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Book of Abstracts

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Book of Abstracts

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Functional characterization of *Phytophthora parasitica var. nicotianae* PpRxLR4 effector protein

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Plant-Pathogen/Insect Interaction

Abstract

Phytophthora parasitica is one of the most destructive plant pathogens, affecting over 255 plant species globally, causing an estimated $5 billion in crop losses annually. *It* secretes numerous effector proteins, including core RxLR effectors (CREs), which are delivered into host subcellular compartments to manipulate host immune responses by targeting core host proteins. While previous studies focus on RxLR effectors from other *Phytophthora* species, the role of RxLRs from *P. parasitica* remains largely unexplored. Previous *in silico* predictions from our laboratory identified 71 core RxLR effectors from *P. parasitica*; however, a large portion of these remain functionally uncharacterized. In this study, PpRxLR4, a candidate CRE, was investigated. Blast searches show PpRxLR4 is conserved in four *P. parasitica* isolates and six *Phytophthora* species. Transient expression in *Nicotiana benthamiana* revealed that the GFP-PpRxLR4 does not induce cell death at 5 days post-infiltration (dpi), indicating it likely functions to suppress host immunity. Confocal microscopy demonstrated that PpRxLR4 localizes to the cytoplasm, nucleus, and nucleolus of *N. benthamiana*. Western blot analysis confirmed stable expression of GFP-PpRxLR4 fusion at 2 dpi. Yeast-Two Hybrid screen with *Solanum tuberosum* cDNA library shows that PpRxLR4 interacts with Ribulose bisphosphate carboxylase/oxygenase activase, Phytochrome B, Lipoamide acyltransferase, Soluble inorganic pyrophosphatase (PPA1) isoform X1, Cyanate hydratase, Carbonic anhydrase 2, and Delta-1-pyrroline-5-carboxylate synthase. Many of these proteins share the same localization as GFP-PpRxLR4 and are involved in regulating reactive oxygen species (ROS), particularly hydrogen peroxide, a key driver of cell death. Overall, findings suggest that PpRxLR4 targets host proteins in the cytoplasm and nucleus to suppress ROS-mediated cell death, facilitating *P. parasitica*'s survival during the biotrophic phase of infection.

Keywords

Host-pathogen interaction, Virulence, Reactive oxygen species, Biotrophic phase, Immunity suppression

Impact of crop rotation and cover crops on soil health in a dryland cropping system in the Eastern Free State, South Africa

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Disease Management

Abstract

Soil health (SH) is defined as the ability to function as a living system while sustaining productivity, improving quality and promoting plant, animal, and environmental health. Analyses for SH include chemical, physical and biological properties. However, soil biology is currently understudied. A region's SH status will influence recommendations that can be made to improve SH, such as the inclusion of cover crops. The aim of this study was to evaluate the effect of crop rotation and cover crop inclusion on SH within a long-term potato rotation system in the Eastern Free State. The trial was divided into two five-year cycles. In Year 7, the four rotation systems included a mixture of five cover crops (R1), sugar beans (R2), soybeans (R3), and sunflowers (R4). In Y8 all rotations were maize, while in Y9 (2024/25), half of the plots in each rotation system were maintained fallow and the other half were planted with the cover crop mixture. Soil biological properties measured at the beginning, middle and end of each season included microbial functionality using Biology EcoPlates (expressed as average well color development (AWCD)), and microbial respiration. Throughout each of the three years studied (Y7 to Y9), AWCD was highest in R1 and R4, as well as in the middle of the season compared to the beginning and end of the season. Similarly, respiration was highest in R1 and R4 over the three years. AWCD and respiration were also higher in the cover crop plots in Y9 compared to the fallow plots for all treatments. Increased activity and functionality in R1 indicate that cover crop inclusion has a positive effect on the biological properties, which may be a result of the different crops included in the mixture. This may alter microbial diversity and related functionality, and by extension, improve soil health.

Keywords

Soil biology, soil ecology, microbial functionality, microbial activity, Eastern Free State

Ten years of plant health research in South African botanical gardens: the SANBI-FABI sentinel project

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Biosecurity

Abstract

In 2016, SANBI and FABI launched the collaborative project "Monitoring Tree Health at Sentinel Sites: Botanical Gardens and Arboreta" under the framework of the International Plant Sentinel Network (IPSN). The project aimed to enhance the surveillance and identification of plant pests in South Africa's plant collections, provide a platform for recording baseline information, and support the listing and regulation of invasive plant pests and pathogens. This initiative was based on the recognition that botanical gardens and arboreta are valuable resources for the early detection of biodiversity threats. The project facilitated the detection of recently introduced pests (e.g., the polyphagous shot hole borer [PSHB], Fusarium wilt of palms), the discovery of novel host-pathogen associations, and the identification of previously undescribed microbial species. The project also raised awareness of pest and pathogen threats to plant collections, assisted with the diagnosis of pests and diseases occurring in these spaces, and contributed to the training of garden staff and students in plant health. It served as a platform for the development of biosecurity best practice guidelines to help botanical gardens manage pest risks in their collections and support restoration activities. Importantly, the project contributed to the recognition and management of plant pests and pathogens as invasive species, an area often overlooked. Three risk analyses were conducted for invasive pests and pathogens present in South Africa (i.e., PSHB, *Cinara* spp., and *Phytophthora cinnamomi*), synthesizing evidence to inform South Africa's invasive species regulations. Finally, the project has highlighted the ongoing and critical need for closer collaboration between plant pathologists, entomologists, invasion biologists, and biodiversity conservation organizations.

Keywords

biosecurity, botanical gardens, invasive pests and pathogens, polyphagous shot hole borer, sentinel sites

Microbial biocontrol of penicillium-induced citrus fruit decay: screening and characterization of bacterial and yeast antagonistic isolates

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Disease Management

Abstract

Penicillium digitatum (green mould) and *Penicillium italicum* (blue mould) cause significant crop losses in citrus postharvest. While conventional synthetic fungicides effectively control these pathogens, consumer resistance and environmental concerns have prompted research into biological alternatives. This study evaluated bacterial and yeast strains isolated from citrus leaves and peels for their ability to control green and blue mould. Of the 102 isolates screened *in vitro*, seven promising candidates were selected for secondary screening. Using molecular tools, the two most effective bacterial isolates, UK37 and SCO13, were identified as *Bacillus amyloliquefaciens* strains. In *in vivo* tests on Navel oranges, some of the microbes demonstrated useful levels of disease control: Strain UK37 inhibited *P. italicum* by 96.9% and *P. digitatum* by 98.8%. Strain SCO13 inhibited the two pathogens by 94.2% and 95.3%, respectively. When tested on Valencia oranges stored at 25°C for ten days, *B. amyloliquefaciens* Strain SCO13 demonstrated promising biocontrol potential, inhibiting *P. digitatum* by 64.1% and *P. italicum* by 80.4%.

Using scanning electron microscopy, it was observed that treatment with the biocontrol agents resulted in shriveled hyphae and reduced numbers of conidia being produced by both pathogens. In contrast, strains of previously isolated *Meyerozyma guilliermondii* yeast were ineffective as biocontrol agents. These findings suggest that the biocontrol efficacy of *B. amyloliquefaciens* Strains UK37 and SCO13 rivals the level of control offered by synthetic fungicides, creating a non-chemical alternative for postharvest disease management in citrus production. Further research is needed to optimize production methods, identify a compatible adjuvant, optimize application methods, and assess the feasibility of applying these biocontrol agents to citrus crops on a large scale. In the longer term, commercialization steps and registration processes will need to be completed before a commercial product can be marketed to citrus farmers.

Keywords

Citrus, *Penicillium* Species, Yeast, Bacteria, Postharvest disease management

From reads to results: evaluating sequencing platforms for citrus virus surveillance

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Pathogen Identification, Characterisation and Taxonomy

Abstract

This study explores the application of high-throughput sequencing technologies for virus detection and discovery in citrus plants, a crop of significant economic importance globally and particularly in South Africa. Viral pathogens and viroids pose a major threat to citrus production, necessitating sensitive, accurate, and cost-effective diagnostic tools. While Illumina sequencing has been widely validated in various crops for pathogen detection, the emerging Oxford Nanopore platform offers advantages such as longer read lengths and real-time analysis, which may enhance virus identification and genome assembly despite lower coverage. Three citrus plants, each graft-inoculated with a well-characterized set of pathogens (including three viruses and three viroids) served as the study material. Total RNA was extracted from these plants and sequenced using both the Illumina and Oxford Nanopore platforms. Data analysis focused on quantifying pathogen-associated reads and evaluating genome coverage to assess detection sensitivity and pathogen profile completeness. Additionally, a financial comparison was conducted considering the South African context, particularly examining cost implications when employing sample pooling strategies on the Oxford Nanopore platform. The results confirmed that Oxford Nanopore sequencing successfully detected all known pathogens, achieving reasonable genome coverage that compensated for its lower read depth through longer reads. Illumina sequencing reaffirmed its high sensitivity and comprehensive coverage, consistent with previous studies. Importantly, nanopore sequencing demonstrated cost competitiveness when pooling samples, making it a viable alternative for diagnostics in time-sensitive settings. This study highlights the complementary strengths of both platforms, with nanopore offering promising potential not only for accurate virus detection but also for discovering novel viral agents in citrus. These findings have significant implications for improving plant health surveillance and managing viral threats in citrus production, ultimately supporting sustainable agriculture and biosecurity efforts in South Africa and beyond.

Keywords

High-throughput sequencing, Virus discovery, Illumina, Nanopore, RNA viruses, Viroids

Branch invaders: investigating the *Botryosphaeriaceae* in South African avocados

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Avocado production in South Africa is increasingly threatened by fungal pathogens from the *Botryosphaeriaceae* family. These pathogens are associated with branch cankers, dieback, and fruit rot, ultimately reducing tree productivity (Avenot *et al.*, 2023). Despite their widespread presence in South Africa (Möller *et al.*, unpublished data), the species involved remain uncharacterised. This study aims to identify *Botryosphaeriaceae* species affecting avocado to understand their potential impact on local production systems. Between August and September 2024, symptomatic and asymptomatic avocado material was collected from key production regions of South Africa, including Limpopo, Mpumalanga, KwaZulu-Natal, and the Western Cape. Fungal pathogens were isolated, and *Botryosphaeriaceae*-like isolates were purified and stored. Genomic DNA was extracted, and the ITS region was amplified for genus-level identification. For selected isolates, *TEF-1α* and *β-tub* genes were also amplified. PCR products were sequenced, edited, and identified via BLAST. Sequences were aligned and subjected to maximum likelihood phylogenetic analysis, with robust bootstrap support, incorporating reference strains and outgroups. A total of 256 *Botryosphaeriaceae* isolates were recovered from avocado tissues. These comprised 16 species from four genera: *Neofusicoccum*, *Lasiodiplodia*, *Diplodia*, and *Botryosphaeria*. The most frequently isolated species were *N. parvum*, *L. theobromae*, and *N australe*. *N. parvum* dominated in Limpopo, Mpumalanga, and KwaZulu-Natal, while *N. australe* was most prevalent in the Western Cape. Several less common taxa were also detected, including *L. crassispora*, *L. iranensis*, *L. mahajangana* and *N. kwambonambiense*. The *Botryosphaeriaceae* population associated with South African avocados is diverse, dominated by a few species alongside several rare taxa. These less common species may contribute to future disease outbreaks under changing environmental conditions. This study provides the first comprehensive assessment of *Botryosphaeriaceae* diversity in South African avocados, generating critical baseline data to support early detection, guide disease management, and mitigate potential losses in industry productivity.

Keywords

Botryosphaeriaceae, avocado, branch canker, dieback

Diversity and distribution of pepper ringspot virus associated with *Helianthus annuus* L. in South Africa

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Ecology and Epidemiology

Abstract

Pepper ringspot virus (PepRSV), a bipartite, nematode-transmitted tobravirus, was recently detected on potato in South Africa, where it causes the economically important disease “spraing.” This constitutes both a host and range expansion for the virus and marks one of the most significant phytosanitary incursions in the country in the past decade. Since 2021, foliar ringspot symptoms consistent with viral infection have also been observed on sunflower (*Helianthus annuus* L.), particularly in the eastern Free State. High-throughput sequencing linked these symptoms to PepRSV infection. Between 2021 and 2022, nine symptomatic sunflower samples from the Free State and North-West provinces were analysed via RNA-seq using an Illumina NextSeq 2000. Viral contigs were identified using BLASTx. An additional ~150 symptomatic sunflower samples were collected in 2024–2025 from the eastern Free State. RT-PCR and Sanger sequencing of the RNA1-encoded replicase gene were used to identify PepRSV variants. Diversity within the p2b gene region on RNA2 was analysed using PacBio amplicon sequencing. Metaviromic analysis confirmed that PepRSV was consistently associated with sunflower displaying ringspot symptoms. Sanger sequencing of RNA1 indicated low genetic diversity among isolates. However, difficulties assembling complete RNA2 segments suggested high variability in the intergenic and p2b regions. PacBio sequencing confirmed this, revealing a hypervariable region in RNA2 that ranged from 82–1039 nucleotides in length, with limited sequence conservation among samples. This represents the first report of PepRSV on sunflower, South Africa’s most important oilseed crop. While the economic impact of PepRSV on this host is unclear, it represents an important host expansion and phytosanitary incursion. The distribution of PepRSV on sunflowers appears to be concentrated in the eastern Free State, and transmission mechanisms remain an area of active research.

Keywords

PepRSV, sunflower, variable RNA2

Translating international standards for phytosanitary measures into practice: strengthening disease management in plant health systems

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Disease Management

Abstract

The management of plant diseases requires integrated strategies that are scientifically sound, risk-based, and internationally acceptable. Plant pathogens, (whether bacterial, fungal, or viral), pose threats to trade, food security and biodiversity. The International Standards for Phytosanitary Measures (ISPMs), developed by the International Plant Protection Convention (IPPC) within the Food and Agricultural Organisation of the United Nations, provide a framework to mitigate these risks while facilitating the safe movement of plants and plant products. The IPPC standards are recognised in terms of the agreement on sanitary and phytosanitary measures of the World Trade Organisation. Pest Free Areas, Pest Free Places of Production and Pest Free Production Sites allow countries to demonstrate the absence of plant pathogens, through surveillance. Where absence cannot be declared, Areas of Low Pest Prevalence offer a useful categorisation for pests with low incidence. A systems approach, as provided for in ISPM 14, combine multiple measures to achieve the appropriate level of phytosanitary protection by integrating multiple, independent and dependant phytosanitary measures. The successful implementation of ISPMs requires close collaboration between National Plant Protection Organisations, scientists and industries. Surveillance capacity, diagnostic capability, early detection and rapid response to pest outbreaks are important requirements. Case studies or scenarios will be presented to illustrate how ISPMs have been applied to develop measures to mitigate plant pathogens in high-value crops to ensure compliance with import regulations to enhance the export of fresh South African fruit.

Keywords

Phytosanitary, surveillance, diagnostics, compliance, collaboration

Evaluating the efficacy of biocontrol agents against *Phytophthora cinnamomi*

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Disease Management

Abstract

Macadamia trees are susceptible to *Phytophthora cinnamomi*—a soil-borne pathogen causing severe root rot and stem cankers. The pathogen can be prevalent in accredited and non-accredited macadamia nurseries within seedbeds containing sand or composted pine bark as growth medium, and in seedling roots. This study evaluated the efficacy of seven commercially available *Trichoderma*- and five *Bacillus*-products for managing *P. cinnamomi* in nurseries. Efficacy of the biocontrol agents was evaluated based on five assays: (i) macadamia seedling root colonisation, (ii) *P. cinnamomi* inoculum reduction in sand, (iii) *P. cinnamomi* inoculum reduction in composted pine bark, (iv) *in vitro* inhibition of *P. cinnamomi* via volatile compounds, and (v*) in vitro* inhibition of *P. cinnamomi* via non-volatile compounds. Among the Trichoderma-products, the volatile inhibition assays had the strongest correlation ($P < 0.01$; $r = -0.530$ to -0.570) with seedbed medium performance. For the Bacillus-products, only the non-volatile assay showed a weak but significant correlation ($P = 0.03$; $r = -0.230$) with the pine bark assay. *Trichoderma harzianum A* and *Bacillus methylotrophicus* were the most effective at suppressing *P. cinnamomi* in both sand and pine bark, with greater efficacy in sand. Both strains also successfully colonised seedling roots. *Trichoderma harzianum*A was also the most effective Trichoderma-product *in vitro* although its volatile inhibition did not differ significantly from *T. harzianum B,* which was ineffective in seedbed media. Among the Bacillus products, *Bacillus methylotrophicus* and *B. subtilis* showed the strongest non-volatile inhibition, however *B. subtilis* was ineffective in seedbed media. All *Bacillus* products were ineffective volatile producers. Based on the current data, reduction of *P. cinnamomi* inoculum in seedbed growth media assays are the most reliable predictors of product efficacy under nursery conditions. Future studies will validate this for a subset of biocontrol products on *P. cinnamomi* inoculated macadamia seedlings.

Keywords

Trichoderma; *Bacillus*, management, *in vitro* assay, nursery growth media.

Whole genome sequence data applied for the first time to interrogate the genetic diversity and population structure of *Chrysoporthe austroafricana*

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Ecology and Epidemiology

Abstract

Chrysoporthe austroafricana is an important fungal pathogen of plantation-grown *Eucalyptus*. It belongs to the family Cryphonectriaceae, which also includes *Cryphonectria parasitica*, the causal agent of chestnut blight that devastated the American chestnut in its native range. Chr*. austroafricana* was first identified (initially as *Cryphonectria cubensis*) in South Africa's KwaZulu-Natal province, where it causes a severe girdling canker disease on susceptible hosts. Following its initial discovery, the pathogen was also found on non-native ornamental *Tibouchina* species and native *Syzygium* species. Its occurrence on indigenous hosts led to the hypothesis that Chr*. austroafricana* is native to southern Africa - a view supported by earlier studies of its genetic diversity. However, this hypothesis had not been rigorously tested using whole-genome data until now. In this study, we analysed whole-genome sequence data from 47 isolates of Chr*. austroafricana*, collected between 1998 and 2002 from *Eucalyptus*, *Tibouchina*, and *Syzygium* in KwaZulu-Natal. Sequencing was performed using the Illumina NovaSeq platform (USA), and genome-wide variant calling using GATK generated a high-confidence SNP dataset. These SNPs were used to investigate genetic diversity, population structure, and potential recombination. We also examined the distribution of mating types among the isolates. The Chr*. austroafricana* population exhibited high levels of genetic diversity. While no distinct population structure was observed across different host species, some weak geographic structuring was evident. Both mating types were detected across the population, regardless of host or location; however, evidence of recombination was limited. These results suggest active movement of Chr*. austroafricana* among *Eucalyptus*, *Syzygium*, and *Tibouchina* in KwaZulu-Natal. Notably, the high gene flow between isolates from *Syzygium* and *Tibouchina* implies that these hosts may act as reservoirs of genetic diversity for the pathogen. This has important implications for breeding disease-resistant *Eucalyptus* species and for the sustainable management of plantations in the region.

Keywords

Canker disease, Cryphonectriaceae, Host-shift, Management, Myrtales

Complexity of Citrus Liberibacter diagnostics and psylloid vector surveillance in Africa

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Biosecurity

Abstract

Huanglongbing (HLB) in Africa is associated with three related bacteria; '*Candidatus Liberibacter asiaticus*' (CLas), '*Ca*. L. africanus' (CLaf) and '*Ca*. L. africanus subsp. clausenae' (CLafCl). CLaf is present in central, eastern and southern African countries and is spread by the indigenous African citrus triozid (ACT), *Trioza erytreae* (Del Guercio, 1918) (Hemiptera: Triozidae). CLas was first reported on the African continent, in Ethiopia, in 2010, followed by reports in Uganda and Tanzania. A South African-led survey found that CLafCl was misidentified as CLas in Uganda and Kenya. CLafCl was detected widely in Uganda, Ethiopia, and Kenya. The presence of CLas in Ethiopia was confirmed, but the report of CLas in Kenya is still not confirmed. CLas is naturally vectored by *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), commonly known as Asian citrus psyllid (ACP) and currently detected in East and West Africa.

Surveillance to detect ACP incursions and to monitor populations of ACT and associated Liberibacters was conducted in citrus environments in southern Africa since 2021. Morphological identification was supported by molecular barcoding. A panel of real-time assays was used for Liberibacter detection, and conventional PCR and Sanger sequencing were done for verification. Molecular phylogeny supported the distinction of seven triozid species, including ACT. CLaf was only detected in ACT, and CLas was not detected in southern Africa. We developed tools to distinguish ACT from closely related non-pest, indigenous species. Similarly, we detected undescribed, indigenous *Diaphorina* spp., morphologically similar to ACP, some of which harbor *Liberibacter* spp. detected by diagnostic assays for CLas and CLaf. Identification of citrus psylloid pests and *Liberibacter* detection is complex in the African context. Assays used to detect HLB pathogens differ in their ability to differentiate citrus pathogens from other, related bacteria present in psylloids and rutaceous host plants and require an integrative assay approach.

Keywords

Diaphorina citri, *Trioza erytreae*, *'Candidatus Liberibacter asiaticus'*, Huanglongbing, disease incursion

Investigating the efficacy of biocontrol agents in combination with fungicides as pruning wound protectants on grapevine rootstock mother blocks

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Disease Management

Abstract

Rootstock mother vine heads can be severely infected with grapevine trunk disease (GTD) pathogens which can move through the vascular tissue to new canes used for grafting. Pruning wound protection on rootstock mother vines is required to prevent initial infection. The aim was to assess fungal and bacterial biological control agents (BCAs) alone and in combination with fungicides as pruning wound protectants. Fungal and bacterial isolates were screened *in vitro* against *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, *Neofusicoccum parvum*, and *Diaporthe ampelina*. Fungal isolates were tested for volatile and secondary metabolite production, and the bacterial isolates were screened through competition growth, secondary metabolite assays and hypersensitivity reaction. The sensitivity of the pathogens toward boscalid, DDAC, fluazinam, pyraclostrobin, tebuconazole and thiophanate-methyl were assessed at various concentration ranges. The best-performing fungicides were evaluated against 24 BCA isolates. BCAs, fungicides and combinations were assessed on Richter 99 detached shoot assay. A total of 121 fungal and 96 bacterial BCA isolates were screened. Eight *Trichoderma*, three *Penicillium*, two *Aureobasidium*, one *Clonostachys*, and nine bacterial isolates exhibited strong inhibitory activity *in vitro* against four GTD pathogens. Selected fungal isolates had more than 60% inhibition of all GTD pathogens. However, one bacterial isolate had a hypersensitive reaction on tobacco and was therefore excluded. Tebuconazole, fluazinam and DDAC were effective against all four GTD pathogens and were further assessed to be used in combination with shortlisted BCAs. Thiophanate-methyl was not effective against *Pm. minimum* or *P. chlamydospora.* The detached shoot assay is currently underway. Several fungal and bacterial BCAs with potential (alone and in combination with fungicides) to control four GTD pathogens were identified. A pruning wound protectant could improve the phytosanitary status of propagation material by preventing pathogen infection and minimising the risks of spreading GTD pathogens through infected propagation material.

Keywords

Grapevine trunk disease, integrated disease management, secondary metabolites, propagation material, phytosanitary status

Genetic diversity of banana wilt associated phytoplasmas in Papua New Guinea and the Solomon Islands

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Biosecurity

Abstract

Lethal yellowing of cooking banana and coconut in Papua New Guinea has resulted in devastating losses of these crops to local growers since the early 1990s. The disease is associated with a novel phytoplasma '*Candidatus Phytoplasma noviguineense*' in the Madang Province. Other closely related banana wilt associated phytoplasmas (BWAPs) occur in different regions of PNG and the Solomon Islands. Symptoms in banana include severe leaf wilting and necrosis of vascular tissue. Losses are attributed to failure to produce fruit bunches, undeveloped fruit, wilting and plant death. In coconut, yellowing of leaves that droop and lead to early nut fall and tree mortality. The BWAPs from other regions of PNG and the Solomon Islands are only associated with disease in banana. Many phytoplasma disease of vegetable crops that result in vegetative malformations have been studied and effectors of these phytoplasmas have been defined. Although several phytoplasmas associated with lethal yellowing have been described, little research has been done regarding their pathogenicity. Very few phytoplasma diseases of banana have been described, and these have also received little attention. The differences in host range of the BWAPs is also not understood. To address this, whole genome sequencing and assembly of representatives of each BWAP was performed. Annotated genome assemblies were used to identify putative effector genes, genes that are conserved within BWAP taxa, and genes that can explain the host divergence among the different BWAPs. The results obtained from putative effector gene identification will contribute to knowledge regarding pathogenicity genes that have not been studied in phytoplasmas associated with lethal yellowing type diseases. Comparative genomics of the different BWAPs elucidate unique biological features associated with this group of phytoplasmas, and differences identified among the three BWAPs explain variation in the host range in different regions where BWAPs occur.

Keywords

wilt disease; taxonomy; comparative genomics; bacteria; 16S group

Assessing the efficacy of the Alter-Rater risk model in managing Alternaria brown spot of citrus in South Africa

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Disease Management

Abstract

Alternaria brown spot (ABS) of citrus, caused by Alternaria alternata in South Africa, is a serious disease that affects tangerines and their hybrids. Severe infections may lead to leaf and fruit drop, and significant losses in marketable fruit and yield. Control of ABS relies heavily on fungicide applications and cultural methods to reduce disease severity. This study aims to validate the Alter-Rater risk model, developed in Florida, USA, to accurately predict A. alternata infection periods and time fungicide sprays for ABS control under South African conditions. The accuracy of infection periods predicted by Alter-Rater was evaluated over three seasons by placing 'Nova' mandarin bait trees in a 'Nova' mandarin orchard in the North-West province. Bait trees were removed after each infection period, and the observed disease incidence and severity data were correlated with the predicted infection periods and weather data collected from a weather station placed near the orchard. An orchard spray trial was carried out to compare the effectiveness of sprays based on Alter-Rater to standard calendar-based and biological control spray programmes. While calendar sprays followed typical grower practices, Alter-Rater sprays were done just before predicted infection periods. Disease severity on leaves and fruit was assessed after infection periods and before harvest, respectively. Correlations were made between the weather data, the predicted infection periods, and the severity ratings throughout the season. Results showed that the Alter-Rater spray timing aligned closely with calendar programmes during a hot, humid season conducive to ABS infection, and the same number of sprays was applied. The Alter-Rater model could possibly reduce the number of sprays in seasons that are not as conducive for ABS. This study has validated the Alter-Rater model and its strong potential to aid South African citrus growers to optimise timing of fungicide applications, ultimately improving ABS control and fruit yield.

Keywords

Alternaria alternata, risk-predicting model, spray programme, 'Nova' mandarin orchard, weather

Shifts in the potato soil microbiome under changing temperature and CO_2 levels

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Ecology and Epidemiology

Abstract

Climate change poses a significant threat to agroecosystems, possibly adversely affecting plant health and productivity. The soil microbiome is vital to soil health, ecosystem resilience, and productivity, but has been shown to be sensitive to climatic shifts, particularly elevated temperature and carbon dioxide (CO_2) levels. This study investigated taxonomic shifts in the potato soil microbiome under elevated temperature and CO_2 levels in the context of climate change in South Africa, where average temperatures are projected to rise twofold compared to global averages. Two growth chamber trials were conducted with four temperature-CO_2 treatment combinations, with maximum temperature maintained for one hour in the first trial and for four hours in the second. Alpha diversity remained largely unaffected, but significant shifts in beta diversity revealed changes in microbial community composition. Elevated temperature showed enrichment of taxa that might have thermo-tolerant traits, such as Gemmatimonadota and Bacillota, while elevated CO_2 was associated with increased relative abundance of genera such as *Saccharomonospora* and *Flavobacterium*. Previous literature has reported these genera to be both beneficial to ecosystem functions, such as nutrient cycling and plant growth promotion, as well as pathogenic to crops. Fungal taxa showed a less pronounced response, but genera such as *Cladosporium* and *Penicillium* appeared to show increased relative abundance under combined elevated temperature and CO_2 conditions. However, agriculturally significant genera like *Fusarium* and *Aspergillus* tended to exhibit reduced abundance under these conditions. These findings suggest that while microbial richness and evenness remained stable, shifts in community composition may reflect a microbial response to environmental stress, potentially supporting resilience to changing climatic conditions. Elevated CO_2 emerged as a primary driver of microbial community composition. However, long-term implications for nutrient cycling and soil productivity require further investigation. This study highlights the importance of understanding soil microbiome dynamics under climate change to develop resilient agroecosystems.

Keywords

Microbial community composition, Microbial diversity, Climate change, Soil Health, Potato agroecosystem

Comparing citrus RNA extraction methods to boost virus and viroid detection accuracy

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Pathogen Identification, Characterisation and Taxonomy

Abstract

RNA extraction methods have a critical role in the detection of citrus pathogens, specifically viruses and viroids. Accurate detection is vital for citrus certification programs to ensure the propagation of healthy, pathogen-free plants, and effective disease management. Ten citrus plants were inoculated with a defined set of viruses and viroids, including citrus tristeza virus (CTV), citrus virus A (CiVA), and three viroids (CEVd, CDVd, HSVd). RNA was extracted using four protocols: CTAB:LiCl (CL), CTAB:Ethanol (CE), Trizol:LiCl (TL), and SDS:Isopropanol (SI). An additional experiment was conducted that involved five plants infected with the same pathogen profile, from which RNA was extracted using three methods: SI, adjusted CE, and CTAB:Isopropanol (CIs). Extracted RNA underwent RT-qPCR to quantify pathogen RNA concentration and Illumina high-throughput sequencing to assess influence of bioinformatic detection. The extraction method significantly influenced detection efficiency of the pathogens differentially as determined by RT-qPCR and HTS. The CL protocol yielded the highest CTV RNA copy numbers, while SI was most sensitive for CiVA detection. Viroid detection varied, with CE and CL providing reliable results for CEVd and CDVd, and CE performing best for HSVd. In additional comparison with five plants, SI outperformed in virus detection and produced viroid results comparable to the other protocols. Variability in viroid detection across protocols likely reflects uneven viroid distribution within plant tissues, emphasizing the need for representative sampling and RNA extraction methods that maximize recovery. Despite variation across RNA extraction methods, all target pathogens were detected, showing that current approaches are effective and that adjustments can ensure detection. Optimizing RNA extraction protocols is essential for improving diagnostics in citrus certification schemes. Identifying effective extraction methods enhances pathogen detection reliability, ensuring disease-free propagation material for sustainable citrus cultivation. This study provides a foundation for refining diagnostic workflows in citrus pathology.

Keywords

RT-qPCR, High-throughput sequencing, HTS, Citrus certification, Citrus tristeza virus

Identification and characterisation of a putative virulence effector from *Puccinia graminis* f. sp. *tritici*

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Plant-Pathogen/Insect Interaction

Abstract

Puccinia graminis f. sp. *tritici* (*Pgt*) is a destructive fungal pathogen of wheat. Central to its virulence is a repertoire of secreted effector proteins that manipulate host immunity to promote fungal development. Once delivered to the host cells, effector proteins suppress plant defence mechanisms to facilitate fungal colonisation. This study aims to identify and characterise a putative *Pgt* effector gene that promotes virulence in *Pgt*-inoculated wheat. Transcriptomic analysis identified five effector-like genes that were significantly upregulated in *Pgt*-inoculated wheat. After cloning the five genes, *in vitro* synthesised double-stranded RNA was used to silence the expression of each gene in the *Pgt*-inoculated susceptible wheat Line 37-07 using Spray-Induced Gene Silencing (SIGS). Two genes that, when silenced, led to significantly reduced disease symptoms in treated plants, were selected for further bioinformatic and expression analyses. Silencing of two of the five candidate genes resulted in a significant reduction in disease severity compared to the untreated control plants. Both silenced genes demonstrated a decrease in the number and size of uredinia. These genes were selected for further characterisation coded for a predicted Class II aldolase/Adducin N-terminal domain protein and a GTP-binding nuclear protein. Two putative virulence effector genes were identified from *Pgt*. Further analysis would provide important insights into their role in the *Pgt* virulence response in susceptible wheat.

Keywords

Puccinia graminis f. sp. *tritici*; wheat; effector proteins; virulence; Spray-Induced Gene Silencing (SIGS)

The potential of anaerobic soil disinfestation for managing apple replant disease in South Africa

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Disease Management

Abstract

Apple replant disease (ARD) threatens apple production globally, causing stunted growth and reduced yields in young apple trees replanted on the same site. Although pre-plant soil fumigation is currently the standard treatment, alternatives are needed to control soilborne pathogens. Anaerobic soil disinfestation (ASD) is a strategy that has shown efficacy for controlling ARD and soil-borne pathogens at levels similar to that produced by fumigation. ASD efficacy is highly influenced by the type of labile carbon source utilised. ASD has not been evaluated in South Africa; therefore, our research aims to evaluate the efficacy of ASD using different, locally available carbon sources. Carbon sources, evaluated in independent trials, were externally sourced (sourced-in; fruit pomace and hay) or field-grown (triticale, Japanese radish and black oats). The carbon sources were incorporated into two orchard soils, placed in pots, watered to field-capacity and tarped with plastic. Following completion of the ASD process the pots were planted with apple seedlings. ASD efficacy was evaluated by assessing anaerobicity and apple seedling growth responses. Both orchard soils amended with carbon sources became anaerobic whereas unamended treatments remained aerobic. Efficacy based on seedling assays for the sourced-in carbon sources differed between the two soils, and in trial repeats. ASD conducted using the sourced-in carbon sources showed promise, albeit inconsistently. The ASD trial utilising field-grown carbon sources, which requires additional replication, showed that black oats significantly improved seedling growth, but only in one orchard soil. The degree of anaerobicity did not correlate with ARD treatment efficacy. Ongoing work aimed at quantifying ARD marker causative agents may provide insights regarding differences in observed carbon source dependent efficacy of ASD between the two orchard soils and trial repeats. Variations in soil type and the soil microbiome may have also contributed to the variable efficacies.

Keywords

Sustainable disease management, Soilborne disease

Phylogenetic analysis of South African isolates of *Puccinia striiformis* f. sp. *tritici* and *P. graminis* f. sp. *tritici*

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Disease Management

Abstract

New races of both *Puccinia striiformis* f. sp. *tritici* (*Pst*) and *P. graminis* f. sp. *tritici* (*Pgt*) are regularly detected in South Africa (SA), which may result from either local mutational adaptations of existing races or through foreign incursion events into the country. The latter is a major concern for wheat production in SA, since the associated risk factors of such new incursions are unknown. The aim of this study is to develop a standardised screening protocol to assess risk factors associated with unknown field isolates of *Pst* and *Pgt* in SA using MARPLE diagnostics. Isolates of *Pst* collected in SA, Lesotho and Zimbabwe, as well as isolates of *Pgt* collected in SA, Zimbabwe and a reference isolate from Uganda, were genotyped to determine their genetic relationships with a panel of international isolates. Using MARPLE diagnostics (https://marple-diagnostics.org/), 387 *Pst* and 281 highly polymorphic *Pgt* gene regions were sequenced with the Oxford Nanopore MinION platform to produce a maximum likelihood tree. The *Pst* isolates formed two distinct genetic clades separate from most of the international isolates. Isolates of *Pst* races 142E30A+, 7E22A- and 6E22A+ grouped with isolates from Kenya (PstS1) and Azerbaijan (PstS2), whereas isolates of *Pst* races 6E16A-, 6E22A- and 142E30A+ grouped with an isolate from Sweden (PstS4) in the second clade. While re-typing of some isolates is needed to confirm their race identity, the study suggests that considerable genetic diversity exists within races. The *Pgt* isolates also formed two distinct genetic clades with one consisting of isolates of Ug99 race variants and the other of non-Ug99 race variants. This confirms the division of the *Pgt* population as found in previous microsatellite marker analyses. These findings provide valuable information for future surveillance studies by providing a baseline reference source to identify unknown field isolates.

Keywords

Puccinia striiformis f. sp. *tritici*, *Puccinia graminis* f. sp. *tritici*, wheat, MARPLE diagnostics, fungicide sensitivity

Epidemiology of dry flower disease on macadamia in South Africa

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Ecology and Epidemiology

Abstract

Dry flower disease of macadamia, caused by *Neopestalotiopsis* and *Pestalotiopsis* species, has been reported to cause significant yield loss. This study aimed to investigate the epidemiology of dry flower disease of macadamia in South Africa. Research was conducted during the 2022 and 2023 flowering season in Mpumalanga province at three different locations (Barberton, White River, and Hazyview) and on three different cultivars ('A4', 'Beaumont', and 'HAES 849'). The effect of temperature on colony growth of *Neopestalotiopsis* spp. was determined *in vitro*. Weekly spore trap samples were evaluated microscopically and macroscopically to count *Neopestalotiopsis*/*Pestalotiopsis* conidia and colony-forming units. The timing of infection and disease incidence was assessed fortnightly on inflorescences. Samples of inflorescences and putative *Neopestalotiopsis/Pestalotiopsis* isolates (obtained from inflorescences and spore traps) were subjected to molecular techniques to detect and identify *Neopestalotiopsis* and *Pestalotiopsis* spp. The interaction between temperature and *Neopestalotiopsis* isolates was statistically significant (P = 0.006). Optimal colony growth of *Neopestalotiopsis* spp. was observed at 20°C and 25°C. Airborne conidia of *Neopestalotiopsis/ Pestalotiopsis* spp. were detected in all the orchards before flowering, with the highest occurrence observed on the 'Beaumont' at Hazyview. Few conidia were present during bud development/raceme elongation; however, time of infection evaluations detected *Neopestalotiopsis*/ *Pestalotiopsis* spp. in immature, non-symptomatic inflorescences before symptoms were visible, indicating that infection can occur as early as bud development. Symptoms were observed at all stages of flowering. Conidia and DNA of *Neopestalotiopsis*/ *Pestalotiopsis* spp. were detected at all stages of flowering, with the highest occurrence observed during peak flowering/anthesis. Findings from this study are useful for identifying critical infection periods, developing accurate predictive tools, and developing effective control strategies.

Keywords

Pestalotioid fungi, blossom blight, pathogenic ecology, tree nut, conidia abundance

Unidirectional Mating-Type Switching in *Ceratocystis*: A Unique Sexual Strategy Shaping Pathogen Adaptation

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Sexual reproduction is a key evolutionary force shaping pathogen diversity and adaptability. In filamentous ascomycetes such as *Ceratocystis* species, sex is governed by the structure of the mating-type (*MAT1*) locus. Isolates can be self-fertile or self-sterile, depending on the genetic composition of their *MAT1* locus. Intriguingly, all known *Ceratocystis* species reproduce via a rare strategy known as unidirectional mating-type switching, where the self-fertile state is unstable and lost through irreversible deletion of specific *MAT* genes. This results in progeny pools that include both self-fertile and self-sterile individuals. This review explores what is currently known about this unusual mode of sexual reproduction. Research in this field spans from the early recognition of ascospore dimorphism linked to differences in mating ability to recent molecular studies that implicate direct repeat-mediated homologous recombination as the driver of switching. Two direct repeat regions flanking the deleted *MAT* genes are believed to facilitate these permanent genomic changes. While self-fertility can promote rapid reproduction via haploid selfing, the emergence of self-sterile progeny through unidirectional switching could play a critical role in facilitating outcrossing. This has significant implications for hybridisation between species, an increasingly observed phenomenon in *Ceratocystis* pathogens of plantation grown trees. Notably, interspecific hybrids, often the result of outcrossing between compatible *MAT* types, have been associated with heightened aggressiveness and host range expansion, posing serious threats to economically important tree crops such as *Eucalyptus*. By interrogating the mechanisms and evolutionary implications of unidirectional mating-type switching, we highlight how sexual strategy influences the adaptive landscape of plant pathogens. A deeper understanding of this process will significantly aid in predicting pathogen emergence, hybridisation risk, and ultimately, for managing disease outbreaks in forestry and agriculture.

Keywords

Fungal reproduction, forestry pathogens, evolutionary adaptation, self-fertility, self-sterility

Utilising plant health-promoting *Bacillus* strains from cactus pear to mitigate drought stress and *Fusarium* induced damping off and root rot in wheat

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Disease Management

Abstract

Wheat is ranked as the second most consumed grain globally. However, its cultivation is threated by diseases, drought stress and other environmental factors. *Fusarium* spp. are soilborne pathogens that cause damping off as well as root and crown rot of wheat in South Africa. Plant health-promoting micro-organisms associated with plant-microbiomes present a viable strategy for sustainable agriculture. This study aimed to use beneficial *Bacillus* strains isolated from cactus pear to alleviate drought stress and *Fusarium* damping off and root rot in wheat. Briefly, the soil was inoculated with $1*10^6$ spores/g of *F*. *oxysporum* and *F*. *graminearum*. Wheat seeds were bioprimed with $1*10^8$ CFUs of *B*. *subtilis* and *B*. *halotolerans* separately while distilled water served as control. Germination percentage, mean germination rate, rate of emergence, and root rot incidence and severity were assessed. To evaluate drought stress alleviation, wheat seedlings were soil drenched with the *Bacillus* spp. following that, the soil was kept at 10% water holding capacity for three weeks to induce drought stress. The seedlings were assessed for chlorophyll fluorescence, pigment content (Chlorophyll a&b and carotenoids), soluble sugars, stored carbohydrates, protein content, and antioxidant enzyme activity. The *Bacillus* treatments significantly ($P<0.05$) improved the germination parameters and reduced root rot incidence and severity compared to control seeds for both pathogens. Chlorophyll fluorescence (Fv/Fm) in *B. subtilis* treated seedlings showed 0.76, while *B*. *halotolerans* had 0.75 and the positive control was 0.77 for positive control which were higher than the negative control's 0.63 ($P=0.00005$). The treatment seedlings had significantly higher levels of carbohydrates, pigment content and antioxidant enzymes activity while glucose levels were higher in negative control. The *Bacillus* strains used in this study promote wheat health under abiotic and biotic stress conditions. They can be incorporated in an integrated pest management program during wheat production.

Keywords

Biocontrol, Plant growth promotion, Biostimulant, Microbiomes, Beneficial microbes

Assessment of the level of tree dieback in mature apple orchards through remote sensing techniques

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Disease management

Abstract

Dieback of fruit trees is caused by canker and wood rot pathogens, leading to twig blight, cankers, and wood decay. Older trees more often show symptoms and affected branches are removed. Although production declines in ageing orchards, no research has been linked to the occurrence of dieback. The aim was to assess yield loss, financial loss and use remote sensing techniques to assess dieback on apple trees. In nine apple orchards with different levels of dieback, 25 trees were classified using a disease scale (Class 1 to 5, with 1 being healthy). Apples were harvested and the total yield per tree per disease class was determined. An infected branch from Classes 2 to 5 were sampled, isolations made from internal symptoms and fungal species identified through sequencing. Multispectral images were obtained with drone flights of four Golden Delicious orchards and analysed to assess changes in canopy health and identify symptoms such as sectoral dieback or missing trees. Branches that were sampled from diseased trees showed typical wood discolouration and rot associated with dieback. Fungi identified from the isolations include *Basidiomycota*, *Botryosphaeriaceae*, *Diaporthales*, *Diatrypaceae* and *Togniniaceae*. Yield data declined as dieback increased. For the green cultivar, the average yield per tree was 143 kg (Class 1), 87 kg (Class 2), 78 kg (Class 3), 46 kg (Class 4) and 17 kg (Class 5). For the red cultivars, the average yield was 88 kg (Class 1), 61 kg (Class 2), 55 kg (Class 3), 34 kg (Class 4) and 13 kg (Class 5). Drone images were taken of three orchards, of which the analysis is pending. Trees showing dieback cause a loss in yield. A protocol was developed to evaluate the level of dieback of an orchard using remote sensing.

Keywords

Canker, yield loss, drone images, satellite images

Tackling the HLB threat to citrus in southern Africa

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Biosecurity

Abstract

Huanglongbing (HLB; Asian Citrus Greening), associated with '*Candidatus* Liberibacter asiaticus' (CLas) and vectored by the Asian Citrus Psyllid (ACP, *Diaphorina citri* Kuwayama) (Psyllidae), is the most feared disease of citrus worldwide. Southern Africa is free from HLB and ACP, but ACP is present in East and West Africa, and HLB has been reported from Kenya and confirmed in Ethiopia. Natural range expansion of ACP and eventual HLB incursion into southern Africa are considered inevitable. In preparation, South Africa developed an HLB/ACP Action Plan, based on scenario planning and world best-practice. The Action Plan was recently updated after scenario planning exercises to re-evaluate its practical feasibility and took into consideration the HLB and ACP experiences from other countries, with adaptation to local circumstances, including capacity constraints in southern Africa. Once ACP and HLB become established in parts of southern Africa, eradication is unlikely, especially in urban areas. However, well-prepared, rapidly deployed, area-wide response actions can be effective in containing the incursions, curtailing the rate of spread, and preventing further long-range spread within the region. Early detection is key to effective control measures, and thus detection surveys are conducted throughout the region, focusing on likely introduction pathways. HLB and ACP surveillance in southern Africa is complicated by the occurrence of endemic *Diaphorina* species that look like ACP, the endemic occurrence of a milder form of HLB, African Citrus Greening associated with *Candidatus* Liberibacter africanus' (CLaf), and subspecies of CLaf that were found to cross-react with CLas PCR primers. These challenges underscore the need for regionally coordinated surveillance systems and control measures, robust diagnostic tools, and sustained awareness efforts to ensure early and accurate detection of ACP and HLB in southern Africa.

Keywords

Biosecurity; Asian Greening; Liberibacter asiaticus

Species boundaries and genetic diversity revealed in archival South African clinical *Sporothrix* strains

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Sporotrichosis is a fungal disease caused by species in the genus *Sporothrix* (*Ophiostomatales*). These fungi include not only human and animal pathogens but also environmental species with historical ties to plant material and woody substrates. South Africa is considered an endemic region for sporotrichosis, with some of the largest recorded outbreaks globally. While the clinical relevance of *Sporothrix* is well established, its evolutionary and ecological connections to plant-associated environments remain underexplored. In this study, we re-evaluated historical *Sporothrix* strains from South Africa, originally collected between the 1990s and early 2000s, and preserved in culture collections. Using molecular techniques, their identities were reconsidered and their genetic diversity analysed. The majority of strains were identified as *S. schenckii* (n = 69), with single representatives of *S. globosa* and *S. pallida*, as well as two strains representing an undescribed lineage. The *S. schenckii* population showed a strongly biased mating type distribution (92% MAT1-2), and microsatellite analysis revealed only four multilocus genotypes; these correspond with distinct clades observed through phylogenetic analyses. One dominant microsatellite genotype accounted for 85% of the population. The findings suggest a clonal expansion of this successful lineage, potentially reflecting selective advantages under specific environmental or epidemiological conditions. The low genetic diversity and skewed mating type ratio also raise questions regarding dispersal mechanisms, persistence in the environment, and overlooked environmental reservoirs of these medically important fungi. The phylogenetic placement of *Sporothrix* in *Ophiostomatales*, an order that includes several saprobic and plant-pathogenic fungi, underscores the need for integrative research that bridges clinical mycology, fungal ecology, and plant pathology. Such a perspective is necessary to fully understand the emergence, evolution, and potential plant-associated reservoirs of *Sporothrix*.

Keywords

Mycoses, Sporotrichosis, environmental fungi, epidemiology, phylogenetics

A surprisingly diverse assemblage of *Botryosphaeriaceae* associated with blue stain of *Pinus* in Mpumalanga, South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The *Botryosphaeriaceae* is a diverse family of fungi of latent pathogens that infect a wide range of woody hosts, including *Pinus* species. These fungi are associated with dieback, cankers, and wood discoloration. In this study, we surveyed the historic records of the Forestry and Agricultural Biotechnology Institute (FABI) culture collection and conducted field sampling in Sabie, Mpumalanga to assess the diversity and prevalence of *Botryosphaeriaceae* in *Pinus* seedlings and blue-stained logs. Historic records revealed that *Diplodia sapinea* is the most frequently isolated species from *Pinus* hosts, with a historic *D. sapinea* to *D. scrobiculata* ratio of 28:1. The field sampling yielded 187 isolates, including two species of *Diplodia* (n=163 isolates), five species of *Lasiodiplodia* (n=14 isolates), and two species of *Neofusicoccum* (n=10 isolates). These results represent the first report of *Lasiodiplodia* and *Neofusicoccum* species from *Pinus* hosts in the Sabie region, and the first association of *D. scrobiculata*, *L. pseudotheobromae*, and *N. parvum* with blue-stained wood in South Africa. Amongst the *Diplodia* isolates, *D. sapinea* to *D. scrobiculata* occurred in a ratio of 1.7:1. Population analyses of *D. sapinea* revealed mating type frequencies of 1.4 *MAT1*: 1 *MAT2* and a varying distribution of both dispensable chromosome 15 and dispensable chromosome 16, reflecting an expected cryptic sexual cycle in the region. Finally, to enable rapid species identification, we developed three PCR based diagnostic primer sets capable of distinguishing *D. sapinea* and *D. scrobiculata*. This study highlights shifting fungal dynamics in *Pinus* plantations and provides essential tools for improved pathogen monitoring and biosecurity in South African forestry.

Keywords

Diplodia spp., *Lasiodiplodia* spp., *Neofusicoccum* spp., Species diversity, Molecular diagnostics

Development of maize lethal necrosis disease (MLND) resistant maize using CRISPR/Cas9 technology

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Disease Management

Abstract

Maize lethal necrosis disease (MLND) is a significant threat to maize production in sub-Saharan Africa and can lead to 100% yield losses in extreme cases. The disease results from the synergistic interaction between maize chlorotic mottle virus (MCMV) and any one of maize infecting potyviruses. Currently, there are no resistant germplasm to control MLND. For infection to occur, potyviruses are known to interact with the host eukaryotic translation initiation factor (eIF) proteins. Targeting these proteins for editing will disrupt the potyvirus infection process, leading to the control of MLND. This study aims to develop MLND-resistant maize lines through targeted genome editing of selected eIF proteins using the CRISPR/Cas9 system. Candidate susceptibility genes involved in viral translation and replication, specifically eIF4G, eIF4E1, and eIF4E2, were identified through literature review and *in silico* analysis of plant-virus protein interactions. Guide RNAs (gRNAs) targeting these genes were designed using *in silico* proteomic studies and recommended software. Using Golden Gate cloning, plasmid vectors containing gRNA arrays, Cas9, and selectable marker sequences to form transformation vectors. The resulting transformation vectors were incorporated into *Agrobacterium tumefaciens* strains LBA4404 and EHA1501 using electroporation. Positive transformants in which Cas9 and BAR gene selectable marker sequences had been successfully integrated were confirmed using colony PCR. Our preliminary results show that MCMV's P50 protein is the only viral protein that interacts with eIF4E proteins. This information was used to design gRNAs to disrupt these sites of interaction. Transformation vectors were successfully electroporated into *Agrobacterium*. The efficacy of the transformation vectors will be evaluated using maize protoplast before transforming selected maize lines using immature embryos as explants. This project will result in the development of maize lines that are resistant to MLND leading to sustainable maize production, ensuring food security for millions who depend on maize as a primary source of food.

Keywords

Plant-virus interaction, potyviruses, proteomics, susceptibility genes, Golden Gate cloning

Evaluating UV-C light as a control measure for *Phytophthora* brown rot of citrus and elucidating the defence response

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Disease Management

Abstract

Brown rot of citrus fruit is caused by *Phytophthora nicotianae* in South Africa. Brown rot first appears as a light brown discoloration of the rind, which spreads and becomes necrotic, leading to a leathery appearance. Latent infections can lead to losses where disease can easily spread to neighboring fruit in the carton. Management of brown rot is achieved through pre-and postharvest fungicide treatments. The continuous scrutiny of fungicide use has led to the exploration of ultraviolet (UV) light, specifically UV-C light as a potential alternative. The aim was to evaluate the potential of UV-C treatment to control postharvest brown rot on lemons and oranges, while also elucidating the pathogen -host interaction through gene expression analysis. To evaluate the curative ability of UV-C, all fruit were first inoculated with *Phytophthora nicotianae* zoospores, followed by UV-C treatment at varying time points after inoculation. For the preventative assessment, fruit were treated with UV-C prior to inoculation with *P. nicotianae* zoospores at varying time points after treatment. The registered fungicide azoxystrobin was used as a positive control in all trials. Expression of different defence-related genes was monitored using real-time quantitative PCR (qPCR). UV-C had a curative effect on lemons at 0 hours, and 24 hours post inoculation achieving 52% and 43% control for the respective hours. UV-C had a curative effect on oranges with the highest control achieved (62%) at 12 hours post inoculation. UV-C had little to no effect as a preventative measure on both lemons and oranges. Gene expression revealed a correlation between disease control and the activation of defence response at the molecular level. These findings contribute to a better understanding of citrus-*Phytophthora nicotianae* interaction and support the potential of UV-C light as a sustainable postharvest disease management strategy.

Keywords

Citrus, *Phytophthora nicotianae*, postharvest, management, qPCR

Fungicide sensitivity of *Alternaria* species causing Alternaria brown spot and core rot of citrus in South Africa

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Disease Management

Abstract

The management of Alternaria brown spot (ABS) of mandarins and Alternaria core rot (ACR) of citrus predominantly relies on the application of fungicides. The fungicide sensitivity status of *Alternaria* species against registered actives is not known in South Africa. The aim was to establish the fungicide sensitivity levels to predict a sensitivity shift or fungicidal resistance. Fungicides subjected to sensitivity testing of *Alternaria alternata* and *A. arborescens* were azoxystrobin, pyraclostrobin, fluopyram and tebuconazole. The mean EC50's for mycelial growth ($EC50_{RMGI}$) and conidia germination ($EC50_{RSGI}$) inhibition was determined. Isolates with varying levels of sensitivity against each active were selected and the *Cytb*, *SdhB*/*SdhC*/*SdhD* and *CYP51* genes sequenced. Single nucleotide polymorphisms (SNP's) were identified and isolates compared to confirm sensitive and tolerant isolates. *Alternaria alternata* had significantly higher $EC50_{RMGI}$ and $EC50_{RSGI}$ values than *A. arborescens* for both fluopyram and tebuconazole. No significant differences were observed in $EC50_{RMGI}$ values among species for pyraclostrobin. However, *A. arborescens* had significantly higher $EC50_{RSGI}$ values than *A. alternata* for both azoxystrobin and pyraclostrobin. The exposed population showed reduced sensitivity to strobilurin fungicides. Fluopyram and tebuconazole were effective to inhibit *Alternaria* species, however, a sensitivity shift was observed for tebuconazole. Molecular resistance was confirmed for QoI and SDHI fungicides. The G143A and *SdhB*-H277Y/*SdhC*-H134R SNP's were predominantly associated with QoI and SDHI resistance, respectively. For DMI molecular resistance, 19 haplotypes were identified of which, h1 and h4 were associated with sensitive isolates and haplotypes h5 and h8 with tolerant isolates. *Alternaria alternata* and *A. arborescens* isolates illustrated reduced sensitivity for QoI's. Fluopyram and tebuconazole were highly effective, however, a noticeable shift was observed against tebuconazole. This is the first report of QoI and SDHI molecular resistance in *A. alternata* and *A. arborescens* associated ABS and ACR in South Africa.

Keywords

Azoxystrobin, pyraclostrobin, fluopyram, tebuconazole, baseline sensitivity, population sensitivity, molecular resistance.

Involvement of non-crop *Poaceae* species in the maintenance of the *P. alienus* leafhopper and the Wheat dwarf virus

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Ecology and Epidemiology

Abstract

Harvest represents a challenge for the persistence of insect-borne viral diseases in agroecosystems. To overcome this challenge, some viruses infect non-crop plants as reservoirs for future introduction of viruses to newly sown fields. The wheat dwarf disease (WDD), one of the most important viral diseases on cereals, is caused by the wheat dwarf virus (WDV) and is transmitted by the leafhopper *Psammotettix alienus*. While the epidemic process of this disease has been studied in crop areas, very few data is available on factors influencing the maintenance of the leafhopper and the virus between growing seasons. To better understand the contribution of non-crop species in the epidemiology of WDD, plant-virus (i.e. infection rate) and plant-vector (i.e. survival and fecundity) interactions were monitored on non-crop Poaceae (NCP) species. Results showed that NCP species can be clustered according to host quality for WDV and/or *P. alienus*. *Bromus hordeaceus* and *Phalaris arundinaceae* (two species with contrasted host quality for WDV and *P. alienus*) were included in multi-host arena experiments to assess the impact of a heterogeneous plant environment on leafhopper preferences and WDV infection. Collected data showed that *P. alienus* prefers *B. hordeaceus* (a poor efficient host for WDV) for oviposition. This could lead to a dilution of viruliferous vectors in environments containing *B. hordeaceus* plants as hosts for WDV. Altogether, results of this work indicate that host quality and the composition of plant populations are important for WDD maintenance in non-cultivated areas.

Keywords

Plant virus, host range, insect-borne virus, transmission, ecology

Occurrence and genetic diversity of *Stenocarpella maydis* and associated Diplodiatoxin in maize grain from commercial and smallholder fields across multiple seasons in South Africa

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Post-harvest Pathology and Mycotoxins

Abstract

Stenocarpella maydis (Diplodia) ear rot remains a significant threat to maize production in South Africa, causing yield losses and compromising grain quality. Historically, research has focused on the fungal pathogen itself; however, the recent isolation and characterization of Diplodiatoxin through a collaborative effort by the Agricultural Research Council-Grain Crops, the University of Pretoria and Southern African Grain Laboratories, has enabled a more comprehensive understanding of its toxicological impact. This study presents findings on the occurrence of *S. maydis* and Diplodiatoxin in maize grain across South African production regions. Maize grain samples were collected from commercial and smallholder farms across all major maize-producing provinces over multiple seasons (2019–2025). Ears were processed, surface-sterilized, and plated to assess *S. maydis* incidence and severity. Since 2019, sub-samples were analyzed using Liquid Chromatography–Mass Spectrometry (LC/MS) to quantify Diplodiatoxin levels. Results showed consistently lower *S. maydis* incidence and Diplodiatoxin levels in commercial maize, while smallholder samples exhibited significantly higher incidence and toxin concentrations. Diplodiatoxin levels ranged from non-detectable to 15,736 µg/kg, with elevated concentrations frequently associated with monoculture, no-till systems in smallholder fields. These findings highlight the influence of agronomic practices on pathogen prevalence and mycotoxin accumulation. To investigate genetic diversity, over 400 *S. maydis* isolates were preserved in the ARC-GC biobank, with 30 selected for whole genome sequencing. This molecular characterization will provide insights into population structure, pathogenicity, and toxin biosynthesis pathways. Information regarding the effects of Diplodiatoxin in animals is limited, and its impact on human health remains unknown. These findings underscore the urgent need for further toxicological studies to assess potential risks, particularly in vulnerable populations, and to inform future regulatory frameworks and public health strategies in maize-consuming regions.

Keywords

Mycotoxin quantification, agronomic practices, Liquid Chromatography - Mass Spectrometry (LC/MS), whole genome sequencing, public health risk

A combined recombinase polymerase amplification CRISPR/Cas12a assay for detection of *Fusarium oxysporum* f. sp. *cubense* tropical race 4

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The soilborne pathogen *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) is currently devastating banana production worldwide. Once introduced, the pathogen cannot be eradicated from soil where it can survive for decades. The only long-term management option is to replace Foc TR4-susceptible with resistant banana varieties. Timely detection of the pathogen, however, is critical to prevent the introduction of Foc TR4 into new areas and limit spread from infested sites. Surveillance based on symptomology alone is often unreliable due to overlap with symptoms caused by other biotic or abiotic stresses. While laboratory-based molecular methods such as PCR and quantitative (q)PCR remain the gold standard for detection, they rely on expensive equipment and skilled personnel. In this study, a rapid, single-tube detection technique was developed by combining recombinase polymerase amplification (RPA) and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas12a technology (RPA-Cas12a) for detection of Foc TR4. The technique provides a simple, single-tube detection alternative that is analytically robust with improved specificity compared to available molecular detection assays and eliminates the need for expensive and sophisticated laboratory equipment. The RPA-Cas12a assay was conducted isothermally, had a sensitivity to detect up to 10 fg target DNA, and did not cross-react with any of the 76 non-target isolates tested. The assay successfully detected Foc TR4 from naturally infected banana samples collected in the field using a simplified DNA extraction method. Signal visualization was achieved via real-time fluorescence using a portable fluorometer or endpoint fluorescence visible with the naked eye under an LED blue light transilluminator. The method can be integrated with inexpensive fluorescent or electronic detection devices to support in-field diagnostics, offering a promising tool to enhance early detection and accelerate containment strategies against Foc TR4.

Keywords

CRISPR/Cas12a, Disease diagnosis, Fusarium wilt of banana, Rapid detection, Recombinase polymerase amplification, Tropical Race 4

Banana bunchy top virus outbreak and control

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Biosecurity

Abstract

Banana bunchy top disease (BBTD), caused by Banana bunchy top virus (BBTV), is the most devastating viral disease of bananas worldwide. BBTV is a quarantine virus and must be absent in imported *Musaceae* propagation material. South Africa has about 13 000 ha under bananas; excluding small-holder farmers. The disease is spread by the banana aphid (*Pentalonia nigronervosa*Coquerel (Hemiptera: Aphididae), and through infected propagation material. In 2015, the detection of the virus was reported from an isolated area in the KwaZulu-Natal (KZN), South Coast region. Delimiting surveys were conducted across banana-producing regions in South Africa, focusing on where the BBTD outbreaks were reported. A total of 1568 plant- and aphid samples were collected on 21 commercial farms and 80 rural households in the KZN region. The distribution of the virus in the KZN region was recorded and the molecular diversity in the plant- and aphid samples determined. The spread of the disease to other commercial farms, 47km from the initial outbreak site in KZN was demonstrated. Management strategies, including removal of infected plants, control of aphid populations and early detection in the aphid populations, have been implemented in areas where positive samples were identified. Awareness campaigns were launched in the rural areas in KZN, but the disease is still spreading in these regions. The continuous surveillance of BBTV forms part of the strategic plan of the Department of Agriculture (DoA). As such, BBTV presence was confirmed in samples collected from the South Coast region and is now also recorded from isolated regions in the KZN North Coast region. BBTD currently has a negative impact on plant health and food security in the KZN region and efforts by the ARC, commercial farmers and the DoA is ongoing to limit the spread of the disease. Will the effort be enough?

Keywords

banana bunchy top virus, detection, food security, KwaZulu Natal

From sequence to symptom — linking small RNAs to leaf bleaching in avocado sunblotch disease

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Plant-Pathogen/Insect Interaction

Abstract

Avocado sunblotch disease, characterised by distinct chlorosis of fruit, stems, and/or leaves of affected trees, has a significant impact on the South African avocado industry. The disease is caused by avocado sunblotch viroid (ASBVd) – a ~250 nt, single-stranded RNA molecule, which is the type species of the family *Avsunviroidae*. Related avsunviroids, carrying particular genome mutations, have been shown to trigger chlorotic symptoms by silencing specific host genes using viroid-derived small RNAs (vd-sRNAs). To elucidate whether a similar mechanism is responsible for the manifestation of sunblotch symptoms, the ASBVd sequence variants and vd-sRNAs present in bleached and asymptomatic leaf tissues from symptomatic avocado trees were examined. The ASBVd variants carrying the bleaching-associated mutation differed in their abundance between green and yellow leaf tissues. The increased viroid titre in yellow tissues led to the generation of numerous ASBVd-derived small RNAs (ASBVd-sRNAs), which accumulated predominantly in yellow tissues. ASBVd-sRNAs containing the bleaching-associated mutation were limited almost entirely to yellow tissues and were predicted to target numerous avocado transcripts for degradation via RNA silencing pathways. Transcriptome analysis showed that 25 potential avocado mRNA targets had decreased expression levels in yellow tissues as compared to green tissues from the same symptomatic trees. One downregulated avocado gene, *PRPL34*, which encodes a protein that functions in host chloroplasts, showed the most potential to be targeted by ASBVd-sRNA-mediated RNA silencing. The correlation between the expression levels of *PRPL34* and its predicted targeting by ASBVd-sRNAs suggests that silencing of this gene may have a role in triggering bleaching symptoms in ASBVd-infected trees. This work represents the first recent exploration into the molecular mechanisms underlying symptom development during ASBVd infection and introduces promising avenues for future research into ASBVd pathogenesis.

Keywords

Avocado sunblotch viroid, ASBVd, viroid-derived small RNAs, RNA silencing, viroid pathogenesis

Airing out the risks: airborne fungal spore fluctuations in South African blueberry packhouses

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Ecology and Epidemiology

Abstract

The South African blueberry industry is rapidly growing due to the high antioxidant and anti-inflammatory properties of the fruit, making it popular as a health food. However, the fruit is highly susceptible to postharvest fungal contamination, particularly by *Botrytis cinerea*, posing a significant challenge to exports. This study monitored the fluctuations in culturable airborne fungal spore concentrations (FSC) in three South African blueberry packing facilities, over the 2024 commercial packing season, through active air sampling and culture-based techniques. Dominant fungal genera were identified as *Cladosporium*, *Penicillium*, followed by various yeasts. Specifically, the main packing area displayed a clear dominance in *Cladosporium* – 73 % [Packhouse A (PA)], 90 % [Packhouse B (PB)], and 60 % [Packhouse C (PC)]. *Botrytis* showed increased sporadic detection as the season progressed, with notable accumulation detected in the main packing area of all facilities as the season progressed; PA (96 %), PB (75 %), and PC (87 %). While overall FSC showed significant fluctuations across areas and time points, often exceeding the threshold for a healthy indoor environment (<1000 CFU/m^3), no clear correlation was observed between increasing fruit volume and spore load. This suggests that inoculum from incoming fruit did not drive airborne fungal spores. Similarly, environmental parameters (temperature, relative humidity, CO_2) showed no correlation between airborne FSC in PAC, while sporadic associations were observed in PB. These findings suggest that unmeasured variables may be influencing the observed fluctuations. Despite the preliminary nature of this study, these findings provide foundational insights into the airborne mycoflora in a fresh fruit packing environment and support further investigation into fungal risk management during blueberry processing. Moreover, the significant abundance of *Cladosporium* and overall mycoflora highlights the need for species-level identification, particularly given the potential health-risks to facility workers, as several *Cladosporium* spp. produce allergens.

Keywords

Postharvest contamination, *Botrytis*, *Cladosporium*, airborne fungi, indoor air quality

Goss's Wilt in maize — we didn't see it coming

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Biosecurity

Abstract

Goss's Wilt, caused by *Clavibacter nebraskensis*, (Davis et al.) Li et al. was first confirmed in South Africa in 2024, presenting a real-life phytosanitary challenge. Previously restricted mainly to North America, Canada and parts of Mexico, the unexpected arrival of this bacterial pathogen exposed vulnerabilities in biosecurity systems, seed movement controls and industry preparedness. Recent amendments to the Control Measures R. 110 (30 May 2025) in terms of the Agricultural Pests Act, (No.36 of 1983) published in the Government Gazette, tabled the causal organism of Goss's wilt as a regulated pest in South Africa under official control. This grants the authority responsible to prescribe control measures as set out in the Pests Act to prevent and combat the spreading of pests. Early outbreaks displayed the characteristic symptoms: water-soaked lesions, typical freckles with bacterial exudate, necrotic streaks, and varnished leaf appearance. The detection prompted urgent regulatory responses, highlighting gaps between pre-existing contingency plans and the realities of rapid pathogen establishment. Beyond the immediate production threats, including yield losses and increased production costs, Goss's Wilt poses longer-term risks to regional trade, market access, and food security. With South Africa's maize seed industry lacking prior exposure and natural resistance in local germplasm, the sector faces heightened vulnerability. This paper reviews the 2024 outbreak, explores possible introduction pathways, and summarizes early epidemiological findings. Importantly, it reflects on lessons learned as paper-based plans confronted real-world challenges, emphasizing the need for stronger integration between biosecurity policy, industry readiness, transparency and applied disease management. The Goss's Wilt incursion underscores the urgent need to strengthen proactive horizon scanning programs, systematically monitoring global pest and disease trends to better anticipate and prepare for emerging threats. Building forward-looking surveillance, rapid diagnostics, resilient seed systems, and multi-stakeholder coordination will be key to mitigating the next unforeseen biosecurity event.

Keywords

Clavibacter nebraskensis, Biosecurity, Phytosanitary, Seed

The hidden sex lives of beetle-associated *Ambrosiella* species

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Fungi in the genus *Ambrosiella* are symbionts of ambrosia beetles and play a central role in the establishment and maintenance of beetle galleries within their host trees. These fungi are introduced by the beetles into plant tissue during boring and proliferate within the xylem, where they serve as the primary food source for the beetles. Several *Ambrosiella* species are linked to economically important tree diseases, either directly as pathogens or indirectly through their beetle vectors. While *Ambrosiella* were long considered asexual, a sexual state has recently been described in one species. In this study, we investigated the genetic basis of sexual reproduction in *Ambrosiella* species. Publicly available genome assemblies of *A. beaveri*, *A. cleistominuta* and *A. xylebori* were mined to identify mating-type (*MAT*) genes and components of the fungal mating pheromone pathway. Each species harbored a complete mating-type (*MAT1*) locus, comprising of four mating-type genes, including *MAT1-1-1* and *MAT1-2-1*, indicative of sexual capability. The organization of this locus was consistent with primary homothallism, suggesting a capacity for self-fertility. The fact that sexual structures have been reported for single isolates of *A. cleistominuta* support these results and provide morphological evidence for homothallic sexual reproduction. We also identified genes encoding the a-factor pheromone and its corresponding receptor, indicating partial conservation of the pheromone-response pathway. However, the α-factor pheromone gene was entirely absent, and its corresponding receptor appears mutated and likely non-functional. These findings suggest that while *Ambrosiella* species are capable of sexual reproduction via homothallism, they may have lost the capacity for mate recognition. Understanding the sexual biology of these fungi offers insights into their population dynamics, adaptability, and potential for long-distance spread through beetle vectors.

Keywords

Mating-type (MAT1) locus, Primary homothallism, Pheromone pathway, Ambrosia beetle symbiont, Fungal genome mining

Effect of foliar fungicides on Fusarium head blight and mycotoxin contamination of wheat

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Disease Management

Abstract

Fusarium head blight (FHB) is an important disease of wheat, caused primarily by *Fusarium graminearum s.s*. FHB results in three-fold losses for producers, namely reduced yield and grain quality, and contamination of grain with toxic secondary metabolites, knowns as mycotoxins, especially deoxynivalenol (DON). To date, no fungicides are registered for the management of FHB and DON contamination in South Africa. Wheat producers, however, routinely apply fungicides to manage foliar diseases. The aim of this study was to evaluate the efficacy of commercial foliar fungicides against FHB by measuring disease incidence, percentage Fusarium-damaged kernels (%FDK), 1000-kernel weight (TKW) and DON levels. A field trial was conducted in 2023 at Napier, Western Cape, with three commercial fungicides (tebuconazole (TebuCure 250 EW), prothioconazole + tebuconazole (Prosaro 250 EC) and azoxystrobin + propiconazole + pydiflumetofen (Miravis® Neo)) applied to twelve wheat cultivars prior to inoculation with *F. graminearum s.s*. The cultivars were selected based on their differing responses to FHB and DON accumulation. A significant cultivar x treatment (fungicide vs control) x inoculation (fungal vs water) interaction was observed for all disease parameters. Generally, fungicide application, followed by fungal inoculation, significantly reduced disease levels. Cultivar SST 0127 treated with tebuconazole and inoculated with F. graminearum had a significantly lower FHB incidence (3.3%) than the control treatment inoculated with the pathogen (65.0%). A significant treatment x inoculation interaction was observed for DON. Apart from the application of Prosaro 250 EC followed by fungal inoculation (0.00765 mg kg^{-1}), Miravis® Neo and TebuCure 250 EW significantly reduced DON levels (0.00296 mg kg^{-1} and 0.0026 mg kg^{-1} respectively) compared to the water-treatment followed by fungal application (0.03391 mg kg^{-1}). This study showed that foliar fungicides can effectively reduce FHB and DON levels, although additional data from different regions over multiple years is needed to confirm these results.

Keywords

F. graminearum, deoxynivalenol, chemical control, cultivar response

A decade of pecan disease research in South Africa

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Disease Management

Abstract

Since the first plantings of pecan (*Carya illinoinensis*) in 1912 in South Africa, it has developed into a R2 billion per annum industry. However, it is only over the last decade that the industry started to recognise the importance of pecan disease research in South Africa. Prior to that, the industry mainly depended on the knowledge of the USA and Mexico as the two biggest producers in the world. The Pecan Health Research Group was established in 2017 at the University of the Free State to identify and study the more important fungal pathogens associated with pecans in South Africa. Initially, six categories of fungal plant pathogens were recognised for further research based on previous experiences. These include fungi such as *Neofusicoccum parvum* (black blotch and die-back), *Cladosporium cladosporioides* (scab), *Alternaria alternata* (Alternaria black spot), *Fusarium* species (overall decline and die-back), *Neocosmospora vasinfecta*, and *Chaetomium* species (black spots on fruit). During seasonal field trips to all the production areas in South Africa, it became evident that other diseases are also of importance. These include Ganoderma root and basal rot, Phellinus heart rot, Schizophyllum trunk rot, overall decline, and post-harvest diseases. This has led to the publication of the book "Fungal diseases of pecans in South Africa" which was presented at the AGM of the South African Pecan Nut Producers Association NPC (SAPPA) in November 2024. The book contains information on the most prominent fungal diseases affecting the health of pecans, including information on the causative agents, geographical distribution, management strategies, and effective use of fungicides. The book serves as a guide for pecan producers in South Africa to recognise and manage diseases, and to use as a discussion tool for agents serving the industry in disease management.

Keywords

Carya illinoinensis, fungal pathogens, book, review, pecan health

Assessing grain mold prevalence and farmer awareness in South African Sorghum Production Systems

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Post-harvest Pathology and Mycotoxins

Abstract

Grain mold is a complex fungal disease that affects sorghum, especially under humid conditions during and after grain fill. The disease compromises grain quality and poses significant food safety risks through potential mycotoxin contamination. Despite its importance, field-level data in South Africa remains limited, particularly regarding farmer awareness and preparedness. From 2023 to 2025, disease surveys were conducted in 70 sorghum fields across five provinces during the grain fill stage. Grain samples were collected, morphologically identified, and Sanger sequenced. Furthermore, grain was sent for mycotoxin analysis (LC-MS/MS) and next-generation sequencing (Illumina sequencing) to identify mycotoxigenic fungi (results pending). Additionally, 33 sorghum producers (both commercial and smallholder) were interviewed using a structured questionnaire to assess their awareness of grain mold, mycotoxins, and associated health risks. Grain mold was observed in 17% of the fields (n = 12). In Limpopo, one locality sampled at the harvest stage showed >80% incidence, while in other regions, mold symptoms were linked to bird-damaged panicles during grain fill. *Alternaria alternata, Epicoccum sorghinum* and *Curvularia kusanoi* were the most prevalent fungi identified with Sanger sequencing. Farmer interviews revealed that among smallholders, 42% were unaware of mycotoxins, and only 6% were familiar with their health impacts. Furthermore, 45% could not recognise the risks associated with moldy grain. In contrast, commercial producers indicated greater awareness, with 27% having knowledge of mycotoxins and 36% could distinguish between healthy and moldy grain. Although field-level mold incidence was generally low, severe cases and poor awareness among smallholders raise concerns for food safety. Improved timing of surveillance, post-harvest analysis, and farmer education are crucial. Addressing these gaps, through targeted training and accessible diagnostic tools, can reduce mycotoxin exposure risks and promote food safety.

Keywords

Epidemiology, Mycotoxins, Disease intensity, Farmer perceptions

The use of bioproducts to control agriculturally significant diseases

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Disease Management

Abstract

The South African Bioproducts Organisation is a non-profit organisation that represents all stakeholders of bioproducts in the South African agricultural industry including researchers, manufacturers, distributors, trialists growers and retailers. Our mandate is to promote awareness of bioproducts and how they can be integrated into crop management systems to ensure sustainable agriculture. SABO wishes to use the platform of the SASPP to highlight bioproducts and their critical role in disease management in South African agriculture. The loss of numerous pesticides due to their inherent hazardous status or through resistance development has driven the need for suitable biological alternatives in the South African market. However, our climatic conditions are unfavourable for the broad-spectrum use of certain bioproducts - high temperatures and low rainfall. Thus, increased research focus is needed to identify potential solutions but also to ensure that these solutions are well suited to farm-level adoption. There are already a number of micro-organisms and plant extracts which have been registered as biocontrol agents locally. The vast majority of these are organisms and products developed outside of South Africa. However, there is a need to increase the number of locally sourced actives and to facilitate more on-farm identification of potential biocontrol agents. Supplementing local populations will be the precursor for a stronger ecosystem. SABO has been working hard to become a platform to connect different role players within the industry. By connecting researchers with the industry, SABO hopes to encourage more collaboration that makes meaningful contributions to our industry.

Keywords

Biocontrol agents, Sustainable Agriculture, Alternative control measures, Bio-products, Collaborative research

Identification and characterisation of *Alternaria* spp. and *Cladosporium* spp. causing postharvest decay in late mandarin fruit during cold storage periods in USA markets

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The South African Citrus Industry is the second largest exporter globally. Key export markets include Europe, Asia and North America. Cold chain practices are crucial for maintaining fruit quality. During the second half of the 2023 export season, mandarins exported from the Western Cape region exhibited irregular black spotty lesions on the rind, including brown, watery lesions after prolonged storage at -0.6°C. Characterised symptoms were consistent with sooty spot. *Alternaria* spp. and *Cladosporium* spp. were isolated from brown lesions and black lesions, respectively. This was the first report of sooty spot on South African exported mandarins, highlighting an emerging concern for export quality. This study aimed to identify the pathogens involved and assess their pathogenicity. A two-year survey was conducted in four orchards that experienced the most losses due to sooty spot. From February to July, fruit was sampled to monitor seasonal fluctuations in *Alternaria* spp. and *Cladosporium* spp. Frequencies of these pathogens were correlated against rainfall (mm) and relative humidity (RH%). Isolates obtained from the survey were identified using relevant barcoding genes. Pathogenicity trials were conducted using *Alternaria* spp. and *Cladosporium* spp. Wounding with sterilized sand paper proved to be the most effective inoculation method. Fruit rinds were wounded and inoculated with each pathogen, alone and in combination. *Alternaria alternata* and *Cladosporium cladosporioides* were frequently isolated across all surveyed orchards. No significant correlation was observed between the frequency of isolates and weather conditions. Both species were confirmed to be pathogenic when inoculated individually and in combination. This study identified *Alternaria* spp. and *Cladosporium* spp. as causal agents of sooty spot, on exported mandarins, with no clear link to environmental conditions. These results highlight the need for targeted postharvest disease management strategies and further investigation into latent infections and cold storage effects to safeguard fruit quality in export markets.

Keywords

Cold chain, postharvest decay, sooty spot

Novel oomycides evaluated for systemic performance and defence induction on citrus rootstocks

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Disease Management

Abstract

Phytophthora root rot is a destructive disease affecting citrus production in the orchard and nursery. In recent years, fungicides with novel modes of action have been developed targeting oomycete pathogens, but many of these compounds are not yet registered on citrus in South Africa; notably mandipropamid, zoxamide and ethaboxam. In this study, we investigated the uptake of these oomycides by Rough lemon and Carizo citrange rootstocks and their translocation into stems after soil or foliar application and compared their mobility and transport *in planta*. Further investigation focused on hormonal indicators of resistance in planta after application, to evaluate defence induction potential. Liquid chromatography-tandem high resolution mass spectrometry was used to detect and quantify active ingredients in tissues, and all compounds investigated were confirmed to be absorbed into roots and acropetally transported to stems. However, ethaboxam showed relatively limited acropetal mobility into stems compared to mandipropamid and zoxamide. Zoxamide had acropetal mobility when applied as a root drench and good basipetal mobility into stems, but minimal basipetal transport into roots. Oomycide xylem mobility *in planta* can be predicted using the logP and pKa values for the compounds. The oomycides were predicted to have medium systemic mobility limited to the xylem, and our results support the model's predictions. The compounds were shown to be taken up by the roots yet demonstrated differing xylem mobility. Zoxamide applied as a foliar application moved readily into the stem but minimally into roots; this could suggest good overall xylem mobility yet reduced movement in the phloem. These findings support the acropetal mobility of the three compounds investigated and suggest zoxamide being optimally xylem mobile and moderately ambimobile. Furthermore, multivariate analysis of metabolome strongly associated the modes of action with phosphite application, potentially signaling a similar role in resistance induction.

Keywords

Oomycide mobility, Citrus rootstocks, Phytophthora root rot, Defence induction, Resistance markers

Combating a globally important biosecurity threat: the *Fusarium* wilt Tropical Race 4 case study

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Biosecurity

Abstract

Cavendish bananas have increasingly been affected by *Fusarium oxysporum* f. sp. *cubense* tropical race (Foc TR4) since it was first identified in Southeast Asia. The pathogen has since spread to more than 20 countries in the Indian subcontinent, Middle East, Africa and Latin America, causing large-scale damage. The rapid spread is often linked to the expansion of monoculture commercial production with either contaminated plant material, soil, tools and machinery. Exclusion remains the most effective means to prevent Fusarium wilt TR4. Yet, exclusion depends on effective awareness programs among stakeholders and appropriate or applicable biosecurity systems, both at country borders and at farm level. Early and accurate diagnosis is essential for the containment of Foc TR4 but is difficult as the pathogen is often introduced well before large-scale damage becomes evident. Containment seldom succeeds due to the significant financial and human capital investment required. A generic approach to manage Foc TR4 does not exist. Specific considerations for each Foc TR4 incursion include plantation history, topography, the time of introduction and pattern of spread, resource availability and, most importantly, the economic viability and sustainability of available mitigation measures. The basis of a successful and integrated disease management strategy involves the planting of resistant or tolerant varieties, along with the reduction of inoculum build-up and spread. The status of Fusarium wilt TR4 globally will be discussed, with emphasis on efforts and available strategies to mitigate its impact.

Keywords

Fusarium wilt, banana, Tropical Race 4, management, biosecurity

Evaluation of optimum detection times for avocado sunblotch viroid (ASBVd) during phenological growth stages of avocado

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Disease Management

Abstract

Avocado sunblotch disease (ASBD) is an economically important disease of avocado, caused by avocado sunblotch viroid (ASBVd). The disease can cause yield losses, especially in asymptomatic trees. Limited information is available on the effect of time of sampling and tissue type (hardened-off leaves, new leaf flush and flower buds) on ASBVd diagnostic detection. These factors are important for reliable ASBVd detection and can increase the sensitivity of tests when pooled tree samples are tested. This study aims to evaluate the effect of sampling time during phenological growth stages of avocado in mature avocado trees; and to evaluate the effect of tissue type (hardened-off leaves, new leaf flush and flower buds) on ASBVd detection. A dataset of 17 'Fuerte', 21 'Edranol', and 16 'Hass' ASBVd positive trees were selected for this study. The average Ct values of selected trees were used to determine the titre of ASBVd infection over time. Average Ct values in 'Fuerte', 'Edranol' and 'Hass' trees showed detection throughout the phenological growth stages of the trees. However, in trees with an initial low ASBVd titre, ASBVd was not detectable during the period when fruit is on the tree. In these trees with low ASBVd titre, the older leaves were a more reliable source for detection in 'Fuerte' and 'Edranol' trees and in 'Hass', both young and older leaves resulted in reliable detection. The trees with low ASBVd titre were infected with the symptomatic ASBVd strain detected in avocado orchards. Fruit from symptomatic trees display typical ASBVd symptoms and are known to occur at a low titre in leaves and have an uneven distribution within a tree. Findings from this study, thus far, indicate that the time of sampling for ASBVd indexing will have an influence on accurate molecular detection results and can have an impact on disease management.

Keywords

avocado sunblotch viroid, ASBVd, optimal detection, phenological growth stage

Winter cover crop phenology-dependent allelopathic effects against *Phytophthora nicotianae* in a citrus glasshouse study

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Disease Management

Abstract

Citrus production in South Africa is increasingly constrained by tree dieback caused by *Phytophthora nicotianae*, which is a destructive oomycete frequently isolated in nurseries and orchards. The use of cover crops as an ecologically sensible disease management approach has been shown to be effective in reducing root rot severity on woody perennials. However, cover crops have not been investigated for their ability to suppress *P. nicotianae* population densities in a citrus rhizosphere. A completely randomized factorial glasshouse experiment was conducted to evaluate the curative effect of winter cover crops on a rough lemon rootstock artificially infected with *P. nicotianae*. Treatments included Saia oats, barley, common vetch, rapeseed, tillage radish, Japanese radish, a cover crop mixture, an oomycide standard (mefenoxam), and two controls (inoculated untreated and uninoculated untreated). Destructive sampling was done at cover crop emergence, flowering and senescence stages. Data was analysed using a linear mixed model. Significant effects of treatments were observed for root surface area, feeder root sloughing off percentage and fresh biomass ($p < 0.001$), while the destructive sampling stage significantly influenced the root surface area ($p = 0.001$) and feeder root sloughing off percentage ($p < 0.001$). Cover crop treatments did not significantly differ from the inoculated control when severity of feeder root rot was considered. In contrast, trees treated with mefenoxam were able to progressively regenerate feeder roots despite the tap root damage. Feeder root sloughing averaged 84,68 % across all cover crop and mixture treatments, compared to 64,17 % in the mefenoxam-treated trees and 87.60 % in the inoculated untreated control. Preliminarily, none of the winter cover crops and their mixture improved rootstock health once infection was established, suggesting that their allelochemical production thresholds were insufficient to suppress *P. nicotianae* in rootstocks with a high disease pressure, irrespective of when they were terminated.

Keywords

Root rot, rough lemon, biological suppression, oomycete, destructive sampling

Critical fruit protection period against citrus black spot infection in South Africa

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Ecology and Epidemiology

Abstract

Citrus black spot (CBS), caused by *Phyllosticta citricarpa*, is an important disease of citrus. The disease severity has been associated with *P. citricarpa* infection of fruit in their young stages, and the duration of their susceptibility to infection appears to be influenced by inoculum levels and regional climate. A study was conducted in orchards across two South African provinces to assess the intensity of CBS under two conditions: (i) unsprayed fruit of Valencia sweet orange, Nova, and Empress mandarins inoculated with *P. citricarpa* at 10^1, 10^3, and 10^5 pycnidiospores/ml at various intervals after fruit set, from October (petal fall stage) to March (for mandarins) or June (for sweet orange), and (ii) non-inoculated Valencia fruit from orchards subjected to spray programs ranging from one to nine monthly applications between October and June. A higher average disease severity index (DSI ~0.94) was observed when Valencia oranges were inoculated between November and January, whereas inoculations from February to June resulted in significantly lower severity (DSI ~0.07). In Nova mandarins, fruit inoculated in November or December showed higher CBS intensity, while a higher CBS intensity was observed on fruit inoculated in December for Empress mandarins. The absence of fungicide applications from October to December during the 2017–2018 season led to increased CBS intensity that was comparable to that of non-treated control trees. Leaving sweet orange trees unprotected from January onwards did not significantly increase the CBS intensity. These findings indicate that the dark green, early fruit stage (approximately 15–30 mm in diameter), particularly between November and January, is the most critical period for CBS infection in South Africa. Accordingly, citrus growers should protect fruit during this period to effectively manage CBS fruit infections.

Keywords

Phyllosticta citricarpa, fungicide sprays, inoculation, ontogenic resistance

Ultrastructural examination of the fungus-to-fungus interactions of *Lecanicillium uredinophilum* and *Phakopsora pachyrhizi*

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Disease Management

Abstract

Asian soybean rust (ASR) is caused by the biotrophic fungus *Phakopsora pachyrhizi* Syd. & P. Syd. and is one of the most important diseases of soybean [*Glycine max* (L.) Merr.], with recorded yield losses of up to 100%. The fungus *Lecanicillium uredinophilum* isolate PP2018-001, originally isolated from wild strawberry rust pustules, revealed hyperparasitic abilities on the rust urediniospores of soybean and frangipani (*Plumeria* spp.). This study examined the fungus-to-fungus interactions of the hyperparasite (*L. uredinophilum*) and *P. pachyrhizi* using confocal laser-scanning microscopy (CLSM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) to reveal whether *L. uredinophilum* isolate PP2018-001 employed mycoparasitism as its mechanism of action. The CLSM used an *Agrobacterium tumefaciens*-mediated transformation (AGTM) of *L. uredinophilum* isolate PP2018-001 with the green fluorescence protein (GFP) gene to clearly track the hyperparasite infection process. The SEM and TEM investigations used both *in vivo* and *in vitro* co-inoculations to examine the extent and type of damage caused by *L. uredinophilum* on the urediniospores of *P. pachyrhizi.* Confocal microscopy revealed the ability of *L. uredinophilum* to penetrate and intensely colonise urediniospores within 36 hours. In SEM studies, *L. uredinophilum* hyphae extensively coiled around urediniospores after both the *in vivo* and *in vitro* co-inoculations, with clear penetration and damage of outer urediniospore walls, which with time, produced visible perforations and loss of cell integrity. TEM revealed the infection of urediniospores by *L. uredinophilum,* penetrating hyphae, germ tube pores and the collapse of urediniospores. This study captured the parasitic nature of *L. uredinophilum* on *P. pachyrhizi* fungus-fungus interactions. It showed that mycoparasitism definitely occurred, and possibly enzymatic activity occurred, resulting in the degradation of urediniospore outer walls and germ tubes. The results are important for the potential registration of isolates of *L. uredinophilum* for the biological control of soybean rust.

Keywords

biotrophic, *Glycine max*, urediniospores, hyperparasite, mycoparasitism

Machine learning-based prediction of leaf wetness duration: a framework for evaluating biocontrol deployment feasibility in soybean fields

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Ecology and Epidemiology

Abstract

Leaf wetness (LW) plays a crucial role in the development and spread of fungal phytopathogens and their hyperparasites. Its influence is particularly significant for both *Phakopsora pachyrhizi*, the Asian soybean rust (ASR) pathogen, and its fungal biocontrol agent, *Lecanicillium uredinophilum*. Climatic and microclimatic conditions, especially LW, can impact the effectiveness of biological control in the field. This study utilised automatic field weather stations in Greytown and Cedara (KwaZulu-Natal Province, South Africa) to monitor LW and leaf wetness duration (LWD) in soybean canopies. Six machine learning (ML) algorithms—Random Forest (RFM), CatBoost (CBM), Gradient Boosting (GBM), Extra Trees (ETM), AdaBoost (ADM), and Decision Tree (DTM)—were evaluated for predicting LWD using eight explanatory variables: relative humidity, air temperature, dew point, wet bulb temperature, day_night, day_part, wind speed, and wind direction. Leaf Wetness was defined using resistance measurements below 501 kOhms. Model performance was assessed with confusion matrices and F1 scores, and variable importance was interpreted using SHAP (Shapley Additive exPlanations) values. Machine learning models were trained on data from three canopy-level sensors (0.45 m, 0.60 m, 0.90 m) at both sites. All six algorithms demonstrated strong predictive capability, with F1 scores ranging from 0.82 to 0.95. At Greytown, ADM performed best across all sensor levels, with F1 scores of 0.94 (LWS1), 0.90 (LWS2), and 0.89 (LWS3). At Cedara, multiple models shared top performance: LWS1 (0.94), LWS2 (0.95), and LWS3 (0.96). Relative humidity consistently emerged as the most influential variable. These findings suggest ML models can reliably predict LW and LWD, supporting not only disease forecasting but also optimised timing for deploying biocontrol agents like *L. uredinophilum*, even with relatively short-term, high-resolution data collection.

Keywords

Leaf wetness duration, biological control, mycoparasite, machine learning, algorithms, *Lecanicillium uredinophilum*

Transcriptomic analysis of *Cercospora zeina* effectors reveals the expression of necrosis-inducing secreted protein 1 (NIS1) homologs during the mid stage of maize infection

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Plant-Pathogen/Insect Interaction

Abstract

Cercospora zeina is the causal agent of gray leaf spot disease on maize (*Zea mays L*.) in South Africa. The disease is characterised by the formation of rectangular lesions that reduce the plant's ability to photosynthesise, resulting in poor yields. Proteins called effectors are thought to mediate this process by modifying the host to facilitate the pathogens lifestyle. Although some *C. zeina* effectors have been studied, their expression patterns and roles in host colonization remain largely unknown. To address this, transcriptomics data from glasshouse trials in combination with genome-guided protein annotation, structural modelling, and *Agrobacterium*-mediated transient transformation (ATT) assays were used to identify effector homologs, evaluate expression profiles and investigate aspects of their molecular functions. Effector expression clustering revealed a predominance of effectors with peak expression at the mid stage of maize infection. Within these clusters, two homologs of the necrosis-inducing secreted protein 1 (NIS1) effector from *Colletotrichum incanum* were identified and named CzNIS1a and CzNIS1b. During infection, CzNIS1a is expressed at a higher-level relative to CzNIS1b and is co-expressed with the necrosis-inducing extracellular protein 2. Interestingly, homologs of NIS1b are double the size of typical NIS1 effectors and seem to be restricted to members of the Mycosphaerellaceae and Dissoconiaceae. Domain analysis predicts a b-sheet domain at the N-terminus of CzNIS1a and CzNIS1b that has structural and sequence similarity to *Colletotrichum* NIS1 effectors. CzNIS1b also contains an extended C-terminal domain consisting of four a-helices with unknown function. Similar to *Colletotrichum* NIS1 effectors, CzNIS1a and CzNIS1b trigger necrosis in *Nicotiana benthamiana* ATT leaf assays. Biochemical assays show reactive oxygen species generation and callose deposition in areas expressing CzNIS1a and CzNIS1b, altogether suggesting that they activate plant defences in *N. benthamiana*. These effectors are therefore promising targets for host-pathogen research in the maize-*C.zeina* pathosystem.

Keywords

Gray leaf spot disease, Alphafold, Agroinfiltration, Pattern-triggered immunity, Tobacco

Unravelling the endophytic maize grain mycobiome of grey leaf spot diseased plants: Assessing the seed-borne potential of *Cercospora zeina*

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Plant-Pathogen/Insect Interaction

Abstract

The endophytic maize grain mycobiome has received relatively little attention compared to the rhizosphere and phyllosphere, despite its critical role in post-harvest quality, seed health, and disease transmission. The presence of *Cercospora zeina* [the causal agent of grey leaf spot (GLS)] in maize grain therefore remains unconfirmed. Moreover, the influence of GLS diseased plants and geographical variation on the grain mycobiome is poorly understood. This study investigated the endophytic grain mycobiome of grey leaf spot diseased plants using the same maize hybrid. Grain from plants with low and high GLS disease (on account of different fungicide treatments) were collected from two different locations (Cedara and Bergville, South Africa) for fungal community profiling. Full-length internal transcribed spacer (ITS) region metabarcoding (PacBio Sequel IIe system) with the use of peptide nucleic acid to block maize ITS amplification was utilised to profile the maize grain fungal community. The ecological roles were inferred using FUNGuild. A total of 148 fungal amplicon sequence variants (ASVs) were detected. Fungal community composition significantly varied across locations and treatments, with Bergville samples exhibiting significantly lower overall diversity. Functional guild analysis showed that 54 % of fungal ASVs were classified as plant pathogens. Maize plants with low and high GLS infection levels showed no significant differences in fungal composition and diversity. Importantly, no *C. zeina* was identified in maize grain, suggesting it is not a seed-borne pathogen, reducing concerns about vertical transmission of GLS. Overall, the results suggest that geographical variation influences grain mycobiome composition, likely attributed to abiotic factors such as soil physicochemical properties, soil type, elevation and climate, while localised variation may be due to fungicide efficacy shaping the grain fungal community composition and diversity. The findings about *C. zeina* being absent in maize grain confirm our current understanding of the disease cycle for GLS.

Keywords

PacBio, seed-transmitted, PNA clamps, Metabarcoding, seed-health

Fungicide sensitivity of *Botrytis cinerea* isolates from South African lemon orchards

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Disease Management

Abstract

Botrytis cinerea is an economically important pathogen that causes petal necrosis, premature flower fall and rind distortion in lemons. Its high genetic plasticity makes it a high-risk pathogen for fungicide resistance development. Persistent application of fungicides has led to the emergence of resistant *B. cinerea* isolates in various crops. This study aimed to determine the genotypic diversity and the *in vitro* sensitivity of *B. cinerea* isolates from lemon orchards in South Africa to azoxystrobin, benomyl, fludioxonil, pyrimethanil and a combination of fludioxonil and pyrimethanil. A total of 114 *Botrytis cinerea* isolates were collected from lemon orchards in the Limpopo, Eastern Cape, and Western Cape provinces of South Africa during the 2022/23 to 2024/25 seasons. Genetic diversity in all the isolates was determined using PCR to detect transposable elements, *boty* and *flipper*. The sensitivity of thirty *B. cinerea* isolates selected based on location was tested against the previously mentioned fungicide active ingredients. The results showed that the dominant genotypes identified were *boty* (44.35%) and *transposa* (36.65%), with *vacuma* (13.91%) with *flipper* (6.09%) being less prevalent. Fungicide sensitivity assays revealed that azoxystrobin exhibited EC_{50} values ranging from 0.0617 to 2.4328 ppm, whereas benomyl demonstrated EC_{50} values between 0.0083 and 0.0331 ppm, pyrimethanil between 0.0514 and 0.8210 ppm, fludioxonil between 0.0215 and 0.1601 ppm, and the combination of pyrimethanil and fludioxonil between 0.0071 and 0.2011 ppm. There was no clear relationship between sensitivity and genotypic diversity, as all isolates were generally sensitive to the tested active ingredients. However, one outlier *transposa* isolate showed low sensitivity to azoxystrobin, with an EC_{50} value of 2.4328 ppm. These findings provide evidence that *B. cinerea* isolates from South African lemon orchards are genetically diverse. This could be a potential challenge for disease management, so rotating the active ingredients is advisable to reduce the risk of resistance development.

Keywords

Grey mould, fungicide resistance, genetic diversity, transposable elements, citrus

Functional insights into plant immune modulation by the *Phytophthora parasitica* effector PpRxLR55

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Plant-Pathogen/Insect Interaction

Abstract

Phytophthora parasitica is a destructive oomycete pathogen that infects over 255 plant species, including economically important crops such as potato. Central to its pathogenicity is the secretion of RxLR effectors, which are delivered into the host cytoplasm to manipulate plant immunity. Although several RxLR effectors have been characterized in other *Phytophthora* species, the functions of those from *P. parasitica* remain largely unexplored. In this study, we investigated the role of PpRxLR55 in manipulating host immunity during *P. parasitica* infection. Transient expression of PpRxLR55 in *Nicotiana benthamiana* did not induce immediate programmed cell death (PCD) at 5 dpi, but delayed PCD was observed at 10 dpi, accompanied by early accumulation of reactive oxygen species and enhanced callose deposition, suggesting activation of basal immune responses. Subcellular localization revealed that PpRxLR55 targets the nucleus, nucleolus, and endoplasmic reticulum (ER), with its accumulation in the ER causing visible structural disruption. To determine the proteins that PpRxLR55 might interact with, we performed yeast two-hybrid (Y2H) screening. This analysis revealed several interacting host proteins involved in key cellular processes, including ER stress regulation, vesicle trafficking, and immune signaling. Among these were Membrane Steroid-Binding Protein 2 (MSBP2), Phenylalanine Ammonia-Lyase (PAL), RNA-binding proteins, Exocyst component SEC5A, and TORTIFOLIA1. MSBP2 and PAL, the most abundant interactors, were further validated by pairwise Y2H assays, confirming their direct interaction with PpRxLR55. Collectively, these findings reveal that PpRxLR55 might contribute to *P. parasitica* virulence by targeting multiple host pathways, disrupting ER homeostasis, and interfering with immune signaling to promote disease development.

Keywords

Phytophthora parasitica, RxLR effector, Plant immunity, Endoplasmic reticulum stress, Protein-protein interaction

Fungal Déjà Vu: Two decades of *Immersiporthe knoxdaviesiana* (Cryphonectriaceae) canker epidemics driven by clonal pathogen lineages

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Ecology and Epidemiology

Abstract

Immersiporthe knoxdaviesiana (Cryphonectriaceae) causes an aggressive canker disease on native Cape Beech (*Myrsine melanophloeos*) in South Africa. First reported in the early 2000s in the Harold Porter National Botanical Gardens, Western Cape, the disease is characterised by cankers originating around branch junctions, girdling stems and top dieback. Nothing is known regarding the origin or drivers of this serious disease. To investigate the population biology of the pathogen, we developed 15 polymorphic microsatellite markers using genome sequences for two *I. knoxdaviesiana* isolates. A collection of 90 isolates from historical and recent outbreaks at Harold Porter, Kirstenbosch National Botanical Garden, and Haga Haga in the Eastern Cape was genotyped. The results revealed remarkably low genetic diversity including only three multilocus genotypes (MLGs). A single genotype (MLG1) was dominant (76.7 % of all isolates) in historical and recent collections at Harold Porter. The Haga Haga population exhibited higher genetic diversity, comprising two distinct MLGs, isolated from trees with fruiting bodies (MLG2) and healthy trees (MLG3). Notably, MLG3 was also found in isolates from healthy tissues of trees in Kirstenbosch. The sexual state of *I. knoxdaviesiana* was found for the first time on asymptomatic branch from Kirstenbosch and Haga Haga, but microsatellite data provided no evidence of random mating. The clonal structure and low diversity in the epidemic population in Harold Porter is typical of invasive species undergoing population bottlenecks through a founder event. However, evidence from this study showing that *I. knoxdaviesiana* exists as an asymptomatic endophyte in healthy trees suggests that it could be a native pathogen exhibiting increased aggressiveness under changing environmental conditions. Further sampling across the native range of *M. melanophloeos* in sub-Saharan Africa will help to trace the geographic origin of *I. knoxdaviesiana* and determine whether it represents an introduced species or a native pathogen undergoing a shift in biology.

Keywords

botanical gardens, emerging disease, *Myrsine melanophloeos*, native tree, riparian forests

Safeguarding the soybean export market: a national surveillance approach to sudden death syndrome in South Africa

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Biosecurity

Abstract

South Africa remains a competitive soybean exporter with 150 245 tonnes of soybean exported in 2025. Unfortunately, sudden death syndrome (SDS), which is a serious soilborne disease of soybean, has been reported in the country in 2013. The disease is caused by different *Fusarium* spp. with *Fusarium virguiforme* regarded as the most important. The disease, with special reference to *F. virguliforme*, has not been reported in China. Currently, China has emerged as an important soybean export destination for South Africa and demonstrating the absence of *F. virguliforme* has become a phytosanitary priority. A multi-year SDS surveillance initiative was launched during the 2021/2022 season through collaboration between Grain South Africa, the South African Department of Agriculture, the soybean industry, and the Agricultural Research Council. The surveillance strategy focused on farms within a 100 km radius of major export silos in the eastern Free State, KwaZulu-Natal, and Mpumalanga. The surveillance program was designed to support phytosanitary compliance with Chinese export requirements. Annual field surveys included sampling of symptomatic and asymptomatic plants, roots, and soil. Morphological identification and molecular diagnostics were employed to detect SDS-associated *Fusarium* spp., with special focus on *F. virguliforme*. Soybean seed samples collected from silos intended for export, in collaboration with the South African Grain Laboratory, were also analysed. Over the surveillance period, SDS symptoms were observed in fields across all three provinces. Multiple *Fusarium* isolates resembling SDS pathogens were isolated and identified as *F. brasiliense* and an undescribed *Fusarium* species. *Fusarium virguliforme* was not detected in any of the samples analysed from soil, plants, or seed to date. This surveillance program demonstrates the value of coordinated long-term monitoring in supporting export readiness and national biosecurity. The South African SDS model serves as a case study for collaborative government–industry–research responses to phytosanitary challenges and export-driven disease surveillance.

Keywords

Fusarium virguliforme, molecular detection, roots, soil, seed

Cross-pathogenicity of species within the *Fusarium graminearum* species complex obtained from different field crops produced in South Africa

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Post-harvest Pathology and Mycotoxins

Abstract

Maize, wheat, sorghum and soybean are economically important crops often used in rotation. Pathogens negatively impact these, especially mycotoxigenic *Fusarium* fungi. The *Fusarium graminearum* species complex (FGSC) is the main causal agent of Gibberella ear rot of maize, Fusarium head blight of wheat, and are major components in Sorghum grain mold and Fusarium root rot in soybean. These fungi produce harmful metabolites, mycotoxins, that pose a significant food safety threat. Understanding the ability of FGSC species to cause disease and produce mycotoxins across these crops will provide clarity on the influence of crop rotation and Conservation Agriculture on subsequent disease development and mycotoxin contamination when these host crops are rotated. Isolations from maize, wheat, sorghum and soybean seed from South African producers yielded several FGSC species including *F. graminearum* s.s., *F. boothii, F. meridionale, F. acacia-mearnsii* and *F. cortaderiae*. Mycelial suspensions of representative isolates (n=16) were used to inoculate the crop from which it was isolated as well as other host crops under greenhouse and field conditions. The disease severity was subsequently rated, and mycotoxin contamination was measured while re-isolations were performed to confirm causal pathogen identity. All the FGSC species caused disease when cross-inoculated onto different crops. *Fusarium meridionale* isolates from soybean were the most pathogenic on maize and wheat, compared to positive control treatments. Disease severity under greenhouse conditions was significantly higher compared to disease symptoms under field condition. Mycotoxins Deoxynivalenol, Nivalenol and Zearalenone were found in the grain of all crops. The study clearly indicates that FGSC species from different crops can cross-infect, thus rotation with host crops can contribute to subsequent disease and mycotoxin contamination and should be avoided.

Keywords

Fusarium graminearum, *Gibberella, maize, wheat, sorghum, soybean*, *mycotoxins*

The potential of plant extracts as biostimulants and biofungicides on potatoes (*Solanum tuberosum*)

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Disease Management

Abstract

Potatoes (*Solanum tuberosum)* are important globally and is the largest fresh vegetable crop in South Africa. Challenges smallholder farmers face with potato production include lacking the resources to acquire synthetic fungicides and fertilizers. Furthermore, these chemicals can result in environmental pollution and can negatively impact human and animal health. Agrobiologicals offer sustainable alternatives. In this study, fermented aqueous plant extracts from *Lantana camara, Moringa oleifera,* and *Melia azedarach*, as well as commercial products Ecklomax, K-sil, Fighter were evaluated for their biofungicidal and biostimulant activities on potato plants under an uncontrolled greenhouse environment. In addition, Biocult (a commercial product) was applied to all treatments to test whether its application could enhance the effects of the other treatments. This comparison was referred to as Biocult vs non-Biocult. Biostimulatory effects were monitored by assessing canopy cover and yield. No difference was noted between the treatments for canopy cover and yield parameters. All non-Biocult treatments had a higher average tuber weight than Biocult treatments. Biofungicidal activity was measured by assessing the disease percentage in a given area, focusing on symptoms of brown spot, early blight and late blight. The following treatments did not differ significantly from one another for brown spot and late blight percentages respectively: *L. camara* (20.9%, 3.2%), *M. azedarach*(21%, 4.0%), *M. oleifera* (19.9%, 4.3%) and Fighter (chemical control) (22.6%, 3.3%). However, K-sil (non-treated control) showed significantly higher disease levels, with 33.1% for brown spot and 8.8% for late blight. For early blight there was no significant differences between treatments. The research showed that plant extracts only exhibit biofungicidal activity against late blight and brown spot and not biostimulatory activity. The success of this research could benefit smallholder farmers as plant extracts are relatively easy to make, pose little risk to human health and are biodegradable.

Keywords

brown spot, disease suppression, fermented extracts, late blight, smallholder farmer

Seasonal occurrence of *Pseudopithomyces toxicarius* in South African dairy pastures

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Post-harvest Pathology and Mycotoxins

Abstract

Facial eczema (Sporidesmin toxicity) is a seasonal liver disease in ruminants caused by the ingestion of sporidesmin A, a mycotoxin produced by *Pseudopithomyces toxicarius* (*Pt*). While well-documented in New Zealand, Facial eczema is an emerging and underrecognized issue, particularly in the Eastern Cape province of South Africa. This study represents the first targeted survey of *Pseudopithomyces* species in Eastern Cape pastures. The aims were to: (i) identify which *Pseudopithomyces* species are present; (ii) monitor *Pt* spore levels throughout the year using both spore counts and spore traps; and (iii) assess the ability of local isolates to produce sporidesmin A. Grass samples were collected every four to six weeks from five Eastern Cape farms. Spores were counted using haemocytometer-based washing methods, and airborne spores were monitored weekly using spore traps. Isolates were identified using morphology and sequence data. Sporidesmin A production was assessed using UHPLC-QTOF-MS. Only *Pt* was detected across all sites, and 70% of the isolates were confirmed to produce sporidesmin A. Haemocytometer-based spore counts peaked during summer, with concentration ranges rising from 3000-12500 spores/g grass in January, to 6000-545000 spores/g in February, before slightly decreasing in April to 41000-345000 spores/g. Data from airborne spore counts indicate the absence of *Pt* in the off-peak winter season, with a gradual increase observed at the start of summer. This seasonal peak highlights a potential risk period, however a direct link to observed reductions in milk yield and to clinical Facial eczema in South Africa requires further validation. This study establishes baseline data on *Pseudopithomyces* presence and seasonal dynamics in South Africa and confirms the toxigenic potential of local *Pt* strains. Continued ecological and toxigenic monitoring, including population-level studies, will enhance forecasting tools and inform management practices to reduce the risk of Facial eczema in ruminant livestock.

Keywords

Facial eczema, spore trap, sporidesmin A, surveillance, spore count, mycotoxin

Symbiotic bacterial metabolites from entomopathogenic nematodes for biocontrol of *Rhizoctonia solani* and *Botrytis cinerea*

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Disease Management

Abstract

Soilborne infections, including damping-off of seedlings, stem cankers, root and stem rots produced by *Rhizoctonia solani,* adversely affect crop yield and quality, significantly impacting food security. Similarly, *Botrytis cinerea*, which is responsible for grey mould, impacts several fresh produce pre- and post-harvest. Due to chemical control posing issues associated with safety, chemical residues and development of resistance, the use of biological control is a safer alternative management strategy for these pathogens. Five *Photorhabdus* and *Xenorhabdus* bacterial symbionts of entomopathogenic nematodes (EPN) that produce antimicrobial agents were selected for evaluation against *R. solani* and *B. cinerea*. Metabolites were incorporated into the culture media to evaluate their efficacy on the mycelial growth inhibition (*R. solani* and *B cinerea*) as well as the spore germination inhibition of *B. cinerea*. The findings showed potency of bacterial metabolites from a *Xenorhabdus* sp. isolated from *Steinernema yirgilamense,* resulting in growth inhibiting rate of 75 % (*R. solani*) and 31 % (*B. cinerea*) respectively as well as 92% *B. cinerea* spore germination inhibition. Although tests were applied *in vitro,* metabolites from the symbiotic bacteria of *S. yirgalemense* showed potential for use as biological control agents against *B. cinerea* and *R. solani*. The next step is testing these metabolites *in vivo* against these pathogens.

Keywords

Antimicrobial metabolites, *Photorhabdus,* plant disease, spore germination inhibition, *Xenorhabdus.*

Evaluation of alternative spray programs for the control of pre- and post- harvest diseases on avocado

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Disease Management

Abstract

Avocado production in South Africa is heavily affected by pre- and post-harvest diseases, with the most serious threats being *Pseudocercospora purpurea* (Cercospora spot), *Colletotrichum* species (anthracnose), and species within the *Botryosphaeriaceae* complex (stem-end rot). These pathogens cause substantial economic losses, especially under the warm, wet summer conditions that favour their development. Current management strategies rely predominantly on copper-based fungicide spray programmes, which remain among the most effective control measures. Although alternative fungicides have been evaluated, effective suppression of these diseases also requires the correct selection of application equipment, accurate timing, and thorough coverage. Over the past two decades, research at Westfalia Fruit Estate in Limpopo Province has focused on developing alternative and more sustainable management strategies. The subtropical climate of this area, with annual rainfall ranging from 800 to 1300 mm and occasionally exceeding 1800 mm, creates highly favourable conditions for disease incidence. Research efforts have therefore prioritized reducing orchard inoculum, preventing latent infections, and optimising pre-harvest spray programmes to balance efficacy with sustainability. Recent studies have evaluated reduced copper application rates through novel formulations and application technologies. Preliminary results indicate that alternative spray technology, in combination with a new copper formulation, provides control comparable to the current commercial standard in controlling avocado diseases. These results demonstrate the potential to optimise copper fungicide use in avocado orchards without sacrificing efficacy, while contributing to reduced production costs, lower environmental impact, and improved sustainability of disease management practices.

Keywords

Pseudocercospora purpurea, *Colletotrichum* species, *Botryosphaeriaceae*

Establishing the *in vitro* sensitivity of currently used oomycides and determining baseline sensitivity to new proposed oomycides to manage *Phytophthora* on macadamia in South Africa

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Disease Management

Abstract

Phytophthora root rot and stem cankers (PRRSC), caused by the oomycete pathogen *Phytophthora cinnamomi*, is a major disease affecting macadamia production in South Africa. Effective management of PRRSC relies on an integrated disease management approach, including cultural and chemical practices. This study aims to assess the *in vitro* sensitivity of *P. cinnamomi* isolates to both currently registered (mefenoxam and phosphonates (phosphite)) and new prospective oomycides (oxathiapiprolin, fluoxapiprolin, propamocarb hydrochloride and fluopicolide). High-throughput screening methods, specifically a 24-well microtiter spectrophotometer assay, were optimised to assess the sensitivity of *P. cinnamomi* isolates from nurseries and orchards. Isolates were selected from the screening results to determine the EC_{50} using the gold standard (GS) amended agar method. Screening of the *P. cinnamomi* population against mefenoxam did not identify any resistant isolates. The average EC_{50} of a subset of the isolates using the GS method was 0.208 mg/L. Screening against phosphite identified *in vitro* tolerant isolates, with the average phosphite GS EC_{50} for a subset of isolates being 167.82 mg/L. Screening of new oomycide actives, namely oxathiapiprolin and fluoxapiprolin, indicated a high degree of *in vitro* sensitivity of the *P. cinnamomi* population. The baseline EC_{50} values are still being determined. Screening of fluopicolide and propamocarb hydrochloride using the 24-well assay, followed by GS baseline EC_{50} estimations is still ongoing. The results from this study highlight the sensitivity of the *P. cinnamomi* population towards mefenoxam, with a shift in *in vitro* tolerance towards phosphite. The relevance of the *in vitro* phosphite tolerance for *in planta* control will be investigated in a future study. Establishment of the baseline sensitivity of new oomycide actives and determination of the sensitivity to currently used oomycides contribute to the development of sustainable chemical management strategies for PRRSC in macadamia production.

Keywords

P. cinnamomi, mefenoxam, phosphonates, oxathiapiprolin, fluoxapiprolin, fluopicolide.

The biosecurity surveillance trapping network in southern Africa and citrus *Liberibacter* detection

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Biosecurity

Abstract

Huanglongbing (HLB) is a devastating citrus disease associated with '*Candidatus* Liberibacter asiaticus' (*C*Las). African citrus Greening is a similar, but less aggressive disease associated with '*Ca*. L. africanus' (*C*Laf) and '*Ca*. L. africanus' subsp. clausenae (*C*LafCl) in Africa. However, the occurrence of *C*Las in East Africa is a significant threat to the southern African citrus industry. *C*Laf has likely been present in South African citrus orchards since the 1920s and was first observed in the North West Province, and later in Mpumalanga Province. The disease is regulated in the country to protect declared Greening-free and low disease prevalence areas, with ongoing monitoring programs and protocols in place to detect and prevent its spread in these areas. The disease can spread by natural movement of the African citrus triozid (ACT) vector, *Trioza erytreae* (Del Guercio, 1918) (Triozidae), or by the movement of infected plant material. With the potential incursion of both *C*Las and its primary vector, *Diaphorina citri* Kuwayama (Psyllidae), effective surveillance and detection methods are crucial to protect the citrus industry. A biosecurity trapping network was established to monitor for *D*. *citri* incursion, to monitor ACT populations, and to test for the presence of citrus Liberibacters. Yellow sticky traps have been placed in citrus production regions, including commercial farms, residential areas, nurseries, and around North Eastern borders of South Africa. The traps are serviced by Citrus Research International (CRI), the Department of Agriculture (DoA), and citrus growers. All traps are sent to CRI for psylloid identification for vectors and Liberibacter testing. Data for ACT abundance and citrus Liberibacter detection from the various trapping regions are presented for the period 2020-2025. While *C*Laf is widely distributed in South Africa, *C*Las and its vector *Diaphorina citri* have not been detected.

Keywords

Huanglongbing, African Greening, Yellow sticky traps, *Trioza erytreae, Diaphorina citri*

Stressed out: how environmental challenges shape gene expression in *Fusarium oxysporum* f. sp. *cubense*

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Ecology and Epidemiology

Abstract

Fusarium oxysporum f. sp. *cubense* (Foc), the causal agent of Fusarium wilt in banana, poses a major threat to global production. Foc races are classified based on host range: race 1 (R1) affects banana cultivars such as Lady Finger, Gros Michel, Silk, and Pome; subtropical race 4 (STR4) affects R1 susceptible cultivars and Cavendish under environmental stresses; and tropical race 4 (TR4) affects Cavendish under any condition along with other Foc susceptible cultivars. In subtropical regions, both the pathogen and plant host encounter environmental stressors that may alter Foc pathogenicity and host susceptibility, such as events of low temperature. Symptom expressions associated with STR4 are believed to be linked to abiotic stressors; however, this assumption has not been validated under controlled conditions. This study hypothesizes that temperature, salinity, pH, and hypoxia influence the expression of pathogenicity-related genes in STR4. Quantitative PCR was used to assess gene expression in multiple Foc R1 and STR4 strains exposed *in vitro* to varying levels of these environmental factors. Data on the differential gene expression will be presented. These findings provide insights into the environmental modulation of Foc STR4 pathogenicity and may inform disease management strategies for subtropical banana production.

Keywords

Plant Pathology, Fusarium wilt, Banana

The Polyphagous Shot Hole Borer invasion in South Africa: where are we now?

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Biosecurity

Abstract

The invasive Polyphagous Shot Hole Borer beetle (PSHB, *Euwallacea fornicatus*) is a devastating pest that, together with its symbiotic fungus *Fusarium euwallaceae*, can kill a wide range of native and exotic woody hosts and cause significant economic damage. Originally from Asia, this pest invaded Israel, the USA, South America, Europe, Australia, and South Africa. Ongoing research shows that PSHB poses a significant threat to native forests, urban and agricultural trees. More than 300 tree species can be infested by the beetle, some of which develop disease symptoms and may die. Here, cumulative information on the invasion history, biology, ecology, and management of this destructive pest, gathered from various studies and researchers in South Africa, is provided. Historical records indicate the presence of PSHB in South Africa as early as 2012, but it was not reported until 2017 after its discovery in infested trees in a botanical garden. This delay meant that the pest could establish and rapidly spread across the country, mainly due to the transport of infested materials. PSHB now has major impacts in leafy urban areas across the country. The economic and ecological consequences of this still need to be fully realised. Impacts in fruit orchards are currently isolated to stone fruit trees, pome fruit trees, and nut trees. For many of these, South Africa is the first country globally to experience PSHB infestations. The beetle has also established in multiple native forest ecosystems across the country; the ecological effects of which are only starting to emerge. Chemical management options are limited and may not be effective. Therefore, research into possible biological control options has been initiated. These include investigations into the use of entomopathogenic fungi, mites, nematodes, and parasitoid wasps. Initial results of these investigations will be highlighted.

Keyword

symbiotic fungus

In vitro efficacy of phosphonates and novel oomycides to inhibit *Phytophthora nicotianae* from South African citrus nurseries and orchards

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Disease Management

Abstract

Phytophthora nicotianae is an important soil- and water-borne oomycete phytopathogen causing root rot in citrus nurseries and orchards of South Africa. Management of this oomycete relies mainly on the use of active ingredients such as phenylamides and phosphonates. Phosphonates have a complex and multifaceted mode-of-action, which reduces the risk of resistance development in *P. nicotianae*. Recent reports from various citrus growing regions globally indicate an increase in the insensitivity of *Phytophthora* spp. to other registered active ingredients. This necessitated an investigation to determine if the active ingredients currently registered for the management of *Phytophthora* spp. on citrus in South Africa are still effective, and if products registered for the management of oomycetes on other crops can be considered as alternatives. Potassium- and ammonium phosphonate, along with four novel active oomycides registered for use on different crops (amisulbrom, ethaboxam, mandipropamid, and zoxamide), were screened *in vitro* for their efficacy against *P. nicotianae*. A total of 46 isolates from South African citrus nurseries and orchards were screened *in vitro*. No isolate was found to be insensitive to the evaluated phosphonate products, with no significant differences between products. South African *P. nicotianae* isolates were found to be sensitive to amisulbrom (EC_{50} 0.2758 ppm), ethaboxam (EC_{50} 0.04627 ppm), mandipropamid (EC_{50} 0.00662 ppm) and zoxamide (EC_{50} 0.01123 ppm). While no insensitivity development against phosphonates was observed, an integrated management approach for *P. nicotianae* should be followed to prevent a shift towards reduced sensitivity in the South African citrus population of *P. nicotianae*. The four novel oomycides effectively inhibited *P. nicotianae*, prompting further research to support the registration of these active ingredients as alternatives to phosphonates and phenylamides. The results from this study indicate the sensitivity of the South African *P. nicotianae* population from citrus to currently registered active ingredients and novel oomycides.

Keywords

Phytophthora root rot, amisulbrom, Ethaboxam, mandipropamid, zoxamide

Towards a centralised and accessible disease surveillance system for grain crops in South Africa

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Biosecurity

Abstract

Effective plant disease surveillance is fundamental to sustainable crop production, yet in South Africa, disease prevalence data for grain crops often relies on outdated resources. This gap hinders timely responses to emerging threats and limits the evidence base for decision-making. Field-based disease surveys were conducted during the 2024 and 2025 growing seasons across the National Cultivar Trials of soybean and sunflower. The incidence and prevalence of fungal diseases were recorded using standardised field observations guided by crop-specific disease compendia. Each confirmed disease occurrence was geo-referenced, annotated with host and symptom data, and stored within a digital repository. In parallel, a systematic literature review was undertaken to digitise reports of fungal diseases in grain crops across South Africa. This included first reports and peer-reviewed records of disease occurrence, which were mapped through an interactive Shiny application to visualise trends. The survey revealed a diverse range of diseases across both surveyed crops, including Cercospora leaf blight, Phyllosticta leaf spot, Septoria leaf spot, and bacterial blight on soybean, as well as Verticillium wilt, Alternaria leaf blight, and white rust on sunflower. Several under-reported or misidentified diseases were also detected. Samples of potential viruses were shared with virologists for identification. At the National Biosecurity Summit, stakeholders expressed the need for a more accessible, centralised, and regularly updated platform for plant disease reporting. We propose the development of a national #PlantDiseaseZA dashboard, integrating survey data into a live platform that supports informed action by policymakers, researchers, and producers. A pilot of the dashboard includes diagnostic laboratories, occurrences of diseases, e.g., Goss's Wilt, Pepper ringspot virus and *Puccinia* spp. Revitalising plant disease surveillance is not only a scientific necessity, but it is a strategic opportunity to transform how we respond to disease outbreaks and safeguard South Africa's grain sector under changing climatic and production pressures.

Keywords

Biosecurity, collaborative plant pathology, early detection, digitisation, diagnostic network

Forest biosecurity in Southern Africa - challenges and opportunities

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Biosecurity

Abstract

Non-native microbes and invertebrate pests have caused significant economic losses to the plantation forestry industry in South Africa. Apart from resulting in growth losses and tree mortality, these biological invasions have at great cost, necessitated species and varietal changes. Similarly, native forests in South Africa are under considerable threat, with pathogens such as *Phytophthora cinnamomi* and *Austropuccinia psidii*, and the polyphagous shot hole borer, for example, representing serious threats to the survival of native forest plants. The introduction pathways for forest pests and pathogens into South Africa are diverse, including the import of growing media and live plants (e.g., *Armillaria mellea* and *Rhizina undulata*) and timber / logs (e.g., the pine wasp *Sirex noctilio*). The likely route of introduction for many other non-native forest pests is through the movement of live plant material. However, studies on the pathways and drivers of introduction and establishment of these pests and pathogens are still comparatively limited. Given the potential for host jumps and cross-sector spread, effective management of pest and pathogen incursions into South African forests and plantations is reliant on not only the forestry sector, but also the agricultural and ornamental plant sectors. Collaboration and coordinated action among government agencies, industry stakeholders and the public is essential. Achieving this requires a better understanding of the invasion biology of forest pests and pathogens and greater integration of forest biosecurity issues into national legislative frameworks and the broader invasion science community.

Keywords

Tree pathogens; plantations; invasive pests

Characterisation and management of *Leptosphaeria maculans* causing blackleg of canola in the Western Cape province of South Africa

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Disease Management

Abstract

Blackleg, caused by *Leptosphaeria maculans* is an important disease of canola globally and in South Africa. The pathogen has a high evolutionary potential which enables it to rapidly overcome host resistance. Therefore, investigation of genetic diversity, structure and changes in avirulence (*Avr*) gene frequencies in *L. maculans* populations can provide important information to inform management strategies. In this study, whole genome sequence data was generated for 230 *L. maculans* isolates collected across the canola production regions of the Western Cape (2020-2022). SNPs were identified and used in clustering and population genomic analyses. To assess South African isolates in the global context, data for 171 international isolates was included, and analyses repeated. The genome data was also utilised to genotype *Avr*-genes of isolates, and phenotypes were inferred from published data. The clustering analyses revealed the genetic differentiation between the Swartland and Southern Cape regions. ANOVA suggested a shallow population structure with only 3.71% of variation occurring between regions. For the international isolate collection, results showed a high similarity between Australian and Swartland isolates, while isolates from the Southern Cape formed a unique genetic cluster. Low frequencies of *AvrLm1* (6.4%), *AvrLm4* (0.3%) and *AvrLm6* (36%) suggest that the pathogen population overcame the corresponding *R*-genes, *Rlm1*, *Rlm4* and *Rlm6*, present in cultivars which were extensively planted in the last decade. High frequencies of *AvrLm7* indicate that the efficacy of the recently deployed *Rlm7* is still intact. Field trials conducted in the Swartland and Southern Cape (2021-2023) supported these *Avr*-gene frequencies, showing that *Rlm7* in cultivars is associated with lower blackleg severity. These results highlight the need for an *R*-gene rotation strategy to ensure the longevity of *R*-genes deployed. This research provides the foundation to understanding blackleg in South Africa to ultimately aid the development of effective integrated management strategies.

Keywords

Genetic population structure, Next generation sequencing, Major gene resistance, Resistance breakdown, Race structure

Mapping maize ear rots and their toxins in South Africa

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Post-harvest Pathology and Mycotoxins

Abstract

Maize ears are susceptible to various Fusarium species, which produce mycotoxins that compromise both grain yield and quality. Mycotoxin contamination poses a threat to food security since there are no cultivars that show complete resistance to the casual pathogen, and there are no registered fungicides to control the disease or mycotoxins. Thus, the aim of the study was to 1. perform surveillance of ear rot diseases and identify associated fungi in maize-producing localities of S.A and 2. to determine the incidence and level of ear rots and mycotoxins in maize grain. Maize kernels were collected for two consecutive seasons (2022/23 and 2023/24) and were visually inspected for ear rot symptoms. Grain was subjected to fungal isolation on potato dextrose agar with antibiotics for ±4 days at 25℃. Isolated fungi were identified based on morphological characteristics, and identification was confirmed by sequence comparison of the elongation factor gene region. For mycotoxin analysis, 250g of maize kernels were processed, and 10g of the resulting flour was used for multi-mycotoxin analysis. During the 2022/23 season, a total of 397 *Fusarium* isolates representing 11 species were obtained from 12 localities across five of the seven maize-producing regions. During the 2023/24 season, 653 *Fusarium* isolates representing six species were obtained from 19 localities across six regions. In both seasons, *Fusarium verticillioides* (n=112 and 378) and *F. graminearum* (n=90 and 176) were the most frequently isolated species, respectively. Despite the high prevalence of mycotoxigenic *Fusarium* species, the concentrations of fumonisins and deoxynivalenol were low and below the regulatory limits of 2000 µg/kg and 1,000 µg/kg intended for human consumption, respectively. The presence of diverse mycotoxins at varying concentrations across different regions underscores the influence of environmental conditions on mycotoxin contamination in maize and highlights specific regional risks for mycotoxin exposure in S.A. maize production.

Keywords

Maize, maize ear rots, mycotoxigenic fungi, *Fusarium*, mycotoxins

Integrative analysis of genetic and metabolic resistance mechanisms to *Fusarium* and *Gibberella* Ear Rot of maize

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Post-harvest Pathology and Mycotoxins

Abstract

Fusarium verticillioides and *F. graminearum s.l.* are major pathogens responsible for Fusarium ear rot (FER) and Gibberella ear rot (GER) of maize, respectively. Infection of kernels reduces grain yield and quality as well as contamination with harmful metabolites, mycotoxins, produced by these fungi. Mycotoxins are associated with serious, irreversible health effects in humans and animals, posing a significant food safety concern. Incorporating resistance to multi-mycotoxigenic fungi into locally adapted maize germplasm represents the most sustainable and environmentally friendly approach to disease management. This study aimed to identify quantitative trait loci (QTLs) associated with resistance to FER, GER, and their associated mycotoxins in South African-relevant maize material, while also investigating the metabolic response of maize to fungal infection. Double haploid lines (n=200), developed from a well-characterised resistant line (CML390) and a susceptible line (R2565Y), was evaluated for disease resistance over two seasons. Genomic characterisation was conducted using Diversity Arrays Technology (DArT) and QTL mapping identified DNA markers on chromosome 2, 4, 5, 9 and 10. In parallel, untargeted metabolomic analysis was performed on maize lines with known resistance to ear rot, aiming to identify metabolic biomarkers associated with resistance. Several molecules were identified as significant (Phytoalexins, fatty acids and amino acids), however only a few were deemed highly significant with toxin accumulation in inoculated grain. For fumonisins, di- and triterpenoids were significantly associated with samples contaminated with fumonisins whereas linoleic acid and isoleucine and derived molecules were linked to samples contaminated with zearalenone. Integrating metabolomic and genomic data will support the efficient identification and selection of resistant material for breeding programs. Ultimately, this work contributes to plant improvement strategies focused on enhancing disease resistance and reducing mycotoxin contamination in maize.

Keywords

Fusarium verticillioides, F. graminearum, Mycotoxins, Quantitative trait loci, QLT mapping, Metabolomics.

Rootstock sensitivity to citrus viroids - Valencia field and glasshouse trials

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Disease Management

Abstract

Citrus viroids are graft- and mechanically-transmissible and can unintentionally be introduced and spread in nurseries and orchards. Citrus viroids are associated with several diseases dependent on the sensitivity of the rootstock and scion but are frequently latent infections. Citrus dwarfing viroid (CDVd) is associated with tree stunting on sensitive hosts. In South Africa, CDVd is often detected in older, commercial orchards, frequently in combination with hop stunt viroid (HSVd). Adjacent, newly planted orchards are vulnerable to viroid transmission where sanitation of cutting tools between orchards is inadequate. More recently, topworking new scion cultivars to existing rootstocks is a shift in orchard renewal practices from the standard replacement with nursery trees, and necessitated research to investigate the risks pertaining to citrus viroids. A Valencia field trial was planted in Mpumalanga in 2019 to quantify the effect of CDVd as a single infection and as a combined infection with HSVd on a range of nine rootstock cultivars. Trees were patch graft inoculated, and viroid transmissions were confirmed with RT-PCR. Tree canopy volumes and yield were determined yearly. Additionally, five rootstock cultivar seedlings were used for a glasshouse trial to measure the impact of CDVd as a single infection on rootstock and scion growth. Side shoots were removed to allow only one stem to grow. Growth was analysed by measuring stem-length of five growth periods of cut-back and regrowth over a total period of 23 months. Thereafter a Valencia scion was budded, and growth was measured in the same manner. Field trial results show that stunting varied dependent on rootstock and was more pronounced with combined infections. Reduced production correlated with the dwarfing effect. In the glasshouse trial, rootstock growth was not affected by CDVd infection, but scion growth was reduced on two rootstocks which supported the field results.

Keywords

Graft-transmission, stunting, growth, production

Fungicide sensitivity and characterisation of mutations associated with reduced QoI and SDHI sensitivity of grapevine powdery mildew

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Disease Management

Abstract

Erysiphe necator, the causal agent of grapevine powdery mildew, causes significant yield losses in South Africa. Reduced fungicide sensitivity has been suspected and the incidence of chasmothecia observed in vineyards has increased over the past decade. This study aimed to evaluate the level of sensitivity of powdery mildew isolates to QoI and SDHI fungicides and characterise mutations associated with reduced sensitivity. Single *E. necator* colonies were sampled from 29 vineyards in the Western and Northern Cape Provinces. DNA was extracted, the G143A mutation (confer QoI resistance) determined and *MAT1* idiomorph identified. Leaves were inspected for chasmothecia. Seven vineyards were resampled, and isolates were single-spored onto leaf disks. The EC_{50} values were determined for QoI and SDHI fungicides, *Cytb* and *SDH* genes were sequenced and SNPs identified and linked to resistance. QoI resistance in a total of 528 colonies were evaluated from 29 vineyards, of which the majority (66.34%) were the mutant type (MT) individuals. Of these, 19 vineyards were MT dominant, and seven vineyards had even ratio of MT:WT (wild type). Either both mating types were identified or chasmothecia were observed, confirming sexual reproduction. Three vineyards from the Overberg region were WT dominant of which one vineyard only had WT individuals, only *MAT1-2* idiomorph and no chasmothecia, indicating sexual reproduction is not occurring. The EC_{50} values were determined for 60 isolates and mutations identified. The SDHI subunit genes were sequenced for isolates from 29 vineyards once the mutations were known. The majority of *E. necator* populations exhibited reduced sensitivity to QoI and SDHI fungicides with mutations characterised and EC_{50} values determined. Notably, sexual structures were present in all populations except one, where only WT individuals were detected, suggesting that the absence of sexual reproduction in this vineyard limited the emergence of resistant genotypes.

Keywords

Erysiphe necator, TaqMan probe assay, mutant type, wildtype, chasmothecia

Puccinia graminins f. sp. *tritici* remains an evolving threat to wheat production in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is an important disease of wheat in South Africa (SA). Genetic resistance provides effective control of stem rust. However, new *Pgt* races frequently emerge and overcome resistance in existing cultivars. Monitoring the *Pgt* population is essential for early detection and management of the threat posed by new races. Wheat and triticale stems infected by *Pgt* were collected from commercial fields and rust trap nurseries across the production regions of SA between 2021 and 2024. The virulence diversity of *Pgt* isolates was assessed using standard differentials, and commercial cultivars and breeding lines were evaluated for their seedling response to new races. Twelve *Pgt* races were identified from 391 isolates pathotyped including seven new ones: 2SA25, 2SA46, 2SA67, 2SA18/24, 2SA34, 2SA36/24 and 2SA125. Race 2SA125 is virulent to the resistance genes *Sr25* and *SrTmp,* which were previously effective in SA. Further, the three new races detected in 2024 were virulent on *Sr24* and may exert pressure on South African wheat germplasm carrying this gene. Race 2SA36 (new) exhibited virulence to 69% of the 138 wheat entries evaluated, making it comparable to the highly virulent race 2SA107, which infected 70% of the entries. The percentage virulence of the remaining new races ranged from 5% (2SA46) to 47% (2SA125). While current commercial cultivars remain effective, the new races pose a risk to some breeding lines and one cultivar in the pipeline for commercialization, which have shown susceptibility. Virulence and genetic analysis suggest that the new races may have developed through both local evolution and incursion. These findings indicate continued variability of *Pgt* in SA, emphasizing the need for ongoing surveillance and breeding efforts to mitigate the impact of the evolving stem rust pathogen.

Keywords

Triticum aestivum, stem rust*,* new races, and wheat

A new leaf spot disease of kiwifruit caused by a species of Sclerotiniaceae

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Leaf spot symptoms were observed on kiwifruit (*Actinidia chinensis*) leaves in two orchards in KwaZulu-Natal and Limpopo, South Africa. Symptoms included circular, light brown spots with dark brown borders containing tuft-like fungal structures visible to the naked eye on the abaxial and adaxial leaf surfaces. Microscopic examination showed that the propagules consisted of filamentous hyphae aggregated in erect, pale brown columns that expanded under moist conditions. This study aimed to isolate and identify the causal agent of the disease. Eleven morphologically similar isolates were recovered by plating propagules of the fungus directly onto culture media. Phylogenetic analyses of DNA sequences for the ITS, LSU, and *RPB*2 regions showed that the fungus resides in the Sclerotiniaceae. Sequences for the isolates formed a distinct cluster closely related to *Microstrobilinia castrans,* which*,* interestingly, is a fungus known to infect spruce (*Picea* spp.) cones. Pathogenicity tests were performed by inoculating asymptomatic detached leaves of *A. chinensis* with propagules collected from naturally infected leaves and mycelium plugs taken from freshly grown cultures of two representative isolates. The inoculated leaves developed lesions within five days with abundant propagules, similar to those found in natural infections, produced within these lesions. The fungus was successfully re-isolated from the symptomatic tissue and developing structures. Future studies will focus on performing pathogenicity tests on living *A. chinensis* plants and providing an appropriate species name for the pathogen.

Keywords

Actinidia chinensis, Microstrobilinia, multi-locus phylogeny, mycopappus-like, novel taxon

Revisiting the taxonomy of *Sclerotinia*: phylogenetic and phylogenomic insights into a fungal plant pathogen

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Accurate taxonomy underpins effective plant pathogen identification and disease management. *Sclerotinia*, a genus of necrotrophic fungal pathogens with global agricultural significance, has not undergone formal taxonomic revision since 1979. That revision, based solely on morphology, recognised only three species – *S. sclerotiorum*, *S. minor*, and *S. trifoliorum* – leaving many described taxa unresolved or unverified. Leveraging modern molecular tools, we conducted a taxonomic study of *Sclerotinia* using two complementary datasets. First, we extracted DNA from 186-year-old *S. sclerotiorum* lectotype to generate an Internal Transcribed Spacer 5.8S rDNA (ITS) sequence. Analysis of this sequence alongside ITS sequences from various *Sclerotinia* species confirmed that ITS alone is insufficient for robust species delimitation. We then constructed two multi-gene phylogenies: one using 883 single-copy orthologs from 63 whole-genome assemblies, and another based on eight commonly used gene regions (*CAL*, *G3PDH*, *HSP60*, ITS, *LCC2*, *MCM7*, *RPB2*, and *TEF1*) compiled from public databases and genome data. Both approaches consistently resolved a core *Sclerotinia* clade that includes eight species. Several historically misclassified or doubtful names, such as *S. bulborum* and *S. sativa*, were reinstated or reclassified. Some synonymies (e.g. *S. matthiolae* with *S. sclerotiorum*) were supported, while others were contradicted. Notably, single-gene analysis revealed that *G3PDH* and *LCC2* outperform ITS in terms of distinguishing between the evaluated species. These findings help refine the taxonomy of *Sclerotinia*, identify high-resolution molecular markers for diagnostics, and highlight the need for a molecular revision of the genus. This work enhances the accuracy of fungal pathogen identification, supporting more effective disease diagnostics and management in agriculture.

Keywords

DNA barcoding, fungal identification, phylogenomics, species delimitation, multigene phylogeny

Ganoderma species associated with *Acacia crassicarpa* planted in lowland areas of Sumatra, Indonesia

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Acacia crassicarpa is widely planted in Indonesia where it grows vigorously and provides an important source of wood pulp. Ganoderma root rot caused by *Ganoderma philippii* has resulted in serious damage to *Acacia mangium* trees planted in mineral soil in Indonesia, but little attention has been given to the disease on *A. crassicarpa*. Recent disease surveys of newly established *A. crassicarpa* plantations in lowland areas of Sumatra revealed the occurrence of a diversity of *Ganoderma* fruiting bodies on the stumps of previous rotation trees, and these were occasionally associated with basal stem infections and root rot. The aim of this study was to identify the species of *Ganoderma* collected from three different compartments planted to *A. crassicarpa*. Phylogenetic analyses of sequence data for the ITS, mtSSU, nLSU, nSSU, *rpb2, Bt1a* and *tef1* regions of isolates revealed the presence of four species, including *G. australe, G. ellipsoideum, G. orbiforme* and *G. williamsianum. Ganoderma philippii* was not found in any of the compartments surveyed. Two of the species, *G. orbiforme* and *G. williamsianum,* were isolated from roots and basal stems of dying *A. crassicarpa* trees. Although the species found in this study have previously been reported from Indonesia, our results represent the first record of their association with *A. crassicarpa.* Pathogenicity trials are underway to determine the relative importance of these *Ganoderma* species to *A. crassicarpa* plantation forestry.

Keywords

Basidiomycetes, forestry, Ganoderma root rot, phylogenetics

Patterns of local Ganoderma root rot spread in *Eucalyptus* plantations in Sumatra

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Ecology and Epidemiology

Abstract

Ganoderma root rot (GRR) is a serious disease on various tree hosts in tropical regions of Southeast Asia. In Indonesia, *Ganoderma philippii* was the primary pathogen causing GRR in *Acacia mangium* plantations. Due to combined losses from Ceratocystis wilt (caused by *Ceratocystis manginecans*) and GRR, *A. mangium* have been replanted with *Eucalyptus* clones and hybrids. Currently, GRR hotspot areas are developing in these *Eucalyptus* plantations. Affected trees show leaf yellowing, rapid wilting, and often mortality. Characteristic signs on infected roots include red melanized layers on the bark surface, yellow-white mycelium under the bark, and red streaks within the roots. We hypothesise that GRR spreads by root-to-root contact, with infected stumps from previous rotations potentially acting as an important source of inoculum for new infections. In this study, we identified the *Ganoderma* species causing the recent GRR outbreaks on *Eucalyptus and* assessed whether infected stumps from previous rotations are a source of inoculum for new infections. Approximately 200 stumps were excavated in three compartments affected by GRR. The *Eucalyptus* trees planted adjacent to these stumps were also examined for GRR, and isolations were made from infected roots of those that displayed symptoms. During the course of three years, 185 *Ganoderma* isolates were collected. Root-to-root contact was observed between infected stumps and dying trees adjacent to them. All isolates from newly planted *Eucalyptus* were identified as *G. philippii* based on DNA sequence data. Of the 194 excavated stumps, all were confirmed to still harbour inoculum of *G. philippii* in the roots. The results confirmed that *G. philippii* is the single cause of GRR in the surveyed plantations. It was also shown that the pathogen spreads actively by root-to-root contact to adjacent uninfected trees. The outcome of this study will be used to implement management strategies to reduce the impact of the disease.

Keywords

Basidiomycetes, Forestry, *Ganoderma philippii,* Ganoderma root rot, Root disease

Sources of *Phytophthora nicotianae* in citrus nurseries

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Ecology and Epidemiology

Abstract

Intermittent outbreaks of Phytophthora-related diseases, such as root rot in citrus nursery trees, suggest the presence of *Phytophthora* spp. in citrus nurseries. These diseases persist despite existing management practices, including water sanitation, selection of tolerant rootstocks, routine testing, and the use of oomycete-targeting fungicides. This study aimed to identify infection pathways and potential inoculum sources of *Phytophthora nicotianae* in South African citrus nurseries. *Phytophthora nicotianae* is considered the most significant species impacting citrus production in the country. Twelve commercial citrus nurseries located across South Africa's key citrus-growing regions were surveyed. Inoculum of *P. nicotianae* was consistently recovered from soils around nursery tunnels, potting media in use, and unused growing media. Additional inoculum reservoirs included gloves and boots worn by nursery staff, cement benches supporting potted plants, moss and plant debris in or near pots, filled and used seedling trays, wheelbarrows, tractor tyres, and shade netting. At the time of sampling, irrigation water from all nurseries tested free of *Phytophthora* inoculum, indicating it was not a source of contamination. However, the nursery environment harboured *P. nicotianae* inoculum, which could be disseminated to plants via mechanical vectors such as workers' gloves and boots, as well as equipment like tractors and wheelbarrows, all of which tested positive for the pathogen.

Keywords

Phytophthora root rot, citriculture, inoculum, saplings, soil-borne diseases

Biocontrol of citrus soilborne diseases: A multifactor analysis of growth promotion and defence gene expression

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Disease Management

Abstract

A deeper understanding of the multiple facets of disease suppression employed by biological control agents (BCA) is key to designing more efficient biological disease management programs. This study aimed to examine the priming ability of three possible BCA's (*Bacillus subtilis* N19 (N19), *Pseudomonas fluorescens* N83 (N83) and *Trichoderma Hamatum* P4 (P4)) *in planta* against common soilborne pathogens *Phytophthora nicotianae* (PN) and *Pythium irregulare* (PI), when applied both preventatively and curatively under greenhouse conditions. Preliminarily, the individual effects of the BCA's and pathogens were evaluated at 24 hours, 48 hours, and one week post-application, followed by the curative and preventative trials. In the preventative trials, seedlings were pre-emptively treated with a BCA and subsequently challenged with the pathogens after two, four or six weeks. In the curative trials, seedlings were first inoculated with the pathogens before receiving either a single, double or three-time application of the BCAs. Leaves were collected four weeks after the last application of the treatments, flash-frozen and used for cDNA library preparation. The priming effect of the BCAs was evaluated through select defence-related genes (NPR1, PR5, PAL, CHS, LOX) along with two stable housekeeping genes (Cyclophilin and Beta-Tubulin) using reverse-transcription quantitative PCR (RT-qPCR). Preliminary results indicated that the N19-treated seedlings increased the expression of PAL and PR5, while N83 upregulated the expression of PAL, PR5 and LOX. The P4-treatments showed increased expression of PR5, NPR1 and LOX. The PAL, NPR1 and PR5 genes all form part of the Systemically Induced Resistance pathway, while LOX forms part of the Induced Systemic Resistance pathway, both of which play a role in preconditioning against pathogenic infection through the process of priming. Understanding this mechanism aids in the optimisation of BCA treatments as well as making them more efficient and economically favourable.

Keywords

priming, greenhouse, curative, preventative, RT-qPCR

A LAMP assay to rapidly detect *Ganoderma philippii* in Indonesian *Eucalyptus* plantations

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Disease Management

Abstract

Ganoderma philippii is an important root rot pathogen affecting *Eucalyptus* in Indonesian plantations. Effective disease management requires rapid and accurate detection of the pathogen. In this study, we developed a sensitive and specific Loop-Mediated Isothermal Amplification (LAMP) assay targeting a terpene synthase-type gene unique to *G. philippii*. The sensitivity of the assay was evaluated using serial dilutions of genomic DNA (1 ng to 0.001 ng), while the specificity was assessed against 13 closely related *Ganoderma* species and three other saprotrophic fungi commonly found on *Eucalyptus*. A regularly available plant extraction kit was used to extract DNA to detect *G. philippii* spores as well as the presence of the pathogen in infected root and soil samples. The assay was validated across 24 *G. philippii* strains. The LAMP assay was highly sensitive detecting as little as 0.01 ng of *G. philippii*DNA. The specificity tests confirmed exclusive amplification of *G. philippii*, with no amplification observed among other related fungi. Furthermore, the assay was able to detect the pathogen in root samples. This is the first isothermal diagnostic assay developed for *G. philippii*. Its high specificity, sensitivity and ability to detect the pathogen directly from environmental samples, highlights its potential to provide a rapid field diagnostic tool. This should significantly improve monitoring and management of *G. philippii* in *Eucalyptus* plantations.

Keywords

Ganoderma philippii, Loop-Mediated Isothermal Amplification, Diagnostic assay, Root-rot disease, Plantation forestry

Incidence of heart rot in pomegranates under alternative treatments in conjunction with registered azoxystrobin

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Disease Management

Abstract

Pomegranates (*Punica granatum*L.) are cultivated as a minor crop in South Africa, with it expanding over the past five years due to increased demand driven by its health benefits and export potential, particularly during the northern hemisphere's off-season. Heart rot infections of pomegranates, primarily caused by latent infections of *Alternaria* spp. during flowering, remains a major pre-harvest concern. To investigate the potential of alternative treatments in assisting in heart rot infections, an orchard trial was conducted evaluating L-Phenylalanine (LPA) and silver hydrogen peroxide (SHP), applied in conjunction with the standard registered fungicide azoxystrobin. The trial was conducted on two cultivars, 'Acco' and 'Wonderful'. Each treatment comprised three replicates of three trees each (except for one control replicate for 'Acco'). Treatments included: a positive control (1 mL/L of a product containing 200 g/L azoxystrobin and 125 g/L difenoconazole), 1.8 g/L LPA, and 100 mL/L SHP (50% hydrogen peroxide stabilized with 0.01% Ag+). Three spray applications were applied during flowering at two-week intervals. Prior to harvest, fruit were harvested, counted, and assessed for heart rot symptoms. In 'Acco', heart rot incidence averaged 1.17% for SHP, 2.33% for LPA, and 0.94% for the control. In 'Wonderful', SHP-treated fruit had 0.42% incidence, LPA 0.69%, and controls 1.04%. Preliminary pathogen isolations identified *Alternaria*, *Aspergillus*, and *Penicillium* spp. While control treatments had the lowest incidence in 'Acco', likely influenced by the limited control sample size, SHP consistently resulted in lower incidence compared to LPA across both cultivars. These findings suggest potential for SHP as a supplementary treatment in managing heart rot in pomegranate.

Keywords

Alternaria, *Punica granatum*, disease management, pre-harvest, minor crop

Penicillium diversity across soils of the Western Cape

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Penicillium is a diverse genus, with species found in many habitats. The Western Cape province of South Africa is particularly species-rich. Of 598 currently accepted *Penicillium* species, 162 have previously been reported from South Africa, with 47 described as new. However, previous surveys indicate that many *Penicillium* species remain undiscovered and we hypothesize the Western Cape is a potential hotspot for the genus. Our aim was to conduct a comprehensive survey of soils across the Western Cape to uncover hidden *Penicillium* diversity at the southern tip of Africa. We also aimed to develop a pipeline for accurate species-level identification using a metabarcoding approach, by comparing the detected communities to a traditional culture-dependent approach. As part of the survey, 15 soils were collected from a diverse range of bioregions across 5 sampling sites in Cape Town. DNA was extracted and long-read sequences of the partial beta-tubulin gene region (*BenA*) were generated using the PacBio Sequel II platform. A curated reference database for *Eurotiales* was incorporated into the pipeline to identify Amplicon Sequence Variants (ASVs) to species. In parallel, *Penicillium* strains were isolated from the same soils using Dichloran 18% Glycerol (DG18) as a selection medium. These strains were identified to species based on *BenA* sequences. The metabarcoding approach yielded 10363 reads, representing 26 species across 7 sections and 16 series. The culture-dependent approach yielded 119 strains, representing 39 species, 7 sections and 21 series. Five species could not be identified and represent putative new species. Our study is the first comprehensive overview of the diversity and distribution of *Penicillium* across the Western Cape, and we reveal both known and putative novel species using an integrated metabarcoding and culture-dependent approach whilst identifying regions to be focused on for future more in-depth collections.

Keywords

ASVs*, Eurotiales*, Metabarcoding, PacBio, Taxonomy

The South African Emergency Plant Pest Response Plan: a strategic and plant biosecurity approach to early detection and rapid response

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Biosecurity

Abstract

The South African Emergency Plant Pest Response Plan (SAEPPRP) presents a comprehensive, standards-compliant framework designed to enhance early detection and facilitate rapid response to plant pest incursions. Grounded in the principles of the International Plant Protection Convention (IPPC), including sovereignty, risk management, transparency, pest reporting and non-discrimination, the SAEPPRP ensures that national pest management measures are scientifically justified and based on sound evidence. It aligns with the international standards of the IPPC, particularly those related to surveillance, pest status, containment, eradication and pest reporting. The plan enables the implementation of national legislation, namely Regulation R110 of the Agricultural Pests Act (Act No. 36 of 1983) and provides for a coordinated and more efficient response to pest outbreaks by the executive officer of the Act. The SAEPPRP emphasises collaboration among government agencies, research institutions, industry stakeholders, and local communities to prevent the spread of diseases such as Citrus Greening, *Xylella fastidiosa* and other quarantine pests. It integrates risk assessment, surveillance, and diagnostic capacity through stakeholder collaboration to mitigate potential economic impacts while ensuring trade and market access stability. It also provides for a pest reporting mechanism through the International Phytosanitary Portal (IPP). The implementation of the SAEPPRP started in 2013 and became under review in 2024 through a series of stakeholder-inclusive workshops. The key objectives include early detection and rapid response, pest containment and eradication, coordination and communication, risk assessment, effective resource mobilisation, capacity building and public awareness: By aligning with international standards and regulatory frameworks, the SAPPRP exemplifies a proactive, coordinated methodology essential for sustainable pest management in the context of global trade and climate change.

Keywords

International Standards, National Legislation, Early Detection, Rapid Response, Stakeholder Collaboration

Uncovering new *Ficus* hosts for *Phakopsora nishidana*

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Disease Management

Abstract

Commercial fig trees (*Ficus carica*) are susceptible to rust disease caused by *Phakopsora fici* (syn. *Cerotelium fici*; *Kuehneola fici*; *Uredo fici*). Historic reports attributed fig rust in South Africa to *P. fici* infection. The recent detection of rust on edible fig trees (*F. carica*; 2020), ornamental creeping fig (*F. pumila*; 2023) and a coastal strangler fig tree (*F. natalensis*; 2023) near Hermanus in the Western Cape Province of SA, led to the hypothesis that all three hosts are infected by the same rust species, and that the latter two could act as inoculum sources for the economically important edible fig. The rust-causing isolates were identified using sequence analysis of the ITS2-28S rRNA locus, while cross-inoculation studies confirmed the compatibility of the pathogen and three host plants. Microscopy was used to examine fungal morphology. Using phylogenetics, all isolates collected from symptomatic leaves of the three host plants, were identified as *Phakopsora nishidana*. These isolates grouped separate from three *P. fici* reference isolates in the phylogenetic tree, confirming *P. nishidana* as the causal agent of fig rust in SA. Their identity was further confirmed using uredinium, telium and spore morphology, which established that it was distinct from *P. fici*. Cross-inoculation studies confirmed that all three host plants are susceptible to inoculation with *P. nishidana*. This study represents the first report for *P. nishidana* on *Ficus* trees in Africa. It further confirmed that both the creeping and coastal strangler figs, as susceptible hosts, provide inoculum that could significantly impact commercial fig production in SA. Further studies are needed to determine the host status of other South African *Ficus* sp. to *P. nishidana* infection.

Keywords

Phakopsora nishidana, fig rust, *Ficus carica*, *Ficus pumila*, *Ficus natalensis*

Rooted in protection - Deciphering *Trichoderma's* arsenal against *Dematophora necatrix* destruction

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Disease Management

Abstract

White root rot (WRR) caused by *Dematophora necatrix* poses a significant threat to avocado orchards, mainly in South Africa, Israel and Spain. Various studies have focused on the biological control of *D. necatrix* including investigating bacteria, fungi and mycoviruses, to suppress the colonization ability of the fungus. Some fungi, specifically, *Trichoderma* spp. have shown an antagonistic effect against *D. necatrix*. Therefore, unravelling the mechanisms by which *Trichoderma* spp. antagonise *D. necatrix* may reveal a promising avenue for WRR management in avocado orchards. The aim of this study was to investigate the antagonistic modes of action of various *Trichoderma* spp., both *in vitro* and *in planta*, to inhibit the growth of *D. necatrix.* Using a dual culture assay, all isolates were classified as effective antagonists (>75%) and five isolates demonstrated significant inhibition towards *D. necatrix*. Scanning electron microscopy revealed direct mycoparasitic interactions, including hyphal coiling, penetration, and subsequent lysis of pathogen hyphae. These findings highlight the role of physical antagonism in *Trichoderma*'s biocontrol mechanism. Antibiosis assays were performed using filtered liquid cultures of *Trichoderma* spp. to assess the potential production of non-volatile and diffusible metabolites. The growth inhibition observed ranged from 7.76 – 56.82%. An inoculation trial was also performed to evaluate the efficacy of a commercial *T. atroviride* (Eco-77®) formulation on the avocado rootstock Dusa® under greenhouse conditions. The uninoculated plants (treated and untreated) appeared healthy and showed no leaf wilting or necrotic root symptoms. Inoculated plants which were pre-treated with Eco-77® displayed notably reduced leaf wilt at 6-weeks post-inoculation, compared to both untreated and post-treated inoculated plants. These results establish a foundation for *Trichoderma*-mediated suppression through mycoparasitism, while the *in planta* trial seeks to bridge laboratory findings with practical disease management strategies.

Keywords

white root rot, avocado, biocontrol

Insights from the shared infection strategies of plant- and insect- pathogenic fungi

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Plant-Pathogen/Insect Interaction

Abstract

Pathogenic fungi, regardless of their preferred hosts, utilize multiple common strategies for host specificity, recognition, and infection. In some instances, these similarities reflect a common evolutionary history, with cross-kingdom host shifts occurring due to the shared environmental niches of vastly different hosts. For example, some entomopathogenic fungi in the *Hypocreales* have evolved directly from plant-associated fungi, likely due to the close ecological relationship between their insect and plant hosts. In other instances, fungi that are only distantly related to plant pathogens, for example those accommodated in the *Entomophthorales*, exhibit similar infection mechanisms. This can likely be attributed to convergent evolution, highlighting how comparable ecological pressures may drive the emergence of similar strategies. Here, we consider how insights from entomopathogenic fungi may be interpreted in the context of plant pathogens, and vice versa. One such example lies in the use of Pth11-like G-protein coupled receptors. These proteins, enriched in both plant- and insect-pathogens, mediate the early stages of host recognition and have established roles in virulence in plant pathogens like *Magnaporthe oryzae* and insect pathogens such as *Metarhizium robertsii*. The utilization of effectors is also emerging as a shared strategy across fungal pathogens. This cornerstone of plant pathology is informing the discovery of related proteins in entomopathogenic fungi, revealing conserved modes of host manipulation. Similarly, the production of secondary metabolites is strongly linked to virulence in both plant and insect pathogens, with these compounds functioning as toxins, immune suppressors, and signalling molecules, regardless of the host. Whether through shared evolutionary histories or convergent evolution, recognizing these common strategies and identifying where they differ from others a powerful lens through which to investigate the evolution of fungal pathogens. This allows for a deeper understanding of pathogenic fungi and their ecological roles, leading to more integrated management strategies in the long term.

Keywords

Entomopathogens, Plant pathogens, GPCRs, Effectors, Secondary metabolites

Progress towards testing and identifying pesticides for sustainable management of nursery and plantation pests and pathogens

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Disease Management

Abstract

In South Africa, the use of pesticides is an important component of an integrated management strategy for insect pests and pathogens in nurseries and plantations. Due to increased concerns around environmental and biological safety, more stringent global and national legislation regarding pesticide-use has resulted in a reduction in the number of active ingredients considered safe for use. As a solution, a Forestry South Africa and Department of Science and Innovation funded project was initiated in 2023 by the Institute for Commercial Forestry Research in collaboration with forestry companies and the Timber Industry Pesticide Working Group. The objectives of the project are to identify and prioritize key nursery and plantation pests and pathogens, test and identify effective pesticides for sustainable control of pests and pathogens and develop standard pesticide testing protocols for registration purposes. A survey conducted to identify insect pests and pathogens of economic importance identified *Botrytis cinerea, Calonectria* sp., *Rhizoctonia* sp. in rooting tunnels, and *Quambalaria eucalypti*, powdery mildew, red spider mite and thrips in hedge plants as priority eucalypt nursery pests and pathogens. Pesticide testing protocols were developed based on national regulatory requirements and in consultation with pesticide companies. In 2023-2024 seven pesticide pre-screening trials were conducted, three trials testing 20 products against *Q. eucalypti*, three trials testing 27 products against powdery mildew, and one trial testing 13 products against rooting tunnel pathogens. Three fungicides were found effective against *Q. eucalypti* while 17 fungicides were found to be effective against powdery mildew and two products showed potential against the rooting tunnel disease complex. A second pre-screening trial is currently underway to confirm findings from the first rooting tunnel disease complex trial. Results obtained from this pesticide screening project will be used to select products for label extension trials to further promote sustainable management of forestry nursery pathogens.

Keywords

Botrytis cinerea, forestry nursery, pesticide screening, powdery mildew, *Quambalaria eucalypti*

Transmission studies to determine the susceptibility of alternative host plants in the transmission of banana bunchy top virus in South Africa

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Plant-Pathogen/Insect Interaction

Abstract

Banana bunchy top virus (BBTV) is transmitted through infected planting material and by its vector, *Pentalonia nigronervosa* (Hemiptera: Aphididae). This study investigated whether *Colocasia esculenta* (taro), *Alocasia macrorrhizos* (elephant's ear*), Alpinia zerumbet* (shell ginger) and *Strelitzia reginae* , serve as reservoirs of the banana aphid in South Africa, and if these plants are potential alternative hosts of BBTV. A transmission experiment was conducted using a BBTV-infective and BBTV non-infective *P. nigronervosa* colonies. Twenty aphids sourced from banana plants were transferred to six plants of each test species using a fine brush: three replications from the infective colony and three from the non-infective colony. The trial was monitored for 88 days post-inoculation and aphid colonization rates and BBTV symptom development were recorded at weeks 5, 9 and 12. A qPCR protocol was optimized to test for BBTV in plant materials and aphids at each time interval. Additionally, *Canna indica, C. esculenta* and *S. reginae* growing in close proximity to BBTV-symptomatic plants were sampled from the South Coast of KwaZulu-Natal during surveys to determine if they are hosts of BBTV. In the transmission experiment, aphid populations declined on *S. reginae, A. macrorrhizos* and *C. esculenta* until the plants had no aphids left by week 5 while aphids established on *A. zerumbet* and Musa spp, until the end of the trial. From BBTV transmission studies, all plant species tested positive for BBTV except *A. macrorrhizos*, at the end of the trial following qPCR assays. Furthermore, BBTV was not detected on *C. indica*, *C. esculenta* and *S. reginae* plants found growing near BBTV-infected banana plantations. In conclusion, the study suggests that *A. zerumbet* spp., is an alternative host plant of *P. nigronervosa* and BBTV in South Africa. Furthermore, *S. reginae* and *C. esculenta* are assumed to be intermediary hosts of the virus vector.

Keywords

Banana bunchy top virus, BBTV, transmission studies, alternative host plants, Vector

The *Fusarium* mycotoxin cocktail: diversity and contamination in maize from Eastern Cape

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Post-harvest Pathology and Mycotoxins

Abstract

Maize is a staple crop in South Africa but is vulnerable to Fusarium ear rot (FER) and Gibberella ear rot (GER), diseases caused by mycotoxin-producing *Fusarium* species. Fumonisin B1 (FUM B1) was first reported in the Eastern Cape in 1988. Recent surveys suggest a decline in its prevalence. This study examines *Fusarium* diversity and mycotoxin contamination in maize over three growing seasons: 2023, 2024, and 2025. Samples were collected from smallholder farms, targeting maize showing symptoms of FER and GER as well as healthy cobs. *Fusarium* isolates were identified through morphological features and DNA sequencing. Mycotoxins were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). In 2023, 196 samples from five farms yielded 245 *Fusarium* isolates. The dominant species were *F. temperatum* and *F. verticillioides*. Mycotoxins detected included FUMs, deoxynivalenol (DON), zearalenone (ZEA), beauvericin (BEA), fusaric acid (FA), and moniliformin (MON). Many samples contained mixtures of these toxins, increasing health risks. The 2024 survey expanded to seven farms, collecting 302 samples. Farms in OR Tambo had FUM-positive symptomatic maize, some exceeding safety limits. Emerging mycotoxins like diacetoxyscirpenol (DAS) and enniatins were also found. *Fusarium* species diversity increased in 2024, including new species from *Fusarium fujikuroi* complex. The 2025 sample collection occurred in May, with results to be shared at the upcoming meeting. This study is the first comprehensive three-year survey of *Fusarium* and mycotoxin contamination in Eastern Cape maize. It reveals a shift in *Fusarium* diversity, possibly due to climate change, and highlights the increasing risk of mycotoxin mixtures. Further research is needed to assess economic impacts and support food security strategies.

Keywords

Corn, deoxynivalenol, fumonisins, *Fusarium verticillioides, Fusarium boothii*

Posters

Screening of citrus rootstock seedlings for tolerance to combined bicarbonate salt and *Neocosmospora solani*

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Plant-Pathogen/Insect Interaction

Abstract

The detrimental Valley Bushveld Citrus Decline has received increasing attention recently. Symptoms associated with the syndrome include root and trunk decay, reduced yield and tree death. Among other pathogens, *Neocosmospora* species have been identified in affected trees, particularly in trees predisposed to abiotic stress factors such as elevated soil electrical conductivity (EC). As a sustainable management strategy, identifying alternative rootstocks with improved tolerance is critical. This study aimed to evaluate the morpho-physiological and biochemical responses of citrus rootstocks under combined stress from exogenous bicarbonate salt application and *Neocosmospora solani* infection. Carrizo citrange, Rough lemon, and US812 seedlings were evaluated in a greenhouse trail for morpho-physiological responses combined bicarbonate salt stress and *Neocosmospora solani* infection. Growth parameters measured included plant height, shoot and root mass, root length, and root volume. Leaf tissue from the citrus rootstock were utilized to quantify hydrogen peroxide levels and the extent of lipid peroxidation. The antioxidant activity of ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase was also assessed. Good performing rootstock candidates were subsequently selected for single nucleotide polymorphisms (SNPs) analysis. Exposure to simultaneous bicarbonate salt stress and *Neocosmospora solani* infection had a significant negative effect on the morpho-physiology of all screened citrus rootstocks. Differential antioxidant enzyme activity in response to the under combined stress conditions were observed. Despite overall stress sensitivity, certain seedling candidates demonstrated superior performance and were selected further. These findings identify potential biomarkers, providing insights into the biochemical responses of citrus rootstocks with varying tolerance/susceptibility to simultaneous stress conditions. Understanding the mechanisms involved with increasing the tolerance of citrus rootstocks to biotic and abiotic stress factors is vital for achieving extended tree health.

Keywords

bicarbonate soil, combined stress, morpho-physiology*, Neocosmospora solani,* ROS metabolism

Diversity of *Rugonectria* species from alien and indigenous hosts in South African botanical gardens

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Rugonectria is a genus of ascomycetous fungi in the Nectriaceae, commonly associated with perennial cankers on trees. Most species are known from Asia (e.g., *R. castaneicola*, *R. sinica*, *R. neobalansae*) and a few have also been reported from Australia (*R. wingfieldii*) and North America (*R. rugulosa*). Some species have been linked to large perennial cankers on hosts such as *Castanea crenata* (Japan) and *Quercus robur* (China, Australia), and to the rapid decline of *Macadamia* in Hawaii (USA) in association with beetles, while others appear to be saprobic. Prior to the present study, no *Rugonectria* species were known to occur in Africa. Recently, during tree health monitoring activities in botanical gardens as part of a sentinel project led by SANBI and FABI, fruiting structures resembling those of *Rugonectria* were observed on various tree species, and isolations from perennial cankers yielded characteristic *Cylindrocarpon*-like colonies. The sampled trees included the alien species *Albizia chinensis*, *Hymenaea verrucosa*, and *Cassia fistula* in the Durban Botanic Gardens, and the indigenous *Commiphora neglecta* at the same site, *Sclerocarya birrea* in the Lowveld National Botanical Gardens, and *Searsia dentata* in the KwaZulu-Natal NBG. The extent of cankers differed markedly on the different host species, with the largest lesions found on *A. chinensis* and *H. verrucosa* where they covered large areas of the main trunk. Infections on *C. neglecta* and *S. birrea* were typified by localized but deep cankers and only small lesions were found on *C. fistula* and *S. dentata*. Preliminary identifications from sequence data for the ITS gene region confirmed that the isolated fungi were species of *Rugonectria* and likely represent three new taxa all closely related to the recently described *R. wingfieldii*. The ongoing study is focused on resolving the identity of these fungi and conducting pathogenicity tests.

Keywords

biosecurity, novel species, perennial cankers, sentinel sites, tree pathogens

Phytophthora diseases on indigenous hosts in South African botanical gardens

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Pathogen Identification, Characterisation and Taxonomy

Abstract

More than 260 species of *Phytophthora* have been described, many of which are serious plant pathogens, ranking among the most important threats to commercial crops, ornamentals and native ecosystems. Several of these species are important invasive pathogens that have caused severe damage to natural environments around the world. These for example include *P. cinnamomi* in southwestern Australia, *P. ramorum* in western USA and *P. agathidicida* in New Zealand. Although numerous *Phytophthora* species are known to occur in South Africa, little attention has been given to the diseases they cause on native flora. A sentinel site project led by SANBI and FABI, monitoring tree health in botanical gardens, has resulted in the first detection of several alien *Phytophthora* species in the country, as well as the description of the new species, *P. mammiformis*. This study focused on *Phytophthora* species isolated from native trees displaying symptoms of infection. They included *P. cinnamomi* from *Podocarpus latifolius* and *Myrsine melanophloeos*, *P. niederhauserii* from *Harpephyllum afrum* and *Searsia lancea*, as well as *P. citrophthora* and *P. pseudocryptogea* from *Celtis africana*. While these *Phytophthora* species had previously been reported in South Africa, all of the above-mentioned host–pathogen combinations are new records. Pathogenicity tests are underway to assess the ability of these *Phytophthora* species to infect their respective hosts, providing a preliminary indication of their aggressiveness and relative importance. This is particularly because all plant species considered in this study are both native and widely used as ornamentals, raising concerns regarding the potential future threat these *Phytophthora* species may pose.

Keywords

biosecurity, dieback, Koch's postulates, sentinel sites, tree pathogens

Evaluating the impact of environment, maize cultivar, and *Stenocarpella maydis* isolate diversity on Diplodiatoxin production in maize grain

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Post-harvest Pathology and Mycotoxins

Abstract

Maize is a staple crop in South Africa, vital to both food and feed systems. Its production is increasingly threatened by *Stenocarpella maydis*, the causal agent of Diplodia ear rot, which not only reduces yield but also produces Diplodiatoxin—a neurotoxin that poses health risks to livestock and humans consuming contaminated grain. This study aimed to assess the impact of cultivar × isolate × environment interactions on Diplodiatoxin production. Field trials were conducted during the 2024/2025 season at two research sites: Vaalharts (Northern Cape) and Cedara (KwaZulu-Natal). A randomized complete block design was used, with three replicates of five maize cultivars inoculated with nine *S. maydis* isolates via sterilized, mycelia-colonized toothpicks. Post-harvest assessments included visual disease severity ratings and quantitative measurements of ear and grain weights. Disease severity ranged from 0% to 100% across both sites. Cultivar DKC78-78BR × isolate ADM194.14d (Vaalharts) and CAP9-262RR × isolate MO36 (Cedara) showed tolerance, while SC701 × isolate ADM434 exhibited high susceptibility at both locations. Cob and kernel weights varied significantly, ranging from 358-405g and 428 - 530g in Vaalharts, and from 0–445g and 0–373g in Cedara, indicating substantial differences in disease impact and yield potential. Preliminary results suggest that environment, cultivar, and isolate interactions significantly influence *S. maydis* infection severity and yield outcomes. LC/MS analysis of harvested grain is underway to quantify Diplodiatoxin levels and further clarify the role of these variables in mycotoxin risk. These findings will contribute to breeding and management strategies aimed at reducing Diplodia ear rot and safeguarding maize production in South Africa.

Keywords

Mycotoxin contamination, Disease tolerance, Yield loss assessment, Field trial methodology, LC/MS analysis

Screening of maize (*Zea mays* L.) cultivars for sources of charcoal rot (*M.phaseolina*) resistance

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Plant-Pathogen/Insect Interaction

Abstract

Charcoal rot is one of the more serious and destructive diseases affecting maize (*Zea mays* L.), causing significant yield losses. The disease is characterized by premature yellowing and drooping of leaves, discoloration of stalks, lodging, and tilting of ears. Resistance to charcoal rot is a critical component in managing the disease, especially in warm and dry regions where it thrives. The aim of this study is to evaluate charcoal rot (*M. phaseolina*) resistance of 20 commercially available cultivars. The cultivars were tested in field trials planted mid November 2025 in the Northern Cape (Vaalharts) and Kwa-Zulu Natal (Cedara) using a randomised complete block design with 3 replications. A tooth-pick inoculation method was used to infect the plants at the second internode above the soil with *M. phaseolina* isolate (MP3) before silking. Destructive sampling of the inoculated plants was done, and disease ratings were conducted when the plants reached soft dough stage. A rating scale of 1-9 was used where 1-2 is highly resistant, 3-4 is resistant, 5-6 is moderately resistant, 7 is moderately susceptible and 8-9 is susceptible. The combined analysis of variance revealed a significant difference ($p < 0.001$) among the test genotypes for the assessed charcoal rot in both Vaalharts and Cedara. Mean disease ratings ranged from 2.53 (moderate resistance) to 5.20 (susceptible) in Vaalharts, and from 1.60 (resistance) to 5.80 (susceptible) in Cedara. Of the 20 cultivars, SC701, P2885WBR and AG97Y02BR exhibited high resistant reaction to the disease in Vaalharts while P1975PW, DE303(MF) and LG31.752BR exhibited high resistant reaction to the disease in Cedara. This indicated changes in host preference and fungus response to the environment. Furthermore, this revealed specific adaptation of the genotypes suggesting that they can be recommended for environments where they exhibited optimal performance.

Keywords

Charcoal rot, Genotypes, Interaction, Pathogenicity, Resistance

Characterisation of the mating-type locus in *Diplodia* species

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Several phytopathogenic fungi belonging to the genus *Diplodia* are known to cause disease on a wide range of economically significant tree hosts. Species such as *Diplodia sapinea*, *D. scrobiculata*, *D. corticola*, *D. intermedia*, *D. seriata*, and *D. mutila* are associated with cankers, shoot blight, and dieback symptoms, often leading to substantial losses in forestry and agriculture. Despite their importance, little is known about the genetic basis of their mating systems. In this study, we characterized the mating-type (*MAT*) locus of these six species using whole-genome sequences. This involved analyzing their genomes and leveraging the known *MAT* idiomorph sequences of *D. sapinea* to facilitate comparative characterization of the *MAT* locus across the species. The organisation of the *MAT* locus and flanking genes was relatively conserved, except in *D. mutila*, where differences in synteny were observed. All species exhibited a heterothallic mating system, characterised by the presence of either a *MAT1-1* or *MAT1-2* idiomorph per isolate. The *MAT1-1* idiomorph was defined by the *MAT1-1-1* gene, encoding a transcriptional regulator with a conserved α-box domain. Similarly, the *MAT1-2* idiomorph was defined by the *MAT1-2-1* gene, encoding a protein with an HMG-box domain. In addition to the *MAT1-1-8* and *MAT1-2-5* genes present in the *MAT1-1* and *MAT1-2* idiomorphs respectively, the same syntenic cluster of six open reading frames (ORFs) and a putative integral membrane protein (*PIM*) were found flanking the *MAT* locus in all species. In *D. mutila*, this cluster of ORFs was in the opposite orientation. These ORFs include DNA lyase (*APN2*), Cytochrome c oxidase subunit VIa (*Cox-VIa*), Anaphase-promoting complex subunit 5 (*APC5*), Complex I intermediate-associated protein 30 (*CIA30*), Mitochondrial carrier protein (*MCP*), and Phosphoribosylaminoimidazolesuccinocarboxamide Synthetase (*SAICAR syn*). The results enhance our knowledge of the genetic basis of mating systems in these fungi and will contribute to further research on their population biology.

Keywords

Phytopathogenic fungi, *MAT* idiomorphs, heterothallism, synteny, mating systems

Characterization of *Alternaria* leaf blight diseases associated with prominent vegetable crops in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Alternaria species are associated with a wide range of plant diseases that affect different agricultural crops worldwide. Vegetable crops such as potato, sweet potato, tomato, carrot and onion are important sources of food, nutrition, and cash income for both resource-poor and commercial farmers in South Africa. *Alternaria* can have devastating effects on the production of these crops, leading to increased production costs, reduction in crop yield and quality. In recent years, leaf lesion outbreaks have been reported from vegetable growing areas in South Africa, with some species reported for the first time The aim of this study was to evaluate the pathogenicity and identities of *Alternaria* species that cause foliar blight diseases in South Africa.

Morphological identification of *Alternaria* isolates was conducted from representative isolates using Synthetic Nutrient Agar (SNA) and Potato Dextrose Agar (PCA). The sellotape technique was used to prepare microscope slides and Lacto-phenol was used as mounting fluid. Characteristics noted were spore branching patterns, number of spores in chains, and the spore dimensions, ornamentations, and septa patterns. Molecular identification of *Alternaria* species was done by sequencing the *RPB2, GAPDH* and ITS gene regions.

Pathogenicity was evaluated using twelve *Alternaria* species from different crops. Susceptible disease-free seeds and vine cuttings of sweet potato were inoculated with spore suspension of 2.5 x 10^5 spores/ml concentration from each species. Each crop had four replicates, and the fifth pot was used as the control. Disease development and progression were observed and monitored weekly by observing the emergence of ALB disease symptoms in the glass house trials and percentage disease incidence (DI) and disease severity (DS) index were recorded.

Keywords

Characterisation, Leaf blight, Vegetable

Extracellular vesicles in soft rot pathogens: production, cargo, and clues to virulence

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Plant-Pathogen/Insect Interaction

Abstract

Extracellular vesicles (EVs) are increasingly recognised as carriers of functional biomolecules, yet their roles in plant-pathogenic bacteria remain poorly characterised. *Dickeya* dadantii and *Pectobacterium brasiliense*, are soft rot pathogens that cause tissue maceration in a wide range of plants by secreting pectate lyases. This study aimed to characterise EV production in these species and explore how EV traits respond to stress and contribute to virulence. Using electron microscopy, we observed that both species release EVs from the cell surface. The EVs produced had either single or double membrane layers, indicating outer membrane vesicles (OMVs) and outer-inner membrane vesicles (OIMVs). We isolated these EVs and determined their size distribution. *D. dadantii* produced larger EVs than *P. brasiliense*, but at the same growth stage and cell density, *P. brasiliense* produced more EVs. Under chemical stress conditions, the size of EVs did not differ significantly; however, vesicle yield and protein profiles varied, indicating a regulation of EV cargo to respond to stress. To investigate the molecular cargo of the EVs, we compared small RNA (sRNA) from EVs to that of whole cells. The sRNA from EVs was shorter in length, implying that sRNA packaging into EVs may be dependent on length. Given the central role of pectate lyases in soft rot disease, we also examined whether these enzymes are exported via EVs. Pectate lyase proteins were detected in EVs, indicating that EVs may serve as vehicles for virulence-associated factors. Together, these findings provide evidence that EVs are produced by both *D. dadantii* and *P. brasiliense*, and that EV production, cargo composition, and potential function may respond to stress conditions. Our study contributes to the growing understanding of EVs in plant-pathogenic bacteria and supports the idea that EVs could play a role in host-pathogen interactions and disease development.

Keywords

Small RNA, Pectate lyase, *Dickeya dadantii*, *Pectobacterium brasiliense*, stress response.

Postharvest decay control using alternative products for cherry fruit

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Disease Management

Abstract

Effective postharvest decay management of cherry fruit is crucial to maintain fruit quality and reduce losses. However, few alternative control products are currently registered for use, and their efficacy against major postharvest pathogens of stone fruit, particularly cherries, remains uncertain. This study aimed to evaluate the effectiveness of existing and novel alternative control products in limiting postharvest decay of cherry fruit.

Initial *in vitro* screening of alternative control products was conducted on potato dextrose agar against three key postharvest pathogen, *Botrytis cinerea*. The three most effective liquid treatments were selected for *in vivo* evaluation on artificially inoculated cherry fruit. Treated fruit were stored under regular atmosphere (RA), RA + ozone, controlled atmosphere (CA), and CA + ozone. Following cold storage, fruit were subjected to shelf-life conditions before assessing decay incidence and severity. Peracetic acid and hydrogen peroxide and hypochlorous acid (HOCl) and NaCl consistently demonstrated high efficacy (>90%) against *B. cinerea*, significantly reducing both decay incidence and severity compared to the untreated control. *Bacillus amyloliquefaciens* subsp. *plantarum* also showed a significant reduction in decay caused by *B. cinerea*, with efficacy ranging from 15.8% to 66.2%, depending on storage conditions. Peracetic acid and hydrogen peroxide and HOCl and NaCl show strong potential as alternative postharvest treatments for managing decay caused by *B. cinerea*. *Bacillus amyloliquefaciens* subsp. *plantarum* may be a suitable option against *B. cinerea.* Ozone, whether used with RA or CA storage, did not provide significant additional decay control.

Keywords

plant pathogens, storage conditions, efficacy, shelf-life, ozone

Identification and functional characterisation of antimicrobial activity of fungal endophytes against *Exserohilum turcicum*

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Disease Management

Abstract

Northern corn leaf blight (NCLB) is one of the most destructive foliar diseases of maize, caused by *Exserohilum turcicum*. Severe infections can lead to premature death of the leaves, further reducing the grain yield. Given the environmental risks and high costs associated with application of synthetic fungicides, there is a growing need for sustainable alternatives such as biological control agents, which offer eco-friendly and effective disease management. The main aim of this study was to identify fungal endophytes from maize seeds and to evaluate their antimicrobial activity against *E. turcicum* strain CPR216. A total of 110 fungal isolates were obtained from maize seeds of six cultivars, collectively. The molecular characterisation revealed that these isolates belong to the following genera, *Alternaria* spp. (6), *Sarocladium* spp. (65), *Chaetomium* spp. (13), *Epicoccum* spp. (8), *Phialemonium* spp. (1), *Aspergillus* spp. (3), *Fusarium* spp. (7) and *Verticillium* spp. (7). Eighty-six of the 110 fungal isolates were endophytes; 24 were known maize pathogens. The endophytic fungi were assessed for their potential biocontrol activity against *E. turcicum* strain CPR216 through a dual-culture assay and for the production of volatile and non-volatile compounds. Fourteen out of 86 fungal endophytes had the most effective antagonistic activity against *E. turcicum* strain CPR216. Furthermore, three *Chaetomium cochliodes* isolates, i.e., SRT4, SRT13, and SRT59 had the highest mycelial inhibition percentage of 62.5%, 60.1% and 60.1%, respectively. Furthermore, only 9- and 13 isolates, out of the 14 antagonistic fungal endophytes, were able to produce non-volatile and volatile compounds, respectively, which inhibited mycelial growth of *E. turcicum* strain CPR216. The findings from this study highlight the biological control potential of these fungal endophytes isolated from maize seeds against NCLB, and suggest that they are promising candidates for sustainable and eco-friendly alternatives to synthetic fungicides.

Keywords

Northern corn leaf blight, maize, foliar diseases, disease control, sustainable agriculture

A first look at the mycovirome of South African *Sclerotinia sclerotiorum* isolates

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Mycoviruses are gaining increasing attention due to their diverse evolutionary origins and potential in biological control. While some induce hypovirulence in fungal hosts, many remain cryptic, producing no obvious symptoms yet potentially modulating host fitness, stress responses, and biotic interactions. These latent infections may offer insights into virus–host dynamics and the hidden diversity of fungal viromes. *Sclerotinia sclerotiorum*, a globally important plant pathogen, causes severe yield losses in oilseed crops and is of particular concern in South Africa. Despite its economic impact, the mycovirome of South African *S. sclerotiorum* has not been previously investigated. This study aimed to characterise mycoviruses associated with South African *S. sclerotiorum* isolates. Total RNA was extracted from four *in vitro*-cultured isolates, pooled, and sequenced using Illumina-based, ribo-depleted RNA-Seq. A publicly available transcriptome dataset of a single *S. sclerotiorum* isolate was also included in our analyses. Following quality control and *de novo* assembly, a dedicated bioinformatics pipeline was used to identify viral sequences. Ten positive-sense single-stranded RNA (+ssRNA) mycoviruses were identified across the datasets. From the pooled dataset, four viruses belonging to *Mitoviridae*, one to *Botourmiaviridae*, and fragmented sequences showing similarity to *Tymoviridae* were identified. Four mitovirus species were found in the public dataset, including one also present in the pooled sample. This virus was novel but closely matched a virus from *Monilinia fructicola*, suggesting possible cross-species transmission. RT-PCR validation showed that the mycoviruses were distributed across the four isolates used for pooled sequencing, while the *Botourmiaviridae* virus was detected in all isolates, suggesting widespread distribution. This study provides the first baseline of mycoviral diversity in South African *S. sclerotiorum* and reveals cryptic infections in local populations, highlighting an underexplored dimension of fungal biology. Further investigation into the ecological roles and biocontrol potential of these viruses are warranted for sustainable plant disease management.

Keywords

Mycovirus, cryptic viruses, fungal virome, fungal transcriptome, South Africa

Transmission routes of pepper ringspot virus associated with *Helianthus annuus* L. (sunflower) in South Africa

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Ecology and Epidemiology

Abstract

The recent emergence and host range expansion of pepper ringspot virus (PepRSV) (genus: *Tobravirus*) in South Africa potentially threatens the economically important sunflower industry. Tobraviruses are primarily spread via soil-inhabiting trichodorid nematodes, although the transmission of PepRSV to sunflower has not yet been determined experimentally. Pea early browning virus, a tobravirus known for high rates of seed transmission to pea, although this capacity among other members of the genus remains unknown. This study set out to establish the seed and nematode transmissibility of PepRSV to sunflower. Direct testing of 10 seeds was performed, 5 of which were surface sterilised, using a PepRSV-specific RT-PCR. Field (n=500 plants) and pot (n=100 plants) trials were conducted using seeds from potentially PepRSV-infected plants and monitored for symptoms. Soil transmission was achieved in a growth chamber, through growing plants in soil collected from around the rhizosphere of infected sunflowers in the field. The presence of PepRSV was tested using RT-PCR. Direct test of seeds resulted in the detection of PepRSV in 3 of 10 seeds, and of these, 2 were surface-sterilised seeds. No symptoms were observed on plants in either field or pot trials, and RT-PCR testing of a subset of asymptomatic samples was negative. Soil transmission was successfully demonstrated, whereby plants developed symptoms consistent with PepRSV infection in infested soil. This was confirmed with RT-PCR. This is the first experimental transmission of PepRSV to sunflower and has also fulfilled Koch's postulates for this pathosystem. While currently seed-transmission seems unlikely, the field and phytotron trials were limited by access to seeds from infected sunflower, and a larger trial is planned to determine whether PepRSV can be transmitted at very low rates. This study has laid a foundation for understanding the transmission capacity of PepRSV in sunflower production systems.

Keywords

PepRSV, seed transmission, nematode transmission

Isolation and characterisation of plant growth-promoting rhizobacteria (PGPR) from *Cannabis sativa*

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Disease Management

Abstract

Cannabis (*Cannabis sativa*) is a versatile and economically valuable crop widely used in sectors such as textiles, energy, construction, bioplastics and other industries. Due to its end-use applications, there is increasing demand for improved cultivation methods to enhance yield. Plant growth-promoting rhizobacteria (PGPR) offer a sustainable alternative to synthetic fertilisers and have shown potential to improve plant development. However, there is limited research on the impact of PGPR on cannabis growth in South Africa. This study aimed to isolate, characterise and evaluate the PGPR from the rhizosphere soil and roots collected from three provinces - KwaZulu-Natal, North West and Free State. A total of 180 isolates were obtained and characterised using phenotypic and biochemical tests, including potassium hydroxide (KOH) test, motility, endospore staining, catalase activity and biofilm formation. Functional traits assessed included phosphate solubilisation, siderophore production and indole acetic acid (IAA) production. Among these, 58% tested positive for the KOH test and were classified as Gram negative, 94% tested positive for the production of catalase, 50% were able to form biofilms, 97% were motile, 11% produced endospores, 12% had the ability to solubilise inorganic phosphate and 80% were able to produce siderophores. Additionally, all the isolates were able to produce IAA at a concentration ranging from 7.33 µg/ml to 338.07 µg/ml. Based on the *in vitro* screening, 25 of the most promising isolates were selected for a greenhouse trial to assess their plant growth-promoting potential on cannabis. Statistical analysis using ANOVA and post hoc tests revealed one isolate, KZN 131, significantly enhanced root length ($p < 0.05$) compared to all other treatments. These findings confirm that KZN 131 possesses strong growth-promoting potential and can be utilised as a promising bio-inoculant to improve cannabis growth.

Keywords

Cannabis sativa, Plant growth-promoting rhizobacteria (PGPR), bio-inoculant, greenhouse trial

Antifungal efficacy of *Moringa oleifera* leaf extracts against *Alternaria alternata*

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Disease Management

Abstract

Alternaria species cause diseases in many economically important crops which result in economic losses, affecting human and animal health. *Alternaria alternata* causes black point disease in wheat which takes over its spike, resulting in its black or brown discoloration. The disease is managed by using chemical fungicide treatments and crop waste management. However, improper management of the disease can pose health risks. This study examines the effect of *Moringa oleifera* leaf extracts on *Alternaria alternata*. *A. alternata* was isolated from diseased common wheat (*Triticum aestivum*) on potato dextrose agar (PDA) and identified using AaltFor and AaltRev primers. The *M. oleifera* leaf extracts were prepared by using solvents such as methanol (MLME), ethanol (MLEE) and water (MLWE). Phytochemical analysis of the extracts was conducted with quantitative assays such as the determination of total phenol, flavonoid, alkaloids, saponins, and tannins to compare the concentrations of the compounds in the extracts. The percentage inhibition was determined by growing *A. alternata* on infused potato dextrose agar (PDA) plates and in infused potato dextrose broth (PDB) cultures, to report on the reduction of mycelia and spores. The fungus was tested with the extract at different concentrations (10mg/ml, 25mg/ml, 50mg/ml, 75mg/ml, 100mg/ml). The change in antioxidant activity of *A. alternata* was tested with biochemical assays. The change in mycelial structure was visualised with scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Preliminary results show comparison in concentrations of phytochemical compounds of the various extracts, inhibition on plates, reduction in total dry mass of fungus, morphological and structural changes in the spores and mycelia of fungi grown in infused broth. Moringa oleifera leaf extracts (MOLE) can be used as safer alternatives to the control of *A. alternata* in wheat crops.

Keywords

Biocontrol agent, Wheat, Scanning electron microscopy, Transmission electron microscopy, Pathogenicity

Fungi associated with overall decline in pecan orchards in the Northern Cape, South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Pecan (*Carya illinoinensis*) is native to North America and has a long and interesting history. It was first cultivated by Native Americans, who used it as a source of food and medicine. Over time, pecan trees were brought to other parts of the world, including South Africa. Today, pecan trees are an important crop in South Africa, providing economic and health benefits. South African pecan producers struggle with numerous diseases and pests, which affect the optimal production of nuts. A constant emerging problem in the industry is overall decline. Symptoms are mostly noticeable in pecan trees six years and older. Affected trees exhibit underdeveloped leaves, which are very subtle at first but are visible when compared to adjacent trees. The general deterioration affects trees sporadically in an orchard. This study focused on collecting stem and root tissues from overall decline pecan trees in the Northern Cape and the isolation of the associated fungi. A total of nine trees showing overall decline symptoms were sampled for potential pathogens. Various subsamples were taken, including three stem disks at various sections of the tree, root samples, and soil. Growth of fungi occurred on culture media in the laboratory. Fungal isolates were identified by Sanger sequencing, and from these results, isolates were selected for pathogenicity trials on pecan seedlings. Six of the most prominent fungi, including *Fusarium oxysporum*, *Fusarium equiseti*, *Fusarium solani*, *Neofusicoccum vitifusiforme*, *Coprinellus micaceus*, and *Cladosporium cladosporioides,* were used to determine their conformity to Koch's postulates. Six-month-old pecan tree seedlings of the Wichita and Ukulinga cultivars were used. Results show that the tested isolates contribute to the development of stem discolouration and leave shedding. Preliminary results indicated that Wichita resisted lesion development better than Ukulinga. This study confirms that overall decline as a disease complex in pecans.

Keywords

Molecular identification, pathogenicity trials, pecan seedlings, fungi, elongation factor

Fungal diversity in dairy cow forage production systems with special emphasis on mycotoxigenic fungi

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The South African dairy industry is reliant on fodder and forage crops to provide nutrition for cows during the nutrient intensive lactation period. Feed production is the largest expense on a dairy farm, due to the nutrient requirements needed for high quality milk yields. Consequently, reduced milk yields or contaminated milk can have dire consequences for producers. Mycotoxigenic fungi produce toxic secondary metabolites known as mycotoxins and is a possible contaminant of fodder and forage crops. These toxins can have serious health consequence for humans and animals. This study aimed to investigate the fungal diversity of forage crops grown on dairy farms, with a special focus on mycotoxigenic fungi. Forage plants were sampled from one location in the Eastern cape (EC) and another location in the Western Cape (WC). Fungi were isolated and purified; DNA was extracted and representative fungal isolates (40 from the EC and 76 from the WC) were identified to species level through sequencing. Notable mycotoxigenic fungi were isolated from both sampling locations and the percentage prevalence was recorded; *Alternaria alternata* (2%), *A. tenuissima* (7%), *Fusarium cerealis* (1%), *F. culmorum* (3%), *F. equiseti* (10%), *F. incarnatum* (7%), *F. graminearum* (1%) *and F. oxysporum* (57%). High levels of mycotoxigenic fungi were isolated from perennial ryegrass (EC: 35%), lucerne (EC: 28%, WC: 22%) and tall fescue (WC: 22%). These fungi can produce *Alternaria* toxins, beauvericin, deoxynivalenol, nivalenol, and zearalenone. Cow manure sampled from the EC contained *Aspergillus flavus*, a mycotoxigenic fungi of great importance due to its ability to produce highly toxic and carcinogenic mycotoxins known as aflatoxins. This study showed that mycotoxigenic fungi are present in forage pastures and cow manure, highlighting the growing concern of mycotoxin contamination of forage crops. More comprehensive research is needed to determine the extent of mycotoxigenic fungi and mycotoxins in dairy pastures.

Keywords

Mycotoxins, Dairy Industry, *Fusarium*, *Aspergillus,* Sequencing

The impact of different fungicide spray programmes on wheat foliar pathogen diseases

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Disease Management

Abstract

Foliar diseases like rust diseases (*Puccinia* species), the leaf spot disease complex (various fungal pathogens), and powdery mildew (*Blumeria graminis* f. sp. *tritici*) threatens sustainable production of wheat in South Africa. Host resistance and fungicides form part of the integrated approach to combat these diseases, but the effect of different fungicide programmes on a range of cultivars remains largely unknown. The aim of this study was, therefore, to assess the impact of different fungicide spray programmes on eight wheat cultivars in different production regions of the Western Cape, and to record the distribution and frequency of foliar fungal pathogens throughout the province. Wheat cultivars were planted in identical field trials at four locations in the Western Cape during 2024, which received the same treatments, namely control (no fungicides), a lower cost fungicide programme and a more expensive programme. Visual disease assessments were performed every ten days from symptom onset to evaluate the incidence and severity of foliar diseases. Infected leaf samples were collected from all cultivars and treatments at each location. Fungal pathogens were isolated from these samples and are currently being identified with morphological and molecular analyses to confirm their identities and ascertain their occurrence and frequency at different locations and treatments. Visual assessments showed high disease levels at all locations (50–90% on the disease scale), with surprisingly little variation between treatments, likely due to unusually high rainfall across the province and widespread bacterial infection. Fungal diseases observed at all locations included leaf rust, the leaf spot disease complex, and powdery mildew. This study will establish a foundation for understanding the composition and dynamics of the foliar disease complex affecting wheat, and the effect of different wheat cultivars and fungicide programmes on disease development.

Keywords

Identification, Surveillance, Severity, Resistance, Cultivar response

A survey of fungal pathogens affecting cannabis in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Cannabis has a long history of medicinal use in many cultures, dating back thousands of years. The African continent produces approximately 38,000 tonnes of cannabis per year, according to United Nations estimates. Cannabis plants contain 70 unique compounds, collectively known as phytocannabinoids, which are popular for use in medicines, beauty products and recreational use. The legalisation of cannabis in South Africa has led to increased production and consequently, increased reports of disease. The aim of this project was to conduct a survey of fungal diseases of cannabis in South Africa. Samples of diseased cannabis plants were collected during the 2023-2024 and 2024-2025 growing seasons from Gauteng, Limpopo and North West provinces, and limited samples were obtained from KwaZulu-Natal and Eastern Cape provinces. Symptoms ranged from bud rot and foliar diseases to dieback and root rots. A variety of fungi were identified from leaves including *Golovinomyces* sp. causing powdery mildew, as well as various leaf spot pathogens including *Alternaria* spp., *Bipolaris* spp., *Cercospora* sp. *Curvularia* spp., *Didymella* sp. *Epicoccum* sp., *Exserohilum* sp., *Septoria* sp. and *Setosphaeria* sp. Pathogens associated with bud rot were *Botrytis* sp. and *Fusarium* sp. Root rot pathogens isolated from symptomatic material included *Globisporangium* sp., *Pythium* spp., *Rhizoctonia solani*, *Athelia rolfsii* and various *Fusarium* species. During the 2024-2025 season, severe die-back was observed in commercial cannabis fields in North West and Limpopo provinces, leading to total crop failure on two farms. The causal agents were *Neofusicoccum parvum* and *N. kwambonambiense*. As little information on pests and diseases of cannabis in South Africa have been published, most of the fungi identified in this study represent first reports from cannabis in South Africa. Further studies to characterise these fungi and conduct pathogenicity trials are underway. These studies will help to inform effective disease management strategies for cannabis in South Africa.

Keywords

Cannabis, *Neofusicoccum*, powdery mildew, leaf spot, root rot

Preserve, identify, protect: the role of the national collection of fungi in plant pathology and biosecurity

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Biosecurity

Abstract

The National Collection of Fungi (NCF) plays a critical role in supporting plant pathology research, diagnostic capacity, and biosecurity in South Africa. As the country's official repository for fungal cultures, the NCF preserves and curates pathogenic and non-pathogenic fungi of agricultural, forestry, and environmental importance. This work highlights the strategic value of depositing fungal isolates into the NCF, particularly in the context of accurate species identification, long-term preservation, and compliance with regulatory frameworks such as the National Environmental Management: Biodiversity Act (NEMBA) and the Nagoya Protocol on Access and Benefit-Sharing. By integrating molecular identification tools (e.g., ITS and TEF1-α barcoding), the NCF ensures reliable taxonomic resolution essential for plant disease diagnostics and surveillance. Moreover, deposition into a national repository promotes data standardization, reference availability, and responsible access to genetic resources. This presentation outlines recent efforts to strengthen the NCF's role in safeguarding fungal biodiversity, addressing underrepresentation in key taxonomic groups, and enhancing its service to researchers, diagnostic labs, and regulatory authorities. Strengthening culture deposition practices is not only a scientific imperative but a national biosecurity priority.

Keywords

plant pathology; fungal diagnostics; culture deposition; NEMBA; Nagoya Protocol; fungal biodiversity

Influence of storage conditions and packaging in decay control and quality maintenance of table grapes during extended storage

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Disease Management

Abstract

Postharvest fungal pathogens such as *Botrytis cinerea*, *Penicillium expansum*, *Alternaria alternata*, *Cladosporium* spp., and other pathogens, are common in table grapes, particularly under prolonged cold storage and fluctuating postharvest conditions. Recent logistical delays in the supply chain have extended grape storage periods beyond eight weeks in some consignments, often resulting in increased decay and quality deterioration. Although SO_2 is widely used to limit decay, its efficacy depends on proper application, packaging combinations, and environmental conditions. Table grapes were harvested and subjected to three wetting treatments (dry, wet-packed, wet-dried) using sterilized rainwater. Grapes were packed with moisture-absorbing material (MAM) into clamshell or open-top punnets and sealed in outer bags of different perforation configurations. SO_2 sheets of different properties were included. In a separate trial, the same packaging combinations were exposed to four temperature abuse scenarios: no abuse, 4h at ambient, 48h at 7.5 °C, and a combined treatment involving three interruptions during cold storage. Air temperatures were logged, and decay occurrence, SO_2 damage, berry splits, and postharvest quality defects were evaluated. Decay was generally higher in wet-packed grapes when packed in bags of higher perforated area. Outer bags of low perforated area improved decay control, however, SO_2 damage and berry splits, particularly under moisture-rich or abusive temperature conditions, were increased. Temperature abuse increased decay and quality losses, with the most extreme regime significantly increasing losses. Packaging type, wetting regime, and temperature stability, critically influence SO_2 efficacy and grape quality during prolonged storage. While outer bags of lower perforated area enhance SO_2 preservation, they increase SO_2 related damage and is less tolerant to moisture and temperature abuse. An optimized SO_2–packaging–temperature maintenance strategy is essential to maintain grape quality during prolonged storage of table grapes.

Keywords

postharvest, SO_2, fungal pathogens, temperature abuse, moisture

The who's who of the South African *Capsicum* virome

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Capsicum spp. are economically important crops that contribute significantly to global food security and nutrition. However, these crops are vulnerable to rapidly evolving and emerging viral and viroid pathogens that can substantially reduce crop yields and threaten food security. A survey was conducted to determine the diversity and distribution of viruses and viroids infecting *Capsicum annuum* L. cultivars across Gauteng, Limpopo, Northwest and Mpumalanga provinces. Samples were collected from commercial, smallholder, and subsistence farms. Leaves and fruit samples were obtained from plants displaying virus-like symptoms, such as necrosis, chlorosis, mottling, stunting, and pitting. Total nucleic acids (RNA and DNA) were extracted and screened for viruses and viroids using a combination of RT-PCR with genus- and species-specific primers, as well as high-throughput sequencing (Illumina). Viruses and viroids belonging to the following genera were identified; *Alfamovirus*, *Caulimovirus, Ilarvirus, Ophiovirus, Pospiviroid, Potyvirus, Polerovirus, Solendovirus*, and *Tospovirus*. The data generated from this study will inform farmers about the identity, distribution, and prevalence of these pathogens, thereby improving disease monitoring and management strategies. Additionally, this study serves as the first report of three viruses infecting *C. annuum* in South Africa.

Keywords

viral pathogens, Solanaceae, RT-PCR, metagenomics, disease monitoring

Ganoderma lucidum production and nutritional content analysis using readily available raw materials in KwaZulu-Natal

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Ecology and Epidemiology

Abstract

This study investigated the cultivation of *Ganoderma lucidum*, a medicinal mushroom, with the focus on comparing fruitbody yields, phytochemicals and nutrient content from different substrate compositions using readily available agricultural waste in KwaZulu-Natal. The research aimed to identify sustainable and cost-effective raw materials for producing *G. lucidum* given the increasing demand within the province and the interest from local farmers. Six different substrate formulars were tested to determine the most effective composition, which had the following substrate proportions: T1: 37% sugarcane bagasse, 25% hominy chop, 20% wheat bran, 15% sawdust and 3% lime. T2: 50% sugarcane bagasse, 47% sawdust and 3% lime. T3: 50% sugarcane bagasse, 22% wheat bran, 25% sawdust and 3% lime. T4: 50 % teff grass, 47% sawdust and 3% lime. T5: 50% teff grass, 25% sawdust, 22% wheat bran, and 3% lime. Control: 90% sawdust 8% wheat bran and 2% lime. A total of 300 mushroom packs were produced, with each treatment having five replicates of ten packs. Results from the two production cycles showed that all treatments produced mushroom fruiting bodies. T2 showed the highest biological efficiency (128.4%), followed by the control (116.1%), T3 (104.7%), T1 (91.5%), T4 (72.6%) and T5 (38.4%). The highest average mushroom yields were from T3 with 258.4g. T3 also showed the highest levels of anthocyanin, phenolic acid, protein, iron and zinc. Treatments containing teff grass (T4 and T5) performed poorly in terms of mycelium strength and yields in the first production also illustrating high moisture at the bottom of the packs. This study showed that high yields and rich nutrient profiles including phytochemicals in *G. lucidum* can be achieved using local readily available raw materials with T3 producing better yields and nutrient content than the control. Future work can focus more on the impact of environmental conditions and enzyme activity.

Keywords

Biological Efficiency, Phytochemicals, Substrate composition

Sensitivity determination and assessment of *Exserohilum turcicum* to the succinate dehydrogenase inhibitor (SDHI) fungicide pydiflumetofen

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Disease Management

Abstract

Maize production, which is critical to both the food and economic security of South Africa, is persistently threatened by Northern Leaf Blight (NLB), a devastating foliar disease caused by *Exserohilum turcicum* that can reduce yields by 37 – 72%. The succinate dehydrogenase inhibitor (SDHI) fungicide, pydiflumetofen, was recently registered in South Africa for the management of NLB in maize; however, no published baseline sensitivity studies of *E. turcicum* against pydiflumetofen are available for African populations. This study aimed to evaluate the sensitivity of 26 *E. turcicum* strains from Kenya (n = 12), South Africa (n = 7) and the United States (n = 7) to pydiflumetofen using a poison plate mycelial growth assay. Of these, three strains were selected for biochemical and morphological tests to evaluate the effect of pydiflumetofen on conidial production and germination, and cell membrane permeability. Results showed that the effective concentration of pydiflumetofen causing a 50% reduction (EC_{50}) in mycelial growth varied amongst strains, ranging from 0,0123 to 0,9012 µg/mL. Significant differences in mean EC_{50} ($p < 0,05$) were observed between strains from South Africa (0,0441 µg/mL) and the United States (0,2060 µg/mL). Pydiflumetofen exposure resulted in significant reductions ($p < 0,05$) in mycelial growth, conidial production (41,7 – 85,4% at EC_{50}, and 81,7 – 90,6% at 10 × EC_{50}) and conidial germination (35 – 65,2% at EC_{50} and 43,3 – 69,4% at 10 × EC_{50}). In addition, increased cell membrane permeability was observed (-0,3 – 4,4% at EC_{50}, and 2,2 – 16,5% at 10 × EC_{50}) upon pydiflumetofen exposure. Overall, these results revealed that pydiflumetofen had notable effects on the morphology and biological processes of *E. turcicum in vitro*, suggesting its effectiveness in managing NLB in the field. The findings herein will aid in monitoring for *E. turcicum* sensitivity shifts against pydiflumetofen in South Africa and will inform effective spraying program development.

Keywords

Northern Leaf Blight, Maize (*Zea mays*), Baseline sensitivity, Fungicide resistance and *Setosphaeria turcica*

Sensitivity of *Pseudocercospo*ra spp., the causal agents of kiwifruit spot and rot, to fungicides from different chemical groups

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Disease Management

Abstract

A fruit spot and rot, caused by *Pseudocercospora* spp., is currently the most economically important disease affecting kiwifruit in South Africa and poses a significant threat to kiwifruit production. As this is a newly emerging disease, no information is currently available regarding effective control strategies for the fungal pathogen causing the disease. This study aimed to (i) determine the baseline sensitivity of *Pseudocercospora* spp. to azoxystrobin, boscalid, chlorothalonil, cyprodinil, difenoconazole, and fludioxonil in *in vitro* conditions; and (ii) to establish the EC_{50} values for each fungicide. Thirty *Pseudocercospora* isolates obtained from diseased kiwifruit were used to assess their sensitivity towards the chemicals. The isolates were obtained from three provinces, nine host cultivars, and represented eight different ITS haplotypes. The individual and mean EC_{50} values for the fungicides tested ranged from 0.0003–0.0139 ppm (0.0488 ppm) for difenoconazole, 0.018–0.754 ppm (0.243 ppm) for azoxystrobin, 0.016–1.01 ppm (0.385 ppm) for chlorothalonil, 0.1336–1.570 ppm (1.013 ppm) for cyprodinil, 1.049–2.738 ppm (1.550 ppm) for fludioxonil and 0.089–17.017 ppm (5.677 ppm) for boscalid. The mean EC_{50} values demonstrated that difenoconazole, azoxystrobin, and chlorothalonil were the most effective at inhibiting mycelial growth. Fludioxonil also showed effectiveness, although its effectiveness was limited to 75% of the fungal isolates screened, suggesting a potential risk of resistance development. The results indicated that substantial variation in fungicide sensitivity exists within the *Pseudocercospora* spp. isolates with no apparent correlation with ITS haplotype, host cultivar, or geographic origin. This study provides valuable insights into the effectiveness of fungicides against *Pseudocercospora* spp. and contributes to optimizing fungicide application and resistance management. These findings are crucial for developing field trials, establishing long-term spray programs, and facilitating the registration of active ingredients for use in South African kiwifruit orchards.

Keywords

Baseline sensitivity, EC_{50} values, Fungicide resistance, Kiwifruit (*Actinidia chinensis*)

Host range determination of Pepper ringspot virus (PepRSV), the latest threat to the South African potato industry

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Ecology and Epidemiology

Abstract

Over the last four years, a Tobravirus, named Pepper ringspot virus (PepRSV) was found to infect potato crops (*Solanum tuberosum*) in South Africa (SA). To date, PepRSV has only been reported from Brazil, where it infects pepper, tomato, artichoke and *Bidens* weed species, but not potatoes. In SA, PepRSV cause brown arcs and flecks in the tubers, a symptom also referred to as spraing. In light of the importance of the commercial potato industry in SA and its use as a subsistence and cash crop for many households, the PepRSV disease is an important priority for investigation. During this DOA funded research project, widespread testing between 2023 and 2025 revealed the presence of PepRSV in 8 of the 9 provinces of SA. The potato cultivars affected includes Mondial, Sifra, Panamera, Allison, Tyson, Avalanche, UTD Sababa and Lanorma. Nematode transmission trials in pots found that PepRSV is being transmitted by *Nanidorus minor*, a trichodorid found widespread in SA. Two biological repeats of a host range study using nematode transmission in pots were carried out in 2024 and 2025. The study included 32 species, including vegetable crops (tomatoes, peppers, spinach, cucumber, beans, peas, beetroot, onion, turnip, tobacco) and other crops regularly intercropped with potatoes on farms in SA (sunflower, maize, soya, sorghum, rhodes grass, weeping lovegrass, sun hemp, fodder peas, oats, lucerne, mustard, barley). The results of the host range study will be discussed.

Keywords

Pepper ringspot virus, Tobravirus, *Nanidorus minor*, *Solanum tuberosum, host range*

Essential oils against pear postharvest pathogen, *Diplodia seriata*, tested *in vitro* and *in vivo*

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Disease Management

Abstract

The pear industry in South Africa has large economic contributions, and the postharvest decay of pears is of great concern. The identification of pathogens causing disease is important to implement effective management strategies. *Diplodia seriata* was previously confirmed to be the causal organism of black rot postharvest disease of 'Forelle' pears in the Western Cape. Fludioxonil and pyrimethanil are the registered fungicides for postharvest diseases in South Africa. There are concerns over the use of fungicides in industry; therefore, there is a need to identify alternative control methods. Essential oils have been found to possess antimicrobial properties; their use, as part of management strategies, could help prolong the shelf-life of fruits. Thyme, orange and clove oil were tested *in vitro*, on the mycelial growth of *D. seriata* at nine different concentrations. The results showed that thyme oil (200uL/L) effectively inhibited 100% of mycelial growth, followed by clove oil (300uL/L). Orange oil was not able to inhibit 100% of the mycelial growth at the tested concentrations but showed a steady decrease in mycelial growth. Thyme oil (0.5 mL/L) worked better curatively, *in vivo,* compared to clove oil (4 mL/L); thyme oil decreased the mean severity by 33.90% after 12 hours and by 19.25% after 18 hours. Clove oil had a slight decreased severity (3.67%) after 12 hours. The application of these products, preventatively, *in vivo,* showed better results; thyme oil (1 mL/L) was able to decrease the mean severity by 22.23% and clove (4 mL/L) by 6.11%. Thyme oil was more phytotoxic on the peel than clove oil was. The use of essential oils, as part of postharvest management, could prevent decay, and this study was the first step in determining the efficacy of three essential oils in the prevention of black rot of pears.

Keywords

Pests, postharvest diseases, integrated control, alternative management

Fungal diversity in South African gold mines: a hidden reservoir of novel taxa

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Ecology and Epidemiology

Abstract

South African gold mines are unique and isolated subterranean environments in which the fungal diversity remains largely unexplored. These ecosystems, often several kilometres underground, are characterised by elevated temperatures, high humidity, and low light. The widespread use of timber support structures, at various stages of decay, establishes unique microhabitats conducive to fungal colonisation. As part of an ongoing survey of cycloheximide-tolerant fungi, initially motivated by the recovery of *Sporothrix* species from mine-associated environments, we collected timber and dirt samples in active gold mine shafts in South Africa and made isolations using dilution-to-extinction methods on selective media. Identifications were based on ITS sequencing, with additional loci (LSU, *BenA* and *CaM*) used to enhance phylogenetic resolution where required. The findings revealed a remarkably rich and taxonomically diverse fungal community, comprising at least 48 genera across 16 orders. Notably, more than half of the recovered species appear to represent novel taxa. Identifying fungal species from this unique environment has contributed to our understanding of their ecological distribution and potential environmental reservoirs, particularly for taxa of clinical relevance such as species of *Sporothrix*. The results highlight the value of exploring selective and understudied environments for fungal discovery, revealing species of potential importance to plant, animal, and ecosystem health, as well as to ecological, evolutionary, and biotechnological research.

Keywords

Fungal diversity, pathogen ecology, environmental reservoirs, gold mines, underground ecosystems

Room-temperature dried leaf storage preserves citrus samples for reliable detection of *Candidatus Liberibacter* spp.

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Pathogen Identification, Characterisation and Taxonomy

Abstract

One of the most economically damaging diseases of citrus, Huanglongbing (HLB), is associated with phloem-limited bacteria in the genus *Candidatus Liberibacter*, including '*Ca. L. asiaticus*' (CLas), '*Ca. L. americanus*' (CLam), and '*Ca. L. africanus*' (CLaf). The detection of the disease relies on visually assessing trees for characteristic symptoms, such as leaf mottling, along with performing molecular diagnostic assays. Reliable diagnostics depend on high-quality nucleic acids extracted from input plant material. Often, leaf samples are collected from remote sites where cold storage is unavailable, and delays between sample collection and laboratory processing can range from days to weeks. This leads to poor sample preservation and degraded nucleic acids, thereby compromising diagnostics. This study assessed whether drying leaves in brown paper envelopes at room temperature offers a practical alternative for preserving leaves when immediate processing is not feasible. Leaf samples displaying symptoms of CLaf were collected from trees in an orchard, with each sample divided into two: (1) dried for a month and (2) freshly processed. Total nucleic acids (TNA) were extracted from the midribs and petioles using a modified CTAB protocol, and the yield, purity, and integrity were evaluated with spectrophotometry and gel electrophoresis.

Real-time PCR targeting *Candidatus Liberibacter* spp. was performed using cytochrome c oxidase (COX) as an internal control to determine diagnostic sensitivity. Results indicated no significant differences in DNA yield, quality, or cycle threshold (Ct) value between dried and freshly processed leaves, suggesting that drying leaves is a viable method for preserving field-collected plant material.

Keywords

Huanglongbing, Nucleic acid extraction, Molecular diagnostics, Field sampling, Sample preservation

Identification and characterisation of the mating-type locus from the genome sequences of diverse *Sclerotinia* species

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Fungal plant pathogens in the genus *Sclerotinia* infect and kill a wide range of economically important crops worldwide. While the reproductive biology of the three core species – *S. sclerotiorum*, *S. minor*, and *S. trifoliorum* – has been studied extensively, relatively little is known about mating-type diversity across the broader genus. This knowledge gap limits our understanding of the population dynamics, genetic diversity, and potential for disease emergence in the lesser-known species. The aim of this study was therefore to characterise the mating type (*MAT1*) locus and associated genomic features across *Sclerotinia* species. To achieve this, 16 genome assemblies representing 14 *Sclerotinia* species were obtained from public databases and annotated. The *MAT1* locus was identified using tBLASTn searches with known *Sclerotinia* MAT protein sequences, and the surrounding regions were examined for direct or inverted repeat elements previously implicated on structural rearrangements of the mating-type locus. The *MAT1* locus was successfully identified in all analysed species and consisted of between two and four complete *MAT* genes. The genomes of most species harboured genes from both the *MAT1-1* and *MAT1-2* idiomorphs, consistent with a homothallic (self-fertile) reproductive strategy. In several genomes, the presence of direct or inverted repeats suggested potential for gene deletions or inversions within the mating-type region, paralleling observations in *S. sclerotiorum, S. minor*, and *S. trifoliorum*. However, some species displayed only a single *MAT* idiomorph, indicative of heterothallism (outcrossing). These findings expand current knowledge of reproductive diversity in *Sclerotinia*, demonstrating substantial variation in mating-type structure across the genus. Given the role of sexual reproduction in shaping pathogen populations and contributing to their adaptability, this work provides important insights that may inform disease management strategies for this agriculturally significant group of fungi.

Keywords

fungal pathogens, fungal reproduction, homothallism, *MAT* alleles, mating-type genes

Evaluation of the spatial and temporal distribution of '*Candidatus Liberibacter africanus*' in citrus host plants

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Huanglongbing (HLB) is the most significant biological threat to global citrus production, leading to substantial economic losses through yield reduction, diminished fruit quality, and progressive tree decline. The disease is associated with three phloem-limited bacterial species: '*Candidatus* Liberibacter *asiaticus*' (CLas), *Ca.* L. *americanus* (CLam), and *Ca.* L. *africanus* (CLaf). The latter is responsible for the milder form of HLB known as African Greening, which is transmitted by the insect vector *Trioza erytreae*. Although CLaf has been present in South Africa for over a century, key aspects of its biology remain insufficiently characterised, particularly regarding its long-term impact on host health and its epidemiological dynamics in commercial orchards.To address some of these knowledge gaps, a commercial orchard was surveyed, with visual assessments of symptomatic trees conducted across multiple seasons. Bark and leaves were sampled from various points on selected diseased trees to investigate intra-plant pathogen distribution. Furthermore, three citrus cultivars were evaluated to compare CLaf concentrations across various tissue types to determine the spatial distribution in seedlings. Quantitative PCR was employed to determine CLaf concentration, allowing for detailed analysis of pathogen localisation and persistence over time. Despite its longstanding endemic status, information on CLaf remains limited. This study aimed to advance the characterisation of African Greening by elucidating spatial and temporal patterns of CLaf distribution. The findings contribute to a more comprehensive understanding of pathogen behaviour, informing the development of improved diagnostic methodologies, targeted disease management strategies, and ultimately supporting the sustainability of citrus production in regions affected by African Greening.

Keywords

CLaf, Phloem-limited bacteria, African Greening, Huanglongbing, HLB

Callose synthases fortifying avocado defence against *Phytophthora cinnamomi*

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Plant-Pathogen/Insect Interaction

Abstract

The globally significant fruit crop avocado (*Persea americana*) is threatened by *Phytophthora cinnamomi*, a hemibiotrophic oomycete causing Phytophthora root rot (PRR). Despite the economic importance of avocado and the threat posed to the industry by PRR, the molecular mechanisms underlying resistance to the disease remains poorly understood. Callose depositions in the plant cell wall is a well-recognised plant defence mechanism which physically blocks pathogen penetration, with callose synthases responsible for callose production. Confocal microscopy was previously used to demonstrate the timely production of callose in a partially-resistant avocado rootstocks in response to *P. cinnamomi* infection. The current study aimed to characterise avocado *callose synthase* genes (*PaCalS*) and to evaluate their role in defence using dual transcriptome data. Additionally, a callose quantification assay was utilised to assess the role of callose in defence in rootstocks of varying resistance to PRR. Using the West-Indian pure accession avocado reference genome, nine *PaCalS* genes were identified and characterised *in silico* through analysis of their gene - and predicted protein features. Phylogenetic analyses of protein sequences revealed *PaCalS* homology with *CalS* orthologues from other plant species. This, combined with expression profiles during *P. cinnamomi* infection, strongly implicated four genes in avocado defence. Defence-responsive *cis*-acting elements identified in the candidate gene promoters suggest the involvement of complex regulatory pathways. These findings provide evidence that *PaCalS* genes are integral to avocado's defence against *P. cinnamomi*. This research advances our understanding of immunity in this non-model fruit crop, while also identifying promising molecular targets for breeding programs aimed at enhancing disease resistance.

Keywords

Persea americana; callose deposition; *CalS*; Gene characterisation; Crop resistance

Sexual reproduction potential of *Cladosporium cladosporioides*, the causative agent of pecan scab in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

South Africa is the third-largest pecan nut producer in the world. The fungal pathogen *Venturia effusa* causes pecan scab globally. Until recently, it was believed that this fungus also caused pecan scab in South Africa. However, preliminary sequence data indicated that members of the *Cladosporium cladosporioides* species complex were the causal agents of pecan scab in South Africa. There is a lack of knowledge on this phytopathogen such as its distribution, sexual phase and population dynamics as it was only recently determined to be the causal agent of pecan scab in South Africa. This study aimed to determine the population difference in the eastern South African scab-stricken areas, including Limpopo, the Eastern Cape, KwaZulu-Natal, and Mpumalanga. The different mating types and the possibility of sexual recombination were determined in this fungus. The species of *Cladosporium* associated with pecan scab were determined by isolating and culturing the fungi from scab lesions, followed by DNA extraction and identification using sequencing of the ITS, TEF, and actin gene regions. The designed mating type primers were used to amplify and sequence the mating type genes of the fungi. *In vitro* growth studies were used to determine if sexual reproduction is possible. Preliminary results indicated that *C. cladosporioides* and *C. pseudocladosporioides* were frequently isolated from pecan scab lesions in South Africa. *Cladosporium cladosporioides* was found to be heterothallic, representing two mating types that occur in equal frequencies in nature. The authors allude to *C. cladosporioides* being the causal agent of pecan scab in South Africa, however pathogenicity test need to be performed to confirm this. The knowledge gained in this study would aid in understanding of the disease cycle of this fungus. Eventually, this could lead to a more effective management strategy to combat this disease and the registration of targeted fungicides in South Africa.

Keywords

Mating types, *Cladosporium pseudocladosporioides*, DNA sequencing, heterothallism, distribution

Exploring mycoviral diversity and curing strategies in plant pathogenic *Ceratocystis* species

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Disease Management

Abstract

The fungal genus Ceratocystis harbours important plant pathogens responsible for substantial losses in forestry and agriculture. While chemical fungicides remain the primary means of control, their impact on environmental and human health has prompted interest in more sustainable alternatives. Mycoviruses (viruses that infect fungi) have emerged as potential biocontrol agents. However, studying their effects requires virus-free fungal cultures, which are often challenging to obtain. This study aimed to identify both novel and known mycoviruses in isolates of *C. fimbriata*, *C. manginecans*, and *C. eucalypticola*, and attempt to eliminate mycoviruses from *C. fimbriata* to facilitate future functional studies. Five rounds of hyphal tipping were performed in the presence of ribavirin, cycloheximide, ampicillin, kanamycin, and rifampicin. In selected cases, both asexual and sexual spore isolations were conducted. Total RNA was extracted from treated cultures to assess viral presence. RNA sequencing was performed on fifteen different *Ceratocystis* isolates from diverse geographic regions to screen for novel mycoviruses, while RT-PCRs were used to detect previously reported viruses in other isolates. Numerous isolates shared similar viral profiles, although some variation was observed based on species and geographic origin. While complete viral elimination was not achieved, ribavirin treatment and sexual spore isolation effectively reduced viral loads for certain viruses. Antibiotic treatment in combination with hyphal tipping also had limited success. This study expands current knowledge of mycovirus diversity within *Ceratocystis* spp. and identifies methods that may aid in generating virus-free fungal cultures. These findings support future efforts to explore mycoviruses as biocontrol agents in plant pathology.

Keywords

Mycovirus; Biocontrol; RNA-seq; mycovirus elimination; *Ceratocystis fimbriata*

The effect of shade netting on the prevalence of *Alternaria* brown spot and core rot diseases of citrus in South Africa

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Ecology and Epidemiology

Abstract

The implementation of shade nets in citriculture is focused on improving fruit quality and yield. Previous research showed diverse effects derived from the application of shade nets such as changes in microclimate, tree physiology, carbohydrate accumulation, phenology, and fruit quality of citrus. However, the effect of shade nets on plant pathogens and disease development are not known. The aim of this study was to assess the disease incidence and -severity of Alternaria brown spot (ABS) and -core rot (ABR) of citrus for open and shade-net-covered orchards. A survey was conducted in the major citrus-producing areas, which included the Eastern Cape, Mpumalanga, Limpopo and the Western Cape. Alternaria brown spot was assessed on mandarins by recording the number of infected trees and infected fruit per tree, and by assessing the severity of the disease by investigating the canopy and fruit. Alternaria core rot was assessed on oranges and mandarins by recording the number of infected fruits per tree. Additionally, the ACR tree incidence was recorded. Both ABS and ACR showed higher prevalence in the northern producing areas of South Africa, with little prevalence in the Western Cape. *Alternaria alternata* and *A. arborescens* were the dominant species isolated. Shade-net-covered orchards had higher tree- and fruit incidences of ABS compared to open orchards. Similarly, shade-net-covered orchards had higher canopy and fruit disease severity indices for ABS compared to open orchards. Nadorcott, Orri and Tango cultivars were the least severely affected by ABS disease and Nova and Leanri the most severely affected cultivars. There were no significant differences in disease incidence for ACR between open orchards and shade-net-covered orchards. Shade nets alter an orchards microclimate which could favour certain plant pathogens. The use of shade netting promoted ABS infections and disease development, but not ACR.

Keywords

Survey, disease incidence, disease severity, *Alternaria alternata*, *Alternaria arborescens*

Phytophthora palmivora: a serious threat to papaya production in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Phytophthora palmivora is an Oomycete pathogen widespread in tropical and subtropical regions, causing severe diseases such as fruit rot, bud rot, blight, cankers, and root rot. In South Africa, papaya is grown by both smallholder and commercial farmers, particularly in the Limpopo and Mpumalanga provinces. In 2019, widespread symptoms of crown and root rot were observed in South African papaya orchards, leading to significant tree collapse and loss of fruit production. This study aimed to identify and characterize *Phytophthora* species infecting papaya in South Africa, as well as to investigate the distribution and aggressiveness of identified species. Samples were collected from six commercial orchards, and diseased stems and soil samples were analysed, resulting in the isolation of only one *Phytophthora* species. Molecular identification using rDNA-ITS sequencing confirmed the species identification as *P. palmivora,* and pathogenicity tests revealed the pathogen's aggressiveness on papaya fruits and trees. This is the first report of *P. palmivora* in South Africa, where it is a regulated pathogen. Given its destructive potential, *P. palmivora* poses a serious threat to the papaya industry and other crops such as macadamia, citrus, and avocado. Understanding the distribution of *P. palmivora* and its potential introduction routes is critical for implementing effective management strategies to protect South African agriculture from further damage.

Keywords

Phytophthora, *Carica papaya,* Disease

First report of cannabis diseases in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The rapid expansion of cannabis production for medicinal purposes in South Africa has led to increasing concerns regarding the prevalence and impact of significant disease challenges, particularly with regard to fungal and Oomycetes pathogens. This study aimed to identify and confirm the pathogens causing wilting, yellowing, and white and grey mould symptoms on cannabis. Samples were collected from an aeroponic cannabis facility in South Africa. The resulting isolates were identified based on DNA sequence data from the rDNA-ITS and *tef1-α* loci, and the pathogens were confirmed as *Botrytis cinerea, Fusarium oxysporum, Pythium myriotylum* and *Sclerotinia sclerotiorum*. Pathogenicity trials further confirmed that all these species were capable of causing the observed symptoms on cannabis plants. Notably, this study represents the first report of these pathogens causing disease symptoms on cannabis in South Africa. These findings highlight the urgent need for proactive disease monitoring and the development of effective management practices to mitigate the impact of fungal pathogens on the country's growing cannabis industry.

Keywords

Cannabis, hydroponics, wilting, root rot, pathogen

Taking a look at Tospovirus diversity in Solanum crops in South Africa.

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The genus *Orthotospovirus* comprises 26 plant-infecting species characterized by a tripartite RNA genome. Historically divided into serogroups I, II, and III (TSWV, WSMoV, and IYSV), species classification is now based on amino acid sequence identity of the nucleocapsid (N) protein, resulting in five phylogenetic clades: the Tomato spotted wilt virus (TSWV) clade, *O. glycininecrovenae* (formerly soybean vein necrosis virus, SVNV) clade, IYSV clade, WSMoV clade, and *O. arachiflavamaculae* (formerly groundnut yellow spot virus, GYSV) clade. Among these, TSWV is a major pathogen infecting over 1,000 plant species, including tomatoes, peppers, lettuce, peanuts, and potatoes, causing yield losses ranging from 50% to 100%. This study aimed to investigate the diversity and occurrence of orthotospoviruses infecting vegetable crops in South Africa. Samples were collected during 2023 and 2024 from the Northern provinces from symptomatic vegetables, including potato tubers showing necrosis, pepper plants exhibiting ringspot symptoms on leaves and fruit, and tomato leaves showing chlorosis. In addition, ten archived samples collected between 1991 and 2014 and stored in the Virology Collection at the ARC-PHP, previously found by electron microscopy to contain tospovirion particles, were included to provide historical context. All samples were screened using RT-PCR with Tospo genus specific primers, and positive samples were further analysed by high-throughput sequencing (HTS) on the Illumina platform. Preliminary results confirmed the presence of orthotospoviruses in several recent and archived samples. Sequence and phylogenetic analyses are ongoing to determine strain diversity, clade distribution, and the possible emergence of novel variants in South African vegetable crops. The study provides new insights into the molecular epidemiology and diversity of orthotospoviruses in the region, contributing to improved surveillance and management of these economically significant plant pathogens.

Keywords

Orthotospovirus diversity, High throughput sequencing, Vegetable crop pathogens, Tomato Spotted Wilt Virus (TSWV), RT-PCR screening

Efficacy of baculoviruses against *Plutella xylostella* (Diamondback Moth) in larval bioassays

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Plant-Pathogen/Insect Interaction

Abstract

Baculoviruses are DNA viruses that infect very specific insect hosts as a natural way to control insect populations. As such, they play important roles in agriculture as biological control agents. Globally, brassica crops are threatened by insect pests such as *Plutella xylostella* (Lepidoptera: Plutellidae), also known as the diamondback moth (DBM). Critically, DBM has developed resistance to chemical pesticides, calling for alternative control agents such as baculoviruses to be further explored as a biological control method. The aim of the study was therefore to test the efficacy of two baculoviruses, namely *Ostrinia furnacalis* nucleopolyhedrosis virus (OfNPV) and *Mamestra brassicae* nucleopolyhedrosis virus (MbNPV), at different viral concentrations against the larvae and pupae of DBM. To inoculate the larvae, cabbage leaf discs were submerged in different viral concentrations (0, $1x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$) of each of the respective viruses. A single DBM larva was carefully placed on a leaf disc in separate wells of a twelve-well plate. The larvae were monitored daily for 14 days for mortality. The number of days elapsed before mortality occurred and the percentage mortality after 14 days were recorded. Both OfNPV and MbNPV induced dose-dependent mortality against DBM. The $1x10^4$–$1x10^7$ OfNPV treatments induced 25%–58,3% larval mortality, while MbNPV induced 19,4%–38,9%, respectively. For pupal mortality, OfNPV treatments induced 13,8%–25% mortality, while MbNPV induced 5,6%–16,7%. The average days elapsed before larval mortality occurred in OfNPV was five days, and eight days for average pupal mortality. For MbNPV, 3 days elapsed before average larval mortality occurred and 9 days for average pupal mortality. Occlusion bodies were isolated from dead larvae and pupae and analysed using PCR and Sanger sequencing to confirm infection by the viruses. Further work will determine the efficacy of the viruses on DBM in vivo in pot trials.

Keywords

Biological control, brassica crops, Baculovirus, Lepidoptera, bioassays

Testing entomophagous fungi against Diptera and Lepidopteran pests

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Disease Management

Abstract

Insect pests are responsible for 18% of the losses faced by agricultural production worldwide. Unfortunately, insect pest control programs are faced with the development of multi-insecticide resistance developing within insect populations and persistent chemical residues on grains, fruits and other food sources. As a sustainable alternative, entomophagous fungi (EPF) are gaining prominence in integrated pest management (IPM) strategies. EPF based products accounts for only 35% of the world biological pesticide market, and they rank second after bacterial pesticides. The current study aims to access insecticidal activities of South African isolated EPF isolates against the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae) and leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae), which are important pests on fruits and tomatoes, respectively. To achieve this, different life stages of insects were screened under laboratory and glasshouse settings with EPF strains from the National Collection of Fungi. Prior screening, growth and sporulation rates of the strains were quantified and compared as key production attributes using different media and phylogenetic analyses will be conducted on the EPF strains to depict their evolutionary relationships. Results from this study will help in decision making when selecting an EPF to control a specific order of insects. In addition, locally isolated EPF will limit the import of foreign strains into the country.

Keywords

Biocontrol, Integrated Pest Management, Fruit fly, Leaf miner, Pesticides

Characterisation of *Bipolaris*, *Curvularia* and *Septoria species* associated with leaf spots on cannabis

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The expanding cultivation of *Cannabis sativa* for medicinal and industrial purposes in South Africa in recent years, has been accompanied by an increase in foliar diseases, particularly leaf spots observed in various regions of South Africa. However, the causal agents responsible for the disease remain unknown. In this study, symptomatic leaf samples were collected from five provinces of South Africa including Eastern Cape, Limpopo, Northwest, Free State and Gauteng. A total of 101 fungal isolates were obtained, PCR reactions and sequencing of the ITS regions were conducted for all the isolates. Preliminary BLAST analysis using the obtained ITS sequences identified 46 isolates belonging to *Curvularia*, 28 to *Bipolaris*, and 27 to *Septoria*. To confirm species level identity, multilocus sequencing and phylogenetic analyses were conducted on seven gene regions (ITS*, TEF1-α, LSU, RPB2, TUB, CAL, and ACT*) for *Septoria,* on two gene regions (ITS and *GADPH)* for *Bipolaris* and on three regions (ITS*, GADPH* and *TEF*) for *Curvularia*. Based on phylogenetic analyses, all 27 *Septoria* isolates were identified as *Septoria cannabis*, with beta-tubulin offering the best species level resolution. Sequencing and phylogenetic analyses are being conducted on the remaining *Bipolaris* and *Curvularia* isolates. Pathogenicity tests are also being conducted to fulfill Koch's postulates. This study provides the first confirmed report of *Septoria cannabis* associated with cannabis in South Africa and contributes valuable knowledge to our limited understanding of fungal pathogens affecting this emerging crop.

Keywords

Cannabis sativa, Molecular analysis, *Septoria cannabis,* Phylogenetic analysis, Pathogenicity tests.

Pepper ringspot virus (PepRSV) in South Africa – How widespread does this nematode-transmitted tobravirus occur in potato production regions of South Africa?

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Ecology and Epidemiology

Abstract

Potatoes (*Solanum tuberosum*) are a vital resource for South Africa (SA) in both food security and economic growth. Potatoes are the fourth most consumed food in SA and support an industry estimated to be valued at R33 billion as of 2021. As Africa's third-largest potato producer, SA harvests approximately 2.5 million tonnes of tubers annually. However, the nematode-transmitted Tobravirus *Pepper ringspot virus* (PepRSV), first detected in 2020, poses a significant threat to potato production in SA. PepRSV was first identified in the solanaceous crops tomato and artichoke and *Bidens* weed species native to Brazil in the 1960s. In potatoes, PepRSV is known to cause the sparring and flecking of tubers and mosaic chlorosis of leaves, which can lead to significant crop loss. This project carried out a virus screening survey, to determine geographical distribution and prevalence of PepRSV in potato production regions of SA. This information will allow for improved management and control of the viral outbreak. Potato tubers were collected from the Johannesburg fresh produce market as well as the Cape Town fresh produce market between June 2023 to June 2025, and screened for PepRSV using a reverse transcriptase polymerase chain reaction (RT-PCR) protocol that detects the PepRSV viral coat protein gene (RNA 2). A total of 1758 samples were screened, of which 37% (548) tested positive for PepRSV, but only 2.6% (38) of the positive samples displayed symptoms of PepRSV infection in the tubers. To date, PepRSV was found in potatoes produced in 8 of the 9 provinces, with the western and eastern production regions of the Free State exhibiting the highest infection rate.

Keywords

Solanum tuberosum, Geographical distribution, Virus screening, Crop loss, *Pepper ringspot virus*

Understanding orange rust (*Puccinia kuehnii*) in South Africa: early observations and research priorities

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Disease Management

Abstract

Orange rust, caused by the fungus *Puccinia kuehnii* (W. Krüger) EJ Butler, was historically considered a minor sugarcane disease in Australia. However, an apparent genetic shift in the pathogen between 1999 and 2001 caused devastating yield losses in the widely cultivated Q124 variety, with an estimated A$200 million economic impact. Since then, the disease has spread widely across the global sugarcane industry. In South Africa, orange rust was first detected on spore traps in Komatipoort, Mpumalanga, in 2016, and was subsequently observed on sugarcane on the KwaZulu-Natal North Coast in 2022. The disease has since spread throughout the country's sugarcane-growing regions.

The main factors influencing the development of orange rust and its impact on production are varietal susceptibility, environmental conditions and inoculum pressure. Research on these factors are in progress, to estimate the effect of orange rust on production in the South African industry and to refine the current rust management strategy.

Preliminary variety screening and field observations indicate that N12, N52, N60, N63, and N70 are the most frequently and severely affected by orange rust. In the first large-plot field trial established at Umfolozi, the recoverable value (RV) yield (tons/ha) was 19% lower in variety N60 when orange rust was not managed with fungicide. In laboratory experiments, urediniospore germination was highest at 17^{o}C and 100% relative humidity, and lowest at 31^{o}C. From field observations over the past three years, orange rust symptoms develop from late December or early January and tend to decline from May onwards. Preliminary findings suggest that field infections are favoured by periods of at least nine hours with temperatures between 23° and 27^{o}C, leaf wetness exceeding 294 mV, a relative humidity higher than 95%, as well as intermittent light rainfall. Further research is underway to confirm these findings.

Keywords

biosecurity, incursion, sugarcane diseases, yield loss

Proteomic analysis of the plant-pathogen interaction between the B45 (resistant) and 'Royal Gala' (susceptible) apple cultivars

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Plant-Pathogen/Insect Interaction

Abstract

Venturia inaequalis is a fungal ascomycete pathogen responsible for causing apple scab, one of the most critical and globally occurring diseases found in apples (Malus x domestica). The disease is mainly controlled by fungicides, but still crop losses occur every season. During the plant-pathogen interaction, the proteins secreted by fungi play an important role in the defence mechanisms used by plants to help overcome host resistance. To comprehend the interactions and connections between pathogens and plants, understanding these interactions at a proteomic level is important. 'Royal Gala' (no resistance gene) and B45 (Rvi8 resistance gene) were spray-inoculated with a mixture of *V. inaequalis* conidial spore suspension and leave samples collected at 4 different time points post-inoculation (dpi) for protein extraction. B45 accession displayed a more rapid and robust proteomic response to *V. inaequalis* infection compared to the 'Royal Gala' cultivar. Distinct proteomic profiles between B45 (724 proteins) and 'Royal Gala' (1,310 proteins) were revealed. Throughout the infection timeline, B45 showed a significant increase in stress response proteins as early as 2 days post-inoculation (dpi), highlighting it's ability to swiftly activate defence mechanisms against the pathogen infections. In contrast, the 'Royal Gala' exhibited a delayed stress response, with a notable increase in stress proteins only observed at 10 dpi, indicating a slower activation of defence pathways. This delay in response in 'Royal Gala' may account for its higher susceptibility to *V. inaequalis* infection. B45 showed a higher number of proteins involved in photosynthesis, transport, and stress response, which are critical for maintaining cellular homeostasis and activating defence mechanisms during pathogen attack. The Royal Gala cultivar, displayed lower numbers of these proteins, correlating with greater susceptibility to infection. The unique proteins identified at various dpi time points in both cultivars suggest specific molecular pathways that are activated in response to the infection.

Keywords

Venturia inaequalis, apple scab, plant-pathogen interaction, proteins

in silico prediction and characterisation of secondary metabolites in *Exserohilum* species associated with maize

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Plant-Pathogen/Insect Interaction

Abstract

Maize (*Zea mays*) is an important staple crop in sub-Saharan Africa (SSA). However, fungal pathogens such as *Exserohilum turcicum* and *Exserohilum rostratum* pose a major threat to maize production, causing up to 72 % yield losses and threatening food security for millions. Secondary metabolites (SMs) from fungal biosynthetic gene clusters (BGCs) are key virulence factors, though the role of several SMs in coordinating host-pathogen interactions remains unresolved. This study aimed to employ *in silico* analyses to annotate, predict and functionally characterize fungal SM-encoding BGCs within the *E. turcicum* (Et28A strain) and *E. rostratum* (LWI strain) reference genomes that potentially play a role in host pathogenicity. The study involved genome quality assessment, structural annotation and SM functional prediction. A total of 48 BGCs were identified across 21 chromosomes of *E. turcicum* and 54 BGCs across 16 chromosomes of *E. rostratum*. These BGCs are associated with major SM chemical classes, including type 1 polyketides (T1PKs), non-ribosomal peptides (NRPs), hybrid NRP-T1PKs, and terpenes. Conserved polyketide BGC domains linked to the production of phytotoxins, monocerin and alternapyrone were detected in both species. Notably, *E. rostratum* uniquely harbored T1PKs associated with the mycotoxins fusarin C and fumonisin B1. Interestingly, the NRP BGC domains associated with the mycotoxin apicidin were identified exclusively in *E. turcicum*. Conversely, a terpene BGC predicted to encode the mycotoxin clavaric acid was identified only in *E. rostratum*. These results demonstrate that the secondary metabolite BGCs in *Exserohilum* species vary in orientation, organization and chemical diversity, both within and between species. These variations potentially influence pathogenicity and provide new information on the infection mechanisms of these *Exserohilum* species. This knowledge informs the development of disease management strategies, which are essential for enhancing agricultural productivity and food security in SSA.

Keywords

Exserohilum turcicum, Exserohilum rostratum, Biosynthetic gene clusters, Phytotoxin, Mycotoxin

Macrophomina phaseolina associated with cotton seedlings in South Africa

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Ecology and Epidemiology

Abstract

Macrophomina phaseolina is an important pathogen of cotton in many countries, causing charcoal rot in older plants. The pathogen has not been recorded on cotton in South Africa, with no information on the incidence thereof on cotton seedlings locally. To ascertain whether *M*. *phaseolina* may be problematic on cotton seedlings in South Africa, surveys were conducted during 2023 and 2024 to investigate the fungi/oomycetes associated with diseased seedlings. Samples were collected from 20 locations each in 2023 and 2024. Sampling sites were located across the KwaZulu-Natal, Limpopo, Northern Cape and North West provinces and included dryland and irrigated fields. Information on cropping and irrigation practices were collected from farmers. A total of 30 four- to six-week-old diseased seedlings were collected from each field and isolations were conducted from cotyledons, hypocotyls and roots. Disease symptoms recorded include lesions on cotyledons, pre- and post-emergence dying-off, stunting, wilt and hypocotyl and root rot. Numerous fungi and oomycetes were obtained including *M. phaseolina*. The identification of the pathogen was confirmed with sequencing of the translation elongation factor 1 alpha gene region. During the 2023-season, *M. phaseolina* was obtained from seedlings from 11 fields from three provinces (KwaZulu-Natal, Limpopo, Northern Cape) and during the 2024-season from 13 fields from all four provinces. *Macrophomina phaseolina* was isolated more frequently from roots and hypocotyls than cotyledons. The pathogen was obtained from seedlings sampled from both irrigated and dryland fields, irrespective of production practice (monoculture or rotation with barley, maize, oats, sunflower or wheat), and from fallow lands and virgin fields (previous grassland). Future pathogenicity tests will determine which symptoms observed on seedlings are associated with the pathogen under field conditions.

Keywords

Surveys, diseased seedlings, disease symptoms, hypocotyls, roots

Diaporthe species associated with sudden death of rooibos in plantations

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Ecology and Epidemiology

Abstract

Sudden death is a serious disease of rooibos in plantations and can cause yield losses of up to 30%. Disease symptoms occur during February/March as moisture stress increase and plants can die within a week after symptoms become visible. Surveys to investigate the incidence of fungi/oomycetes associated with sudden death were conducted on five farms each in the low, medium and high rainfall rooibos production areas of the Western Cape during 2024. Plants exhibiting symptoms of sudden death as well as visually healthy plants were collected, and isolations were performed from the lower part of the stems and crowns, and the upper, middle and lower parts of the main roots. Tissue discolouration was more prevalent in the stem, crown and upper root parts in plants with sudden death than visually healthy plants. Numerous fungi/oomycetes were obtained from diseased and healthy plants, including *Diaporthe* spp. Representative isolates were molecularly identified by amplifying the translation elongation factor 1-alpha gene region. *Diaporthe* spp. were isolated significantly more frequently from diseased than healthy plants and more frequently from stems, crowns and upper root parts compared to middle and lower roots. Significantly higher incidences were obtained from the stems (9.44%) and crowns (4.72%) of plants obtained from the high rainfall area compared to plants from the medium (1.94% and 1.67%) and low (0.83% and 0.56%) rainfall areas. *Diaporthe* spp. identified include *D. aspalathi*, *D. ambigua* and *D. woodii*, with *D. ambigua* and *D. woodii* the most prevalent. Infection of rooibos plants by *Diaporthe* spp. may disrupt water flow from the roots to the stems during periods of moisture stress, thereby contributing to sudden death of rooibos. However, the results of surveys thus far indicate the involvement of a complex of pathogens in sudden death of rooibos in plantations, which necessitates further research.

Keywords

Diaporthe ambigua, *Diaporthe aspalathi, Diaporthe woodii*, surveys, Western Cape

Sex in fungal pathogens – how weird can it get?

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Sexual reproduction is fundamental to the survival and spread of fungi, including many plant pathogens. It enables the production of sexual spores, which often play a key role in dispersal and host infection. Equally important, sexual outcrossing drives genetic recombination, generating variation that can influence traits such as host range, survival, and resistance to control measures. Fungi are remarkably diverse in how they reproduce sexually. Most species follow one of two well-known strategies: heterothallism, where individuals require a compatible partner to mate; and primary homothallism, where individuals are self-fertile. However, a growing number of fungi, many of them plant pathogens, display far less conventional mating systems. At the heart of all sexual strategies lies the mating-type (*MAT1*) locus, a genomic region that governs mating identity and compatibility. In fungi with unusual sexual systems, the *MAT1* locus often exhibits equally unconventional features, including changes in size, gene content, and structure. These atypical configurations can provide important clues to hidden or cryptic modes of reproduction. While such genetic peculiarities may seem abstract, they can have real-world implications for understanding how pathogen populations reproduce, persist, and spread across hosts or environments. Despite their potential importance, these "weird" aspects of fungal sex remain relatively understudied.

In this presentation, we explore examples of unusual mating strategies and *MAT1* architectures in fungal pathogens. By examining how fungi bend the rules of sex, we gain new insights into their biology and possibly uncover new avenues for disease management.

Keywords

Cryptic sex, Genetic recombination, Mating-type (*MAT1*) locus, Mating strategies, *Pezizomycotina*

Peeling back the layers: the isolation and characterisation of the pathogens responsible for onion bulb disease in The Gambia

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Onions are important for food security and economic resilience globally. The crop forms an integral part of the sub-Saharan African agricultural sector, but its production is increasingly at risk due to diseases and climate change. Onion bulb rot is one of the most serious biotic threats, decreasing yield and quality. A disease locally referred to as "Jabaa tolo" emerged in 2021 in The Gambia, exhibiting characteristic symptoms of onion bulb rot disease and resulting in substantial losses. The objective of this study was to identify and characterise the causal agents of onion bulb rot in the country. A total of 100 mature onion bulbs showing bulb rot symptoms were collected from farms in The Gambia. Primary isolations were made, and bacteria and fungi were purified. Bacteria were identified by sequencing of the 16S rRNA gene region and MALDI-TOF MS. The fungal isolates were identified based on their morphology and by sequencing of the TEF-1α gene. Pathogenicity tests were performed on onion scales and bulbs, verifying the disease-causing potential of each isolate. The results revealed that bacterial soft rot was predominant in onion samples and that bacteria were more frequently isolated than fungi. *Burkholderia*, *Enterobacter*, *Klebsiella*, *Xanthomonas*, and *Pseudomonas*, were among about the bacterial genera isolated. Among them, isolates identified within *Burkholderia* were aggressive pathogens of onion, causing extensive tissue decay. Fungal species included *Fusarium proliferatum*, *F. acutatum*, and *F. oxysporum* as well as the post-harvest pathogen *Aspergillus niger*. Integrated disease management approaches such as clean seed systems, sanitation, optimised irrigation, and improved harvesting practices should be applied to reduce the impact of the disease.

Keywords

Bacterial Bulb Rot, *Burkholderia*, *Fusarium*, Pathogenicity Trials, Integrated Disease Management

Grapevine crown gall in table grape mother blocks in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Grapevine crown gall is an important disease worldwide, incurring financial losses to nurseries with symptomatic grapevines unfit for sale. Plant certification scheme rules require grapevines to be visually free of crown gall. The causal agent, *Allorhizobium vitis*, survives systemically in vascular bundles allowing the pathogen to be easily disseminated via asymptomatic propagation material. Recently, new evidence has determined *All. vitis* to be a species complex, with the description of a new species, *All. ampelinum*. Therefore, the aim of the project was to survey grapevine mother blocks in South African table grape production areas and to investigate the genetic diversity of *All. vitis* isolates. During summer 2023 and 2024, surveys were conducted in the table grape producing areas of the Berg (Western Cape) and Orange (Northern Cape) River regions for crown gall. Bacterial isolates were screened via PCR for pathogenic *All. vitis*, with positive isolates subjected to rep-PCR. Pathogenic *All. vitis* was detected in 25% of grapevines, while non-pathogenic *All. vitis* was detected in 22% of samples. Fifty-seven percent of grapevines tested negative for pathogenic *All. vitis* and *Agrobacterium* spp. Examination of the galls showed that pathogenic *All. vitis* was present in only 41% of galls. Preliminary rep-PCR analysis of *All. vitis* isolates originating from the Northern and Western Cape differentiated two genetic profiles for pathogenic isolates from these two sites, as well as another two profiles for non-pathogenic Western Cape isolates. These results indicate the presence of the crown gall pathogen in grapevine mother block material which appears to be the result of a heterogenous *All. vitis* population. MLSA characterization is underway to further examine the genetic diversity of these isolates. This will determine if the rep-PCR differences are due to inter- or intraspecies differences providing greater insight into crown gall in South Africa.

Keywords

Allorhizobium vitis, PCR, MLSA, survey

Early detection of Valley Bushveld Decline in citrus orchards using visible-near infrared spectroscopy

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Plant-Pathogen/Insect interaction

Abstract

Valley Bushveld Decline (VBD) is a major issue affecting the Sunday's River valley, leading to significant losses in citrus orchards. The disease is characterised by withering, yellowing leaves and gradual tree death. Conventional detection methods, such as nucleic acid and serology-based assays, are laborious and costly, suggesting that there are rapid, non-destructive alternatives. This study aims to evaluate the potential of near-infrared (NIR) hyperspectral imaging for early detection of VBD in citrus orchards. We employed the Specim-IQ hyperspectral sensor to collect leaf reflectance data and analysed three plant groups: healthy, asymptomatic, and diseased. Our experiments, conducted at two sites in the Sunday's River valley, involved citrus trees on Carrizo citrange rootstock. A comprehensive statistical analysis of the Specim-IQ sensor's 400–1000 nm images enabled discrimination between healthy and VBD-affected plants based on their reflectance spectra patterns. Although further validation is required, this approach shows promise as a non-invasive, cost-effective method for in-field diagnosis and mapping of VBD, indicating a major advance in precision agriculture. Early detection is essential for timely intervention to prevent disease spread throughout orchards. The study's findings emphasise the capabilities of NIR hyperspectral imaging to improve VBD identification and management, providing a basis for further research and practical use in commercial citrus farming.

Keywords

NIR, Thermography, SpecimIQ, Spectrophotography, Carrizo Citrange

Analysis of seed surface disinfection techniques for the detection of seed-borne bacterial pathogens in underutilized crops

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Disease Management

Abstract

Underutilized crops are often propagated using seed from informal markets, where seed health is rarely assessed. These seeds may harbor seed-borne pathogens, yet standardized methods for detecting bacterial pathogens in such crops are lacking. This study evaluated different surface disinfection methods to improve the detection of seed-borne bacterial pathogens. Seeds of *Amaranthus* spp., *Corchorus olitorius*, *Cleome gynandra*, *Vigna subterranea* (Bambara groundnut), *Vigna unguiculata* (Cowpea), and *Cannabis sativa* (Hemp) were obtained from an informal market in Marabastad, Pretoria, and from the Agricultural Research Council – Vegetable, Industrial and Medicinal Plants (ARC–VIMP) seed collection. Seeds were treated with bleach, ethanol, and hydrogen peroxide for 2, 5, or 10 minutes, followed by three washes with sterile distilled water. Treated seeds were plated on Nutrient Agar (NA) and incubated at 30°C overnight. After incubation, seeds were assessed for bacterial contamination. Treatments that produced contamination-free seeds were considered effective and seeds from those treatments were ground in a sterile blender, serial diluted and plated on NA and incubated at 30°C overnight to isolate internal bacteria. Resulting colonies were purified, and DNA was extracted for 16S rRNA gene amplification and sequenced. Sequence identities were confirmed through BLAST analysis against the NCBI database. Surface disinfection using 1% bleach for 10 minutes was found effective for *Amaranthus*, Bambara groundnut, and Hemp, while both 70% ethanol and 1% bleach for 10 minutes were effective for *Cleome* and Cowpea. Although no bacterial pathogens were detected, endophytic genera such as *Priestia*, *Sphingomonas*, and *Terribacillus* were identified. These findings provide baseline information to support seed health testing of underutilized crops.

Keywords

Seed testing; Underutilized crops; Seed-borne pathogens

The distribution, diversity and virulence of *Clavibacter nebraskensis* causing Goss's bacterial wilt and leaf blight in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Clavibacter nebraskensis (Cn), the causal agent of Goss's bacterial wilt and leaf blight (GBWLB), was first reported in South African maize in January 2024. GBWLB primarily affects maize (*Zea mays*), a key staple food and economic crop in South Africa, making the disease a major threat to both food security and the national economy due to its impact on yield. As an emerging pathogen, the distribution, genetic diversity and virulence of Cn strains circulating in the country has not yet been determined. This study aims to identify, characterize, and assess the virulence of Cn isolates from different provinces in South Africa. Symptomatic maize leaves were collected from six different provinces, surface sterilized, homogenized, and cultured on *Corynebacterium nebraskensis* selective medium (CNS) at 28°C for four to seven days. Pale yellow-orange colonies on CNS were purified on nutrient broth yeast extract (NBY) medium, Gram stained and subjected to Bacterial deoxyribonucleic acid (DNA) extraction using the Zymo Research Quick-DNA™ Kit. DNA quality was assessed with a Nanodrop spectrophotometer ($A_{260/280}$ and $A_{260/230}$). Species confirmation was performed using conventional polymerase chain reaction (cPCR) with the CMN01184 primer set. A total of 349 isolates from fifty different maize fields across six different provinces were tested for Cn using cPCR. Of these, three from KwaZulu-Natal, four from Eastern Cape, three from Mpumalanga, six from Gauteng, nine from North-West, and 25 from Free State tested positive for Cn. This study confirms the presence and distribution of Cn in six South African provinces, highlighting its expanding presence and potential threat to national maize production. Results from this study will be critical for guiding future disease management strategies and breeding programs.

Keywords

Maize, food security, DNA extraction, conventional Polymerase Chain Reaction (cPCR), disease management

Characterising the *Aspergillus* species diversity associated with groundnuts produced in South Africa: working towards developing a biocontrol product

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Post-harvest Pathology and Mycotoxins

Abstract

Aspergillus flavus and *A. parasiticus* are highly pathogenic fungi that affect various economically important crops worldwide. These fungi cause oilseed quality losses by the production of harmful metabolites, known as aflatoxins, that cause acute illnesses, including hepatocellular carcinoma and liver cirrhosis. In South African groundnut production, *A*. *flavus* and *A*. *parasiticus* are the predominant fungal species of concern due to their ability to produce aflatoxins. However, there is a notable lack of comprehensive data regarding their occurrence, geographic distribution across production regions, and toxigenic potential. Therefore, the aim of the study was to characterise toxigenic and atoxigenic fungal strains of *A. flavus* and *A*. *parasiticus* from groundnuts kernels cultivated in South Africa. More than six hundred groundnut kernel samples were collected across six provinces from commercial-, subsistence farming, and processing facilities. These represented every production area in SA with samples collected per annum from 2023/24 and 2024/25. Numerous isolates of *A. flavus* and *A. parasiticus* strains were obtained from kernels and identified morphologically and molecularly. Fungal strains were phenotypically evaluated for their toxigenic potential using coconut milk agar, presence of aflatoxin biosynthesis genes (aflQ and aflD) confirmed by PCR, and quantification of aflatoxins via high-performance liquid chromatography (HPLC). Atoxigenic *A. flavus* and *A. parasiticus* fungal strains identified in this study will be used to determine their ability to suppress aflatoxin-producing strains using an intra-competitive mechanism. Strains showing a significant reduction in toxigenic potential will further be assessed for vegetative compatibility to determine their ability and usefulness as biocontrol agents to prevent aflatoxins in groundnuts.

Keywords

Atoxigenic, toxigenic, aflatoxin, *Aspergillus* flavus and *Aspergillus parasiticus*

Incidence and characterization of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* in South African dry bean fields

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Sclerotinia sclerotiorum cause white mold under a wide range of hosts and produces sclerotia, hardened melanized structures that can persist in soil for years, making management challenging. *Sclerotium rolfsii* causes southern blight, typically in warmer climates and is known for rapid colonization and aggressive infection. It produces sclerotia and abundant mycelium, often seen at the base of infected plants. There is a paucity on recent literature specifically focused on *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* affecting dry beans in South African farmer fields. This study aimed to isolate, characterize, and determine the distribution of these pathogens across different agro-ecological zones in South Africa. Field surveys were conducted at ARC research sites (Cedara (1), East London (1), and Bethlehem (1)), commercial farms (Mahikeng (1), Naboomspruit (6), Taung (1), Tzaneen (1)), and a small-scale farm in Venda (1). In Cedara, *S. rolfsii* and *S. sclerotiorum* were present in 40% and 15% of the field, respectively. East London showed 30% incidence of *S. rolfsii*, while in Bethlehem, Taung, and Venda neither of the pathogens occurred. In Mahikeng, *S. sclerotiorum* was observed at 35%. All six fields in Naboomspruit were infected with *S. sclerotiorum*, with incidence ranging from 15 – 70%. Tzaneen showed a 20% incidence of *S. sclerotiorum*. Preliminary morphological characterization showed that *S. sclerotiorum* produced white, cottony mycelium with black sclerotia arranged peripherally. In contrast, *S. rolfsii* developed fast-growing, dense mycelium with light brown, round sclerotia distributed both centrally and peripherally, consistent with diagnostic features. Ongoing work includes detailed morphological and molecular analysis to aid disease management and breeding programs aimed at improving dry bean resilience in South Africa.

Keywords

Agro-ecological zones, Field survey, Sclerotia, Southern blight, White mold

Proteomic analysis of banana bunchy top virus and *Musa acuminata* protein-protein interactions for development of virus-resistant banana

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Plant-Pathogen/Insect Interaction

Abstract

Banana bunchy top virus (BBTV) is the causative agent of banana bunchy top disease, a serious threat to banana production in South Africa and globally. The virus hijacks host cellular machinery by establishing critical protein–protein interactions (PPIs). Therefore, comprehending these interactions is essential for engineering virus resistance. Traditional breeding has limited potential due to banana sterility and low genetic diversity, highlighting the need for innovative approaches. Therefore, this study seeks to identify and disrupt banana genes encoding BBTV susceptibility proteins using CRISPR/Cas9 to produce BBTV resistant banana. Banana RNA was used to construct a yeast two-hybrid (Y2H) cDNA library of GAL4-activation domain (AD) prey fusion proteins. Additionally, prey fusions of elongation factor 1-alpha, dolichyl-diphosphooligosaccharide, rhodanese-like domain, 26S proteasome, THO-complex subunit 4A, histone H2B, uncharacterized protein and serine hydroxymethyltransferase, reported to interact with plant DNA viruses, were also explored as candidates. *In silico* analysis of putative interactions between these and BBTV proteins was performed using AlphaBridge, but indicated low confidence prediction of the protein structures in AlphaFold. However, the interactions are being evaluated experimentally using Y2H and co-immunoprecipitation assays. The BBTV protein ORFs were cloned into pGBKT7 Gal4 binding domain plasmid for expression of viral proteins as bait. Screening of the banana AD protein library and the other candidate interacting proteins by mating with the BBTV proteins is ongoing. Upon validation of the PPIs by Y2H and co-immunoprecipitation, identified non-essential banana proteins will be knocked out using CRISPR/Cas9. The knockouts in the identified susceptibility genes will potentially confer BBTV resistance, thus alleviating challenges posed by the pathogen in South African banana industry.

Keywords

CRISPR/Cas9, Yeast-two-hybrid, co-immuniprecipitation, AlphaBridge, AlphaFold

Detection of *Sweet potato chlorotic stunt virus* and its relationship with potyviruses: the South African experience over the past 10 years

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Sweet potato (*Ipomoea batatas* (L.) is an important food and nutrition crop in South Africa. One of the production challenges is low production, attributed to viral co-infection perpetuated by its vegetative nature. The aims of the study were to determine the prevalence of crinivirus, *Sweet potato chlorotic stunt virus* (SPCSV), its relationship with potyviruses, and to assess the responses of sweet potato breeding lines and varieties to multiple viral infections through greenhouse experiments. The prevalence of SPCSV and potyviruses in the Limpopo, Mpumalanga, Eastern Cape, Western Cape and Gauteng provinces was determined by conducting field surveys in smallholder and emerging farmers' production areas. Serological and nucleic acid-based methods (DNA sequencing, next generation sequencing-Illumina MiSeq platform, etc.) were used to detect and genetically characterize SPCSV, and the potyvirus *Sweet potato virus 2* (SPV2). Detection methods, including analysis of part of the coat protein (CP) encoding nucleotide (nt) sequences (phylogeny) showed that the South African isolates of *SPCSV* correspond to and are distinct from the other East African (EA) strains of *SPCSV* used as reference sequences. Published and designed primers successfully detected and confirmed the prevalence of SPV2 in Limpopo, Mpumalanga and Gauteng provinces and the virus was detected in co-infections with *Sweet potato feathery mottle virus* (SPFMV). The pairwise comparison of the amino acid (aa) sequences encoding the CP gene region of SPV2 confirmed that isolates in this study shared 94% to 99% CP aa identity with those originally reported in South Africa. Analysis of NGS generated data (CLC Bio Genomics Workbench) also confirmed the presence of SPCSV, SPFMV and SPV2, generating near complete genome sequences. Assessment of varieties and breeding lines' response to viral infection identified promises varieties for inclusion in future breeding programs as sources of parent material for virus tolerance.

Keywords

SPCSV, SPV2, SPVD, Detection, RT-PCR, Cloning, Sequencing and NGS.

Description and pathogenicity of the *Cladosporium cladosporioides* species complex causing pecan scab in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The pecan (*Carya illinoinensis*) is indigenous to North America where it grows in the wild along the Gulf of America. It was imported to South Africa in the late 1800's. South Africa is the third largest producer of pecans with a market value of more than R2 billion per annum. Pecan scab is recognised as a major threat to the quality and yield of pecan nuts, especially in the eastern parts of South Africa. Recently, it was indicated that the pecan scab fungus in America, *Venturia effusa*, has not been found in South Africa. Instead, members of the *Cladosporium cladosporioides* species complex is regularly isolated from scab lesions on fruit, leaves, and twigs. A study was conducted to determine which species in this complex can cause pecan scab. A phylogenetic tree was constructed based on genetic sequencing of the ITS, TEF, and ACT gene regions of 20 selected *Cladosporium* cultures. These have been isolated from the fruit, leaves, and air from pecan orchards in scab-stricken areas such as Limpopo, Kwazulu-Natal, and the Eastern Cape. Results indicated that at least three species representing the *C. cladosporioides* species complex, including *Cladosporium cladosporioides sensu stricto*, *C. anthropophilum* and a potential new species, were found to cause typical scab lesions in a detached fruit pathogenicity assay. In addition, *C. sphaerospermum sensu stricto*, representing the *C. sphaerospermum* species complex was also found to cause typical scab lesions. This study is further confirmation that *Venturia effusa* is not the causative agent of scab in South Africa. It also shows that *Cladosporium cladosporioides s. str.* as the dominant causative agent of pecan scab, although other species in the complex can also cause this disease. Therefore, the South African pecan industry should approach the management of scab differently than their counterparts in the USA.

Keywords

Phylogenetics, pathogenicity, *Cladosporium sphaerospermum*, *Venturia effusa*, *Carya illinoinensis*

Evaluation of fungicides against the most prominent fungal pathogens of pecan diseases in South Africa

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Disease Management

Abstract

A wide variety of fungal pathogens are known to cause disease in pecan orchards in South Africa. There is a significant challenge for producers to select fungicides for disease control without promoting pathogen resistance due to the continuous use of the same fungicides. The pecan industry is, therefore, continuously looking for fungicides that can be alternately used with the existing registered fungicides. For this purpose, a study was undertaken where 21 fungicides were screened against 12 of the most important fungal pathogens known to cause disease in pecans. The tested fungi include *Alternaria alternata* (Alternaria black spot), *Aspergillus flavus* (post-harvest decay and aflatoxin production), *Chaetomium globosum* (post-harvest decay), *Cladosporium cladosporioides* (pecan scab), *Coprinellus micaceus* (overall decline and brown rot), *Fusarium euwallaceae* (PSHB-associated damage), *F. graminearum*, *F. oxysporum*, and *F. solani* (overall decline and die-back), *Neofusicoccum parvum* (black blotch and die-back), *Trichoderma harzianum* (beneficial fungus against pathogens), and *Trichothecium roseum* (pink mould). Results indicated that fungicides such as Sporekill and Tebuconazole 250 EW were effective against all fungi tested at EC_{50} values below 0.04 µl/ml and EC_{90} values below 0.5 µl/ml. Other fungicides that show relatively good effectiveness against most fungi, except some *Fusarium* species, are Amistar Top, Coptin, Nativo SC300, Score, and Tilt/Bumper. Fungicides showing promise against the scab fungus, *C. cladosporioides*, include Ag-Tin, Amistar Top, Bravo 720, Coptin, Sporekill, Tebuconazole 250 EW, and Tilt/Bumper. Those that are effective at EC_{90} levels against *Neofusicoccum parvum* include Amistar Top, Bravo 720, Coptin, Cungfu 538 SC, Nativo SC300, Score, Sporekill, Switch, Tebuconazole 250 EW, and Tilt/Bumper. Based on the results in this study, producers work alongside their agrochemical agents and can use this information to generate fungicide application programmes with flexibility to combat fungal resistance to the traditional fungicides.

Keywords

Carya illinoinensis, EC_{90}, fungicide resistance, Tebuconazole 250 EW, Sporekill

Postharvest management of wet core rot infections in apple

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Disease Management

Abstract

Core rot of apple is a complex of symptoms caused by fungal infections that begin in the core region of the fruit and expand into the outer fruit tissue, resulting in decay. Wet core rot (WCR) is one of the symptoms and is characterised by a soft, wet, light brown lesion that rapidly spreads into the outer fruit tissue and during storage, this can result in the infection of adjacent fruit. Globally, WCR is reported to be caused by several pathogens, including *Penicillium* and *Fusarium* species. The efficacy of current postharvest management strategies against this important postharvest decay is unknown. In this current study, pathogens isolated from WCR symptomatic fruit were used in fungicide drench trials to determine the efficacy of the application of fungicides, fludioxonil and pyrimethanil, as well as cold storage in the management of decay in 'Fuji' and 'Top Red' apples. Results indicated that the application of fludioxonil reduced both decay incidence and severity in both cultivars, compared to inoculated control apples. Additionally, pyrimethanil significantly increased both decay incidence and severity in 'Top Red' apples. Furthermore, the results indicated that the cold storage (-0.5°C) of inoculated fruit that had been treated with either fungicide significantly reduced both decay incidence and severity in 'Fuji' apples. Results from this study have shown that the postharvest application of fludioxonil combined with cold storage can effectively manage decay caused by WCR pathogens.

Keywords

Cold storage, Fuji, Fludioxonil, *Fusarium*, Pyrimethanil

Reconsidering cellulose in the cell walls of the Ophiostomatales

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The prevailing view in fungal cell wall biology is that fungal walls are composed primarily of chitin, with cellulose mostly not considered relevant. This perspective, however, overlooks various studies going back to the 1960s that reported the presence of both cellulose and chitin in the cell walls of species in the Ophiostomatales. In this study, we revisited that question by investigating the presence of cellulose in several species in this order using a combination of microchemical and molecular methods. Cell walls from selected isolates were extracted and tested for the presence of cellulose using Lugol's solution and sulphuric acid. To explore the genetic basis of cellulose synthesis, a gene believed to encode cellulose synthase (*cesA*), was functionally characterised in *Ophiostoma novo-ulmi* using a CRISPR-Cas9 mediated gene editing system, which was applied for the first time in an *Ophiostoma* species. Phylogenetic analyses of *cesA* homologues across multiple Ophiostomatales species revealed the presence of at least three distinct gene variants involved in cellulose synthesis. The results provide clear evidence that cellulose is a consistent component of the cell walls in members of the Ophiostomatales. The successful application of gene editing in *O. novo-ulmi* opens new possibilities for functional genomics in this group. Furthermore, insights into cell wall composition may inform future strategies for disease control and fungal diagnostics.

Keywords

Structural polysaccharides, CRISPR-Cas9 editing, *cesA* homologues, fungal pathogen, chitin

The feasibility of High-Throughput sequencing for subsistence farmers in resource-constrained environments

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Plant-Pathogen/Insect Interaction

Abstract

Subsistence or smallholder farmers face significant challenges related to crop failure, soil nutrient depletion, and climate conditions. These Sub-Saharan Africa farmers employ traditional breeding techniques and diagnostic methods that are frequently ineffective, time-consuming, and unavailable because of the lack of infrastructure and financial limitations. The potential application of metabarcoding using high-throughput sequencing (HTS) to profile soil microbial populations may assist subsistence, smallholder and commercial farmers. Increased knowledge of soil microbial diversity and profiling the occurrence of beneficial and damaging microbes can help farmers improve production and increase plant health. This project intends to bridge the technology division between smallholders and commercial farmers at scalable options for enhancing agricultural output. This will be accomplished by validating two sequencing platforms on the different samples and to investigate the development of cost-effective sequencing-based solutions that address the challenges faced by smallholder farmers Soil samples were analysed from 288 small holdings obtained from three regions in the Limpopo province. Preliminary findings indicate a high diversity of phytopathogens with wide distribution in the Limpopo province. Gaining insight into microbial diversity will support subsistence farmers in preventing crop failure, increasing yield and improve soil management through early detection of fungal pathogens.

Keywords

Subsistence farmers, Smallholder farmers, Commercial farmers, high-throughput sequencing (HTS), Metabarcoding

Field-based disease phenotyping of sorghum genotypes in three contrasting South African environments

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Disease Management

Abstract

Sorghum is a key cereal crop for food, feed, and climate resilience in South Africa. However, its productivity is constrained by several fungal diseases, including rust (*Puccinia purpurea*), anthracnose (*Colletotrichum sublineola*), grain mold (a complex of fungal pathogens), and historically, ergot (*Claviceps africana*). Identifying resistant genotypes is critical for integrated disease management and sustainable production. This study forms part of the Sorghum Cluster Initiative: Prebreeding Programme, funded by the Department of Science and Innovation and hosted at the University of the Free State.

A total of ~144 sorghum genotypes were evaluated under natural infection conditions across three contrasting agroecological zones during the 2024/25 cropping season. Locations included Pietermaritzburg (Cfb), Bloemfontein (BSk), and Krugersdorp (Cwb), representing distinct climatic conditions. Disease incidence and severity were recorded for major fungal diseases at each site, across ~ 30 plants per genotype.

Disease pressure varied markedly by location. Pietermaritzburg experienced the highest overall fungal disease incidence, particularly due to Rust, which was only observed at this site. Anthracnose incidence was also high in both Pietermaritzburg and Krugersdorp, while Bloemfontein showed minimal disease expression, likely due to a later planting date and drier growing conditions. Fourteen genotypes displayed low Anthracnose incidence across the affected sites, and 29 genotypes remained symptom-free for Rust in Pietermaritzburg. These genotypes represent potential candidates for advancing disease-resistant breeding lines. Grain mold assessment was limited due to incomplete panicle emergence at the time of scoring, and further evaluations are required. Ergot was not observed, but its historical significance justifies future screening.

This multi-location trial revealed promising sorghum genotypes with resistance to rust and anthracnose, especially under high-pressure environments. The results contribute to breeding efforts focused on developing resilient cultivars, ultimately supporting sustainable sorghum production and plant health.

Keywords

Disease resistance, disease incidence, exotic germplasm,

The study of plant growth-promoting rhizobacteria (PGPR) in South Africa and their effects on potato plants

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Disease Management

Abstract

Potato (*Solanum tuberosum*) is one of the most widely produced crops in the world. The crop is affected by many biotic and abiotic factors, leading to the overuse of chemical fertilizers and pesticides. Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that can promote the growth of plants. The aim of this study was to explore the use of PGPRs, focusing on characterization of the isolates, and determining their effect on the growth of potato plants. Isolates (n=155), previously isolated from healthy potato roots and rhizospheric soil in potato fields were used in this study. These isolates were tested for morphological and biochemical characteristics such as KOH test, catalase activity, biofilm formation and motility, and PGPR traits such as phosphate solubilisation, siderophore production, and indole-3-acetic acid (IAA) production. Based on these results, twenty-five isolates were selected for the greenhouse trial. Preliminary results of the isolates showed that 53% tested positive for the KOH test, 95% tested positive for catalase activity, 28% were positive for biofilm formation, 56% were positive for motility, 39% had phosphate solubilizing ability, and 38% produced siderophores. Twelve isolates have IAA concentrations above 1000 $\mu g.ml^{-1}$ while four isolates, namely B140, B240, B153, and B290 significantly increased tuber production and tuber mass as compared to the control. Isolates B140 and B800 showed significant root length, root mass, and plant height compared to the control. These isolates will be identified on molecular level. Future studies will focus on biofertiliser formulation with these strains. The resulting growth promotion of potato plants, i.e. increase in root and shoot weight, total root length, and tuber mass may enable farmers to produce crops of good quality with higher yields, leading to improved food security and improved nutrition.

Keywords

PGPR, *Solanum tuberosum,* characterization, growth promotion

Screening of fungicide actives and biological control agents against husk rot disease pathogens in macadamia

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Disease Management

Abstract

Husk rot disease is the most important fruit disease globally affecting macadamia. The disease is characterised by soft and necrotic lesions caused by multiple fungal species in the *Diaporthe, Colletotrichum* and *Calonectria* genera. Increased outbreaks of husk rot has motivated research investigating management of the disease, which currently relies primarily on the use of fungicides. The dependence on fungicides is however concerning, highlighting the need to evaluate alternative management strategies as well. Therefore, the study aimed to evaluate the efficacy of fungicide actives and biological control agents (BCAs) against husk rot disease. Eight fungicide actives were screened *in vitro* using the amended media method whilst the BCAs, *Trichoderma asperellum* and *Bacillus subtilis* were evaluated using a dual culture method. The most effective fungicide actives and the two BCAs were subsequently evaluated *in vivo* for their preventative and curative ability in reducing disease severity on detached nuts. The data analysis for all assays was performed using Excel2016 and RStudio. The efficacy of the fungicides varied significantly. The demethylation inhibitor fungicides (DMI), tebuconazole and difenoconazole were the best performing fungicides *in vitro* across the three pathogenic genera with EC_{50} values ranging between 1.37 ± 27.2 and 3.12 ± 18.43 PPM, respectively. *T. asperellum* showed better inhibitory activity when compared to *B. subtilis in vitro*. For the *in vivo* assessments, preventative treatments showed better control compared to curative treatments. The study contributed valuable knowledge regarding potential management options for husk rot disease and showed that combination treatments that contain one of the two DMI fungicides would be appropriate for controlling husk rot disease particularly during the early stages of infection. The study also demonstrated that BCAs represent a viable management option and further evaluation of additional BCAs is recommended.

Keywords

Diaporthe, *Colletotrichum,* tebuconazole, *Trichoderma*, preventative treatments

Screening of endophytic bacteria isolated from apple rootstocks for their potential as biological control agents against crown gall disease of apple

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Disease Management

Abstract

Crown gall, induced by *Agrobacterium tumefaciens* (syn. *Rhizobium radiobacter*), causes significant harm to apple saplings, leading to stunted growth and diminished commercial worth. Abnormal gall-like growths on apple rootstocks, resembling crown gall symptoms, were widely observed in 2022 in nursery plants. The non-pathogenic strain *A. radiobacter*K84 (syn. *Rhizobium rhizogenes*K84) is effective in biological control, although its continued use may lead to decreased effectiveness, and it does not always work for apple rootstocks. This study investigated the potential of endophytic bacteria to control crown gall in susceptible apple rootstocks. Bacterial endophytes were isolated from apple rootstocks and tested for their ability to inhibit the growth of pathogenic *A. tumefaciens in vitro*. Isolates which exhibited antagonism were subjected to 16SrDNA sequencing, and assessed for producing proteases, siderophores, hydrogen cyanide, IAA, and phosphate solubilization. Biocontrol efficacy was investigated using a carrot disk assay as well as inoculation of tomato seedlings. Seven bacterial strains showed antagonistic activity against a South African pathogenic *A. tumefaciens* strain isolated from a gall on apple rootstock CG778. Preliminary identification of these endophytes indicated that four isolates were *Pseudomonas* spp. and two were *Bacillus* spp. All isolates possessed the growth-promoting trait of phosphate solubilization, while only two isolates produced IAA. Isolates varied in their response to protease, hydrogen cyanide, and siderophore production. In the *in vitro* carrot disc biocontrol assay, three isolates significantly reduced gall development. Similarly, in the tomato plant assay, three isolates had a high inhibitory effect against the pathogen. The results indicate that some endophytic bacteria are good candidate biocontrol agents that can be further tested for their efficacy to control apple crown gall disease. The candidate biocontrol agents also have the added benefits of plant growth promotion traits that can be further exploited.

Keywords

Antagonistic activity, *Agrobacterium*, bacterial endophytes, crown gall, growth-promoting trait

The South African Sugarcane Research Institute: 100 years of pathology research

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Disease Management

Abstract

Sugarcane has been cultivated in South Africa (SA) since the mid-1800s, with the first commercial plantings recorded in 1848. Over the years, the industry has faced numerous disease threats, beginning with the first global report of sugarcane smut (*Sporisorium scitamineum*) in 1877. The unregulated import of new varieties in the early 1900s introduced viral pathogens including sugarcane mosaic virus and sugarcane streak virus, leading to severe outbreaks. In response, the industry-funded SA Sugar Experiment Station (now the SA Sugarcane Research Institute) was established in 1925, with an initial focus on breeding disease-resistant varieties and managing pathogen threats. Key to this was the introduction of a quarantine facility at the Durban Botanic Gardens in 1926 to promote the safer importation of foreign varieties. Over the years, a wide range of viral, bacterial and fungal diseases have impacted sugarcane production in SA. Significant progress has been made in disease detection, ranging from the introduction of routine serological diagnostics to the more recent development of pathogen-specific PCR, qPCR, and LAMP assays. Breeding programmes have focused on natural disease resistance, with improved screening techniques and integrated disease management strategies. To strengthen disease surveillance and seedcane quality, Local Pest, Disease and Variety Control Committees were established in 1982. These committees oversee regional monitoring and seedcane certification under the regulatory framework of the Sugar Act and the Sugar Industry Agreement. Furthermore, stringent quarantine protocols—including the use of tissue culture (e.g., Novacane®)—have been developed to minimise the risk of introducing foreign pathogens. This paper highlights historical responses to disease outbreaks that evolved into a proactive approach combining breeding, diagnostics, quarantine, surveillance, agrochemicals, and a recent focus on biocontrol options. This integrated strategy supports the continued productivity and sustainability of the SA sugarcane industry, while ongoing research and monitoring remain critical in mitigating emerging threats.

Keywords

Diagnostics, quarantine, monitoring, disease management

Identification of potential pathogenic traits exhibited by *Rahnella* spp. as opportunistic pathogens of onion bulbs using comparative genomic analyses

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Plant-Pathogen/Insect Interaction

Abstract

Onions are vital to global food security, yet bacterial pathogens threaten their production. *Rahnella* species have been identified in diseased onions, but their pathogenic potential remains unclear. Whole-genome sequencing and comparative genomics were used to determine the potential genetic traits responsible for colonisation, pathogenicity, and antimicrobial resistance. This study presents a comprehensive genomic analysis of five *Rahnella* species/strains, viz. *R. aquatilis* (20CA0197 and 20CA0198), *R. aceris* (20WA0051, 20WA0057), and *R. perminowiae* FS4. The genome sizes of the five sequenced strains ranged from 5.54 to 5.84 Mbp, with GC content between 51.6% and 52.2%. The genome completeness was high, with BUSCO values above 98%. The pan-genome analysis identified a total of 15,175 coding genes, including 3,356 core genes shared amongst 19 species of *Rahnella* genomes and a large number of 8,447 cloud genes. Pathogenicity-related genes were categorised according to their predicted functions: metabolism, secretion systems, motility, adherence, lipopolysaccharide biosynthesis and antimicrobial tolerance. All five *Rahnella* strains possessed type VI secretion system but lacked typical pathogenicity determinants such as type II and type III secretion systems. Thiosulfinate gene cluster was present in *R. aquatilis* (20CA0197 and 20CA0198), and *R. perminowiae* FS4 strains possibly indicating their adaptation to thiosulfinate-rich environments. Biosynthetic gene clusters related to siderophores, quorum sensing, and antimicrobial compounds were common in *Rahnella* strains, indicating the presence of competitive survival strategies. Phage elements were identified in all strains, which may be contributing to the genetic diversity of onion-associated *Rahnella* strains. These findings enhance our understanding of *Rahnella*'s genetic composition and their potential role in onion diseases.

Keywords

In-silico, onion-bacteria interaction

Host-plant mediated selection of beneficial microbes in the presence of plant resistance inducers

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Disease Management

Abstract

Managing plant fungal diseases largely depends on plant resistance and chemical fungicides. However, the widespread use of fungicides can negatively affect non-target organisms and the environment and decrease efficacy due to resistant pathogen strains. Due to these challenges, there is a worldwide movement away from chemical fungicides and towards more sustainable approaches. One such strategy involves priming plants with resistance inducers, which are known to influence the recruitment of beneficial microorganisms by plants. In the novel approach described here, a series of plant resistance inducers were assessed for their effect on sugarcane plantlet survival and growth, and direct impact on the sugarcane pathogens *Sporisorium scitamineum* and *Fusarium verticillioides in vitro*. Host-plant mediated selection of beneficial microbiomes from diverse environmental samples was conducted in the presence of resistance inducers in a specially designed synthetic medium that will kill the plantlet in the absence of beneficial bacteria. After one month of exposure, the "best grown" plantlets were selected based on appearance, and representatives of recruited microbial communities were isolated and assessed for PGP traits and direct biocontrol activity against pathogens. Results demonstrate that certain resistance inducers that are compatible with plantlet growth and survival *in vitro* (e.g. Fluopyram at 250 µM and Imidacloprid at 300 µM) can directly inhibit *S. scitamineum* teliospore germination and *F. verticillioides* mycelial growth. Host-plant mediated microbial selection in the presence of resistance inducers yielded bacteria with plant growth-promoting properties, including phosphate solubilisation, IAA production and ACC (1-aminocyclopropane-1-carboxylate) deaminase activity. Many isolates showed varying degrees of *S. scitamineum* teliospore germination, sporidial mating inhibition, and growth inhibition of *F. verticillioides* mycelium. This methodology increases the likelihood of compatibility between the plant, resistance inducer, and the recruited microbiome. Ultimately, synthetic microbial communities can be designed for further experimentation in the presence of resistance inducers.

Keywords

Fusarium, *Sporisorium*, SynComs, PGPR, Biocontrol

Survey of Potato Late Blight in South Africa to Recap on Mating Types and Assess Fungicide Sensitivity of *Phytophthora Infestans* Isolates

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Disease Management

Abstract

Phytophthora infestans, the causal agent of potato late blight, is a major constraint to potato production globally. In South Africa, only the A1 mating type has previously been reported, with limited genetic diversity due to clonal reproduction. Given the possibility of the A2 mating type being introduced and increasing global concern over fungicide resistance, this study aimed to (i) determine the current mating types of *P. infestans* in South Africa and (ii) assess the sensitivity of local isolates to six commercial fungicides. Surveys were conducted in 2023 and 2024 across five major potato-growing provinces (Limpopo, Gauteng, Free State, KwaZulu-Natal, and Western Cape), where symptomatic leaves were collected. A total of 145 isolates of *P. infestans* were recovered and mating type were evaluated using pairing assays with known A1 and A2 reference strains. All isolates were identified as A1 mating type, as oospore formation was observed in pairings with the A2 reference strain. To assess fungicide sensitivity, six commercial fungicides (amisulbrom, azoxystrobin, chlorothalonil, cymoxanil, fluazinam, Phosphorus acid) were tested using an in vitro assay. Growth inhibition is being evaluated to determine effective concentrations, with testing still ongoing. This study confirmed that only the A1 mating type of *P. infestans* is currently present in South Africa. Continued surveillance is necessary to monitor for the possible introduction of the A2 mating type, which could significantly increase genetic diversity and complicate disease management. The results of fungicide sensitivity testing will support the development of more effective and sustainable control strategies for late blight as this trial will provide insight into the effectiveness of current chemical treatments used on potato.

Keywords

A1 and A2 strains, Oospores, genetic diversity, pairing assay, invitro assay, fungicide resistance

Identification of *Nanidorus minor* as the vector of Pepper Ringspot Virus (PepRSV) in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Pepper ringspot virus (PepRSV), one of three known *Tobravirus* species, was detected in South Africa (SA) in potato-growing regions in 2020. PepRSV was first identified in tomato, pepper and artichoke in Brazil in the 1960s. Due to its previous geographical restriction to Brazil, limited information exists internationally regarding PepRSV. Like other tobraviruses, PepRSV is expected to be transmitted by nematodes from the Trichodoridae family. This study aimed to identify the Throchodorid nematode vector of PepRSV and develop a method to detect the presence of PepRSV in farm soil. Trichodorid nematodes were collected from the rhizosphere of a PepRSV infected potato field displaying symptoms of tuber spraing in Reitz in 2024, as well as from a field in Senekal in 2025, with sunflowers displaying ringspot symptoms on the leaves. A bait trapping experiment was set up, where various hosts were planted in the farm soil in pots in the greenhouse. It was found that spinach, tomato, pepper and sunflowers became infected with PepRSV when planted in the soil from Senekal, displaying ringspot symptoms on the leaves of the sunflowers and tomatoes, but no symptoms on spinach or peppers. In contrast, only tomato, pepper and spinach plants became infected when planted in the soil from Reitz. The presence of PepRSV variants with different host ranges are being investigated. Furthermore, the presence of PepRSV was confirmed in the nematodes *N. minor* by RT-PCR, confirming this species as a vector in SA. Lastly, bait-trapping experiments with spinach as the indicator host were used to determine the presence of PepRSV in farm soil collected from various potato farms around SA. The results of this study are an important step in developing reliable molecular diagnostic tools for confirming PepRSV presence in diverse biological samples and for informing future surveillance, management, and control strategies.

Keywords

Tobravirus, +ssRNA virus, nematode transmission, RT-PCR, bait trapping.

The development of translation initiation factor-based resistance to sweet potato virus disease using Crispr/Cas9 technology

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Disease Management

Abstract

Sweet potato is an important staple food in Africa and many parts of the world for consumption and to generate income. The synergistic co-infection of *Sweet potato feathery mottle virus* (SPFMV, genus *Potyvirus*) and *Sweet potato chlorotic stunt virus* (SPCSV, genus *Crinivirus*) can cause sweet potato virus disease (SPVD) with yield losses of up to 90% having been reported. Potyviruses use the genome-linked viral protein (VPg) to hijack the host's eukaryotic translation initiation factor 4E (eIF4E) to initiate viral translation and infection. Target-specific mutagenesis of the eIF4E gene may lead to resistance against SPFMV and SPVD by disrupting the interaction between VPg and eIF4E. The aim of this study was to knockout the eIF4E gene in sweet potato to generate SPFMV and SPVD resistant lines using CRISPR/Cas9 technology. A 628 base pair segment of exon 1 in the eIF4E was amplified and sequenced using Illumina MiSeq for allelic variation analysis. Two site-specific guide RNAs (gRNAs) were designed within conserved regions of the gene segment. An *in vitro* cleavage assay was used to evaluate the candidate gRNAs, and the CRISPR/Cas9 vector expressing the two gRNAs was constructed using Golden gate modular cloning. The candidate gRNAs were located at conserved positions 179 and 375 within exon 1 of the gene segment. The cleavage assay revealed that both gRNAs were effective in cleaving the target fragment. The Regenerative Activity-dependent in Planta Injection Delivery (RAPID) method, dependent on the clonal regenerative capacity of sweet potato, was used for transformation. PCR tests revealed the presence of the Cas9 gene in 13 samples 10 weeks after transformation. TIDE analysis results suggested the presence of indels in these samples. However, next generation sequencing revealed that there were no indels at the target sites of both gRNAs. Further transformation studies are ongoing to generate SPVD resistant lines.

Keywords

Sweet potato feathery mottle virus (SPFMV), S*weet potato chlorotic stunt virus* (SPCSV), sweet potato virus disease (SPVD), eukaryotic translation initiation factor 4E (eIF4E), virus resistance

Exploring South African oilseed diseases through molecular identification of fungi associated with sunflower and soybean

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Pathogen Identification, Characterisation and Taxonomy

Abstract

"Prevention is better than cure" is often associated with the healthcare sector, although, its relevance to plant pathology cannot be understated. To better understand the fungal disease landscape in oil- and protein-seed crops our research group conducted disease surveys in the National Cultivar Trials of soybean and sunflower across South Africa. Samples from 27 and 14 soybean and sunflower localities were surveyed in 2025, respectively. A variety of symptomatic samples were collected to isolate and molecularly identify fungal pathogens with potential relevance to disease management. Surface-sterilised plant material was plated on half strength potato dextrose agar to isolate potential pathogens. A total of 50 morphologically distinct candidate fungal isolates were selected for molecular identification. Mycelia from pure cultures were used for DNA extractions which were subjected to PCR amplification of both the internal transcribed spacer (*ITS1* and *ITS4*) and transcription elongation factor 1-alpha (*EF1-728F* and *EF1-986R*) regions. Amplified gene regions were subjected to Sanger sequencing and isolate identities were confirmed using NCBI BLAST searches. *Fusarium spp.* and *Sclerotinia sclerotiorum* were among the fungal pathogens identified from soybean, while *Athelia rolfsii, Macrophomina phaseolina, S. sclerotiorum* were isolated from sunflower stems. Molecular identification of fungal isolates obtained from leaves is currently in progress. Accurate molecular identification of fungal pathogens in oilseed crops enhances our capacity to develop targeted disease management strategies, ultimately contributing to more resilient and sustainable agricultural systems in the face of climate variability.

Keywords

Disease surveillance, crop biosecurity, sanger sequencing, *Fusarium* spp., disease management.

Membrane vesicles shape the battle between Pines and *Fusarium*

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Plant-Pathogen/Insect Interaction

Abstract

Understanding the complex dialogue between plants and microbes could transform how we protect forests from disease. Harnessing a tree's own defense strategies may lead to new, eco-friendly ways to control pathogens like *Fusarium circinatum*, the agent of pine pitch canker. In recent years, plant and fungal extracellular vesicles (EVs) have emerged as tiny but potent carriers of biological information that shape these interactions. Our work explores how EVs from *Pinus* species and the pathogen itself affect the growth, metabolism, and resilience of *F. circinatum*, opening the door to sustainable disease management and healthier forests. We combined classical microbiology with advanced analytical techniques, including nanoparticle tracking, transmission electron microscopy for EV characterisation, phenotype microarrays for high-throughput metabolic profiling, and glasshouse trials for in planta validation. A robust size-exclusion chromatography method was developed for isolating EVs from both fungal cultures and diverse *Pinus* species. EVs from *F. circinatum* promoted biofilm integrity and enhanced fungal adaptation to antimicrobial agents and stress conditions, especially within polymicrobial biofilms. In contrast, *Pinus*-derived EVs disrupted fungal metabolism, reduced spore viability, and impaired hyphal growth and biofilm formation. *Pinus* needle-derived EVs caused marked shifts in a range of pathways that affect fungal metabolism, effects that were reproducible across other filamentous pathogens. These findings highlight the central role of EVs in mediating plant–pathogen dynamics and fungal adaptation. Understanding this EV-driven cross-talk offers promising avenues for novel, cost-effective, and sustainable disease management strategies. By aligning forestry and agriculture with nature's own protective mechanisms, we can better defend against significant phytopathogens and help secure a resilient, sustainable future for global plant health.

Keywords

Biofilm, Metabolism, Pathogen-host interaction, Resilience, Therapeutics

Identification and characterization of viruses associated with *Passiflora edulis* in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Passionfruit (*Passiflora edulis*) is a perennial vine that originated in the tropical and subtropical regions of South America. In South Africa, passionfruit is commercially cultivated in the Eastern Cape, Limpopo, Mpumalanga and along the coastal regions of KwaZulu-Natal. The Agricultural Research Council (ARC) has historically been indexing passionfruit to determine whether it is free from Cucumber mosaic virus (CMV), Passionfruit latent virus (PLV) and Cowpea aphid-borne mosaic virus (CABMV). Recently, field samples showing mosaic symptoms on leaves and distortion of the fruit, have tested positive for a *Potyvirus* using reverse transcription polymerase chain reaction (RT-PCR), and subsequent phylogenetic analysis revealed that a few isolates represent a virus strain closely related to the East Asian Passiflora distortion virus (EAPDV). EAPDV is a *Potyvirus* previously reported in Asia which primarily infects passionfruit species and causes similar symptoms. In this study, representative samples were selected and submitted for high-throughput sequencing (HTS) analyses. Quality control of the HTS reads was performed using FastQC, followed by the trimming of low-quality bases and removal of adapters. A hybrid bioinformatics strategy was employed for accuracy and comparative analysis. The first approach involved removing host DNA to reduce background noise and computational load. The second retained the host DNA to confirm host identity and enable host-pathogen interaction analysis. Viral sequences were identified using Kraken2 with both core and virus-specific databases and visualized using Krona. The resulting viral reads were assembled into genomes and assessed for completeness using multiple assembly tools. *Passiflora edulis* cultivar CGPA1 was used as a reference for the bioinformatics pipeline.

Keywords

Passiflora edulis, EAPDV, HTS, detection surveys

Identification of viruses infecting potato (*Solanum tuberosum* L.)in KwaZulu-Natal (KZN): a focus on Potato virus Y(PVY)

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Accurate detection and characterization of plant viruses is important for effective disease management, especially as new virus strains and variants continue to emerge and challenge existing control strategies. In KwaZulu-Natal (KZN), virus infections cause serious yield losses, reduced tuber quality, and higher production costs for both small-scale and commercial farmers. This study aimed to identify and characterize viruses infecting potato in KZN using serological (ELISA) and molecular RT-PCR, SDS-PAGE, and next-generation sequencing (NGS) methods. Leaf samples with typical virus symptoms, such as mosaic patterns, leaf curling, and stunting, were collected from small-scale and commercial farms. Primers targeting the coat protein genes of potato virus Y (PVY), potato virus A (PVA), potato virus S (PVS), potato virus M (PVM), potato virus X (PVX), and potato leafroll virus (PLRV) were used to confirm the presence of these viruses. Strain-specific primers were then used to differentiate PVY strains present in the sampled locations. The results confirmed the presence of potyviruses (PVY and PVA) in all locations and identified PVY^{O} and PVY^{N} strains in Msinga and Howick. PVY was further characterized using SDS-PAGE, which revealed a viral coat protein band of approximately 33 kDa. Sequencing of PCR products for PVY showed evidence of recombination between strains. These results highlight the prevalence of different viruses infecting potato crops in KZN, with a particular widespread occurrence of PVY. Indicating a need for early detection of these viruses as a basis for developing appropriate measures to control/manage the diseases they cause on both small and large-scale farms.

Keywords

Detection, PVY strains, NGS, recombination

Confirmation of virus seed transmission and the effects on wet and dry weight of Swiss chard and mustard spinach

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Disease Management

Abstract

Smallholder and emerging farmers in the Gauteng Province of South Africa produce leafy vegetables such as Swiss chard *(Beta vulgaris* L) and mustard spinach (*Brassica rapa*) for household consumption and income generation. These are included in food diversification programs that are aimed at improving food and nutrition security. Detection of Beet mosaic virus (*BtMV*) and Turnip mosaic virus (*TuMV*) compelled that a study is conducted to determine possible transmission of viruses through seeds and their effects on the dry and wet weight of Swiss chard and mustard spinach. For seed transmissibility tests, seeds (Swiss chard and mustard spinach) were sown in polystyrene seedling trays (no virus inoculation) with sterilized hygromix potting soil and evaluated (visual observations) for symptoms development 1 to 3 weeks post emergence. Effects on the wet and dry weight was determined by planting greenhouse trials with the Swiss chard and mustard spinach. These were mechanically inoculated with *BtMV* and *TuMV*, respectively. Virus infection was confirmed by RT-PCR. Two weeks post emergence, symptoms were observed on Swiss chard seedlings believed to be disease free and RT-PCR detected BtMV from the seedlings. The wet weight of the virus treated mustard spinach was affected and significant differences were observed in trial one. However, the wet weight of Swiss chard was moderately affected by the virus treatment. There was a reduction in the dry weight of mustard spinach on the treated plants versus the control and significant difference was observed in the first trial. The dry weight of inoculated Swiss chard was not significantly different from the control. Further studies should be conducted to confirm the results. Knowledge on seed transmissibility of virus species and their effects on crops contribute to the development of control strategies that will reduce virus dissemination.

Keywords

Leafy vegetables, Beet mosaic virus, Turnip mosaic virus, seed transmission, effects of viruses on plant growth

Identification of seed-borne fungi in underutilized crops: bambara groundnut, amaranth and kenaf

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Underutilized crops play an important role in increasing food security, improving nutrition and promoting environmentally sustainable farming practices. However, seed vigour, viability and storage longevity of agricultural crops are increasingly threatened by seedborne fungal pathogens which can significantly reduce germination rates and influence yield. Despite their importance in African countries, limited research is available on the seed health of *Amaranthus* spp. Bambara groundnut (*Vigna subterranean*), and kenaf (*Hibiscus cannabinus*). This study aimed to evaluate the presence of seedborne fungi in these underutilized crops and obtained isolates were identified using both morphological and molecular approaches (ITS region sequenced). Seeds were collected and screened for fungal isolates using the culture-based method and blotter method. Preliminary results revealed that *Fusarium*, *Penicillium, Macrophomina*, and *Aspergillus* were abundant on Bambara groundnut*, Chaetomium* and *Colletotrichum* on kenaf; and *Alternaria, Aspergillus*, and *Fusarium* on *Amaranthus*. This study contributes to the limited knowledge on seedborne fungal infections in underutilized crops and will aid in the identification of important seedborne pathogens. Further work will be conducted to determine the effect of the isolated fungi on germination and growth of the plants.

Keywords

Seedborne, Fungal, indigenous crops, seeds, Bambara groundnut, Kenaf, *Amaranthus*.

The antagonistic effect of plant growth-promoting rhizobacteria against soil-borne pathogens of potato

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Disease Management

Abstract

Biocontrol of phytopathogens using antagonistic microorganisms is ecologically sustainable and environmentally safe. Plant growth–promoting rhizobacteria (PGPR) inhabit the rhizosphere and root surfaces of plants, enhancing plant growth and health through nutrient mobilization, phytohormone production, and antagonism toward pathogens. Production of antifungal antibiotics is a major mechanism of antagonistic biocontrol bacteria against fungal phytopathogens. The aim of this study was to assess the *in vitro* antagonistic activity of PGPR isolates against major potato pathogens and identify potential biocontrol candidates. Forty-nine PGPR isolates obtained from potato roots and rhizospheric soil from Gauteng were used in this study. Antagonistic activity was assessed *in vitro* using a dual culture assay. Each PGPR isolate was streaked at the centre of a half-strength potato dextrose agar (PDA) plate, and 6 mm agar plugs of one of five potato pathogens (*Fusarium oxysporum*, causal agent of Fusarium wilt*, Athelia rolfsii,* causal agent of Southern blight, *Verticillium dahlia,* causal agent of Verticillium wilt*, Rhizoctonia solani,* causal agent of stem canker, wilt and black scurf*,* and *Colletotrichum coccodes,* causal agent of black dot) were placed 2.5 cm from the bacterial streak on either side. Plates were incubated at 25 °C for 5–7 days. The zone of inhibition was recorded as the mean distance between the fungal pathogen and the bacterial growth. Most PGPR showed antagonism, with five isolates namely B148, B156, B169, B179, and B207, demonstrating the highest inhibition percentages (≥ 50%) across all tested pathogens. Isolate B169 showed significant inhibition against *A. rolfsii* (65% inhibition), while B207 was most effective against *V. dahliae* (62% inhibition). The dual culture assay indicated that these strains may produce antifungal compounds. These isolates will be further evaluated in greenhouse trials to test their efficacy *in vivo*. Their broad-spectrum activity highlights their potential as versatile biocontrol agents in a disease management strategy.

Keywords

PGPR, Potato, Antagonism, Dual culture, Biocontrol

Genome assembly and comparative analysis of *Alternaria linariae* reveal putative novel genes associated with host colonization and virulence

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Early blight (EB) is one of the most economically devastating diseases affecting tomato production, leading to significant yield losses. Traditionally, EB has been associated with the fungus *Alternaria solani*. However, recent evidence indicates that *A. linariae* (previously known as *A. tomatophila*) is the main causative agent. This revised understanding improves diagnostic accuracy and underscores *A. linariae* as a valuable resource for studying host-pathogen interactions, host adaptation, and evolutionary mechanisms within the *Alternaria* genus due to its distinct pathogenic profile and host specificity. The *A. linariae* strain 25, isolated from EB-infected tomatoes and was confirmed through analyses of its asexual morphs, cultural characteristics, and PCR-based identification. Its genetic factors influencing host specificity in was elucidated using whole genome sequencing. These insights are crucial for understanding its pathogenicity and host adaptation and for developing effective disease management strategies. The genome of *A. linariae* strain 25 has been fully assembled and spans 33.1 Mb. It comprises 18 rRNA genes and 117 tRNA genes, and approximately 272,542 base pairs (0.8%) of repetitive elements. The genome features 11,768 predicted gene models, with 221 genes identified as potential candidate effector proteins that may play a role in host colonization and specificity. Additionally, the genome encodes 573 carbohydrate-active enzymes and 37 secondary metabolite gene clusters, which are likely involved in pathogenicity and interactions with the host. A comparative genomic analysis of effector genes across different *Alternaria* spp. revealed that the *A. linariae* strain 25 possesses unique effectors, which may enhance its ability to colonize and adapt precisely to tomato plants. These findings lay the groundwork for further investigation into the molecular mechanisms that drive host specificity and pathogenicity in *A. linariae* and provide a foundation for developing targeted strategies to control early blight in tomato crops.

Keywords

Alternaria linariae, EB, tomato, host specificity, whole genome sequencing

Molecular identification of *Erysiphe* species causing powdery mildew from vegetable crops in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Powdery mildews are significant foliar diseases of vegetable and leguminous crops worldwide. The causal agents of the diseases are fungi in the *Erysiphaceae*, with the species of *Erysiphe* being the most dominant and widely reported. Although these obligate biotrophs pose major challenges in agricultural and horticultural settings, limited research has been conducted in South Africa. In this study, we aimed to survey for *Erysiphe* species infecting vegetable crops in South Africa. Morphological characteristics of conidiophores and conidia were examined alongside molecular phylogenetic analyses based on sequences from the internal transcribed spacer (ITS), calmodulin (*CAM*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), glutamine synthetase (*GS*), β-tubulin (*TUB*), and the second largest subunit of RNA polymerase II (*RPB2*) gene regions. Pathogenicity tests were done to confirm Koch's postulates. Morphological characteristics of the examined isolates were consistent with those of *Erysiphe*. Molecular data further confirmed the identity of the isolates collected, identifying *E. betae* on Swiss chard and beetroot, *E. heraclei* on carrot, *E. neolycopersici* on tomato, *E. radulescui* on cabbage, kale, leafy mustard and mustard spinach, and *E. vignae* on Bambara groundnut, cowpea and sugar bean. Pathogenicity trials revealed that all isolates were pathogenic to their original hosts. This study provides the first in-depth survey of *Erysiphe* species causing powdery mildew on vegetable crops in South Africa, many of which represent first reports. The result also highlights the need for more comprehensive reference data to enhance species-level classifications of these fungi.

Keywords

Erysiphaceae, morphology, phylogenetics, gene regions, pathogenicity

A new ambrosia beetle species infesting *Combretum erythrophyllum* in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The Combretaceae (Myrtales) includes more than 500 tree species distributed primarily throughout tropical and sub-tropical Africa. The best-known species in Southern Africa is *Combretum erythrophyllum*, which is a popular ornamental planted in gardens and along roadsides. Recently, many trees in the Pretoria area of South Africa have displayed symptoms of die-back and decline. Upon closer inspection, holes typical of infestation by ambrosia beetles (Coleoptera: Scolytinae) were observed on the boles and branches of the trees. Infested plant tissue was collected and placed in emergence cages. The emerging beetles were identified based on morphology and DNA sequences (COX, 28S and CAD). Morphologically, they resided in the *Ipini* (Coleoptera: Curculionidae), close to *Acanthotomicus*, but not conclusively matching this or other genera in the tribe. Phylogenetic analyses of the DNA sequences supported this identification. However, due overlapping morphological characters, we have chosen to treat this beetle as a new species of *Acanthotomicus.* Future studies will consider its identity, biology, microbial associates as well as how the serious infestations might be managed.

Keywords

Combretum, Ipini, native species, new species, Scolytinae

Fusarium populations causing dry and stem-end rot of potato in the Free State

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Fusarium strains are known to cause several disease symptoms on crops, including dry rot, stem-end rot of tubers and the wilting of potato plants. In South Africa, the molecular characterisation of the *Fusarium* strains associated with dry rot and stem-end rot of potato is lacking. In this project, symptomatic potato tubers from the Free State were sampled and associated *Fusarium* strains isolated prior to phylogenetic identification and pathogenicity testing. Symptomatic potato tubers were collected from the Free State Province, South Africa and used to isolate *Fusarium* strains. These strains were purified, single spored and DNA was extracted, prior to targeting the partial translation *elongation factor-1 alpha* (*TEF-1α*) gene for Sanger sequencing and data analyses. The isolated *Fusarium* strains identified were further tested for their pathogenicity on disease-free potato tubers and screened for α-amylase production as indicator of pathogenicity. The *Fusarium* strains isolated from the symptomatic tubers included *Fusarium oxysporum* species complex (FOSC), the *Fusarium solani* species complex (FSSC), the *Fusarium fujikuroi* species complex (FFSC) and the *Fusarium incarnatum-equiseti* species complex (FIESC). FOSC was the most often isolated, with FSSC strains shown to be the most virulent when inoculated on healthy potato tubers. *Fusarium chinhoyiense* (FFSC) was associated with diseased tubers, while three additional species, *Fusarium nirenbergiae, F. protoensiforme* and *F. noneumartii* caused symptoms during pathogenicity testing and consistently tested positive for α-amylase production. Here we report *F. chinhoyiense* for the first time on potato in South Africa. This species, originally described from maize and soil in Zimbabwe and South Africa respectively. Knowledge regarding the different *Fusarium* strains recorded from diseased potato tubers and the conformation of known pathogenic strains from diseased potato tubers can assist farmers in better management of the disease.

Keywords

phylogenetic analyses, pathogenicity, α-amylase production, *Solanum tuberosum*, fungal diseases

Evaluating the efficacy of the CRISPR/Cas9 t-dna vectors for mutating viral susceptibility genes in *Nicotiana benthamiana* and banana protoplasts

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Plant-Pathogen/Insect Interaction

Abstract

CRISPR/Cas9 genes editing has potential in knocking susceptibility genes to confer virus resistance. Transient expression of vectors by agro-infiltration and protoplast transformation is useful for evaluation of CRISPR/Cas9 vectors before stable transformation. A *dynamin-related protein 1C* (*DRP1C*) and a *Banana bunchy top virus* DNA-R (*BBTV DNA-R*) target regions were cloned into a pTRANS212 transformation backbone using Golden Gate cloning. The resultant plasmids pDRP1C, pBBTV_DNA-R, and CRISPR/Cas9 vectors pCas9-DRP1C-ZmUbi1, pCas9-DRP1C-PvUbi1, pCas9-BBTVDNA-R-ZmUbi1, and pCa9-BBTVDNA-R-PvUbi1, were transformed into the *Agrobacterium* strain *LBA4404.* The efficacy of the constructs was evaluated by transient co-expression with the CRISPR/Cas9 vectors expressing the target pDRP1C, pBBTV_DNA-R in benthi, *Nicotiana benthamiana,* leaves. The effectiveness of the *DRP1C* vectors were evaluated on isolated banana protoplasts. Molecular analysis revealed that target regions were successfully expressed into benthi leaves. However, no CRISPR/Cas9 induced mutation was detected by T7 endonuclease I and the restriction enzyme digestion assay. *DRP1C* CRISPR/Cas9 vectors were successfully transformed into banana protoplasts. The *DRP1C* target in the banana cells was Sanger sequenced and analysed using TIDE analysis. DRP1C-2 sgRNA in pCas9-DRP1C-ZmUbi produced a 17 % and 5.4 % total mutation efficiency. The results showed that the *DRP1C* CRISPR/Cas9 vectors can induce mutation at target site, although no mutation was induced in the benthi plant. These vectors will be used to generate banana plants with a knockout of the *DRP1C* gene towards obtaining resistance to the banana bunchy top virus.

Keywords

Banana bunchy top virus (BBTV), Dynamin related protein 1C (DRP1C), BBTV DNA-R, CRISPR/Cas9, Agro-infiltration

Assessing cultivar reactions to *Alternaria* spp. in Sunflower grain: a multi-season study across South African production regions

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Alternaria spp. are opportunistic fungal pathogens associated with foliar and seed infections in numerous crops, including sunflower (*Helianthus annuus* L.), a major source of edible oil and protein-rich animal feed in South Africa. Sunflower is particularly vulnerable to *Alternaria*-induced leaf blight, necrosis, and seed decay, with potential yield losses of up to 90% under conducive conditions. Despite their significance, the distribution, species diversity, and cultivar susceptibility to *Alternaria* spp. in South African sunflower grain remains underexplored. This study aimed to determine the prevalence and identity of *Alternaria* spp. associated with sunflower grain and to identify cultivars with reduced susceptibility. Sunflower grain samples from 10 commercially available cultivars planted at Agricultural Research Council (ARC) experimental farms were collected over three seasons (2022-2025), additionally samples were obtained from farms and collaborators across major production regions of the North-West, Free State, Mpumalanga, and Limpopo provinces. Fungal isolations were performed using half-strength potato dextrose agar (PDA), and isolates were identified based on morphological colony and microscopy. Molecular confirmation was done using qPCR and gene markers including ITS1 & 4, GAPDH, Alt a1, and TEF1-α. Severity and incidence varied by region and cultivar. Cultivars AGSUN 5108 CLP, PAN 7180 CLP, P 65 LL 02 showed reduced seed infection in Potchefstroom, indicating potential resistance or tolerance to *Alternaria* spp. Further analysis will focus on characterizing the sunflower grain population diversity of *Alternaria* spp. and assessing their potential to produce mycotoxins. Accurate identification of these pathogens is essential for developing effective control strategies, including integrated disease management practices such as the use of resistant cultivars, optimized planting schedules, and improved post-harvest sanitation. This research contributes to enhanced disease control, improved food safety, and the promotion of sustainable sunflower production in South Africa.

Keywords

Integrated disease management, Seed-borne fungi, Epidemiological mapping, qPCR, Cultivar resistance, Molecular identification

Hierarchical population structure and micro-spatial genetic diversity of *Exserohilum turcicum*

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Plant-Pathogen/Insect Interaction

Abstract

Exserohilum turcicum, the causal agent of northern leaf blight (NLB), is a major threat to global maize production, with yield losses reaching up to 70% under conducive conditions. While the pathogen's population genetics has been extensively explored at broad geographic scales, its fine-scale spatial genetic structure within individual plants and fields remains poorly understood. This study elucidated the spatial distribution of genetic and genotypic diversity of *E. turcicum* across hierarchical scales: lesion, leaf, plant, and field. A total of 144 *E. turcicum* strains, derived from distinct spatial strata, were genotyped using 11 polymorphic microsatellite loci. Contrary to the null hypothesis of a clonal one-spore-one-lesion origin, we detected the co-occurrence of multiple multilocus haplotypes (MLHs) and both mating types MAT1-1 and MAT1-2) within individual lesions, indicative of sexual recombination potential. Gene diversity ($He \leq 0.57$) and genotypic diversity ($I \leq 1.11$; $\lambda \leq 0.98$) were moderate at the leaf and plant levels. Notably, the North West field exhibited reduced diversity ($He = 0.33$; $I = 0.58$; $\lambda = 0.77$) relative to KwaZulu-Natal and Eastern Cape populations. Clonal MLHs were identified across multiple leaf strata of individual plants, suggesting intra-plant conidial movement, confirming the polycyclic nature of the pathogen. Analysis of molecular variance (AMOVA) revealed significant genetic differentiation among fields ($\Phi PT = 0.162$, $p = 0.001$), accounting for 16 % of total molecular variance, while 84% of variation resided within fields, reflecting substantial gene flow at local scales. These findings highlight the need for integrated management strategies that limit local conidial dispersal and gene flow within fields to slow pathogen adaptation. It also underscores the importance of deploying diverse sources of host resistance to reduce the risk of mixed-genotype interactions that might give rise to more virulent genotypes.

Keywords

Maize (*Zea mays*), Northern leaf blight, *Exserohilum turcicum*, Population genetics, Mating types

Evaluation of fungicide application and Potato (*Solanum tuberosum* L) cultivars against Late Blight (*Phytophthora infestans* (Mont.) de Bary)

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Disease Management

Abstract

Potatoes (*Solanum tuberosum* L.) are an important food crop that are cultivated worldwide. Potato production is limited by several factors, including the occurrence of diseases. Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most important diseases that affects potato production. Eight commercial potato cultivars grown under irrigation were evaluated in a randomized spilt-plot trial for its tolerance against late blight disease. The cultivar was the main plot which was split into sprayed and unsprayed fungicide application. The trial was conducted at the KwaZulu-Natal Department of Agriculture and Rural Development, Cedara Research Station (S29° 32' 15 33", E30° 16' 09 19") which is situated in the KwaZulu-Natal Mist Belt. The potato cultivars were planted at 37 037 plants ha^{-1}. A combination of six fungicides and five insecticides were applied weekly to control diseases and insects. Plants were rated weekly for late blight disease severity and final tuber yield was determined. Statistical results indicated significant differences between the cultivars, spray treatments and the season in terms of disease severity index. In the 2022/23 growing season a higher disease severity index was noted on the sprayed cultivars as compared to the unsprayed cultivars. Overall, the sprayed cultivars had a lower disease severity index as compared to the unsprayed cultivars in both the 2022/23 and 2023/24 growing seasons. Results indicate that the cultivar, Connect, had a lower disease severity index in both seasons in the unsprayed treatment. However, in terms of yield this cultivar performed significantly poorly in both the sprayed and unsprayed treatments in both seasons. Therefore, where no fungicides are applied it would be recommended that cultivars El Mundo and 11 Z4 9A1 be utilized as these cultivars were high yielding and had a low disease severity index.

Keywords

Disease severity index, disease, yield, tolerance, KwaZulu-Natal Mist belt

Optimisation of a viability PCR protocol for the detection of *Xylophilus ampelinus* in grapevine

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Biosecurity

Abstract

Current methods for detection of *Xylophilus ampelinus*, the causal agent of grapevine bacterial blight and necrosis entail either bacterial isolation, and/or PCR. However, bacterial isolation can be time consuming, whilst PCR methods do not distinguish between dead and viable cells. To accurately assess the potential biological risk, it is critical to determine whether plant material harbour viable bacteria especially since *X. ampelinus* is a regulated pest. Therefore, the feasibility of a detection protocol for *X. ampelinus* based on viability-real time PCR (v-PCR) was investigated.

Parameters that may influence v-PCR including PMAxx™ concentration and PMA enhancer reagent were first assessed using pure cultures, followed by the detection of viable cells in mixed cultures containing predefined ratios of viable and heat-killed cells. The assay was further optimised using a *X. ampelinus*-spiked plant matrix before being applied to inoculated shoots subjected to hot water treatment.

The 20 µM PMAxx™ treatment produced the most consistent v-PCR results for the pure cultures, suppressing amplification from >99% of the non-viable (heat-killed) cells. The PMA enhancer reagent for Gram negative bacteria did not improve detection under the conditions tested. v-PCR was able to selectively detect viable cells in mixtures containing predefined ratios of viable and heat-killed cells. In an *X. ampelinus*-spiked plant matrix, the effect of PMAxx on suppression of PCR amplification from dead cells plateaued at 75µM. PMAxx was able to suppress amplification from heat-killed cells in a plant matrix as well as inoculated shoots subjected to hot water treatment.

These results suggest that PMAxx-assisted v-PCR has the potential to distinguish between viable and dead cells *in planta*, although further optimization and validation are needed to confirm its effectiveness. Successfully optimising the v-PCR protocol will enhance the accuracy of determining the phytosanitary status of grapevines with respect to *X. ampelinus*.

Keywords

Bacterial blight, PMAxx, viable cells, v-PCR

A novel *Eucalyptus*-associated *Cryphonectria* (Cryphonectriaceae, Diaporthales) species from North Sumatra, Indonesia

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The Cryphonectriaceae (Diaporthales, Ascomycota) accommodates numerous important fungal pathogens that cause branch and stem canker diseases on woody plants globally. These for example include *Cryphonectria parasitica*, the causal agent of chestnut blight, which caused the demise of *Castanea dentata* (American chestnut) in North America. The profound ecological and economic impact of *C. parasitica* has prompted considerable research interest in the diversity, biology, and pathogenicity of other species in the Cryphonectriaceae, globally. During a 2023 survey of a *Eucalyptus grandis* × *pellita* clone infected with the vascular wilt pathogen *Ceratocystis manginecans* in North Sumatra, fruiting bodies of a fungus resembling the Cryphonectriaceae were observed on the bark of dying trees. DNA sequence comparisons for six loci (ITS+LSU+*TUB1*+*TUB2*+*TEF1*+*RPB2*), as well as morphological observations, showed that the fungus is an undescribed species of *Cryphonectria.* Inoculation trials on four eucalypt genotypes showed that the *Cryphonectria* sp. is only mildly pathogenic and that different eucalypt genotypes differed in their susceptibility to infection. This is the first report of a *Cryphonectria* species associated with trees in the Myrtales, with all previously described species found in the Northern Hemisphere on Fagales trees. The low level of aggressiveness of the fungus and its occurrence on trees predisposed due to infection by *C. manginecans* suggests that it is potentially an endophyte in healthy trees.

Keywords

Myrtales, new taxon, canker, plantation forestry, tree disease

Cryptic *Sporothrix* species associated with native pine bark beetles in Florida, USA

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Plant-Pathogen/Insect Interaction

Abstract

The genus *Sporothrix* (Ophiostomatales, Ascomycota) includes a diverse group of fungi with a broad global distribution across a wide range of climatic regions. These species occupy various ecological niches, with some members of the genus well recognised as human and animal pathogens. Other species occur in a variety of niches including in the infructescences of the Proteaceae, on freshly cut timber, soil, as well as living with bark beetles, ambrosia beetles and mites. During surveys of bark-beetle-associated Ophiostomatales from native *Pinus* spp. in Florida, a large number of isolates resembling species of *Sporothrix* were recovered. The aim of this study was to identify and resolve the phylogenetic relationships between these isolates using morphological characters and DNA sequence comparisons. From a collection of 58 isolates, one previously described species (*S. pseudoabietina*), and five novel taxa were found. All these isolates resided in the *S. gossypina* and *S. stenoceras* species complexes. The remarkable diversity of *Sporothrix* species discovered in a relatively small geographic area and associated with a small number of *Pinus* spp. and bark beetle species was unexpected. The results highlight the rich and largely unexplored fungal biodiversity associated with beetle-host systems. They also emphasise the need for further research considering the ecological roles, evolutionary relationships, and potential functional significance of these fungi in forest ecosystems.

Keywords

Ophistomatoid fungi, native forests, new taxa, Scolytinae, conifer

Towards gene editing for resistance against the banana bunchy top virus (BBTV)

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Disease Management

Abstract

Banana bunchy top virus (BBTV), first detected in South Africa in 2016, is the causal agent of banana bunchy top disease. Its eradication has largely been unsuccessful, and its spread has continued. At present, BBTV is managed through a combination of preventive and eradication measures. However, these methods are unreliable, and traditional breeding is expensive and challenging since bananas are sterile, lack genetic variation, and are produced via vegetative propagation. Additionally, no BBTV-resistant bananas exist, making traditional breeding challenging since it relies on already existing resistant genes. Transforming banana suspension cells is challenging, as it is both time-consuming, sometimes taking up to 26 months to establish a stable suspension, and prone to contamination. Host susceptibility genes aid virus invasion and establishment; therefore, this study seeks to identify host susceptibility genes and knock them out using the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (CRISPR-Cas) system to establish host resistance. Potential protein-protein interactions between BBTV genes and candidate host susceptibility genes were identified using AlphaFold and AlphaBridge from the RNASeq data from BBTV-infected Cavendish cv. Williams (*Musa acuminata*, AAA). The cell-cycle link protein (Clink) was found to interact with dolichyl-diphosphooligosaccharide protein, glycosyltransferase subunit 4A, rhodanese-like domain-containing protein 4, 26S proteasome regulatory subunit 6A, and transcription factor bHLH135; while the rolling-circle replication initiator protein (Rep) interacted with elongation factor 1-alpha. These interactions will be confirmed using the yeast two-hybrid essay. One identified interacting host susceptibility gene will be knocked out using CRISPR-Cas9, delivered by *Agrobacterium* transformation, to confer BBTV resistance. The ability of developmental regulators, WUSCHEL2 (WUS2) and BABY BOOM (BBM), to enhance the efficiency of apical meristem transformation as an alternative to transforming banana cell suspension cultures will be tested. Achieving BBTV resistance will aid in BBTV management in the South African banana industry.

Keywords

Apical meristem transformation, CRISPR-Cas9, Protein-protein interaction, Developmental regulators, *Agrobacterium* transformations

Exploring the link between wattle rust (*Uromycladium acaciae*) and wattle mirid (*Lygidolon leavigatum*): insect-disease complex affecting black wattle plantations

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Disease Management

Abstract

Wattle rust (*Uromycladium acaciae* (Cooke) P. Syd. & Syd) and the wattle mirid (*Lygidolon laevigatum* Reut.) constitute a significant insect-disease complex currently threatening the productivity and sustainability of black wattle (*Acacia mearnsii* De Wild) plantations in South Africa. Field observations have revealed a consistent co-occurrence of rust symptoms and mirid damage on affected trees, with dual infestations often resulting in more severe damage than rust alone. This suggests a possible synergistic interaction between the pathogen and insect. To investigate this potential relationship, a study employing a combination of microscopy and quantitative polymerase chain reaction (qPCR) techniques was initiated to examine the interaction between *U. acaciae* teliospores and the exoskeleton of adult *L. laevigatum*. Rust teliospores were collected from infected wattle tree leaflets and introduced into ventilated containers housing mirid adults. Microscopic examination of the adult mirids revealed the presence of teliospores on the wings and posterior abdomen of the mirid. To confirm the presence rust DNA from the insect surface, a qPCR technique will be employed. These findings indicate a potential role of *L. laevigatum* in facilitating the transmission of *U. acaciae*. A controlled nursery trial is currently underway to determine whether the wattle mirid can actively transmit rust to healthy black wattle plants. This study represents an important step toward understanding this complex insect-pathogen interaction and may have significant implications for integrated pest and disease management strategies in black wattle plantations.

Keywords

Insect-disease interaction, Disease transmission, Integrated pest management, Forestry, Quantitative polymerase chain reaction

Extracellular vesicles modulate growth and stress adaptation in *Fusarium circinatum*

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Disease Management

Abstract

The molecular mechanisms influencing *Fusarium circinatum*, an important pine tree pathogen, remain partially understood. We recently reported a biofilm-mediated response in this fungus, which supports its adaptation to harsh conditions including heat stress. Herein, we report that biofilm extracellular vesicles (EVs) play a key role in this adaptive response. The EVs were purified from planktonic and biofilm cells via differential ultracentrifugation and size exclusion chromatography. Their subsequent application to fungal cells revealed the capacity of biofilm-derived EVs (bEVs) to promote conidial viability and germination. When bEV-treated conidia were cultured in the presence of carbon sources (PM1), nitrogen sources (PM3B), and chemical sensitivity agents (PM21D), a delayed nutrient utilization and adaptation to antimicrobial agents such as nystatin, was observed. Furthermore, exogenous application of bEVs on mono- and polymicrobial biofilms significantly enhanced biomass and matrix production, with EVs derived from heat-stressed biofilm (45 ºC, 1 hour) showing more effectiveness at promoting biomass production and resistance to the antifungal agent, tebuconazole. This is consistent with the biofilm's heat resistance previously reported for *F. circinatum* biofilms. Taken together, our work provides novel insights into the EV-mediated molecular interactions that modulate environmental responses in *F. circinatum*.

Keywords

Biofilm, extracellular vesicles (EVs), microbial resilience, Pine Pitch Canker, *Fusarium circinatum*, polymicrobial biofilms

Plant Biosecurity: considerations when dealing with quarantine pests – a Goss' Wilt case study

David Read

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Biosecurity

Abstract

South Africa, as a signatory to the International Plant Protection Convention (IPPC), is obligated to report incursions of quarantine pests and pathogens via its National Plant Protection Organisation, coordinated by the Department of Agriculture (DoA) and Directorate of Plant Health. While initial detections are often made by university and science council researchers, their formal role in reporting often remains unclear. The recent detection of Goss' wilt in maize (*Zea mays*) highlights the value of collaborative and conscientious reporting within the plant biosecurity framework. In January 2024, symptomatic maize samples were submitted to the University of Pretoria's Grain Research Program diagnostic clinic. The causal bacterium was identified using a subspecies-specific PCR assay. These findings were communicated to the relevant industry body, which reported the findings to the DoA. Concurrently, the University of Stellenbosch's diagnostic clinic independently confirmed the pathogen through isolation and genome sequencing. Cultures from symptomatic tissue were used to fulfil Koch's postulates and confirm the identity of the pathogen. Characteristic leaf lesions, exhibiting a shellac-like appearance and distinct "luminous freckles" under backlighting, were observed. Molecular and genomic analyses identified the causal agent as *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn), the bacterium responsible for Goss' wilt and leaf blight of maize, previously reported in the United States. The confirmed presence of Cmn in South Africa represents a significant phytosanitary incursion. However, the rapid and responsible communication between academic researchers, industry stakeholders, and government reduced the impact and economic consequences. This underscores the importance of clear reporting pathways and coordinated responses in managing plant biosecurity.

Keywords

Clavibacter michiganensis subsp. *nebraskensis*, Phytosanitary incursions, Koch's postulates

Diagnostic clinics, Pathogen reporting

Insights into *Turnip yellows virus* populations, infecting *Brassica napus* L. (canola) in the Western Cape, South Africa

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Ecology and Epidemiology

Abstract

Turnip yellows virus (TuYV), an aphid-transmitted *Polerovirus*, significantly reduces canola yields. Variants cluster into phylogroups that differ in transmissibility, severity and host range. P5–I variants are associated with high aphid transmission efficiency and severe symptoms, while P5–II and P5–III exhibit progressively reduced virulence and transmission capacity. In South Africa, TuYV has remained under detected on canola, with symptoms frequently misdiagnosed as nutrient deficiencies. This study provides a multi-season dataset on TuYV distribution, persistence and diversity in South African canola. Between 2021 and 2023, 289 symptomatic plants, including canola, wild radish (*Raphanus raphanistrum*), and white mustard, were sampled from the Swartland and Overberg regions. RNA-seq was applied to 36 samples collected in 2021. Subsequent samples were tested using RT-PCR targeting ORF3a. Phylogenetic analyses were conducted using complete genomes, as well as ORF3a and ORF5 sequences. TuYV was detected in 275 samples, with complete genomes for 30 variants. TuYV was also identified for the first time in wild radish, a potential alternative host. Variants grouped into two phylogroups (P3a–I/P5–I and P3a–II/P5–II), mirroring those described from Australian canola. Most South African variants belonged to phylogroup-II and despite its potentially lower aphid transmissibility it was the predominant variant in both regions and across all seasons. This study confirms the widespread and sustained presence of TuYV in South African canola fields and reveals a structured viral population dominated by phylogroup-II. The consistent presence of both phylogroup-I and II variants across three seasons suggests long-term viral establishment. The absence of spatial clustering and the detection of TuYV in wild radish further support an ongoing, regional virus circulation. These findings provide a genetic and epidemiological baseline for future studies on TuYV epidemiology and persistence in the Western Cape.

Keywords

TuYV, poleroviruses, viral diversity, metaviromics, RNA-seq

Evaluating the efficacy of new experimental actives against Phytophthora root rot and stem canker on macadamia

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Disease Management

Abstract

The oomycete pathogens *Phytophthora cinnamomi* and *Phytopythium vexans* cause root rot and stem cankers on macadamia, with *Ph. cinnamomi* being more aggressive. In South Africa, mefenoxam is registered against macadamia Phytophthora diseases, whereas phosphonate registration trials are ongoing. Oxathiapiprolin registered against Phytophthora diseases on other tree crops in other countries, is a newer active. Our study aimed to evaluate the efficacy of a new experimental product formulation containing oxathiapiprolin and mefenoxam against macadamia oomycete diseases under orchard conditions. Two orchard trials were established at Camperdown (Kwazulu-Natal) and Barberton (Mpumalanga) in 2023 and 2024, respectively. Treatments included mefenoxam, phosphonates, three dosages of two formulations (1:3 and 1:6 oxathiapiprolin:mefenoxam) of the new experimental product and an untreated control. Treatment efficacy at Camperdown was evaluated one year post-application by assessing (i) increase in stem diameter, (ii) change in foliar health, (iii) oomycete pathogen soil quantities and (iv) oomycete root pathogen quantities. Further assessments at Baberberton and Camperdown are underway. The only parameter that differed significantly among treatments at Camperdown one year post-treatment was the change in foliar tree health. All treatments, except the two lowest dosages of oxathiapiprolin+ mefenoxam (1:3 ratio formulation), improved foliar health similarly and significantly relative to the untreated control. Based on soil baiting analyses, *Ph. cinnamomi* and *Pp. vexans* were present in the Camperdown soil, with *Ph. cinnamomi* more prevalent. Only *Ph. cinnamomi* was detected in macadamia roots, indicating that the main causal organism is *Ph. cinnamomi* at Camperdown. Expanding the range of chemical actives available for managing oomycete diseases in macadamia is important, particularly in orchards where conducive environmental conditions necessitate frequent chemical applications. The new experimental product shows promise as an alternative treatment, reducing the risk of resistance development. Quantifying oomycete pathogen levels in soil remains challenging and our quantification methods require further optimisation.

Keywords

Ph. cinnamomi, *Pp. vexans*, mefenoxam, phosphonates, oxathiapiprolin.

Infection and protection of maize silks against mycotoxigenic fungi with fungicides

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Disease Management

Abstract

Maize is the primary food source for many South Africans. Maize production is, however, threatened by various destructive fungal pathogens, like *Fusarium verticillioides* and *F. graminearum*, which cause Fusarium ear rot (FER) and Gibberella ear rot (GER), respectively. These pathogens result in yield losses but also produce mycotoxins that can cause severe health problems in humans and animals. The primary infection pathway of both pathogens is via maize silks, which are prone to infection for a limited time during flowering. Therefore, the application of a chemical control product to protect silks during this crucial period could potentially safeguard grain from infection. To assess silk infection under field conditions, silks were sampled at seven different field sites in South Africa, at three different growth stages (Young silks "R1", browning silks "R2" and completely desiccated silks). The silks were then surface-sterilised or left unsterilized, and placed onto PDA with antibiotics. Fungal colonies that developed were counted, and putative *Fusarium* species sub-cultured and identified. Significantly more colonies were associated with silk exposed to the environment, compared to those protected by the husk leaves. *Fusarium* species isolated from maize silks and stalks; including F. *verticillioides* and *F. graminearum* species complexes species; were subjected to *in vitro* fungicide efficacy evaluation using fungicide-amended PDA. Eleven fungicides were evaluated, with Product 6 (pydiflumetofen, azoxystrobin, propiconazole) and Product 11 (propiconazole) recording the lowest EC_{50} values. The results of this study demonstrated the value of husk leaves in protecting maize ears from pathogens, and identified fungicides that could protect silk exposed to the environment from mycotoxigenic fungi. These fungicides will be used for greenhouse and field-based evaluation to determine whether commercially available fungicides can protect maize silk against infection under field conditions.

Keywords

Fusarium verticillioides, *F. graminearum*, Mycotoxins, Fungicides, Chemical control

Incidence and distribution of *Sclerotium rolfsii* affecting dry bean (*Phaseolus vulgaris*) in South Africa

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Ecology and Epidemiology

Abstract

Dry bean (*Phaseolus vulgaris*) plays a significant role in ensuring global food security due to its high protein content. However, the sustainable production of these crops is negatively impacted by various abiotic and biotic factors. In South Africa, *Sclerotium rolfsii* may be an important soilborne pathogen that impacts production of dry beans, although the extent of the problem is unknown. Earlier reports identified *S. rolfsii* as a constraint to dry bean production in South and Southeast Asia, the Americas, and African countries like Uganda. To assess the prevalence of this pathogen on dry beans in South Africa, surveys were conducted during 2023/2024 and 2024/2025 in 21 dry bean fields in five provinces (Free State, KwaZulu-Natal, Limpopo, Mpumalanga, and North West). Thirty plants exhibiting signs of white mycelial formation on the collar region and above-ground symptoms of drying leaves, wilting and death, were sampled from each field at two growth stages, namely the seedling and mature plant stages. Isolations were made from the roots and hypocotyls and *S. rolfsii* was identified using morphological and molecular methods (ITS1/ITS4 universal primer). The fungus was found to be widely distributed (17 out of 21 fields sampled) in four of the five provinces, except for Mpumalanga, where it was absent. It was recorded from five fields in the Free State, two in KwaZulu-Natal, six in Limpopo and four in North West. The highest disease incidence on roots (37.0 %) and hypocotyls (29.6 %) was recorded in a field in Limpopo (Thabazimbi). The high incidence and wide distribution of *S. rolfsii*, along with the observed wilting and dying of infected dry bean plants, confirms the importance of *S. rolfsii* as one of the major soilborne pathogens of dry beans and highlights the need to develop effective management strategies for South Africa.

Keywords

hypocotyls, roots, soilborne pathogen, seedlings, mature plants

Long-read RNA sequencing reveals extensive alternative splicing repertoires in fungal plant pathogens

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Plant-Pathogen/Insect Interaction

Abstract

Alternative splicing (AS) is a complex post-transcriptional process enabling a single gene to produce multiple mRNA transcripts, increasing transcriptome and proteome diversity. AS in fungi has been shown to occur at lower frequencies than model animal systems, but this process has been linked to key biological processes, including development, pathogenicity, and environmental adaptation. The *Ceratocystis fimbriata s.l.* complex, comprises several closely related fungal plant pathogens displaying distinct host specificity. Economically important hosts include acacia, eucalypts, mango, pomegranate and sweet potato. This complex thus offers an ideal model for investigating fungal adaptation and host specificity. Here, we present a comparative analysis of AS in two species, *C. eucalypticola* and *C. fimbriata*. High-quality genome assemblies were generated for both species, each consisting of 9 contigs, with genome sizes of 32.37 and 31.73 Mb, respectively. Whole transcriptome sequencing was performed under *in-vitro* and *in-planta* conditions using Nanopore and Illumina sequencing. High-quality gene annotations with alternative isoform predictions were generated using this transcriptomic data. Alternative splicing was observed at a relatively high frequency, with over 42% of multi-exonic genes in C. eucalypticola and over 35% in C. fimbriata undergoing AS. Intron retention was the most common AS type, accounting for 84.14% in *C. eucalypticola* and 85.26% in *C. fimbriata*, followed by alternative 3 prime splicing (9.27% and 8.62%), alternative 5 prime splicing (5.38% and 5.32%) and exon skipping (1.17% and 0.8%). Notably, over 43% of AS genes in *C. eucalypticola* and over 29% in *C. fimbriata* were species-specific. Differences in gene expression and transcript usage of AS genes were observed between conditions and species, including in effectors and CAZymes. These findings suggest that AS provides an additional regulatory layer contributing to pathogen-host interaction and lay the foundation for future functional work to unravel the role of AS in host-specificity and adaptation of these fungi.

Keywords

Adaptation, *Ceratocystis*, host specificity, isoform diversity, pathogenicity

Identification and characterization of *Cannabis sativa* viruses and viroids in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The production of *Cannabis sativa* has significantly increased in recent years, due to its recreational, medicinal and industrial applications. It serves as a commercial crop which has the potential to contribute to the development of the economy and to alleviate poverty. However*, C. sativa* is susceptible to a number of plant viruses and viriods which can negatively affect cannabinoid production in medicinal cannabis and biomass in industrial hemp. This study aims to identify and characterize *C. sativa* viruses and viroids using a combination of next-generation sequencing (NGS), and reverse transcription polymerase chain reaction (RT-PCR). Field surveys were conducted by sampling leaf material from different growing facilities and farms in the Eastern Cape, Gauteng, Free State, North West and Western Cape provinces. Plants exhibiting symptoms such as stunted growth, leaf deformity, wrinkled leaf margins, necrosis, mosaic pattern and chlorosis were sampled for analysis. Total RNA was extracted and subjected to RNASeq to identify the presence of known and novel viral agents. Detected virus included hop latent viriod (HLVd), cannabis cryptic virus (CCV), turnip yellows virus (TuYV), ageratum latent virus (AgLV) and alfafa mosaic virus. PCR primers targeting conserved regions of the assembled viral genomes were developed to validate RNAseq findings in individual samples and to facilitate rapid diagnostics. The RT-PCR amplicons were Sanger sequenced and identities of the samples were confirmed by a BLAST search. Asymptomatic plants were found to have viral infections, which shows the need to routinely screen even in the absence of visible disease symptoms. The findings of this study highlights the widespread infection of *C. sativa* by multiple viruses. By successfully identifying these viruses, we will ultimately contribute to improving agricultural practices with the potential of helping cannabis growers in South Africa to better understand and manage viral infections.

Keywords

Pathogens; *Cannabaceae*; Hemp; RNAseq; RT-PCR

Identification and characterization of viruses infecting cowpea and Bambara groundnut in South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Cowpea (*Vigna Unguiculata*) and Bambara groundnut (*Vigna subterranean*) are the underutilized legumes grown by smallholder farmers in South Africa. These crops are gaining research interest for their potential in increasing food and nutritional security. However, their production and commercialization are limited by a number of factors including viruses such as alfalfa mosaic virus (AMV), cucumber mosaic virus (CMV), bean common mosaic necrosis virus (BCMNV), tomato spotted wilt virus (TSWV), cowpea aphid borne virus (CABMV), bean common mosaic virus-black-eyed strain (BCMV-BICM). This study aimed to identify and genetically characterize viruses infecting cowpea and Bambara groundnut to implement effective control measures and address production losses. Samples collected from the Limpopo Province were tested using double antibody sandwich enzyme-linked immunosorbent Assay (DAS-ELISA), plate-trapped antigen-ELISA (PTA-ELISA), reverse transcription polymerase chain reaction (RT-PCR), cloning and sequencing. To test seed transmission, cowpea and Bambara groundnut seeds collected from ARC-PHP, University of Pretoria and Rooivaal farmsteads were planted in the glasshouse, monitored for symptom development and subsequently tested for seed-borne viruses. BCMV-BICM was the most prevalent, 81% from field samples and 39% from seed samples. TSWV, CABMV and CMV were also detected at lower percentages in both field and glasshouse samples. The results of the RT-PCR, ELISA, cloning and sequencing confirmed the presence of common viruses such as TSWV, CMV, CABMV and viruses belonging to the *Potyvirus, Tospovirus and Polerovirus* groups with co-infections commonly observed in the field. These findings highlight the diversity and prevalence of viral infections affecting underutilized legumes in South Africa and emphasize the need for continued monitoring, seed health testing, and integrated management strategies to minimize production losses. Field surveys in different provinces are ongoing, and more work will be done to confirm seed transmissibility and virus species responsible for infection, and the host-range of viruses detected.

Keywords

Cowpea, Bambara groundnut, Viruses.

Characterisation of oomycetes in olive nurseries in the Western Cape

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Phytophthora species are important root and crown rot pathogens of olive trees globally. A previous survey in the Western Cape identified *Phytophthora* species in only 8 of 103 samples, but at 6 of 13 sites, suggesting a limited incidence, but moderate distribution. Other oomycete pathogens of olive trees include *Globisporangium irregulare*, which is commonly found in Western Cape olive orchards (11 of 13 sites, 43 of 103 samples). The aim of this study was to investigate olive nurseries as a possible source for such oomycete pathogens. Forty-one samples were collected from four olive nurseries in the Western Cape (10–11 per nursery). Oomycete species were recovered by direct isolations onto general and selective media, as well as soilbaiting using lemon leaf pieces and pear fruit. Developing colonies were purified onto 5% carrot agar, grouped based on morphology, and representative isolates were identified using restriction fragment length polymorphisms, sequencing and phylogenetic analyses. Oomycetes were recovered from all soil samples through baiting, but only from 17 plants through isolations from roots. Twenty-eight species or complexes were identified from four genera, including *Globisporangium*, *Phytophthora*, *Phytopythium* and *Pythium*. Four species or complexes occurred in all four nurseries, i.e. *Globisporangium irregulare* species complex (29 samples), *G. perplexum* (18 samples), *Phytophthora capensis* (10 samples) and *Pythium* sp. clade B2a (20 samples). Other *Phytophthora* species that were identified included *P. amnicola, P. chlamydospora*, *P. gonapodyides*, *P. multivora*, *P. pseudocryptogea*, and an unidentified species in clade 2c. Previous research has confirmed the pathogenicity of the *Globisporangium irregulare* species complex, *Phytophthora gonapodyides*, *Phytophthora multivora*, and *Phytophthora pseudocryptogea* towards olive trees. However, most species identified in this study have unknown interactions with this host. These results suggest that olive nursery trees may be contributing to the spread of oomycete pathogens in South Africa.

Keywords

Phytophthora, *Globisporangium*, *Pythium*, root rot

Phytophthora cinnamomi and other oomycetes in blueberry nurseries in the Western Cape

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The South African blueberry industry has experienced rapid growth over the past decade. Nurseries supplying the expanding industry pose a risk of spreading pathogens, such as *Phytophthora*, to blueberry orchards. This risk was investigated by characterising oomycetes on blueberry nursery plants in the Western Cape. Sixty blueberry nursery plants were collected from six blueberry nurseries in the Western Cape during spring/summer of 2023–2024. Oomycete species were recovered by direct isolations onto general and selective media, as well as soilbaiting using lemon leaf pieces and pear fruit. Developing colonies were purified onto 5% carrot agar, grouped based on morphology, and representative isolates were identified using restriction fragment length polymorphisms, sequencing and phylogenetic analyses. Oomycetes were recovered from four of the six nurseries. A total of eight species were identified, of which seven were recovered through soilbaiting and three through direct isolations from roots. *Phytophthora cinnamomi* and *Elongisporangium undulatum* had the highest incidences (16 and 12 samples, respectively) and distribution (three nurseries each). This was followed by the *Globisporangium irregulare* species complex (five samples, two nurseries). The remaining species included a putative new species of *Elongisporangium*, *Phytopythium helicoides*, *Phytopythium vexans*, *Pythium myriotylum*, and *Pythium* sp. clade B2a. These were isolated from single nurseries and only one or two samples each. Seven of the eight species were identified in one of the nurseries, while other nurseries only had up to three species. None of the species recovered during this study have been reported on blueberries in South Africa; however, *P. cinnamomi* and the *G. irregulare* species complex are known as blueberry pathogens elsewhere in the world. Management practices to eliminate contamination by oomycete pathogens need to be improved in some nurseries to reduce the risk of such pathogens being spread through nursery material.

Keywords

Elongisporangium, Globisporangium, Pythium, root rot

Evaluation of essential oils for the control of *Phytophthora* brown rot of citrus

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Disease Management

Abstract

In South Africa, *Phytophthora nicotianae* is the main causal agent of brown rot and can cause enormous crop losses. Although phosphonate applications provide a good level of control, the use of postharvest treatments may reduce further losses during storage and transit. Additionally, fungi have shown to build up resistance to synthetic fungicides with prolonged use. Among the potentially useful alternatives for postharvest management of Brown rot is the use of essential oils. In this study, four essential oils (EOs) (lavender, oregano, thyme and cinnamon bark) have been tested *in vitro* in a pilot study against two Phytophthora brown rot isolates. The sensitivity of two *P. nicotianae* isolates (25 1201 and 25 1192) was tested on Corn meal agar (CMA) media amended with EOs. The media was amended with a range of concentrations (0; 125; 250; 500; 750 and 1000 ppm) of each oil (pure). The plates were then incubated at 29°C and allowed to grow. After 5 days the growth of the pathogen was observed and the growth radius was measured using a caliper. Isolate 25 1201 had growth of 13.5 mm at a concentration of 250 ppm and with no further growth at higher concentrations of lavender oil. Growth of 1.3 mm occurred on thyme amended media at a concentration of 125 with no further growth. Isolate 25 1192 showed growth of 20.9 mm and 2.5 mm on lavender and thyme oil amended CMA at a concentration of 125 ppm, respectively. No further growth was observed at higher concentrations. Neither isolates showed any growth on CMA amended with oregano and cinnamon bark oils. Lavender and thyme oils showed potential for controlling Phytophthora brown rot. However, testing of cinnamon bark and oregano oils will be repeated.

Keywords

Phytophthora nicotianae, lavender, oregano, thyme, cinnamon bark

Survival of the bacterial wilt pathogen

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Disease Management

Abstract

Bacterial wilt (BW), caused by *Ralstonia solanacearum,* is considered one of the most destructive plant pathogenic bacterial diseases. Common symptoms include the wilting of foliage, browning of vessels, and stunting of the plant, ultimately leading to plant death. BW is very difficult to control due to the ability of R. solanacearum to survive for years in soil and water without a host plant. Knowledge regarding the survival of *R. solanacearum* in South African soil under different temperature and moisture conditions is lacking. Topsoil (0-10 cm) and deeper layered soil (ca. 1 m) were collected from two different localities, Sandveld (Western Cape Province) and Dendron (Limpopo Province). One kg of soil was placed in Ziploc bags (to keep a constant moisture percentage. The soil was inoculated with *R. solanacearum*, and the survival of the pathogen was determined over a period of 7,5 years at regular time intervals under different temperature (4°C, 15°C, 25°C, and 35°C) and moisture (15% water holding capacity (WHC), 50% WHC, 70% WHC and 90% WHC) conditions. Results recorded indicated that *R. solanacearum* was still detected in low concentrations after 85 months of incubation. There was an increase in the detection after the soil was incubated in a recovery broth for 24 hours. However, the survival rate fluctuated between the different time intervals, but the general trend showed that *R. solanacearum* survival is higher at lower temperatures in clay soil with a high-water content. With an increase in temperature, there was a decline in the number of *R. solanacearum* cells. Knowledge regarding the survival of a pathogen is essential, as it can aid in the development of control strategies by allowing for the exploitation of the pathogen's weaknesses.

Keywords

Ralstonia solanacearum, persistence, soil microcosms

Identification and characterization of *Fusarium* species from diseased cannabis buds, stems and roots

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The genus *Fusarium* includes a diverse range of plant pathogens that have substantial implications for agriculture, particularly in relation to economically significant crops. These pathogens can result in diminished crop yield over time and present significant threats to food safety. With the recent legalization of cannabis cultivation, there has been an increase in the reports of various fungal pathogens associated with diseased cannabis plants. This study aimed to identify and characterize *Fusarium* species isolated from the buds, stems and roots of affected cannabis plants. Cannabis samples displaying characteristic symptoms of *Fusarium* infection were collected from the Gauteng, Limpopo and North West provinces during the 2023-2024 and 2024-2025 growing seasons. Identification of the isolated *Fusarium* species was performed through sequencing and phylogenetic analyses of the translation elongation factor 1-alpha and β-tubulin gene regions. In addition, morphological characteristics—including colony morphology, pigmentation, growth rate on agar media, and spore morphology were assessed to facilitate comprehensive characterization of the isolates. Phylogenetic analyses allowed for the identification of species within three species complexes namely the *Fusarium incarnatum-equiseti* species complex (FIESC), *Fusarium oxysporum* species complex (FOSC) and the *Fusarium solani* species complex (FSSC). Previous reports from other countries have identified these species associated with disease on cannabis, with *Fusarium oxysporum* and *F. solani* frequently reported. While this study confirms the presence of these species in diseased cannabis plants, ongoing monitoring, sampling efforts and further pathogenicity studies are essential to comprehensively understand their roles and impact on disease within cannabis cultivation system.

Keywords

Diversity, Species complex, Pathogenicity, Sequencing, Phylogenetics,

Post Entry Quarantine (PEQ) of citrus

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Biosecurity

Abstract

The disease management unit at the Agricultural Research Council's Tropical and Subtropical Crops, Nelspruit campus (ARC-TSC) is a role player in the mandate of the Citrus Improvement Scheme (CIS) to ensure the supply of pathogen-free propagation material to the South African citrus industry. The Post Entry Quarantine (PEQ) function, assigned by the Department of Agriculture (DoA) to the ARC-TSC is critical to ensure that no foreign pathogens are introduced via imported budwood. This aligns with international standards established by the International Plant Protection Convention (IPPC) and South Africa's Agricultural Pests Act 36 of 1983. Citrus budwood imports are subjected to shoot tip grafting (STG), to eradicate graft transmissible pathogens, i.e. viruses, viroids and bacteria. Once STG is completed, the material is indexed on biological indicators and molecular tests to detect various pathogens. Seed imports are subjected to grow out tests and subsequent molecular diagnosis for pathogens as per the quarantine requirements. The different phases of the STG process are summarized in Annexure 6 (CIS Procedural Guide) and summarized as follows:

Phase 1: Introduction of citrus budwood

Phase 2: STG phase

Phase 3: Successful STG micrografted to rootstock seedling

Phase 4: Biological indexing phase

Phase 5: Line tested virus-free and CTV inoculation commences

Phase 6: Interim release to CFB

Phase 7: 12-month biological indexing

Phase 8: Final release

PEQ of citrus forms part of the Citrus Improvement Scheme (CIS) in South Africa where quality and standards are monitored to ensure the release of disease-free material to industry. Data collected from all citrus imports processed at the PEQ facility at the ARC-TSC shows how shoot tip grafting (STG) remains a cornerstone technology in PEQ for the safe movement and conservation of high-value citrus germplasm worldwide. Biological indexing on sensitive indicator hosts proved to be crucial in the detection of quarantine pathogens.

Keywords

post entry quarantine, citrus, biological indexing, molecular detection, shoot tip grafting

A new leaf spot disease of kiwifruit caused by a species of Sclerotiniaceae

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Leaf spot symptoms were observed on kiwifruit (*Actinidia chinensis*) leaves in two orchards in KwaZulu-Natal and Limpopo, South Africa. Symptoms included circular, light brown spots with dark brown borders containing tuft-like fungal structures visible to the naked eye on the abaxial and adaxial leaf surfaces. Microscopic examination showed that the propagules consisted of filamentous hyphae aggregated in erect, pale brown columns that expanded under moist conditions. This study aimed to isolate and identify the causal agent of the disease. Eleven morphologically similar isolates were recovered by plating propagules of the fungus directly onto culture media. Phylogenetic analyses of DNA sequences for the ITS, LSU, and *RPB*2 regions showed that the fungus resides in the Sclerotiniaceae. Sequences for the isolates formed a distinct cluster closely related to *Microstrobilinia castrans,* which*,* interestingly, is a fungus known to infect spruce (*Picea* spp.) cones. Pathogenicity tests were performed by inoculating asymptomatic detached leaves of *A. chinensis* with propagules collected from naturally infected leaves and mycelium plugs taken from freshly grown cultures of two representative isolates. The inoculated leaves developed lesions within five days with abundant propagules, similar to those found in natural infections, produced within these lesions. The fungus was successfully re-isolated from the symptomatic tissue and developing structures. Future studies will focus on performing pathogenicity tests on living *A. chinensis* plants and providing an appropriate species name for the pathogen.

Keywords

Actinidia chinensis, Microstrobilinia, multi-locus phylogeny, mycopappus-like, novel taxon

Starships in *Sclerotinia sclerotiorum*: First evidence of actively-transposing transposable elements with potential impacts on pathogenicity

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Understanding how plant pathogens evolve new traits is critical for sustainable plant disease management. Recent discoveries have highlighted *Starships* – a newly discovered superfamily of large, mobile genetic elements – as key vehicles for horizontal gene transfer and genome evolution in fungi. However, their presence and potential roles in individual plant pathogens remain largely unexplored. Here, we present the first investigation of *Starships* in the globally important plant pathogen *Sclerotinia sclerotiorum*, using 29 long-read genome assemblies from isolates spanning North America, Europe, and Australia. We employed *ab initio* gene prediction, functional annotation, and *Starship* detection pipelines to identify and characterise these elements and their cargo genes. The median number of *Starships* per strain was 2 (SD=1.2). We detected 11 distinct *Starship* elements (haplotypes), whose captains are classified into 3 distinct naves that belong to the *Phoenix* and *Moya Starship* families. Several high-confidence elements showed signatures of recent or ongoing transposition. Cargo gene analysis revealed several putative effectors, multiple matches to the Pathogen–Host Interaction (PHI) database, and a recurrent cerato-platanin domain – features suggestive of roles in virulence. These findings provide evidence that *Starships* may contribute to the mobilisation of genes associated with pathogenicity in *S. sclerotiorum*. As the first study to characterise *Starships* in this species, our work opens a novel line of inquiry into the evolutionary dynamics of this pathogen. Given their capacity to mediate horizontal transfer of virulence and antifungal resistance genes—even across species boundaries—*Starships* may play a pivotal role in the emergence of new pathogen variants. These insights have significant implications for plant disease surveillance and the development of durable disease management strategies.

Keywords

mobile elements, fungal effectors, horizontal gene transfer, genomics, cerato-platanin

Investigating the antifungal mechanisms of gallic acid against Fusarium wilt disease

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Disease Management

Abstract

Fusarium wilt, caused by *Fusarium oxysporum, is* a major threat to global food security, leading to significant soybean losses and mycotoxin contamination. The widespread use of chemical fungicides poses environmental and health risk, emphasising the urgent need for safer and more sustainable alternatives. Phenolic compounds such as gallic acid are ubiquitous in plant tissues and exhibit various properties including antioxidant, anti-inflammatory, and antimicrobial. However further research is required to elucidate its antifungal mechanisms. Therefore, the aim of this research is to investigate the efficacy of gallic acid in controlling *F. oxysporum*. The antifungal potential of gallic acid against *F. oxysporum* was evaluated using the food-poison technique, while the effect of the treatment on the morphology of the fungi was monitored using high-resolution scanning electron microscopy (HR-SEM). Additionally, changes in the polysaccharide production, chitin content and various biochemical activities of the treated *F. oxysporum* were evaluated. In addition, liquid chromatography mass spectrometry (LC/MS)-based protein profiling was performed to identify differentially expressed proteins related to stress response, metabolism, and pathogenicity. *In vitro* antifungal study as well as HR-SEM images of the treated fungus showed significant inhibition and changes in the cellular structure. The cell wall components of the fungi were greatly affected by gallic acid, causing significant reduction in the polysaccharide and chitin contents. The fungal mycelia treated with 1 mM gallic acid experienced the highest level of reactive oxygen species induced oxidative damage. Additionally, gallic acid affected the pathogenesis of the *F. oxysporum*, by causing a significant decrease in its β-1-4-glucanase and lipase activity. Using LC/MS analysis, protein abundance of *F. oxysporum* was significantly altered in response to treatment with gallic acid. The results suggest that gallic acid may be a useful antifungal agent to manage and control the incidence of *Fusarium* wilt disease to enhance food security.

Keywords

Phenolic acids, Fusarium root rot, Biocontrol agent, Proteomic analysis, Antioxidant enzymes

The Centre of Excellence in Plant Health at the Agricultural Research Council

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Disease Management

Abstract

The Agricultural Research Council (ARC) in South Africa operates through a network of specialized institutes and divisions, each focusing on key areas of agricultural sciences and innovation. The ARC operates 10 research institutes which are strategically located across the country to address diverse agro-ecological conditions and agricultural needs, of which eight are directly involved in plant health related research and development. To more effectively address the complex challenges of plant health, a multidisciplinary Plant Health Centre of Excellence (PHCoE) was initiated within the ARC in 2025. The PHCoE will unite experts from diverse fields, such as plant pathology, virology, bacteriology, mycology, entomology, nematology, arachnology, molecular biology, weed sciences, soil and water sciences, economics, and policy development. The PHCoE encourages collaborations across the different ARC institutes, other research organizations, agricultural industry and government to develop innovative technologies and solutions. Such collaboration is essential for advancing biosecurity, sustainable agriculture, protecting ecosystems, and ensuring food and nutrition security. Key research areas within the PHCoE include biosecurity, biosystematics, pathogen and pest detection and characterization, the study of host, vector and pathogen interactions, disease epidemiology, mycotoxin control, and development of integrated pest management protocols. Key activities of the PHCoE and challenges with the establishment of the CoE within the ARC will be illustrated.

Keywords

Biosecurity, Characterization, Integrated pest management, Interactions, Sustainable agriculture

Infection of Eureka lemon blossoms by *Botrytis cinerea* and validation of the PhytRisk Botrytis infection model

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Ecology and Epidemiology

Abstract

Botrytis cinerea is responsible for blossom blight and rind distortion in lemons. Understanding the environmental conditions that favour infection is critical for optimising management and fungicide use. This study aimed to validate the CRI-PhytRisk infection model by correlating environmental data, blossom infection, rind distortion and airborne inoculum levels with the prediction model. During flowering, 200 blossoms were sampled weekly for eight weeks from three Eureka lemon orchards in the Western Cape. At full bloom, an additional 20 blossoms per orchard were sampled for three weeks to assess floral part infection. Blossoms were either surface-sterilised or left untreated and incubated in moisture chambers. The floral components were placed on *B. cinerea* selective media. Airborne inoculum was also monitored weekly using Vaseline-coated microscope slides, DNA was extracted, and *B. cinerea* was quantified with qPCR. A total of 1 000 fruit per orchard were collected in April 2025 and assessed for rind distortion. *Botrytis cinerea* flower infection varied among orchards. Farm 1 (Riviersonderend) had 29%, Farm 2 (Buffeljagsrivier) 27%, and Farm 3 (Simondium) 10% infection. Of the flower parts, petals and stamens had higher levels of infection (58% and 55%, respectively), while pistils and sepals showed lower infection (11% and 9%). In addition to *B. cinerea*, various other fungal species were isolated and identified with DNA sequencing, including *Alternaria*, *Cladosporium*, *Colletotrichum*, *Diaporthe*, and *Botryosphaeriaceae* species. Rind distortion incidences were 43% for Farm 1, 34% for Farm 2 and 31% for Farm 3. *Botrytis cinerea* was detected from 11% of Vaseline slides with a range of 183 to 766 conidia per slide. Preliminary comparisons between PhytRisk predictions and observed infection levels indicate promising correlation, supporting the model's potential for assessing *B. cinerea* risk forecasting and disease management.

Keywords

epidemiology, qPCR, rind distortion, disease forecasting

Survey of citrus scabin different citrus production regions of South Africa

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The European Commission classified citrus scab pathogens *Elsinoë australis*, *E. fawcettii*, and *E. citricola* as regulated pathogens in March 2019. In the EU, symptomatic fruit identified among imported fruit are tested using *Elsinoë* genus-specific qPCR primers. If a fruit tests positive, the container is rejected. The aim of this study was to clarify the distribution of citrus scab caused by *E. fawcettii* in citrus production regions of South Africa. Citrus orchards were sampled for symptomatic fruit, leaves and twigs in Limpopo (9 orchards), Mpumalanga (4 orchards), and KwaZulu-Natal (7 orchards) during 2025. Orchards were sampled using a random survey approach inspecting trees in two rows in an orchard. Isolations were made from symptomatic plant material, DNA was extracted and *Elsinoë* isolates confirmed with species-specific PCRs and sequencing of the ITS and *rpb2* gene regions. Additionally, DNA was extracted from the plant material and screened with genus-specific qPCR primers. *Elsinoë fawcetti* was isolated from fruit, leaves, twigs, and flower receptacles in Limpopo (3 orchards) and KwaZulu-Natal (6 orchards). In total 78 isolates from 221 fruit, 93 leaves and 19 other plant parts were identified as *E. fawcettii* with an incidence of 8.7% of Eureka lemons, 4.8% of Red Ruby grapefruit, and 3.7% of Midnight Valencia oranges in samples from orchards in one production region in Limpopo. Eureka lemons in samples from orchards in two production regions in KwaZulu-Natal showed infection levels of 18.8% and 39.2%. Results from the qPCR *in planta* detection of *Elsinoë* are underway. In conclusion, *E. fawcettii* was confirmed in samples from some farms in a production region in Limpopo and two regions in KZN. The results contribute towards clarifying the distribution of *E. fawcettii*, within wider surveys.

Keywords

Elsinoë fawcettii, PCR, qPCR, Eureka lemons

Real-time monitoring of Sclerotinia stem rot development on canola using a novel, low-cost spore sampler and qPCR

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Ecology and Epidemiology

Abstract

Canola stem rot, caused by *Sclerotinia sclerotiorum*, is one of the two most economically important diseases affecting this crop in South Africa. Disease outbreaks are sporadic, underscoring the need for prediction tools in management strategies. Airborne *S. sclerotiorum* ascospores are the primary inoculum source, initiating infection through senescent petals. While petal infestation tests are indicative of infection risk, this method is too laborious to apply in prophylactic intervention strategies. Real-time ascospore monitoring is therefore essential. The high cost of existing commercial spore samplers limits widespread deployment thereof in research and industry. In this study, a novel low-cost inertial spore sampler was designed and field-tested at ten geographically diverse locations across South African canola production regions in the 2023 and 2024 production seasons. Quantitative PCR (qPCR) was used in conjunction with the sampler to detect and monitor *S. sclerotiorum* ascospore release. Silicone-coated acrylic rods were mounted as spore collection surfaces. Rods and inflorescences were sampled weekly during the flowering period to assess airborne inoculum and petal infection. A genomic DNA extraction and qPCR assay were optimised to detect ascospores from the rods and petals. In addition, petals were plated on Steadman's semi-selective media and monitored for signs of *S. sclerotiorum* infection. The pathogen was successfully detected with high sensitivity (~2 target DNA copies) from 25.6% and 77.2% of the sampled rods in 2023 and 2024, respectively. Petal infestation tests confirmed ascospore release and susceptible phenological stage alignment at all locations in 2024. The low-cost spore sampler developed enables mass deployment, improving statistical power in epidemiological and related research. Integration of pathogen and environmental data collected in this study is underway to further investigate disease development and identify outbreak risk factors. These insights may support establishing a regional warning system for Southern Cape canola producers, promoting timely and cost-effective disease intervention.

Keywords

Aerobiology, spore trapping, molecular detection, disease development, risk prediction

Comparison of Pathogenicity of *Fusarium circinatum* on Seedlings and Branches of *Pinus patula*

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Plant-Pathogen/Insect Interaction

Abstract

Pathogenicity trials are important tools used in plant pathology to prove causal relationships and evaluate the relative aggressiveness of pathogens. In the case of the fungus *Fusarium circinatum*, the causal agent of pine pitch canker, such trials inform both disease management strategies and host resistance studies. *Fusarium circinatum* can infect all life stages of *Pinus* spp., with infections in nursery seedlings often leading to high mortality rates due to damping-off, root, and collar rot. In mature trees, infection results in dieback and resinous cankers, significantly reducing timber quality and yield. Six-month-old *Pinus patula* seedlings are commonly used in South African pathogenicity trials. A constraint of these trials is that the plants are usually generated from seed implying high levels of genetic diversity and they can also differ markedly in their stages of development. In this study, we compared the pathogenicity of *F. circinatum* strains on *P. patula* seedlings and detached branches from three-year-old plantation-grown trees. In the branch inoculations, all isolates were tested on branches taken from the same trees, which were thus genetically the same. Lesion development as a result of *F. circinatum* inoculation was assessed across both trial types, using nine isolates with known levels of aggressiveness ranging low to high. While both methods showed consistent trends, lesion size was more variable in seedlings, likely reflecting natural host diversity. In contrast, branch assays produced more uniform results consistent with isolates having been tested on the same *P. patula* genotype. These findings suggest that branch assays offer greater reproducibility in studies where consistency is crucial, such as screening mutant strains or conducting genetic analyses. Seedling assays remain valuable to capture the full range of host-pathogen interactions in genetically diverse populations. This study highlights the complementary strengths of both approaches and contributes to refining pathogenicity testing methods for *F. circinatum*.

Keywords

Fusarium circinatum, Pathogenicity trial, *Pinus patula*, Lesion development, Genetic variability

Microsatellite markers developed to monitor populations of the root-rot pathogen *Ganoderma philippii*

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Amongst the various diseases of *Eucalyptus* in the in Indonesia, *Ganoderma* root rot, caused by the fungus *Ganoderma philippii*, has emerged as a significant problem. There are few genomic resources available to study *G. philippii* at the population level. Consequently, we sequenced the genome of *G. philippii* and developed polymorphic microsatellite markers for future epidemiology and genetic diversity analyses. Using Illumina and Oxford Nanopore sequencing, two draft genomes were produced from two isolates, which were 55.8 Mb and 53.5 Mb in size respectively. From the one genome, 1161 microsatellite regions were identified, and 36 were selected to design primers. Seventeen of these markers were tested across 24 isolates and optimized for use in multiplex PCR. The markers revealed 57 alleles and 14 multilocus genotypes (MLGs), and population analysis showed three distinct groups having different levels of diversity and clonality. Linkage disequilibrium results suggested that the fungus mainly reproduces clonally. This is the first study using whole-genome data to develop microsatellite markers for *G. philippii*, and only the second to produce such markers for this species. These new markers will be applied to study the population structure of the pathogen and to monitor its spread in plantations.

Keywords

Ganoderma root rot, Genome sequence, population genetics, single sequence repeats, plantation forestry

Controlling *Fusarium oxysporum*: mechanistic investigation of caffeic acid against crown and root rot

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Disease Management

Abstract

Crown and root rot, caused by *Fusarium oxysporum*, is a major agricultural challenge that leads to severe yield losses in economically important crops. The widespread use of chemical fungicides to manage this disease raises concerns about environmental sustainability, human health risks, and the emergence of resistant fungal strains. As a result, there is a growing need for eco-friendly and effective alternatives to conventional fungicides. A promising phenolic compound, caffeic acid (CA) has shown to have antioxidant, anti-inflammatory, and antimicrobial potential. This study aimed to evaluate the antifungal potential of CA as a sustainable biocontrol agent against *Fusarium oxysporum*. Using the food poison technique, we assessed the inhibitory effects of CA on fungal growth, while high resolution scanning electron microscopy was employed to examine morphological changes in fungal cells. Additionally, biochemical responses—including oxidative stress markers, antioxidant enzyme activity (superoxide dismutase and ascorbate peroxidase), and cell wall components (chitin and polysaccharides)—were analysed using spectrophotometric assays. The impact of CA on fungal pathogenicity was further investigated by measuring cellulase and lipase enzyme activity, and proteomic profiling was conducted to identify differentially expressed proteins associated with stress response, metabolism, and virulence. Our findings demonstrate that CA effectively inhibits the growth of *Fusarium oxysporum*, particularly at a higher concentration (1 mM). CA disrupts fungal cell membranes, induces oxidative stress, and increases cell death. Furthermore, it significantly reduces the activity of key fungal enzymes linked to pathogenicity, such as lipases and cellulases. Proteomic analysis displayed significantly altered proteins highlighting affected resistance mechanisms within fungal cells. These results suggest that CA possesses strong antifungal properties and holds promise as a natural alternative to synthetic fungicides. Its application in priming wheat seeds could enhance plant resistance to *Fusarium* crown rot infections. Further studies on its implementation in crop-protection strategies could provide practical benefits for sustainable agriculture.

Keywords

Disease, Management, Biocontrol agent, Proteomic analysis, Antioxidant enzymes

Three new grapevine trunk disease (GTD) pathogens discovered in Northern Cape vineyards

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Pathogen Identification, Characterisation and Taxonomy

Abstract

Grapevine trunk diseases (GTD) cause decline of vineyards in all grape-growing regions worldwide. Isolations were conducted from two 35-year-old vineyards in Upington, an area where GTD species are not well documented. Three fungal species were recovered from symptomatic material at high frequencies. The pathogenicity of these species on grapevines is unknown. The aim was to identify the species, determine pathogenicity and cultivar susceptibility. Isolates were identified morphologically and molecularly. Pathogenicity trials were conducted using eight representative isolates per species on green shoots and dormant canes of Cabernet Sauvignon vines. After five months, shoots were harvested, lesions measured, and isolations conducted to determine colonisation percentage. Cultivar susceptibility of nine economically important wine grape cultivars was assessed using the most virulent isolate of each species. *Didymella negriana*, *Pyrenochaeta* sp. and *Stilbocrea banihashemiana* were identified. For pathogenicity assays, one of the two positive controls (*Phaeomoniella chlamydospora*) caused the largest lesions in both green shoots (90.3mm) and canes (168.18mm). From green shoots, 22 isolates caused lesions significantly larger (38.34-56.39mm) than the negative control (*Acremonium* sp.: 26.91mm) and six *D. negriana*, five *S. banihashemiana* and three *Pyrenochaeta* sp. isolates were not significantly different to the other positive control (*N. parvum*: 55.62mm). From canes, all isolates caused lesions (49.15- 98.64cm) significantly larger than *Acremonium* sp. and all *D. negriana* and *Pyrenochaeta* sp. isolates and six *S. banihashemiana* isolates caused lesions similar to *N. parvum* (77.97mm). The cultivar susceptibility study is underway. This is the first report of *D. negriana* as GTD pathogen in South Africa and *S. banihashemiana* globally. A new *Pyrenochaeta* sp. was identified and found to be pathogenic on grapevines. Under-studied areas, such as Upington in the Northern Cape Province, may house other undocumented grapevine pathogens.

Keywords

Pathogenicity testing, *Vitis vinifera*, *Didymella negriana*, *Stilbocrea banihashemiana*, *Pyrenochaeta* sp.

The diversity and distribution of *Pythium* and *Phytophthora* species in South African soils

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Pathogen Identification, Characterisation and Taxonomy

Abstract

The oomycete genera *Phytophthora* and *Pythium* are well-known phytopathogens that cause devastating plant diseases including root rot, collar rot, leaf blight, and damping-off. These phytopathogens infect wide host ranges and pose serious threats to agriculture as their occurrence result in yield losses, economic struggles for farmers and a great impact on food security. This study is barcoding *Phytophthora* and *Pythium* isolates housed at The South African National Collection of Fungi (NCF and isolates collected from undisturbed soils in nature reserves and disturbed soils from agricultural systems in South Africa. Standard soil baiting and isolation of the oomycete were used to obtain isolates, DNA was extracted and their ITS rDNA sequences determined. The results generated in this study will enable a deeper understanding of *Phytophthora* and *Pythium* species diversity and evolutionary relationship.

Keywords

Oomycetes, Phytopathogens, Baiting, ITS*, Phytophthora*

Evaluating the efficacy of *Maerua angolensis* and *Tabernaemontana elegans* application in population dynamics of *Meloidogyne* spp., and their effects on the performance and growth of tomato plants under greenhouse conditions

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Plant-Pathogen/Insect Interaction

Abstract

Tomato (*Solanum Lycopersicon* L.) is amongst the most common vegetable crops grown in South Africa and an essential part of a healthy diet. Unfortunately, its production is negatively impacted by various pests, including the root-knot nematodes, *Meloidogyne javanica* and *Meloidogyne enterolobii*, which cause high yield losses. Plant extracts have been used as an alternative method in managing nematodes, however, the method comes with inconsistent results from several factors, among which is time of application, hence the study determined the effect of application time of *Maerua angolensis* and *Tabernaemontana elegans* on nematode population densities and tomato plant growth under greenhouse. The experiment was a factorial arrangement of 2 x 2 x 3 + 2 with the first factor being two plant extracts; second factor being two nematodes at 5000 eggs and juveniles per/plant; third factor being the application and inoculation times (plant extract applied before nematode inoculation; plant extract applied simultaneously with nematode inoculation and plant extract applied after nematode inoculation; positive (crop guard) & negative control) with 5 replications. At 56 days, plants and nematodes variables were measured. When each factor was examined independently, season significantly impacted J2 in tomato roots, the total number of nematodes in tomato root and the final nematode populations. Similarly, the time of plant extract application influenced J2 in tomato root and soil, total nematode numbers in tomato root, and the final nematode populations. In summer, the time of plant extract application demonstrated variable effects depending on the nematodes and season. The results confirmed that plant extracts should be applied in different application intervals.

Keywords

Solanum Lycopersicon, Root-knot nematodes, Plant extracts, Juveniles and Crop guard



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