection (WAI) for 10 weeks in a complete randomized design (CRD). Data were subjected to statistical analysis using ANOVA and means were separated using Duncan's Multiple Range Test (DMRT) at 5 % level of significance. Ten fungal species were isolated. *L. theobromae* had highest occurrence and severity. Aqueous extracts of *M. indica* (39.23 mm at 25 %) and *A. indica+M. inidica* (39.33 mm at 50 %) had the highest plant height while mancozeb had 34.70 mm and 20.67 mm at 25 % and 50 %. *A. indica+ M. indica+ H. suaveolens* (5.71 mm and 5.57 mm) had highest girth while mancozeb had 4.62 mm and 5.11 mm. Number of leaves in *A. indica, H. suaveolens* and leaf area in *M.indica + A.indica* and *A. indica* were highest. Seedlings treated with *H. suaveolens* and *A. indica+ H. suaveolens* recorded disease incidence till 8 WAI but at a level significantly better than the control (wilted). Plant extracts showed fungicidal ability against *L. theobromae. M. indica* had the highest efficacy across all treatments and competed favourably with mancozeb. Efforts should be geared towards quantification and field evaluation of the extracts in the management of cashew diseases.

AP51

The antagonist potential of *Trichoderma* spp. against the White root rot pathogen *Rosellinia necatrix*

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Abstract

White root rot (WRR) is an economically important disease that affects many woody plants, including avocado, and is caused by the fungal pathogen *Rosellinia necatrix.* Infection damages the roots of the host plant, compromising water and nutrient uptake. The pathogen also produces toxins that affect photosynthesis. WRR is characterized by the yellowing and wilting of the leaves and eventually the death of the tree. Infection is often characterized by white mycelial growth on the roots and bark of the trees. WRR has caused devastating losses to avocado in Spain and Israel and has recently been reported in commercial avocado orchards in South Africa, where the major root disease of note has always been Phytophthora root rot caused by *Phytophthora cinnamomi*. The current industry standard avocado rootstock Dusa®, is partially-resistant to *P. cinnamomi*, but susceptible to *R. necatrix*, making the emergence of WRR in South Africa very concerning. To date, no rootstock tolerant to WRR has been released commercially. Effective control methods against *R. necatrix* are lacking, with no chemical products currently registered for use on avocado. The fungicide fluazinam and several biocontrol agents have however been shown to be effective against *R. necatrix*. The current study investigated the antagonistic potential of two Trichoderma isolates which form part of the biocontrol formulation Eco-T® and Eco-77®, Trichoderma *asperellum Kd* and *Trichoderma atroviridae* 77B, respectively. *In vitro* dual culture assays were performed to determine the growth of the pathogen in the presence of the *Trichoderma* isolates and the percentage inhibition was determined. Both *T. asperellum* and *T. atroviridae* caused a signification reduction in the growth of the pathogen. Based on the results, it can be hypothesized that *Trichoderma* spp. outcompete the pathogen for space and nutrients. Moreover, by the production of chitinase enzymes that degrade the fungal cell wall. Future work will assess the antagonistic potential of the commercial formulations containing the *Trichoderma* isolates against *R. necatrix* during avocado infection in a glasshouse trial. The findings from this study have important implications for the integrated management of WRR disease on avocado.

4.Climate Change

CC1

Investigations into *Fusarium verticillioides*-maize interactions related to fumonisin production under drought stress conditions – an omics approach

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Abstract

Africa is one of the most vulnerable continents to climate variability and change because of multiple stresses and low adaptive capacity. Yields from rain-fed agriculture could be reduced by up to 50 % in certain areas in the near future, which would further intensify the adverse impact of food security and exacerbate malnutrition. There is reason to believe that climate change can severely affect the profile of *Fusarium* species that infect maize and, thereby, levels of fumonisin contamination in maize. The current research focuses on the molecular mechanisms of *F. verticillioides* fumonisin biosynthesis and its interaction with drought stressed maize plants. The *in planta* study was initiated in the open tunnel at Welgevallen Test farm in Stellenbosch where a total of 300 maize kernels (IMP50-10R) were planted in 15-L plastic plant containers filled with organic coconut coir growth medium. *F. verticillioides* MRC 826-E and MRC 826-P (high fumonisin producing isolates in maize patties) and MRC 826-J (low FB producer in maize patties) were selected for the study. The *Fusarium* isolates were inoculated into the maize ears two weeks after silking using macro conidia suspensions ($2x10^6$ per ml) for silk channel ear inoculation. Water stresses were induced the same day as fungal inoculation by reducing the water flow by 80, 60 and 40 %, respectively in three of the rows with the control row at 100 % water irrigation. Maize kernels were harvested 5 weeks after inoculation and immediately flash frozen in liquid nitrogen. To assess fungal colonisation of the maize ears genomic DNA was extracted and qPCR assays were performed. Fumonisin analysis was also performed. Protein extraction methods were adapted and optimised from the existing methodology utilised in the maize patty cultures. Initial principal component analysis of the fungal proteins of three different MRC 826 subcultures indicate clustering of the proteins separating the high and low watering regimes. The current study into proteomic analyses *in planta* will be essential in understanding the regulatory mechanism involved in fumonisin production as well as pathogen-plant interactions and/or responses during fungal infection under drought stress conditions.

5 Host- pathogen/insect interactions

HP1

Emerging postharvest diseases on Forelle pears in the Western Cape

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Abstract

Postharvest diseases on pome fruit result in great economic losses during storage worldwide annually. Postharvest diseases are caused by a wide diversity of fungal pathogens. There is however no single management strategy that can dependably control postharvest diseases in practice. It is important to understand the epidemiology of the pathogens to develop novel disease management strategies. The aim of this project is to identify what the emerging pathogens are that have been suppressed for decades and to investigate what the biggest problems are that industry is facing. Once this problem is known, only then effective management strategies can be implemented. Approximately 400 Forelle pears were harvested from 11 different orchards in the Western Cape, where they were then stored for three to six months at regular atmosphere at -0.5 °C. Thereafter, the fruit was evaluated for disease symptoms and fungal pathogens isolated. After the three- and six-month storage evaluation, different fruit symptoms were observed. Calyx mould was the most prominent symptom type in both evaluations. When comparing the average percentage incidence between the two evaluations, there was a 33.23 % increase. In conclusion, there is a major physiological shift between the three and six months due to the ripening process of pears. It is when pear fruit reach maturity that latent pathogens emerge. The different causal pathogens are yet to be identified.

HP2

Previously unknown gall symptoms associated with *Uromycladium acaciae* infection on *Acacia mearnsii*

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Abstract

Infection by many *Uromycladium* species native to Australia result in galls on *Acacia* spp. The only rust of Australian *Acacia* spp. in South Africa that produces galls is *Uromycladium morrisii*, which was intentionally introduced into the country for biological control of *Acacia saligna*. *Uromycladium acaciae*, accidentally introduced into South Africa, is known to only cause leaf and petiole infections on *Acacia mearnsii*. In recent surveys of *A*. *mearnsii* plantations in Paulpietersburg, well developed galls were found on trees. Brown or grey powdery masses of rust teliospores were found on the surface of these galls. DNA was extracted from freshly collected spore masses and the LSU gene region was sequenced using rust-specific primers. Surprisingly, phylogenetic analyses of these sequence data showed that the rust was *U. acaciae.* This is the first time that gall symptoms have been associated with this rust and further studies must be undertaken to determine the basis for this unusual symptom development in a relatively well-known rust.

HP3

Extracellular Vesicles and biofilms of a maize fungal pathogen, *Fusarium verticillioides*

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Abstract

Extracellular vesicles (EVs) induce significant functional consequences in fungi as they internalize bioactive molecules including proteins, nucleic acids, and secondary metabolites from a source cell. These luminal contents can be launched into the extracellular environment where they can persist or be taken up by recipient cells. In fungi, EVs contribute to the production of virulence factors including biofilms, the matrixed and architecturally complex community that takes advantage of new environmental opportunities. Biofilms also generate EVs, however, both the importance of vesicles and biofilm formation in *Fusarium verticillioides* (an important maize fungal pathogen), remains poorly established. For this reason, this study aimed to characterize biofilm formation and extracellular vesicles derived from *F. verticillioides* (*Fv*). *Fv vesicles* were isolated from biofilms and planktonic cells and characterized following the MISEV2018 guidelines, which included TEM, NTA, and proteomics analyses. A sporulating culture (¼ strength PDA, homogenized (1 min) in filter sterile or autoclaved 1x PBS) was used to form biofilms by incubating the plates at 25 °C for 24-72 hrs under stationary conditions. Biofilms were then characterized using CLSM and SEM. EV-mediated effects of biofilms on *Fv* growth were analyzed using biofilm-derived EVs that were co-incubated with conidia for 30-60 minutes, following which, uptake of vesicles and their impact on fungal morphology was analyzed using CLSM and on PDA, respectively. *Fv* forms biofilms following a typical model previously reported in other filamentous fungi. A mature biofilm comprises of hyphae intertwined in a visible extracellular polymeric substance. TEM analysis confirms formation of EVs from planktonic and biofilms cells, which show a typical cup-like shape of EVs seen in fungi. According to NTA, planktonic cells mostly release EVs with 150 and 200 nm vesicles, while biofilms seem to release slightly larger vesicles (P-value=0,0165). Planktonic and biofilm derived vesicles are identical in size but significantly differed in concentration, with biofilms containing far less EVs when visualized under TEM. Our pending data on proteomics and uptake analyses, as well as XTT assay will give us more insights into the biology of EVs and biofilms derived from *Fv*.

HP4

Unravelling infection by Avocado Sunblotch Viroid

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Abstract

Avocado sunblotch is a disease caused by *Avocado Sunblotch Viroid (ASBVd*) – a circular single-stranded RNA molecule which is approximately 250 nucleotides in length. The presence of *ASBVd* in an avocado host can lead to the appearance of severe symptoms such as the formation of coloured, sunken lesions on avocado fruit, discoloured streaks on young stems, and discolouration and malformation of leaves. *ASBVd* infection may, however, remain asymptomatic for extended periods of time. While previous studies have investigated the physiological effect of infection on avocado trees and fruit, there is no definitive evidence yet to explain how the viroid causes disease at the molecular level. To this end, we performed an RNA-seq analysis to determine genome-wide transcriptomic changes triggered by *ASBVd* infection of avocado. Leaves were harvested from four *ASBVd*-infected and four uninfected Hass/Dusa® grafted avocado nursery trees, and RNA was extracted using a modified CTAB method. Illumina NovaSeq 6000 was used for mRNA-seq of the avocado samples. We then used Gene Ontology and KEGG pathway analysis to determine which avocado processes and pathways were altered by ASBVd infection. Results of RNA-seq analyses indicated that *ASBVd* infection induced significant changes to the avocado transcriptome. Plant defense response and phytohormone signalling pathways were significantly affected by *ASBVd* infection. This indicates that avocado hosts respond to infection by *ASBVd*, despite the non-coding nature of the viroid. These findings represent the first genome-wide transcriptomic study in *ASBVd*-infected avocado. This study not only provides a basis for in-depth analysis of affected pathways in further investigations, but also improved understanding of the molecular basis for avocado sunblotch disease.

HP5

Optimizing rapid hot water treatments and biocontrol yeast antagonists for suppressing postharvest avocado anthracnose and stem-end rot diseases

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Abstract

The South African avocado industry experiences combined post-harvest losses of about 50 % due to anthracnose and stem-end rot diseases. The fungicide prochloraz has been used extensively to minimize losses to these diseases. However, regulatory authorities in the EU, a major market for SA avocados, have severely reduced the acceptable MRL of prochloraz in avocado fruits, from the end of 2023. Hence, there is an urgent need to develop chemical-free control of postharvest avocado anthracnose and stem-end rot diseases. This study aimed to optimize rapid hot water treatment (rHWT) and yeast biological antagonists to control anthracnose and stem-end rot diseases caused by *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*, respectively. Hass avocado fruits were exposed to rHWT in a series of temperature and time combinations of 20, 45, 50, 55, 60, 65, 70, 75, and 80 °C (± 0.1 °C) x 20, 30, 45, 60, 75, 90, 105, 120 and 180 seconds, to evaluate avocado skin sensitivity to heat treatments. Lower temperatures combined with shorter periods resulted in no skin damage. Subsequent trials for disease control used temperatures and time combination of 25 (room temperature), 52, 54, 56, and 58 °C (± 0.1 °C) x 10, 15, 20, and 30 seconds. The obtained data were subjected to the analysis of variance (ANOVA) and the best control was provided at 56 °C x 10 seconds. The causal organisms of anthracnose and stem-end rot diseases were isolated using Koch's postulate. More than 100 yeast isolates were screened against the two primary pathogens; yeast Isolates I; B; and J performed well, as did a yeast isolate B13, which is under commercial development (Andermatt-PHP (Pty) Ltd). The integration of rHWT of 56 °C x 10 seconds combined with any of the four-yeast isolates provided postharvest disease control comparable to that of the fungicide prochloraz.

HP6

Extracellular vesicle secretion and biofilm formation in the pitch canker pathogen, *Fusarium circinatum*

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Abstract

Lipid bilayer nanoparticles (30-150 nm) called extracellular vesicles (EVs) transport bioactive compounds with a complex array of functional effects on target cells and present as potential biocontrol agents. All microbes form biofilms, which they generally use to cope with harsh environmental conditions. Biofilm formation in filamentous fungi is an understudied phenomenon whose lack of understanding may limit our ability to assess its impacts as a function of fungal virulence. Therefore, we also characterize the role of EVs in the context of biofilm development by isolating EVs from *F. circinatum* planktonic and biofilm cells. *F. circinatum* spores were inoculated and subjected to isolation of EVs after 3-days at 25˚C, from stationery (biofilm phase) and exponential phase (planktonic) using the differential ultracentrifugation method. Physical characterization of EVs was performed using TEM and NTA. Biofilm matrix assemblage was assessed by seeding spores in chamber slides for a period of 72 h and were stained and observed through the confocal microscopy, and the 7-day old matrix was visualized using SEM. TEM results indicate that *F. circinatum* planktonic and biofilm cells secrete spherical EVs at a diameter ranging from 50 – 200 nm. *F. circinatum* biofilm at 72 h exhibited pronounced hyphal growth. The SEM analysis displayed a complex aggregated growth of hyphal bundles and layers embedded in a partially visible self-produced and secreted extracellular polymeric matrix. The study relied on the use of physical characterization but remained aligned with EV guidelines. During biofilm matrix formation, in the early phase, no extracellular matrix could be detected, and this may be due to the cells adjusting to not only the subjected environment but actively attaching to the surface and secreting adhesive substance by germinating spores and active germlings. The maturation stage as observed via confocal microscopy involves the formation of a compacted hyphal network including layering of hyphal bundles stuck together by the exopolymeric matrix, which was later observed using SEM. *F. circinatum* secret EVs and form biofilms both of which are suspected to be involved in the virulence of the fungus. Further studies of the EVs may help shed some light on their possible biochemical/molecular roles in the pathogenesis of *F. circinatum*.

HP7

Production of defensive metabolites by *Pinus patula* x *Pinus tecunumanii* hybrids in response to *Fusarium circinatum* infection

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Abstract

The pathogen *Fusarium circinatum*, causal agent of pitch canker disease, is currently one of the biggest fungal threats to pine health worldwide. Symptomatic infections are associated with a high mortality rate, particularly for nursery and established plants. Pitch canker disease results in reduced growth and significant annual losses in the South African forestry industry. Pines respond to insect damage and fungal infection by forming traumatic resin ducts, as well as upregulating the production of defence compounds. These phytochemicals include terpenes, the main chemical constituents of pine resin, and phenolics, produced in specialized cells of the secondary phloem. Many of these compounds belonging to these two phytochemical groups are known to have inhibitory or lethal effects on pine pests and pathogens. Although most *Pinus* species are susceptible to *F. circinatum*, there is variation in susceptibility to this pathogen among the different species and their hybrids. Resistance of a pine species to the pitch canker fungus is a major determining factor in the species' value to the pine industry; however, the underlying mechanisms of resistance are poorly understood. To explore defense responses between resistant and susceptible pines, we used GCMS and LCMS to characterize the phytochemical changes in young *P. patula* x *P. tecunumanii* hybrid clones in response to *F. circinatum* infection. A significant increase was observed in the concentration of terpenes and phenolics between five- and 14-days post-inoculation. More resistant hybrids plants showed moderate disease symptoms as well as lower concentrations of defensive phytochemicals, both constitutively and in response *to F. circinatum* infection. These findings suggest that increased concentrations of terpenoid oleoresin and phenolics are not part of the defence strategy of pine against infection by *F. circinatum*. Understanding *F. circinatum's* ability not only to overcome, but seemingly benefit from a more severe phytochemical defense response, will help explain the enduring pervasiveness of this global pine pathogen.

HP8

Diplodia seriata main causal organism for black rot on 'Forelle' pears in the Western Cape

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Abstract

Black rot on 'Forelle' pears in the Western Cape has shown an increase in reports from producers and packhouses. The causal organisms of black rot have been reported as *Diplodia seriata* and *Botryosphaeria dothidea* and these reports were made in 1984 using only morphological identification methods and not molecular methods as it was not present at the time. To determine the disease incidence, 500 fruit from 11 orchards across the Western Cape were harvested including the four main production regions (Elgin, Koue Bokkeveld, Warm Bokkeveld, and Villiersdorp). Harvested fruit was stored in cold storage at -0.5 °C under regular atmosphere conditions for three months. Black rot was confirmed in 10 out of the 11 orchards surveyed and a minimum black rot incidence of 0 % and a maximum of 9.17 % incidence was achieved after cold storage. A mean 1.7 % incidence of black rot on 'Forelle' pears in the 2021 production season from this survey was recorded. After isolations were done from symptomatic pears to confirm Koch's postulates, cultures were purified, and the species identity was determined using species-specific PCR. The main causal organism for black rot was determined to be *D. seriata* and *D. seriata* was collected in regions previously determined to be free of this pathogen. This study aided in the increase in knowledge of post-harvest disease incidences in the Western Cape 'Forelle' pear production. Results from this study so far also confirm that *D. seriata* is the main causal organism for black rot on 'Forelle' pears in the Western Cape.

HP9

Botryosphaeria dothidea strategies to establish asymptomatic infections

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Abstract

Botryosphaeria dothidea is a well-known plant pathogenic *Ascomycete* with a global distribution. The fungus causes many important plant diseases and has among the largest known host ranges of species in the *Botryosphaeriales*. Disease symptoms in mature woody plants include leafspot, dieback, branch and stem cankers and in some cases, tree death. Like many other *Botryosphaeriaceae, B. dothidea* is capable of infection without resulting in symptoms and occurs endophytically within hosts. The fungus only becomes pathogenic once a host tree is subjected to considerable biotic or abiotic stress however, the molecular basis of how *B. dothidea* establishes asymptomatic infections and escapes host detection is unknown. Recent work indicates modification and sequestration of fungal cell wall components as a mechanism to circumvent host detection during infection by fungi. The aim of this study was to characterize homologs of genes involved in cell wall component modification/sequestration that may be implicated in avoidance of host detection and investigate potential chitin modification using fluorescent microscopy. Genome analysis indicated that *B. dothidea* possess multiple genes related to chitin deacetylation, chitin oligomer sequestration and α-1,3-Glucan synthesis. Fluorescent microscopy showed patterns of deacetylated chitin (chitosan) as hyphal modification strategies used by *B. dothidea* to evade host detection during the early infection phase. These results provide valuable insights into the mechanisms that *B. dothidea* and potentially other members of the *Botryosphaeriaceae* utilise to establish long term infections of their host plants without eliciting defence responses.

HP10

Involvement of the Ste2 pheromone receptor in chemotropic sensing of signals from pine root exudates.

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Abstract

Plant root exudates are well known to impact the rhizosphere and its associated microbiome. However, the nature of the signals and the mechanisms underlying a pathogen's growth towards or away from chemical signals (i.e., chemotropic sensing) associated with root exudates is not well-studied. Previous work on the fungal pathogen *Fusarium oxysporum* showed that its sex pheromone receptor Ste2 mediates chemotropic interactions between the fungus and its host plant. The objective of our study was therefore to determine if this same pathway operates in a related fungus, *Fusarium circinatum*, which causes severe root disease of pine plants cultivated in commercial seedling production nurseries. To achieve this objective, a quantitative plate assay was developed to investigate the chemotropic growth of *F. circinatum* toward the *Pinus patula* seedling root exudates. The results indicated that wild type strains of the fungus produced significantly more germlines growing toward the root exudates than mutant strains in which the Ste2 gene has been interrupted. In other words, *F. circinatum* exhibits a positive chemotropic response to the root exudate of its plant host, a response which is mediated by Ste2 sex pheromone. Future research seeking to uncover the chemical compounds responsible for this response could potentially reveal alternative avenues for managing *F. circinatum*-associated root disease in pine seedling nurseries.

HP11

Fungal diversity of marama bean seeds, leaves and tubers from South Africa.

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Abstract

The marama bean, encompassing both *Tylosema esculentum* ((Burch.) A.Schreib.) and *Tylosema fassoglense* ((Kotschy ex Schweinf.) Torre & Hillc.), has been identified as an orphan crop of southern Africa with a great potential for commercialization, largely due to the wide range of nutritional and medicinal benefits offered by the seeds and tubers. The potential of the marama bean to be developed into a valuable crop poses a question regarding possible biotic constraints, including fungal pathogens and mycotoxigenic fungi. This study delves into the mycoflora and mycotoxins associated with marama bean in South Africa. Various fungal isolates were obtained from marama bean leaves, seeds and tubers collected from eight locations in South Africa, and identified by single- or multi-gene Sanger sequencing and phylogenetic analysis. Multi-mycotoxin analysis was performed on the plant material as well as on isolated fungal cultures *in vitro* using maize patty cultures, focusing on *Aspergillus*, *Fusarium* and *Penicillium*. A total of 116 species spanning 27 families were molecularly identified, several of which have been previously reported on marama beans in Namibia, including various *Alternaria, Epicoccum, Fusarium, Penicillium, Phoma* and *Rhizopus* species. Other notable fungal genera isolated from the South African marama bean include *Aspergillus, Lasiodiplodia, Neofusicoccum, Botryosphaeria, Chaetomium, Diaporthe, Didymella, Colletotrichum, Trichoderma, Actinomucor, Bipolaris, Curvularia, Aureobasidium, Pestalotiopsis, Neopestalotiopsis, Talaromyces,* and *Nigrospora*. Mycotoxins were not detected in any plant material. However, several *Aspergillus, Fusarium* and *Penicillium* species indicated the potential to produce aflatoxin B1 and fumonisins B1, B2 and B3 *in vitro*. This is the first study of the mycoflora associated with the marama bean in South Africa, the first report of several fungal species associated with the marama bean in southern Africa, and the first report of mycotoxin producing fungi associated with the marama bean.

HP12

Characterising the effect of salicylic acid on Sclerotinia stem rot of soybean.

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Abstract

Resistance inducers, such as salicylic acid (SA), activate the inherent defence mechanisms of host plants to reduce disease severity. Salicylic acid is a widely studied resistance inducer that has been shown to lead to the induction of systemic acquired resistance against a range of pathogens. The potential for SA applications to control Sclerotinia stem rot of soybean (SSR), caused by *Sclerotinia sclerotiorum*, was investigated. Glasshouse trials were conducted with two soybean cultivars, RA437 and RA565, to elucidate the effects of a commercial SA based product (Product X) on SSR. Repeated trials were conducted in a randomised complete block design with four replications per treatment and three plants per replicate. Treatments included four foliar applications, one drench and a control. The first and second foliar treatment of Product X were applied at two contrasting concentrations at three growth stages, the V3 and R3 growth stages and 21 days after R3 application. The third and fourth treatments repeated the above application without the V3 growth stage. The fifth treatment was a drench application at the R3 growth stage and repeated 21 days later. Inoculations with the pathogen were performed at the R3 growth stage for all treatments. The effect of SA was evaluated by conducting a series of assessments, including SSR ratings, dry biomass and yield. Quantification of *S. sclerotiorum* DNA present in the soybean root system was performed by qPCR after trial termination. The study revealed that high dose treatments significantly ($P<0.05$) reduced the effects of SSR on yield, root weight and biomass in the plants. These treatments were further found to effectively reduce the quantity of *S. sclerotiorum* DNA present in soybean roots. These results indicate the potential for SA to be used in an integrated pest management system for the control of Sclerotinia stem rot in soybean.

HP13

Biocontrol of postharvest pathogens infecting avocado using endophytic *Trichoderma* spp.

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Abstract

Fungal pathogens are a major cause of plant diseases and result in significant crop losses. Due to these pathogens, pre-harvest fungal infections cause both pre- and post-harvest avocado diseases. Agrochemicals are being lost to the avocado industry due to EU MRL levels being reduced in many countries, including those of the EU, a major market for South African fruit exports. This study aims to assess the use of endophytic biocontrol agents as potential substitute to replace agrochemicals for the management of these pathogens. Therefore, the objective of this study is to isolate and screen endophytic strains of *Trichoderma* spp. to control key fungal pathogens such as *Colletotrichum, Pseudocercospora, Botryosphaeria, Cladosporium* and *Sphaceloma.* The key fungal pathogens were isolated, as well as 17 strains of *Trichoderma* spp. in or on avocado tissues. These were screened for endophytic properties. Ten of these *Trichoderma* isolates were selected as possible biological control agents, based on their endophytic properties. These isolates were tested *in-vitro* and *in-vivo* using various techniques. Seven of these *Trichoderma* isolates were able to control all the fungal pathogens during *in-vitro* screening (at levels of between 70 and 100 %). The best strains are now being tested for *in-vivo* activity on avocado fruit. These trials are to confirm the potential of endophytic strains of *Trichoderma* spp. to control key pre-harvest infections of avocado fruit by fungal pathogens.

HP14

Functional analysis of a root-knot nematode, *Meloidogyne javanica* effector protein

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Abstract

Root-knot nematodes (RKNs), *Meloidogyne* spp., diverged towards a parasitic lifestyle on plants being among the most damaging families of plant-parasitic nematodes. To establish a compatible interaction with their host, these sedentary obligate parasites deploy several strategies to counteract plant defense responses, including effector proteins synthesized from the oesophageal glands. Previously, a large repertoire of putative effector proteins was identified from the genome sequence of *M. javanica*. However, the specific function of these effectors in mediating successful nematode infection processes is unknown. Here, we characterise the function of a *M*. *javanica* cysteine-rich secretory protein (MjCRSP) in nematode parasitism. Firstly, a search for homologues of MjCRSP in NCBI non-redundant protein database and Wormbase Parasite database was performed with BlastP (E≤1e-10), and four homologues were obtained in RKN species including *M. incognita, M. arenaria, M*. *enterolobii*, and *M*. *floridensis*. Our results showed that this effector protein is highly conserved within RKN species. This could indicate its indispensable role in supporting RKN parasitic lifestyle. Subsequently, this effector protein was cloned and functionally characterised using an Agrobacterium transient expression system in *Nicotiana benthamiana*. Transient expression analysis showed that MjCRSP does not induce a hypersensitive response (HR) but rather, it can suppress infestin 1 (INF1) triggered cell death (ICD). Additionally, MjCRSP also suppressed defense responses associated with the pathogen-associated molecular pattern (PAMP) Flg22. In the future, the subcellular localisation and the spatial and temporal expression of MjCRSP will be examined

HP15

Functional screening of the *Cercospora zeina* Sn1 candidate effector in *Nicotiana benthamiana*

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Abstract

Gray leaf spot (GLS) is a serious yield-limiting foliar disease of maize caused *by Cercospora zeina* in South Africa. Effectors produced by *C. zeina* help it to overcome host defences by altering the structure and function of maize cells to facilitate completion of its lifecycle. Studying the effectorome of *C. zeina* to understand how its effectors work to cause disease in maize can facilitate the development of targeted control measures to reduce its impact on maize crops. To address this, we developed a method for *Agrobacterium*-mediated transient expression of the *C. zeina* Sn1 candidate effector in *Nicotiana benthamiana*. Agro-infiltrated leaves were monitored for cell death necrosis resulting from pathogen effector-mediated induction of the hypersensitive response (HR). The coding sequence of the Sn1 candidate effector was isolated from the genome of *C. Zeina*, and inserted into the pTRAkc-ERH binary vector system for *Agrobacterium*-mediated transient expression assays in *N. benthamiana*. The experiments were complicated by natural *Agrobacterium* resistance to the carbenicillin selection marker on the pTRAk vector. This necessitated PCR screening *Agrobacterium* cultures during culturing prior to Agro-infiltration to ensure that the pTRAk-Sn1 plasmid was not lost during culture. It was also found that intra-leaf variation resulting from the proximodistal age gradient in *N. benthamiana* leaves could significantly skew the results of transient expression assays. The effects of intra-leaf variation on HR were negated by syringe infiltrating pairs of samples to be compared into similarly aged tissues on either side of the main vein. Infiltrated tissues were then outlined with a marker and scored using a leaf scoring system. Leaf tissues were Agro-infiltrated with the pTRAk-Sn1, pTRAk-INF1 and pGR106-INF1 plasmids. Transient expression of the pTRAk-INF1 plasmid caused HR in *N. benthamiana*, indicating the pTRAk system could be successfully used to transiently express transgenes *in planta*. Transient expression of the Sn1 candidate effector did not cause HR or supress INF1-induced HR in *N. benthamiana,* likely due the target of the Sn1 effector being absent from the *N. benthamiana* system or that it targets a process not examined in this study. Future work should examine the Sn1 effector in maize to better understand its role in *C. zeina* pathogenicity.

HP16

A population genetics study of *Fusarium euwallaceae* in South Africa, the pathogenic fungal symbiont of the polyphagous shot hole borer

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Abstract

The polyphagous shot hole borer (PSHB) *Euwallacea fornicatus*, is an invasive ambrosia beetle threatening South Africa's urban forests. PSHB is known to vector an assemblage of fungal symbionts. Of these, *Fusarium euwallaceae* has been identified as the primary fungal pathogen responsible for causing Fusarium dieback. Because pest management strategies for PSHB are limited, investigating populations of *F. euwallaceae* on a molecular level will provide insight into the possible introductory routes of the beetle and the pathogen. This could reveal how the fungal counterpart of the mutualism might be contributing towards the invasive success of the beetle. The aim of this study was to determine the genetic variation of *F. euwallaceae* in South Africa, with the objective to discover if a single clone of the pathogen is being spread countrywide, or if the population contains multiple haplotypes. A multi-locus *(ITS, TEF1-α, RPB2)* phylogenetic analysis was performed to confirm the identity of the isolates included in the study. Microsatellite markers were developed to screen populations of *F. euwallaceae* for genetic variation at 14 loci across the genome. All 35 isolates were confirmed to be *F. euwallaceae* based on *TEF1-α* sequences. There was no variation detected in the *TEF1-α* sequences and no polymorphism detected using the microsatellite markers. Mating type screening using primers developed for invasive AFC members showed all isolates to be of a single mating type, *MAT1*. These results confirmed a clonal population of *F. euwallaceae* in South Africa based on the sites examined and suggests a single introduction of the beetle-fungus complex into the country. It was also found that the isolates in South Africa are identical to those in California, Israel, and Hawaii. This shows that the invasive impact of the beetle is dependent upon the very close relationship that exists between PSHB and *F. euwallaceae*. Of great concern is that a second haplotype of PSHB has recently been found in South Africa's urban environments. This beetle could harbour a more aggressive strain of the pathogen and raises the urgent need for improved genotype screening to prevent new incursions of beetle-vectored invasive AFC species, for example *F. ambrosium* and *F. kuroshium*, in the country.

HP17

Planting date and environmental effects on Sclerotinia head rot progression in sunflower.

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Abstract

Sclerotinia head rot (SHR), caused by *Sclerotinia sclerotiorum* (Lib.) Debary, is a significant sunflower disease in South Africa. This disease affects sunflowers at the flowering stage, significantly reducing yield and seed quality. High humidity at the flowering stage increases SHR disease progression. Planting date (PD) has been reported as a possible escape strategy as it influences the flowering date of sunflower. However, there are only a few studies investigating PD and environmental effects on SHR progression in sunflower. This study aims to investigate how PD and environmental conditions influence SHR progression in sunflower. Here we investigate the effect of these factors on SHR progression in a commercial cultivar, PAN 7080. During the 2020/21 field season, SHR was observed in the field only in the November plantings. Isolates retrieved from the previous field season were used to inoculate plants at each planting date in the current season. A positive control simulated optimal infection condition using paper bag coverings and negative control with no inoculum were used. Plants were monitored, 72 hours after inoculation, every three days until day 36 days after inoculation. Disease scoring of 0-5 was used (0- no symptoms, 1- less than 12, 5 % of the head is infected, 2- between 12, 5-25 % of the head is infected, 3- between 25-50 of the sunflower head is infected, 4- between 50-90 % of the head is infected and 5- over 90 % of the head is infected). SHR progression was three days quicker in positive controls, than in test plants and resulted in severe tissue damage and development of sclerotia at day nine. SHR progressed faster on the October and December plantings possibly due to wet weather experienced after inoculation, on 27 January 2021 and 17 March 2021, respectively. At day nine, 30 % of October and 50 % of December plants showed severe symptoms. In the absence of high humidity and precipitation, SHR progression stopped, even after infecting the plant. The results of this study suggest that environmental factor influence the SHR progression and flowering times coinciding with lower humidity would help limit damage by this pathogen.

HP18

Response of five potato cultivars to *Meloidogyne enterolobii* and *Meloidogyne javanica* infections

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Abstract

Potato (*Solanum tuberosum*) is a well-known food that is regarded as an important component in the worldwide battle against hunger and malnutrition. Potato is the world's fourth most significant food crop and the first most important vegetable crop in South Africa (SA). Phyto-parasitic nematodes (PPN) are a serious limitation in the SA potato industry. Potato, being a tuberous crop, yield reduction is mainly due to tuber quality and quantity. In order to evaluate the host status and host sensitivity of five commercial potato cultivars, vis, BP1, Hertha, Larnoma, Mnandi, and Up-to-date to the southern root-knot nematode (RKN), vis, *Meloidogyne enterolobii* and *M. javanica,* a pot experiment in the greenhouse was conducted. Five separate experiments, each with treatments, namely, 0, 500, 1500, 1200 and 3500 of eggs and second-stage juveniles (J2), were arranged in a randomized complete block design (RCBD) with five replicates. Fifty-six days after treatment inoculation, nematode effect on plant yield was evaluated, potato tubers were assessed for root galls and nematodes reproductive factor (RF) was computed. There were significant differences amongst treatments on the following potato growth parameters: plant height, stem diameter, chlorophyll content, fresh shoot mass, dry shoot mass, number of tubers per plant and fresh tuber mass in both *M. enterolobii* and *M. javanica* ($P \leq 0.05$). The RF was above unity in all potato cultivars for both *M. enterolobii* and *M. javanica*; with *M. enterolobii* showing more aggressiveness compared to *M. javanica*. Infection of potato cultivars by the test nematodes had severe effects on growth components of all the cultivars. Results suggested that all five commercial potato cultivars were susceptible to both *Meloidogyne* species. Thus, there is an urgent need for RKN management intervention in the aforementioned cultivars.

HP19

Multi-environment efficacy of fungicides against Fusarium head blight of wheat and mycotoxin contamination

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Abstract

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum s.s.,* is an important disease of wheat globally, resulting in yield losses, reduced grain quality and contamination of grain with mycotoxins. Fungicides can effectively reduce disease and mycotoxin levels. The aim of this study was to determine the efficacy of several fungicides to control FHB and mycotoxin contamination in different production regions of South Africa. Six commercial fungicides (Abacus Advance, Acanto, Aviator Xpro, Folicur, Miravis Aeon and Prosaro) were evaluated during 2021 in two field trials in the Western Cape (Napier) and Free State (Villiers), respectively. Five wheat cultivars, including a susceptible and resistant control, was planted in a randomised block design with three replicates at each location. Fungicides were applied to bunches of flowering wheat heads, two days prior to inoculation with *F. graminearum s.s*. Treatments included a fungicide + fungus and a fungicide + water treatment, while a positive (fungus only) and negative (water only) control was also included per cultivar. The percentage visually blighted heads were determined three weeks after inoculation. No genotype x fungicide interaction was observed at either locality. Treatments with water only and fungicide + water exhibited lower disease incidence than fungus only and fungicide + fungus treatments. Following inoculation with *F. graminearum s.s.,* Sumai 3 and SST 0166 had the lowest disease incidence at Napier (20.9 % and 30.2 %, respectively), while SST0166 and SST0117 had the lowest incidence at Villiers (63.5 % and 69.6 %, respectively). SST8154 had the highest disease incidence at both localities (74 % at Napier and 74.6 % at Villiers). Abacus (pyraclostrobin + epoxiconazole), Folicur (tebuconazole) and Prosaro (prothioconazole + tebuconazole) performed significantly better than the remaining fungicides at Napier, following fungal inoculation, while Acanto (picoxystrobin) had the highest disease incidence. At Villiers, however, Miravis Aeon (ADEPIDYN™ + azoxystrobin + propiconazole) had the lowest disease incidence, with Acanto and Abacus having the highest disease, albeit lower than the fungus only control. The effect of fungicides on the accumulation of mycotoxins in wheat grain is currently being determined. This study shows that foliar fungicides can contribute to management of FHB and mycotoxins in wheat grain.

HP20

PacBio sequencing to elucidate fungal mutualists associated with *Xylosandrus crassisculus*, an ambrosia beetle commonly found in avocado orchards in South Africa

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Abstract

Ambrosia beetles and their fungal associates have emerged as threats to the avocado industry. Well-known examples of diseases caused by fungal symbionts vectored by beetles include Laurel Wilt caused by *Raffaelea lauricola* vectored by the redbay ambrosia beetle, *Xyleborus glabratus* and Fusarium dieback caused by *Fusarium euwallaceae*, the primary symbiont of the Polyphagous Shot Hole Borer, (PSHB) (*Euwallacea fornicatus*). The recent introduction of the PSHB and its rapid spread throughout South Africa has promoted monitoring efforts to detect and monitor its' spread. In collaboration with CropWatch Africa and the South African Avocado Growers' Association, this project aims to survey avocado orchards in South Africa for the PSHB and other potential harmful species of ambrosia beetles. Black interception traps were baited with ethanol, a general attractant used to detect a diverse range of ambrosia beetles, and quercivorol, a lure for the detection of the PSHB. During the survey the most abundant species was the granulate ambrosia beetle, *Xylosandrus crassiusculus* and it was therefore chosen for mycobiome analysis. *X. crassiusculus* is one of the most geographically distributed ambrosia beetles with an extensive host range of more than 200 tree species. It is considered a serious pest of nursery, landscape and ornamental trees, along with young forest plantations and fruit orchards and is reported to be associated with several pathogenic fungal genera. In order to elucidate the fungal communities associated with *X. crassiusculus*, long-read PacBio sequencing of the internal transcribed spacer (ITS) and translational elongation factor 1a (TEF1a) genes were used to achieve species-level resolution. The ITS gene the dataset consisted of 900 904 reads across ten samples with twenty-one operational taxonomic units (OTUs) identified, which included *Ambrosiella hartigii* and *Beauveria bassiana*. For the TEF1a gene, 630 544 reads were generated across ten samples with six OTUs identified, which included *Ambrosiella roeperi*, *Beauveria peruviensis* and *Penicillium brevicompactum*. *A. roeperi*, a documented symbiont of *X. crassiusculus* was found in all 10 samples and had the greatest pathogen potential based on FUNGuild analysis and therefore was chosen for the pathogenicity trial on three cultivars namely Hass, Fuerte and Pinkerton to assess the potential impact to the avocado industry.

HP21

Precision phenotyping of *Pinus* for resistance against *Fusarium circinatum*

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Abstract

Pine trees are globally important agricultural and ecological tree species. South Africa's forestry industry is economically valuable with *Pinus patula* being one of the most important pine species. Natural and commercial pine forests are at significant risk to the pitch canker fungus, *Fusarium circinatum*, which is one of the most threatening pathogens to pine species. In the nursery, infection by *F. circinatum* manifests as a wilt, significantly affecting pine production. Insights into the genetic resistance of pines and the pine-*F. circinatum* interaction provides a means for effective disease management as *Pinus* spp. exhibit a wide range of inter- and intra-specific variation in susceptibility. This could aid in producing *F. circinatum* resistant planting stock and curb the damage caused by this disease. This study aimed to develop and optimize a quantitative real-time PCR phenotyping tool to quantify the fungal load in different families of *Pinus spp*. and *P. patula* × *P. tecunumanii* F1 hybrids. Six-month-old seedlings were artificially inoculated with *F. circinatum* and tissue was harvested at 14-, 21-, and 28-days post inoculation for DNA isolation. Lesion lengths were measured weekly for 8 weeks. The fungal load, determined using *F. circinatum* specific primers relative to the amount of host tissue, was compared to the mean percentage live stem to identify correlations between the two sets of phenotypes. *P. tecunumanii* low elevation showed the highest average percentage live stem, indicating resistance, followed by the high elevation provenance then *P. patula*, which had the lowest percentage live stem, indicating susceptibility. There was variation in the outcomes for different families of the same species. Our tool will allow phenotypic classes to be developed on a continuum from highly susceptible to highly resistant, specifically to timely and accurately class F1 hybrid pines. These results provide an accurate phenotype for capturing the subtle variation in pine responses to *F. circinatum*. Furthermore, it allows a resistance threshold to be created to select resistant pines for breeding. Future studies involve characterizing the genetic architecture and identifying quantitative trait loci governing resistance against *F. circinatum.*

HP22

Biocontrol mechanisms of rhizobacteria against *Fusarium* spp. associated with root rot in maize (*Zea mays* L.)

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Abstract

Biological control using beneficial soil microbes, particularly those referred to as plant growth promoting rhizobacteria (PGPR), has become one of the emerging sustainable alternative control measures against several phytopathogens that cause soil borne infections on economically important crops. This work presents the characterization of rhizobacteria isolated from the rhizosphere of pristine grasses for their efficacy against three *Fusarium* spp. associated with root rot. *In vitro* antagonistic activity against *Fusarium oxysporum, Fusarium graminearum* and *Fusarium verticillioides* using the dual culture method, and a glasshouse gnotobiotic assay were used for screening of seven PGPR strains that resulted in significant antagonistic activity. The inhibition zones formed and the percentages of disease suppression by the PGPR isolates were significant ($p \leq 0.05$). In the glasshouse gnotobiotic assay, inoculation with the PGPR isolates not only suppressed root rot symptoms by the *Fusarium* spp. tested, but also increased biomass of the maize plants compared to those inoculated with the *Fusarium* spp. but not with the rhizobacteria. To further elucidate and confirm the modes of action of the biocontrol activity by these PGPR, a whole genome sequencing using Illumina HiSeq 2500 and analysis of the genome of one of the effective strains, *Burkholderia* sp. Nafp2/4-1b (=SARCC 3049) was conducted. The analysis confirmed that this strain contains at least two essential biocontrol traits: *PhzF*, an 885 bp phenazine antibiotic biosynthesis protein and *PvdF*, an 840 bp pyoverdine synthase that codes for the biosynthesis of siderophores. This work provides valuable information that helps to leverage the recent efforts in South Africa initiated towards the development of microbial commercial inoculants for use as biocontrol agents against soilborne diseases.

6. Digital Technology in Plant Pathology

DT1

Predicting resistance of *Eucalyptus* hybrid clones to *Chrysoporthe austroafricana* using FT-IR.

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Abstract

Eucalyptus grandis trees are a primary source of timber in South Africa, however due to increased global trade and climate change the trees are experiencing increased pathogen attacks thus affecting timber productivity. *Chrysoporthe austroafricana* is an important fungal pathogen of *E. grandis*, causing cankers on the stems. Current disease resistance screening methods are reliant on natural infection and artificial inoculations. Fourier transform infrared (FT-IR) spectroscopy is a technique that can generate chemical fingerprints which can be coupled with machine learning tools to differentiate between resistant and susceptible phenotypes. The objective of this study was to develop predictive FT-IR models to discriminate among *C. austroafricana* resistant and susceptible *Eucalyptus* hybrid clones. Stem tissue harvested from clones was analysed with FT-IR and chemical fingerprints from FT-IR were further analysed with machine learning tools to compare spectra from resistant and susceptible clones before infection as well as after infection. Results showed that FT-IR is able to correctly classify between resistant and susceptible *Eucalyptus* hybrid clones prior and after inoculation with *C. austroafricana*. The constitutive SVM model correctly classified 74 % (repeated CV mean accuracy) of the ramets with the training data while both the induced SVM models' repeated CV mean accuracies were 80 % for clone ID grouping and 81 % for lesion length grouping. The study shows the potential of FT-IR as a tool for disease resistance screening of *Eucalyptus* hybrid clones. Classification models developed from this study provided good proof of concept on the potential of FT-IR coupled with machine learning for discriminating between resistant and susceptible clones. Further studies will focus on improving the models' accuracies and reproducibility in field.