



Maize–*Fusarium* associations and their mycotoxins: Insights from South Africa

Cobus M. Visagie^{a,*}, Hannalien Meyer^b, Neriman Yilmaz^a

^a Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

^b Southern African Grain Laboratory (SAGL), Grain Building-Agri Hub Office Park, 477 Witherite Street, The Willows, Pretoria, 0040, South Africa

ARTICLE INFO

Keywords:

Aflatoxins
Corn
Fungal ecology
Mycotoxigenic fungi
Taxonomy
Trichothecenes

ABSTRACT

For maize, a staple food in South Africa, there is a lack of comprehensive knowledge on the mycotoxin-producing fungal diversity. In this study, a fungal community profile was established using culture-dependent methods for 56 maize seed samples that were also analysed for 13 mycotoxins. The fungal isolates were identified by morphology and DNA sequencing. A total of 723 fungal isolates from 21 genera and 99 species were obtained and characterised. *Fusarium* was the most common genus (isolated from 52 samples), followed by *Cladosporium* (n = 45), *Aspergillus* (n = 41), *Talaromyces* (n = 40), and *Penicillium* (n = 38). *Fusarium* communities were dominated by the *Fusarium fujikuroi* species complex, which includes species such as *Fusarium verticillioides* and *Fusarium temperatum*, while *Fusarium awayx* and *Fusarium mirum* are reported here for the first time from South Africa. As for the deoxynivalenol (DON) producing species, only *Fusarium boothii* and *Fusarium graminearum* were isolated to a lesser extent. DON (n = 37), fumonisins (FUM) (n = 32), and zearalenone (ZEA) (n = 6) were detected. The presence of a particular species did not guarantee the presence of the corresponding mycotoxins, while the inverse was also true. The occurrence of DON and/or FUM in South African maize remains a health concern, so continuous monitoring of both fungal species and their mycotoxins is important.

1. Introduction

Although maize (*Zea mays*) is not native to the African continent, it has become a staple food for South Africans and is one of the country's most important crops. The beginnings of maize cultivation in South Africa can be traced back to Portuguese traders who brought maize seed from the Americas (Jeffreys, 1954; McCann, 2001; Sihlobo, 2016). By 2021, maize was the second most widely grown crop in South Africa after another C₄-carbon fixing grass species, sugar cane (DAFF, 2021). The country grows two main types of maize: white maize, which is mainly intended for human consumption, and yellow maize, which is mainly used as livestock feed. Cultivation extends across all provinces in South Africa, but the Free State, Mpumalanga and North West provinces make the largest contribution, together accounting for around 82% of total production (GrainSA, 2023). Almost 90% of South African maize is grown on dryland, with the remaining 10% produced under irrigated conditions (GrainSA, 2023). In the 2021/22 season, South Africa achieved a total maize production of 15,387,200 tonnes, of which 7,789,750 tonnes were white and 7,597,450 tonnes were yellow maize (GrainSA, 2023). Most of this is consumed locally, which emphasises the

critical importance of the domestic market for the industry. Locally produced maize is used as follows: 37.2% for human consumption, 39.2% in the animal feed industry and the remaining 23.6% for seed and industrial purposes (DAFF, 2021). Maize is one of the most widely consumed crops in South Africa. Ranum et al. (2014) estimated average maize consumption at 222 g per person per day, while Shephard et al. (2007) reported a higher figure of 400 g per person per day. In rural communities, however, household consumption can be as high as 1–2 kg per person per day (Burger et al., 2010).

Maize is susceptible to colonisation by filamentous fungi, with certain species that can produce harmful secondary metabolites known as mycotoxins (Munkvold et al., 2019). These mycotoxins can adversely impact the health of humans, livestock, and other animals, reduce crop yield, economically impact the agricultural and food sectors, and damage trade (Shephard, 2008). Among the more than 400 known mycotoxins (Cinar and Onbaşı, 2019), the few widely regulated internationally are also the most common contaminants and capable of causing acute and sub-acute toxic effects (Shephard et al., 2019; van Egmond et al., 2007). *Fusarium* and *Aspergillus* are the most important mycotoxigenic genera associated with maize (Munkvold et al., 2019).

* Corresponding author.

E-mail address: cobus.visagie@fabi.up.ac.za (C.M. Visagie).

<https://doi.org/10.1016/j.funbio.2024.03.009>

Received 1 January 2024; Received in revised form 26 March 2024; Accepted 27 March 2024

Available online 28 March 2024

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Fusarium species are associated with the production of fumonisins (FUM), trichothecenes like deoxynivalenol (DON), HT2-toxin, T2-toxin, etc., and zearalenone (ZEA) (Miller, 1995; Munkvold et al., 2019; Oldenburg et al., 2017), while aflatoxins are primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Frisvad et al., 2019). However, *Fusarium* can also produce emerging and masked (plant-derived conjugates) mycotoxins in maize (Ekwomadu et al., 2020) like beauvericin, enniatins, fusaproliferin, moniliformin, and monoacetoxyscirpenol (Oldenburg et al., 2017).

Recent studies have shown that the percentage of mycotoxin-contaminated grain has increased worldwide and is between 60 and 80% (Eskola et al., 2020). This has been partly attributed to the improved sensitivity of analytical detection methods and the effects of climate change (Eskola et al., 2020). It is predicted that the atmospheric CO₂ concentrations will double or triple (from 350 to 400 to 800–1200 ppb) over the next 25–50 years and that this will lead to a global temperature increase of 2–5 °C (depending on the level of industrial activity), more frequent droughts and extreme climatic events (Bebber and Gurr, 2015; Medina et al., 2015, 2017). These complex environmental dynamics are likely to have a profound impact on mycotoxigenic fungi and consequently affect mycotoxin production (Medina et al., 2015; Perrone et al., 2020). By 2050, the likelihood of aflatoxin contamination of maize will increase across Europe, particularly in southern regions (Focker et al., 2023). Battilani et al. (2016) have already documented growing regions in Spain, Italy and Greece, where there is a high probability of aflatoxin contamination. It is expected that these occurrences will migrate northwards to regions such as southern France, northern Italy and southern Romania due to the temperature rise of several degrees predicted for the coming decades (Battilani et al., 2016). Yu et al. (2022) have also predicted a similar trend for the USA and pointed out that aflatoxins, which is currently limited to the southern states, is likely to move further north into the Corn Belt.

Global warming is increasing the water-holding capacity of the atmosphere so the relative humidity and/or rainfall in many regions is becoming less predictable and higher or more frequent. All types of microorganisms, including fungi, depend on a sufficiently high water availability (increased relative humidity or high water activity) to proliferate, even for the most xerophilic such as many aspergilli (Pitt, 1975; Stevenson et al., 2015b, 2017a). In regions that become drier during climate change, crops can be stressed by drought and so become more susceptible to fungal infection. Furthermore, mycotoxin production can increase at water activities below those that correspond to the growth optimum of mycotoxigenic fungi (Marin et al., 1995, 2024). Therefore, increases in relative humidity and precipitation, and reduced predictability of weather events will almost certainly lead to increases in the outbreaks of maize-associated fungi.

Limited research has been undertaken to explore the potential impact of future climate change on mycotoxins in Africa. A study from Malawi alarmingly revealed that if climate predictions for the country materialised (hotter and drier), their growing regions were at higher risk of aflatoxin contamination (Warnatzsch et al., 2020). South Africa is characterised by low rainfall and frequent droughts, conditions that are favourable to mycotoxin production to persist in large part because plants are stressed under these conditions (Choruma et al., 2022). Despite aflatoxins generally not considered to be problematic in South Africa, particularly in commercially produced maize, there are signs pointing to a potential shift in the incidence and severity of mycotoxigenic *Fusarium* species and their associated mycotoxins (Meyer et al., 2019).

Research in South Africa has been somewhat limited when it comes to the need to comprehensively characterise the prevalence of mycotoxigenic fungal species and their associated mycotoxins. Previous studies often focused exclusively on mycotoxin occurrences or the *Fusarium* species present in maize. Species identifications were mostly based on morphological observations, but some detections were based on molecular methods such as species-specific real-time PCR tests

(Beukes et al., 2017; Ekwomadu et al., 2021; Meyer et al., 2019; Mngqawa et al., 2016; Rheeder et al., 2016). These approaches present challenges such as potential misidentifications, especially in groups that contain cryptic species, and the failure to account for the co-occurrence of species in diverse *Fusarium* communities (Laraba et al., 2021; Yilmaz et al., 2021). Despite past investigations into mycotoxin contamination of South African maize (Ekwomadu et al., 2020, 2021; Gruber-Dorninger et al., 2018; Janse van Rensburg et al., 2014; Meyer et al., 2019), a gap exists in correlating this with fungal species or overall diversity, while one can argue that knowledge on what species occurs in South African maize is outdated due to recent changes in the taxonomy of, for example, *Aspergillus* and *Fusarium* (Crous et al., 2021; Samson et al., 2014). Continuous surveys documenting what species and which mycotoxins occur in particular maize-growing regions, will be essential to investigate the effects that climate change can have on fungal communities and how that impacts its ability to produce mycotoxins. This paper therefore reports on the fungal species and mycotoxins detected in pre-stored South African maize.

2. Materials and methods

2.1. Sample collection

South African white and yellow maize kernels were collected post-harvest but pre-storage (from the 2018/19 production season) in the second half of 2019 when producers delivered their maize crop at the commercial grain storage facilities. A representative sample from each consignment was taken for grading purposes. Approximately 100 g of each sample was placed in a bin at silos according to the grade awarded. A 2.5 kg subsample of each full bin, was sent to the South African Grain Laboratory (SAGL; ISO/IEC17025 accredited). In total, 350 collected maize samples (500 g each) were analysed for mycotoxins, representing all the production regions and white and yellow maize proportionally. The kernels were milled with a 1 mm sieve (Retsch ZM 200 mill), mixed thoroughly and then kept dry at ~21 °C in tightly sealed containers for mycotoxin analyses in 2020 and fungal isolation. Of the 350 samples obtained, 56 were selected for the isolation of the fungal strains. These were 31 yellow-maize samples and 25 white-maize samples that, collectively, came from seven of the maize-production regions.

2.2. Isolation of fungal strains

For fungal isolations, milled maize samples were sprinkled directly onto Potato Dextrose Agar (PDA; Neogen NCM0018), Dichloran-Glycerol Agar (DG18; Oxoid CM0729), Water Agar (WA; Oxoid LP0011) and *Fusarium* Selective Medium (FSM; Leslie and Summerell (2006)). The water activity of these media is, respectively, ~0.994 (Cray et al., 2016), ~0.966 (Hallsworth et al., 1998), ~1, and 0.995 (Medina and Magan, 2010). The media were supplemented with chloramphenicol (100 ppm) and streptomycin (50 ppm) to prevent bacterial growth. The isolation plates were incubated at 21 °C for 5 d. Fungal growth were subsequently observed using a Zeiss Discovery V8 stereomicroscope (Carl Zeiss CMP, Göttingen, Germany), and transferred into pure culture onto 1/4 strength PDA (*Aspergillus* on DG18 and *Penicillium* on MEA). These plates were incubated at 25 °C for 7 d. The cultures were grouped and, if possible, identified to genus by morphology. Single spore cultures were prepared for *Fusarium* and *Trichoderma* following Leslie and Summerell (2006). The strains were accessioned into the laboratory culture collection of the Applied Mycology group at the Forestry and Agricultural Biotechnology Institute (FABI, University of Pretoria, South Africa) and stored as spore suspensions or agar plugs in 15 % w/v glycerol at –80 °C. Some strains were also deposited in the culture collections (Collection Mike Wingfield (CMW) and Collection Mike Wingfield at Innovation Africa (CMW-IA)) of FABI. The strains, their accession numbers and origin are detailed in [Suppl. Table 1](#).

2.3. DNA extraction, sequencing and identifications

The strains were grown on MEA for 7 d and DNA extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA). Extracted DNA was then stored at -20°C .

PCR amplifications focused on gene regions that are taxonomically informative for the isolated genera, including the nuc rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS), the partial beta-tubulin gene (*BenA*), the partial calmodulin gene (*CaM*), the partial glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*), the partial translation elongation factor 1-alpha gene (*TEF*), the partial RNA polymerase II second largest subunit gene (*RPB2*) and the partial actin gene (*ACT*). *BenA* was used as an identification marker for *Penicillium*, *Nigrospora* and *Talaromyces*, *CaM* for *Aspergillus*, *GAPDH* for *Alternaria* and *Bipolaris*, *ACT* for *Sarocladium*, *TEF* for *Alternaria*, *Cladosporium*, *Clonostachys*, *Fusarium*, *Nigrospora*, *Stenocarpella* and *Trichoderma* and ITS for the remaining genera. For some strains, additional gene regions were sequenced where appropriate (e.g. *BenA*, *CaM*, *RPB2* for some *Aspergillus*, *Penicillium* or *Talaromyces*). Table 1 summarises the sequenced gene regions with the primer pairs and PCR conditions used for amplification. Each PCR was prepared in 25 μl total volumes containing 0.15 μl MyTaq DNA polymerase (Bioline, Meridian Bioscience, Memphis, Tennessee), 5 μl 5x MyTaq Reaction Buffer (BioLine), 0.5 μl of each primer (10 μM), 0.5 μl template DNA and 18.35 μl MilliQ H_2O .

After amplification, Sanger sequencing reactions were performed in both directions using the BigDye Terminator 3.1 Ready Reaction Mix (PerkinElmer, Warrington, UK) using the same primers as for PCR. The sequencing reactions were then analysed at the DNA Sanger Sequencing Facility (Faculty of Natural and Agricultural Sciences, University of Pretoria) on an ABI PRISM 3500xL autosequencer (Applied Biosystems, Foster City, California). Sequence contigs were generated in Geneious Prime v. 2023.2.1 (BioMatter, Auckland, New Zealand).

Initial identifications were based on BLAST search comparisons against the NCBI nucleotide database (<https://www.ncbi.nlm.nih.gov/nucleotide/>) and in the case of ITS against the NCBI Fungal ITS Reference Sequence Targeted Loci Project database (Schoch et al., 2014). Final identifications were based on comparisons with a reference sequence dataset published in taxonomic revisions. These included studies on *Alternaria* (Woudenberg et al., 2013, 2015), *Anthracozytis* (Piątek et al., 2015), *Aspergillus* (Houbraken et al., 2020; Samson et al., 2014), *Cladosporium* (Bensch et al., 2012), *Penicillium* (Houbraken et al., 2020; Visagie et al., 2014), *Sarocladium* (Giraldo et al., 2015; Summerbell et al., 2011), *Stenocarpella* (Lamprecht et al., 2011), *Talaromyces* (Houbraken et al., 2020; Yilmaz et al., 2014) and *Trichoderma* (Bissett et al., 2015). *Fusarium* strains were identified using the FUSARIOID-ID database (<https://www.fusarium.org/>) (Crous et al., 2021). Datasets were aligned using the G-INS-i setting in MAFFT v. 7.490 (Katoh and Standley, 2013) and manually trimmed and adjusted in Geneious Prime where necessary. Each dataset was analysed using Maximum Likelihood (ML) in IQtree v. 2.2.2.7 (Minh et al., 2020) and applying the General

Time Reversible nucleotide substitution model with gamma distribution with invariant sites (GTR + G + I). Datasets were partitioned based on introns, exons and codon positions. Confidence in nodes was calculated using bootstrap analyses with 1000 replicates. Trees were visualised in TreeViewer v. 2.2.0 (<https://treeviewer.org/>) and edited in Affinity Publisher v. 2.2.1 (Serif (Europe), Nottingham, UK). All files related to phylogenetic analyses were uploaded to the University of Pretoria research data archive hosted on Figshare (10.25403/UPresearchdata.24921609).

We assessed the phylogenetic relationship between species previously described in the *Talaromyces funiculosus* species complex, including *T. cucurbitiradicus*, *T. funiculosus* and *T. pseudofuniculosus* (Guevara-Suarez et al., 2019; Su and Niu, 2018; Yilmaz et al., 2014). A DNA reference sequence database was prepared that included ITS, *BenA*, *CaM*, *RPB2*, *RPB1*, *Cct8* and *Tsr1*. *RPB1*, *Cct8* and *Tsr1* were extracted from unpublished genomes of strains generated in our research group. Phylogenies were prepared as explained above and we applied the genealogical phylogenetic species recognition concept (GCPSR) to the species complex (Taylor et al., 2000).

2.4. Mycotoxin analyses

A panel of 13 mycotoxins (aflatoxins B₁, B₂, G₁ and G₂, FUM B₁, B₂ and B₃, DON, 15-acetyl-deoxynivalenol (15-ADON), ochratoxin A (OTA), T2-toxin, HT2-toxin and ZEA) were tested with a validated LC-MS/MS method. A standard (10 $\mu\text{g}/\text{mL}$) was prepared using stock solutions made from solid mycotoxin standards of all four aflatoxins, DON, 15-ADON, FB₁, OTA, T-2, ZEA, HT-2 (Romer Labs Diagnostic GmbH; Tulln, Austria) and FB₂ and FB₃ (Cape Peninsula University of Technology; Cape Town, South Africa). For quantitative analyses maize matrix-matched standards were prepared from the 10 $\mu\text{g}/\text{mL}$ standard using maize samples not contaminated with the analysed mycotoxins (Meyer et al., 2019).

Ten gram subsamples (in duplicate from each sample) were extracted (40 mL extraction solution, methanol/acetonitrile/water (1:1:2, v/v/v)) (MeOH for HPLC, >99.9%; acetonitrile HPLC grade, Burdick and Jackson, Ultra-pure water (<18,2 M Ω cm). The samples were blended for 1 min followed by 15 min extraction (in 50 mL polypropylene centrifuge tubes) on a mechanical shaker in a horizontal position, then centrifuged for 10 min at 3000 rpm. An aliquot (5 mL) of this sample extract was diluted with 5 mL methanol/ H_2O (1:3, v/v). The diluted sample extracts were filtered (13 mm, 0.22 μm nylon syringe filters) into HPLC amber vials for the LC-MS/MS analyses by injecting 5 μL (Meyer et al., 2019).

Liquid chromatography mass spectrometry (LC-MS/MS) analysis was carried out on an ultra-performance liquid chromatograph (Waters Acquity UPLC, Waters Corp. Massachusetts, USA) with a C₁₈ column (Waters Acquity UPLC BEH, 1.7 μm , 50 \times 2.1 mm ID) at 30 $^{\circ}\text{C}$ connected to a tandem (triple) quadrupole mass spectrometer (Waters Acquity TQD). A programmed gradient elution comprising mobile phase A (Milli-Q water with 0.5 mM ammonium acetate (purity $\geq 98\%$),

Table 1

Identification markers used to make species identifications. Included are primer pairs and PCR cycle conditions used to amplify markers.

Identification marker	Genus	Primers	Annealing temp (C)	Cycles	Citation
<i>ACT</i>	<i>Sarocladium</i>	Act1 & Act4	52	35	Voigt and Wöstemeyer, 2000
<i>BenA</i>	<i>Aspergillus</i> , <i>Penicillium</i> , <i>Nigrospora</i> and <i>Talaromyces</i>	T10 & Bt2b	55	35	O'Donnell and Cigelnik, 1997; Glass and Donaldson, 1995
<i>CaM</i>	<i>Aspergillus</i> , <i>Penicillium</i> and <i>Talaromyces</i>	CMD5 & CMD6	55	35	Hong et al., 2005
<i>GAPDH</i>	<i>Alternaria</i> and <i>Bipolaris</i>	GPD1 & GPD2	48	35	Berbee et al., 1999
ITS	General	V9G & LS266	55	35	de Hoog and Gerrits van den Ende, 1998; Masclaux et al., 1995
<i>RPB2</i>	<i>Aspergillus</i> , <i>Penicillium</i> and <i>Talaromyces</i>	RPB2-F1 & RPB2-7 C R_1	48	35	Houbraken et al., 2020
<i>TEF</i>	<i>Alternaria</i> and <i>Fusarium</i>	EF1 & EF2	52	35	O'Donnell et al., 1998
<i>TEF</i>	<i>Cladosporium</i> , <i>Clonostachys</i> , <i>Nigrospora</i> , <i>Stenocarpella</i> and <i>Trichoderma</i>	EF1-728F & EF2	52	35	Carbone and Kohn, 1999, O'Donnell et al., 1998

Sigma–Aldrich/Merck) and 0.1% formic acid (98–100%, Suprapur, Merck), and mobile phase B (acetonitrile with 0.1% formic acid) at a column flow rate of 0.4 mL/min from an A:B ratio of 90:10 to an A:B ratio of 10:90 in 15 min for the separation of the 13 mycotoxins. The sample injection volume was set at 5 μ L. A standard calibration curve with at least six concentrations was constructed (linear, 1/x weighted, origin excluded) for each mycotoxin (Meyer et al., 2019). The samples were analysed in duplicate, and the mean values were reported (SAGL, 2020).

3. Results

3.1. Fungal identifications

Isolations from the 56 pre-stored maize samples resulted in 723 strains accessioned and stored in the culture collections housed at FABI. From these, 964 new DNA reference sequences were generated and submitted to GenBank [ITS (n = 52), *BenA* (n = 226), *CaM* (n = 199), *GAPDH* (n = 6), *TEF* (n = 380), *RPB1* (n = 6), *RPB2* (n = 80), *ACT* (n = 3), *Cct8* (n = 6) and *Tsr1* (n = 6)].

Strains were identified to 99 species representing 21 genera, 17 families, 10 orders and 3 phyla. We represent the fungal community from the 56 maize samples, based on the number of samples that a particular genus was detected from (Fig. 1). At the genus level, *Fusarium* (52/56), *Cladosporium* (45/56), *Aspergillus* (41/56), *Talaromyces* (40/56), and *Penicillium* (38/56) were the most common. *Penicillium* (n = 30) was the most species rich, followed by *Aspergillus* (n = 20), *Fusarium* (n = 13), *Talaromyces* (n = 8) and *Cladosporium* (n = 6) (Figs. 2 and 3). The aflatoxin-producing *A. flavus* and *A. parasiticus* were detected in 15 samples, but only sample MO 401 contaminated by *A. parasiticus* had detectable aflatoxin levels.

Within *Fusarium*, the *Fusarium fujikuroi* species complex (FFSC) was the most common, isolated from 51/56 samples and represented by 256 of the 277 *Fusarium* strains. Six FFSC species were identified with *F. verticillioides*, *F. temperatum* and *F. sugluinans* being the most common, while the recently described *F. awaxy* (Crous et al., 2019) and *F. mirum* (Costa et al., 2022) are reported for the first time from South Africa. The *Fusarium sambucinum* species complex (FSAMSC: 8/56 samples), *Fusarium chlamydosporum* species complex (FCSC: 6/56 samples), *Fusarium*

oxysporum species complex (FOSC: 3/56 samples) and *Fusarium incarnatum-equiseti* species complex (FIESC: 1/56 samples) were isolated in relatively low numbers.

Several strains could not be identified to any known species and likely represent new species in *Anthracoctis*, *Penicillium*, *Stagonosporopsis* and *Thyridium*, while we also found a potential new genus in *Pleosporales*.

3.2. Phylogenetic revision of the *T. funiculosus* species complex

Nine strains isolated from maize were found to belong in the *Talaromyces funiculosus* species complex. Our dataset contained 31 taxa with *T. pinophilus* selected as outgroup. The *BenA*, *CaM*, *Cct8*, *RPB1*, *RPB2* and *Tsr1* alignments were 433, 483, 988, 2079, 907 and 2128 bp long, respectively. Phylogenetic analyses of six gene regions resulted in phylogenies with incongruent topologies between *CaM*, *RPB2*, *Cct8* and *Tsr1*, while *RPB1* showed very little variation between these species (Fig. 4). Applying GCPSR we thus consider *T. cucurbitiradicus* and *T. pseudofuniculosus* as synonyms of the older name *T. funiculosus*.

3.3. Mycotoxins

Of the 56 pre-stored white and yellow maize samples selected from the 2018/19 production season, 47 were contaminated by mycotoxins, with 28 of these samples containing more than one mycotoxin (See Table 2). All samples tested negative for HT2-toxin, T2-toxin and OTA. Of the 25 white maize samples, no mycotoxins were detected in two samples (MO 328 and MO 530). Of the 31 yellow maize samples, no mycotoxins were detected in seven samples (MO 56, MO 68, MO 74, MO 81, MO 113, MO 136, and MO 207).

Aflatoxin B₁ (48 μ g/kg) and G₁ (95 μ g/kg) were detected in a single white maize sample (MO 401) from the Free State province. The most prevalent mycotoxins detected in the analysed maize samples included DON, 15-ADON, FUM (FB₁, FB₂, FB₃) and ZEA, which are commonly produced by *Fusarium* species. DON and FUM co-occurred in 22 samples. A summary of the results with concentration ranges, and mean concentrations is presented in Table 3.

Deoxynivalenol was detected in 19 out of 25 white maize samples, with two of these exceeding the 2000 μ g/kg regulated level for human

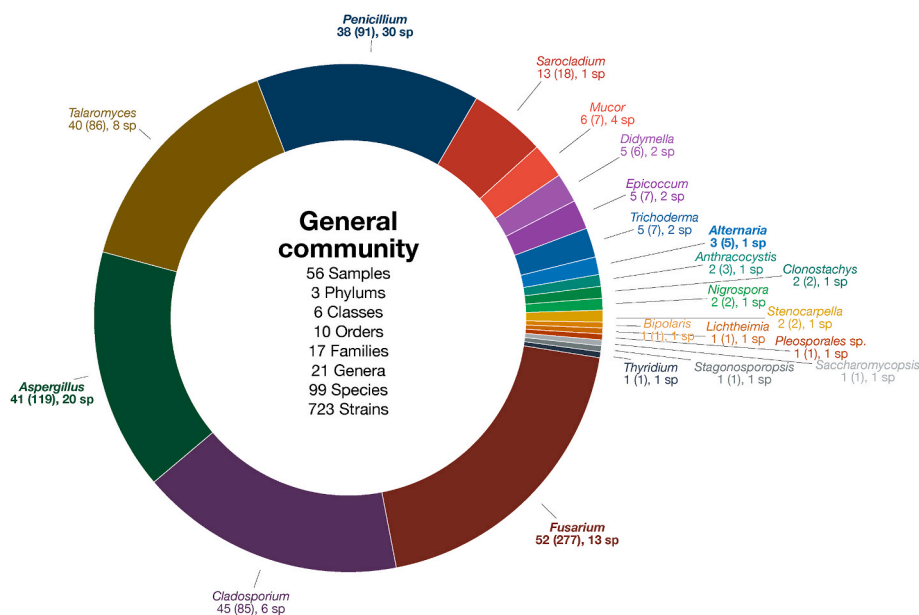


Fig. 1. Pie chart summarising the fungal community associated with pre-stored maize in South Africa. The proportions are based on the number of samples from which each genus was isolated. The numbers in the legends stand for [x (y), z sp] x = number of samples from which each genus was identified, y = number of strains isolated, z number of species identified. Mycotoxigenic genera appear in bold text.

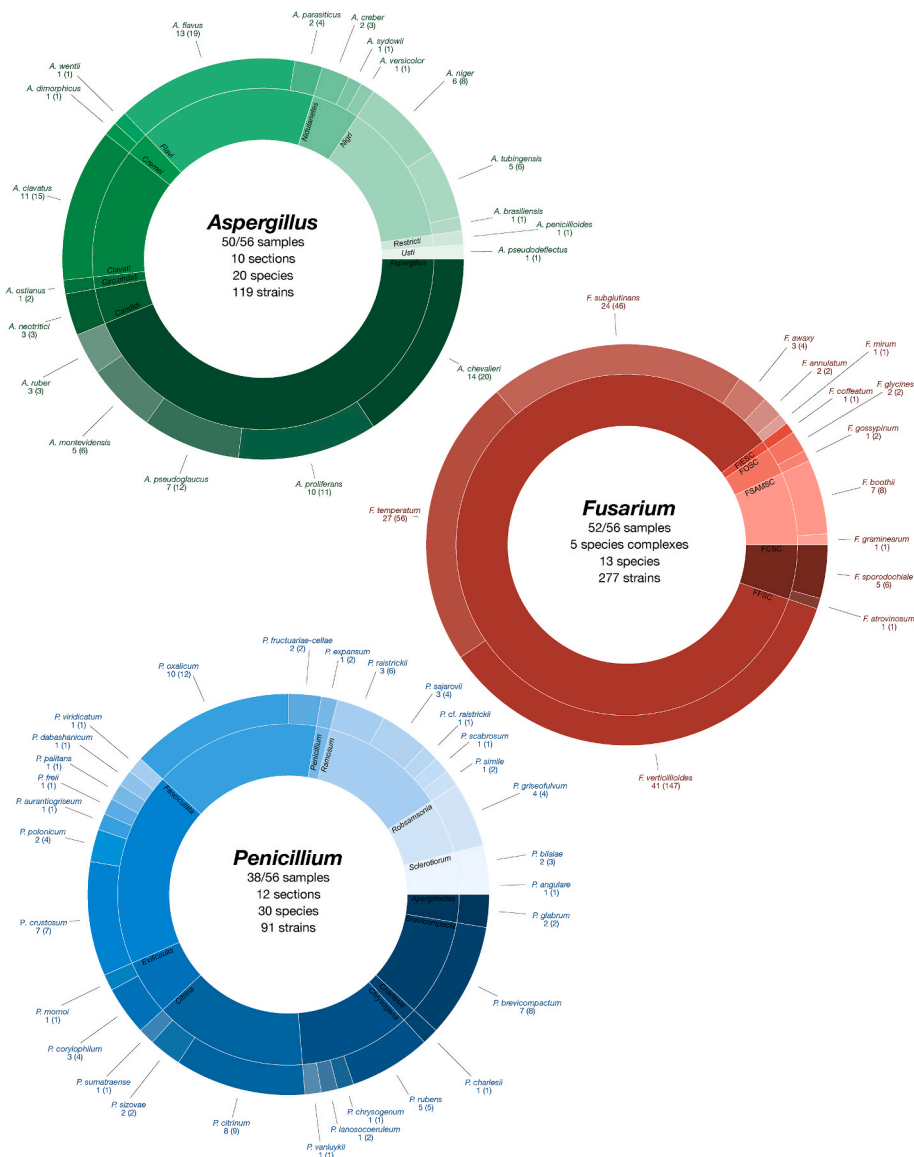


Fig. 2. Pie chart summarising the *Aspergillus* (green), *Fusarium* (red) and *Penicillium* (blue) communities associated with pre-stored maize in South Africa. The proportions are based on the number of samples from which each species was isolated. The numbers in the legends stand for [x (y)] x = number of samples from which each species was identified, y = number of strains isolated. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

consumption (MO 517 = 2410 $\mu\text{g}/\text{kg}$ and MO 603 = 3604 $\mu\text{g}/\text{kg}$). In yellow maize, DON was detected in 18 out of 31 samples, all of which were below the South African regulations. 15-ADON co-occurred in 8 out of 25 DON contaminated white maize samples, all with DON >400 $\mu\text{g}/\text{kg}$. The 15-ADON concentrations ranged from 103 to 593 $\mu\text{g}/\text{kg}$. In yellow maize, 15-ADON was only detected in one sample (MO 552) at a level of 101 $\mu\text{g}/\text{kg}$.

Fumonisins were detected in 32/56 samples, collected from all producing provinces. Twelve of the white maize samples had FUM levels (total = $\text{FB}_1 + \text{FB}_2 + \text{FB}_3$) between 22 and 1303 $\mu\text{g}/\text{kg}$, and 20 yellow maize samples had FUM levels between 23 and 1703 $\mu\text{g}/\text{kg}$, which is below the South African maximum allowable $\text{FB}_1 + \text{FB}_2$ level of 4000 $\mu\text{g}/\text{kg}$ in unprocessed maize intended for human consumption.

Only six samples were contaminated with ZEA, including two white maize (31 $\mu\text{g}/\text{kg}$ (MO 603) and 70 $\mu\text{g}/\text{kg}$ (MO9)) and four yellow maize that ranged from 26 to 957 $\mu\text{g}/\text{kg}$ (MO145, MO8, MO137 and MO86). The six samples with ZEA also contained DON.

4. Discussion

This study reports on the fungal species and mycotoxins in 56 pre-stored maize samples collected in South Africa. The fungal communities were diverse: 723 strains were identified to 99 species and 21 genera. *Fusarium*, *Cladosporium*, *Aspergillus*, *Talaromyces* and *Penicillium* were well represented in the communities. *Fusarium* was present in 52 of the 56 samples, with the FFSC dominating the community. As discussed by Gargouri et al. (2024) in their study of mycotoxigenic fungi of Tunisian maize, *Fusarium* species (especially *F. verticillioides*) exhibit the characteristic traits of microbial weed species (Hallsworth et al., 2023b). In the current study, the most frequently isolated species were *F. verticillioides*, *F. temperatum* and *F. subglutinans*.

The fungal community was largely dominated by xerotolerant/xerophilic species. These fungi can grow at low equilibrium relative humidity or water activity, which is a measure of the amount of available water in a given environment (Flannigan and Miller, 2011; Pitt, 1975; Scott, 1957). There is some debate as to what constitutes a xerophile, but the most widely cited definition is a fungus that grows at a

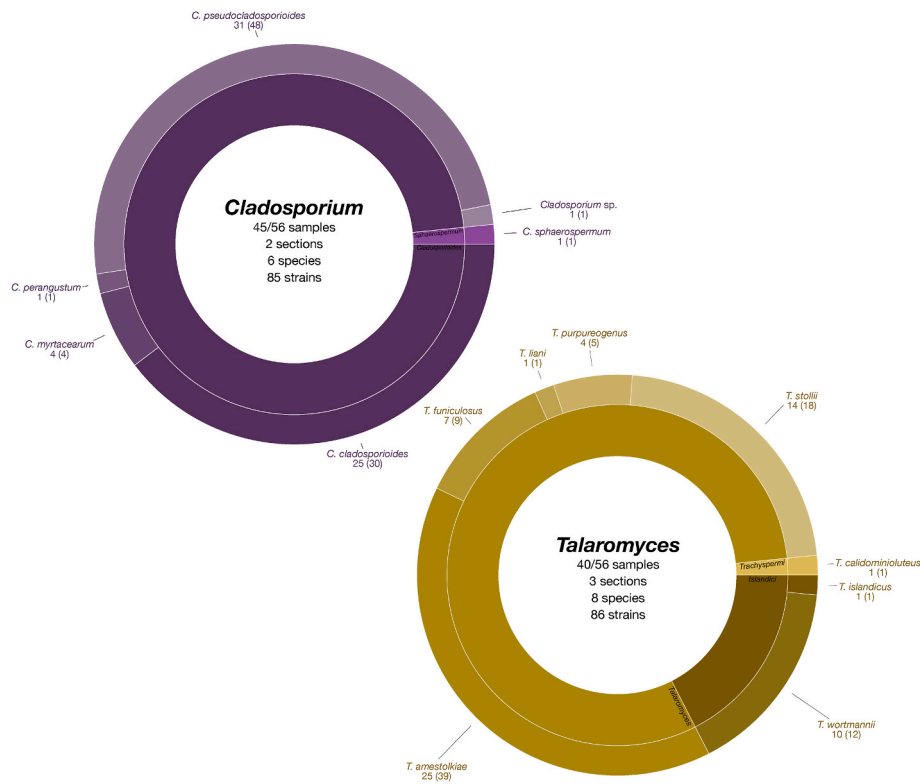


Fig. 3. Pie chart summarising the *Cladosporium* (purple) and *Talaromyces* (olive) communities associated with pre-stored maize in South Africa. The proportions are based on the number of samples from which each species was isolated. The numbers in the legends stand for [x (y)] x = number of samples from which each species was identified, y = number of strains isolated. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

water activity lower than 0.85 (Pitt, 1975). In contrast, evidence suggests that the vast majority of microbes cannot grow below ~0.9 water activity (Brown, 1990). South African maize is usually dried in the field and harvested at a moisture content of 12.5–14% w/w, after which it is stored in silos until it is milled. Even though moisture content and water activity are two different concepts and they are not interchangeable, South African pre-stored maize has little water available. Almost all *Aspergillus*, *Cladosporium*, and *Penicillium* are at least xerotolerant, and many are xerophilic. For example, the *Aspergillus* community was dominated by species from section *Aspergillus* (e.g., *A. chevalieri*, *A. proliferans*, *A. pseudoglaucus*, etc.), which can grow at a water activity of 0.7 or higher (Samson et al., 2019), with only a few aspergilli capable of growth below this (Hallsworth, 2019; Stevenson et al., 2015a, 2017b, 2017c). Other sections identified in our study typically grow at water activity below 0.9 (Samson et al., 2019). This growth characteristic also applies to *Penicillium*, while the commonly isolated *Cladosporium cladosporioides*, and *C. pseudocladosporioides* can grow at water activities of 0.85–0.87 (Segers et al., 2015). *Talaromyces* is generally more sensitive to low water activities (Pitt, 1980; Yilmaz et al., 2014), but the species we identified are considered xerotolerant. *Fusarium* is also not generally considered xerophilic. However, several species associated with food spoilage (e.g. *Fusarium avenaceum*, *F. graminearum*, *F. oxysporum*, *F. verticillioides*, etc.) have been reported to grow at water activity 0.85–0.89 (Samson et al., 2019). This means that the fungi that dominate the communities in our analysed samples are known to be able to grow at the low moisture content at which our maize is harvested which presumably also has a relatively low water activity. The fact that the core community is at least xerotolerant and many of them are xerophilic emphasises the need to use the correct isolation media that will allow the isolation of fungi representative of the communities.

Talaromyces funiculosus (\equiv *Penicillium funiculosum*) was first described by Thom (1910). Its colonies have a characteristic funiculose

texture formed by conidiophores produced on a rope of fertile hyphae. The species is reported to be common in foods, especially cereals, fruits and nuts (Samson et al., 2019). Following the taxonomic redefinition of *Talaromyces* by Samson et al. (2011), Yilmaz et al. (2014) monographed the 88 species accepted at the time and characterised them by morphology and phylogenetics. Since then, *T. cucurbitiradicus* and *T. pseudofuniculosus* have been introduced as close relatives of *T. funiculosus* (Guevara-Suarez et al., 2019; Su and Niu, 2018). Morphologically they were similar, but *T. cucurbitiradicus* was reported to produce chlamydoconidia. Phylogenies based on a limited number of gene regions and strains, resolved strains into three distinct clades representing *T. funiculosus*, *T. cucurbitiradicus* and *T. pseudofuniculosus*. However, in our study, nine strains were isolated from seven samples that belonged to this group. In our phylogenetic analyses, which included more gene regions and a larger sample than used before, we found that only *BenA* supported the three species. *CaM* and *RPB2* resolved some *T. funiculosus* strains (identified based on *BenA*) with strains of either *T. cucurbitiradicus* or *T. pseudofuniculosus*, while *RPB1*, *Cct8* and *Tsr1* showed little variation among strains and thus neither supported nor refuted the three species hypothesis. Applying GCPSR, we accept only one species in this complex, with *T. funiculosus* that has priority over *T. cucurbitiradicus* and *T. pseudofuniculosus*. The description of new species is important and even if we reduce *T. cucurbitiradicus* and *T. pseudofuniculosus* as synonyms of *T. funiculosus*, without their descriptions we would not have the additional morphological, distributional or substrate data that now apply to *T. funiculosus*. Furthermore, the sequence variation now recorded for *T. funiculosus* will also help to facilitate its future identification. Likewise, the large number of sequences we produced and published on NCBI-Genbank will contribute to more robust identifications in future.

Mycotoxins were detected in 47 of the samples, with 28 containing multiple mycotoxins. The levels of mycotoxins detected were below the

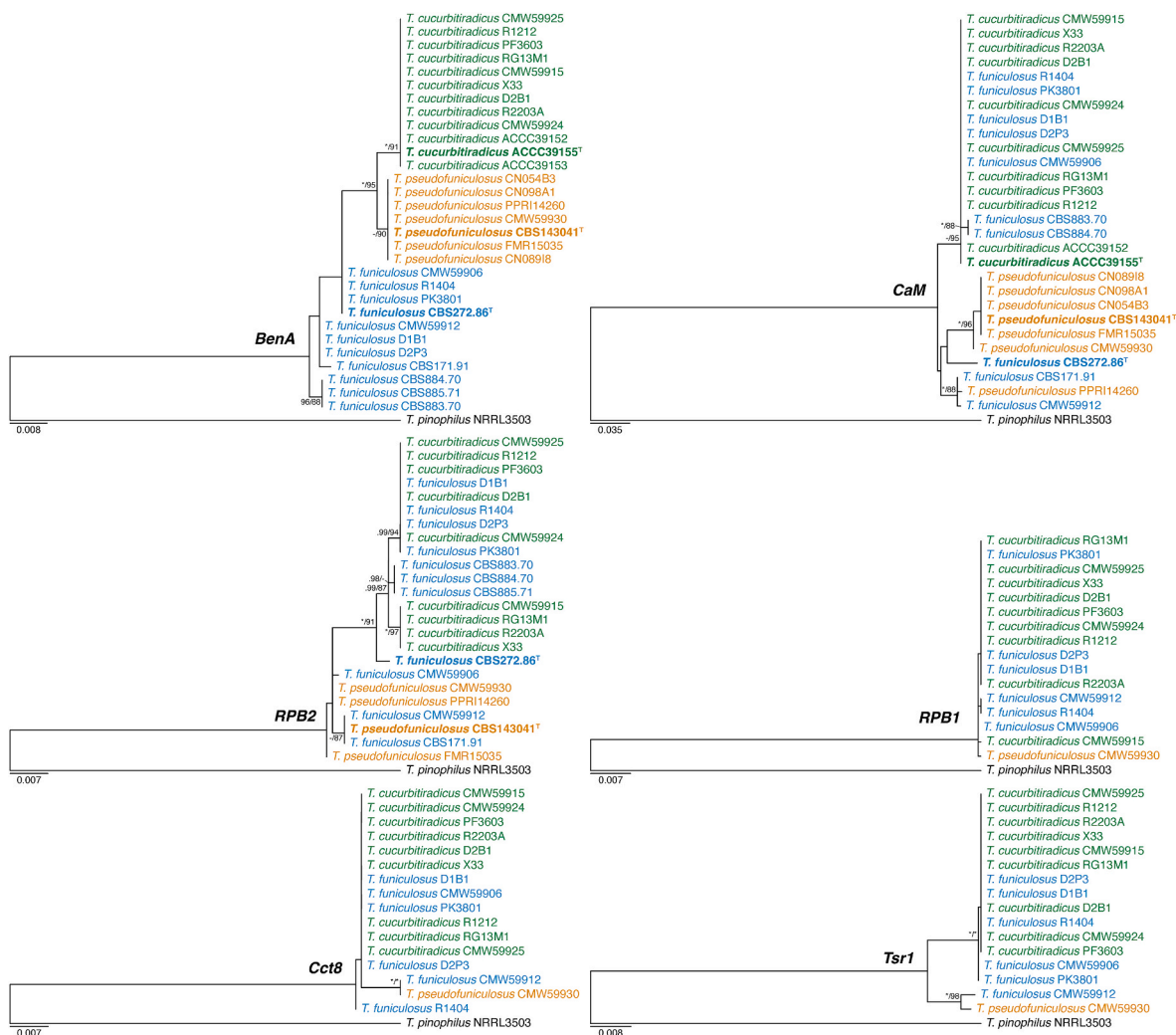


Fig. 4. Phylogenetic trees of the *Talaromyces funiculosus* species complex (including *T. cucurbitiradicus*, *T. funiculosus* and *T. pseudofuniculosus*) based on *BenA*, *CaM*, *RPB2*, *RPB1*, *Cct8* and *Tsr1*. Strains appear in colored text that match their original identification based on *BenA*. Branch support in nodes higher than 80% BS and/or 0.95 PP are indicated at relevant branches (^T = ex-type; * = 100% BS or 1.00 PP; – = support lower than 80% BS and/or 0.95 PP).

maximum allowable levels for South Africa, except for a couple of samples above these limits. We did not detect any HT2-toxin, T2-toxin or OTA in our 2018/19 maize samples. This is similar to Meyer et al. (2019), who also did not detect these during a 4-year study from 2013 to 2017 in South African commercial maize. We also did not identify any fungi known to produce these compounds in maize. Kiš et al. (2021) reported HT2-toxin and T2-toxin from European maize. Ekwomadu et al. (2021) detected OTA in South African maize, reporting contamination rates of 97.8% and 93.0% in small-scale and commercial farming sector samples, respectively.

Aflatoxins pose a significant concern for maize production on the African continent (Gargouri et al., 2024; Meijer et al., 2021), but South Africa is relatively fortunate as our aflatoxin levels in maize are either below the prescribed limits or are rarely detected (Meyer et al., 2019). We identified *A. flavus* and *A. parasiticus* from 13 and two maize samples, respectively, but only one of the 2018/19 growing season samples tested positive for aflatoxins. On the contrary, Ekwomadu et al. (2021) analysed maize from South African markets and storage silos representing both the smallholder and commercial farming sectors and detected aflatoxins in about 25% of the samples. The knowledge that aflatoxin-producing species are present in our maize, but that aflatoxins are not common prior to storage, suggests that improper harvesting and storage practices may have been the cause of the aflatoxin contamination detected by Ekwomadu et al. (2021).

Forty-seven samples contained at least DON, 15-ADON, FUM or ZEA. Fumonins were present in 32 of the 56 samples across all provinces. This corresponded well with the identified fungi. Several species belonging to FFSC is known to produce FUM (Yilmaz et al., 2021), but in our study we only detected *F. verticillioides* that was isolated from 41 of samples. *Fusarium temperatum* and *F. subglutinans* also belong to the FFSC and although some have reported that they produce FUM (Munkvold et al., 2019; Scauflaire et al., 2012; Tagele et al., 2019), others have reported that they do not (Fumero et al., 2020; Pfordt et al., 2020). The Eastern Cape province of South Africa is of historical importance as the first discovery of FUM was made there (Gelderblom et al., 1988). Since its discovery, numerous studies have reported the presence of FUM in South African maize (Ekwomadu et al., 2020, 2021; Gruber-Dorninger et al., 2018; Janse van Rensburg et al., 2014; Meyer et al., 2019; Shephard et al., 2013). A recent ear rot survey did not isolate *F. verticillioides* and did not detect FUM in any Eastern Cape samples (Price et al., 2024). *Fusarium temperatum* and *F. subglutinans* were frequently identified in our study, and together with *F. verticillioides* can also produce emerging mycotoxins such as beauvericin or enniatins (Munkvold et al., 2019; Scauflaire et al., 2012). Although emerging mycotoxins were not investigated in our study, their occurrence should be monitored more closely in the future. We identified two additional FFSC species for the first time in South Africa. *Fusarium awaxy* was described from decayed stalks of *Z. mays* in Brazil and later identified in

Table 2
Mycotoxins present in pre-stored maize samples collected across South Africa.

Sample description		W/ Y ^a	Mycotoxin results (µg/kg)										Mycotoxigenic genera important in maize ^c		
Location	Sample number		Aflatoxins (LOQ ^b 5 µg/kg)					Fumonisin (LOQ 20 µg/kg)				DON (LOQ 100 µg/kg)	15-ADON (LOQ 100 µg/kg)	ZEA (LOQ 20 µg/kg)	
			AFLA B ₁	AFLA B ₂	AFLA G ₁	AFLA G ₂	AFLA Total	FUM B ₁	FUM B ₂	FUM B ₃	FUM Total				
Free State: Allanridge	(18/19) MO 510	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	1007	242	ND	<i>A. flavus</i> , <i>F. subglutinans</i> , <i>F. verticillioides</i>
Free State: Brandfort	(18/19) MO 113	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<i>A. niger</i> , <i>F. sporodochiale</i> , <i>F. subglutinans</i> , <i>F. verticillioides</i>
Free State: De Brug	(18/19) MO 343	W	ND	ND	ND	ND	ND	111	22	ND	133	288	ND	ND	<i>A. flavus</i> , <i>A. niger</i> , <i>F. glycines</i> , <i>F. sporodochiale</i> , <i>F. subglutinans</i> , <i>F. temperatum</i> , <i>F. verticillioides</i>
Free State: De Brug	(18/19) MO 345	Y	ND	ND	ND	ND	ND	197	68	<20	265	ND	ND	ND	<i>A. niger</i> , <i>A. parasiticus</i> , <i>F. boothii</i> , <i>F. verticillioides</i>
Free State: Harrismith	(18/19) MO 342	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	466	ND	ND	<i>F. subglutinans</i> , <i>F. temperatum</i>
Free State: Kransfontein	(18/19) MO 296	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	201	ND	ND	<i>F. subglutinans</i> , <i>F. temperatum</i> , <i>F. verticillioides</i>
Free State: Kroonstad	(18/19) MO 603	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	3604	593	31	<i>F. boothii</i> , <i>F. verticillioides</i>
Free State: Losdoorns	(18/19) MO 493	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	801	112	ND	<i>F. awaxy</i> , <i>F. verticillioides</i>
Free State: Mirage	(18/19) MO 387	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	621	139	ND	<i>F. subglutinans</i> , <i>F. verticillioides</i>
Free State: Senekal	(18/19) MO 328	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<i>F. temperatum</i> , <i>F. verticillioides</i>
Free State: Tierfontein	(18/19) MO 401	W	48	<LOQ	95	<LOQ	143	970	259	74	1303	274	ND	ND	<i>A. parasiticus</i> , <i>F. verticillioides</i>
Free State: Vrede	(18/19) MO 310	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	838	111	ND	<i>A. flavus</i> , <i>F. boothii</i> , <i>F. subglutinans</i> , <i>F. temperatum</i> , <i>F. verticillioides</i>
Free State: Winburg	(18/19) MO 305	W	ND	ND	ND	ND	ND	<LOQ	ND	ND	<LOQ	168	ND	ND	<i>F. boothii</i> , <i>F. gossypinum</i> , <i>F. subglutinans</i> , <i>F. verticillioides</i>
Gauteng: Bloekomspruit	(18/19) MO 127	W	ND	ND	ND	ND	ND	50	ND	ND	50	ND	ND	ND	<i>A. flavus</i> , <i>F. subglutinans</i> , <i>F. verticillioides</i>
Gauteng: Bronkhorstspuit	(18/19) MO 93	W	ND	ND	ND	ND	ND	31	<LOQ	ND	31	205	ND	ND	<i>F. verticillioides</i>
Gauteng: Bronkhorstspuit	(18/19) MO 96	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	207	ND	ND	—
Gauteng: Kaalfontein	(18/19) MO 100	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	847	103	ND	<i>F. subglutinans</i>
Gauteng: Meyerton	(18/19) MO 145	Y	ND	ND	ND	ND	ND	95	27	ND	122	226	ND	28	<i>F. verticillioides</i>
Gauteng: Meyerton	(18/19) MO 146	Y	ND	ND	ND	ND	ND	155	34	ND	189	323	ND	ND	<i>F. graminearum</i> , <i>F. verticillioides</i>
Gauteng: Middelvlei	(18/19) MO 777	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	529	ND	ND	<i>F. subglutinans</i> , <i>F. temperatum</i>
Gauteng: Palmietfontein	(18/19) MO 136	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<i>F. temperatum</i>
Gauteng: Raathsvlei	(18/19) MO 363	W	ND	ND	ND	ND	ND	116	36	ND	152	ND	ND	ND	<i>F. subglutinans</i> , <i>F. temperatum</i> , <i>F. verticillioides</i>
Gauteng: Witfontein	(18/19) MO 102	Y	ND	ND	ND	ND	ND	23	ND	ND	23	ND	ND	ND	<i>A. flavus</i>
KwaZulu-Natal: Dundee	(18/19) MO 5	W	ND	ND	ND	ND	ND	42	<LOQ	ND	42	206	ND	ND	<i>F. temperatum</i> , <i>F. verticillioides</i>

(continued on next page)

Table 2 (continued)

Sample description		W/ Y ^a	Mycotoxin results (µg/kg)										Mycotoxigenic genera important in maize ^c				
Location	Sample number		Aflatoxins (LOQ ^b 5 µg/kg)					Fumonisin (LOQ 20 µg/kg)				DON (LOQ 100 µg/kg)				15-ADON (LOQ 100 µg/kg)	ZEA (LOQ 20 µg/kg)
			AFLA B ₁	AFLA B ₂	AFLA G ₁	AFLA G ₂	AFLA Total	FUM B ₁	FUM B ₂	FUM B ₃	FUM Total						
KwaZulu-Natal: Paulpietersburg	(18/19) MO 271	Y	ND	ND	ND	ND	ND	68	30	ND	98	175	ND	ND	—		
KwaZulu-Natal: Vryheid	(18/19) MO 9	W	ND	ND	ND	ND	ND	38	ND	ND	38	780	ND	70	<i>F. awaxy</i> , <i>F. temperatum</i> , <i>F. verticillioides</i>		
KwaZulu-Natal: Vryheid	(18/19) MO 8	Y	ND	ND	ND	ND	ND	84	<LOQ	<LOQ	84	1137	ND	957	<i>F. temperatum</i> , <i>F. verticillioides</i>		
Limpopo: Nylstroom	(18/19) MO 512	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	100	ND	ND	<i>F. verticillioides</i>		
Limpopo: Nylstroom	(18/19) MO 514	Y	ND	ND	ND	ND	ND	283	88	<LOQ	371	369	ND	ND	<i>F. temperatum</i> , <i>F. verticillioides</i>		
Limpopo: Settlers	(18/19) MO 378	Y	ND	ND	ND	ND	ND	316	90	<LOQ	406	ND	ND	ND	<i>F. atrovinosum</i> , <i>F. subglutinans</i> , <i>F. verticillioides</i>		
Mpumalanga: Arnot	(18/19) MO 152	Y	ND	ND	ND	ND	ND	184	46	<LOQ	230	340	ND	ND	<i>F. verticillioides</i>		
Mpumalanga: Bakenlaagte	(18/19) MO 111	Y	ND	ND	ND	ND	ND	167	50	<LOQ	217	ND	ND	ND	<i>F. subglutinans</i> , <i>F. temperatum</i>		
Mpumalanga: Kendal	(18/19) MO 137	Y	ND	ND	ND	ND	ND	765	315	131	1211	296	ND	75	<i>F. temperatum</i> , <i>F. verticillioides</i>		
Mpumalanga: Kendal	(18/19) MO 139	Y	ND	ND	ND	ND	ND	781	241	79	1101	366	ND	ND	<i>F. temperatum</i> , <i>F. verticillioides</i>		
Mpumalanga: Kinross	(18/19) MO 116	Y	ND	ND	ND	ND	ND	874	220	94	1188	114	ND	ND	<i>F. boothii</i> , <i>F. subglutinans</i> , <i>F. temperatum</i> , <i>F. verticillioides</i>		
Mpumalanga: Maizefield	(18/19) MO 207	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<i>F. temperatum</i> , <i>F. verticillioides</i>		
Mpumalanga: Panbult	(18/19) MO 340	Y	ND	ND	ND	ND	ND	29	ND	ND	29	261	ND	ND	<i>F. temperatum</i>		
Mpumalanga: Platrand	(18/19) MO 56	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<i>F. subglutinans</i> , <i>F. temperatum</i>		
Mpumalanga: Sandspruit	(18/19) MO 81	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<i>F. subglutinans</i> , <i>F. temperatum</i>		
Mpumalanga: Standerton	(18/19) MO 74	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<i>F. temperatum</i> , <i>F. verticillioides</i>		
Mpumalanga: Vogelvallei	(18/19) MO 68	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<i>A. flavus</i> , <i>F. verticillioides</i>		
North West: Barberspan	(18/19) MO 533	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	323	ND	ND	<i>F. boothii</i> , <i>F. sporodochiale</i> , <i>F. temperatum</i> , <i>F. verticillioides</i>		
North West: Brits	(18/19) MO 91	W	ND	ND	ND	ND	ND	504	104	58	666	ND	ND	ND	<i>A. flavus</i> , <i>A. niger</i> , <i>F. annulatum</i> , <i>F. verticillioides</i>		
North West: Gerdau	(18/19) MO 517	W	ND	ND	ND	ND	ND	22	ND	ND	22	2410	467	ND	<i>F. subglutinans</i>		
North West: Gerdau	(18/19) MO 519	Y	ND	ND	ND	ND	ND	67	<LOQ	ND	67	344	ND	ND	<i>F. coffeatum</i> , <i>F. mirum</i> , <i>F. subglutinans</i> , <i>F. verticillioides</i>		
North West: Hibernia	(18/19) MO 550	W	ND	ND	ND	ND	ND	100	21	ND	121	288	ND	ND	<i>A. flavus</i> , <i>F. sporodochiale</i> , <i>F. subglutinans</i> , <i>F. temperatum</i> , <i>F. verticillioides</i>		
North West: Hibernia	(18/19) MO 552	Y	ND	ND	ND	ND	ND	65	<LOQ	ND	65	469	101	ND	<i>A. flavus</i> , <i>F. boothii</i> , <i>F. subglutinans</i> , <i>F. temperatum</i> , <i>F. verticillioides</i>		
North West: Lusthof	(18/19) MO 534	W	ND	ND	ND	ND	ND	195	82	25	302	ND	ND	ND	<i>F. temperatum</i> , <i>F. verticillioides</i>		
North West: Ottosdal	(18/19) MO 527	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	181	ND	ND	<i>A. flavus</i> , <i>F. verticillioides</i>		

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Table 2 (continued)

Sample description Location	Sample number	W/ Y ^a	Mycotoxin results (µg/kg)										Mycotoxigenic genera important in maize ^c		
			Aflatoxins (LOQ ^b 5 µg/kg)			Fumonisin (LOQ 20 µg/kg)			DON (LOQ 100 µg/kg)	15-ADON (LOQ 100 µg/kg)	ZEA (LOQ 20 µg/kg)				
			AFLA B ₁	AFLA B ₂	AFLA G ₁	AFLA G ₂	AFLA Total	FUM B ₁				FUM B ₂		FUM B ₃	FUM Total
North West: Rostraville	(18/19) MO 530	W	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<i>F. subglutinans</i> , <i>F. temperatum</i>
Northern Cape: Barkley West	(18/19) MO 224	Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	112	ND	ND	<i>A. flavus</i> , <i>A. niger</i> , <i>F. subglutinans</i> , <i>F. verticillioides</i>
Northern Cape: Douglas	(18/19) MO 86	Y	ND	ND	ND	ND	958	237	68	1263	686	ND	ND	26	<i>A. flavus</i> , <i>A. niger</i>
Northern Cape: Hartswater	(18/19) MO 42	Y	ND	ND	ND	ND	268	65	24	357	ND	ND	ND	ND	<i>F. verticillioides</i>
Northern Cape: Magogong	(18/19) MO 352	W	ND	ND	ND	ND	368	124	34	526	509	194	ND	ND	<i>F. awaxy</i> , <i>F. glycines</i> , <i>F. subglutinans</i> , <i>F. temperatum</i> , <i>F. verticillioides</i>
Northern Cape: Magogong	(18/19) MO 354	Y	ND	ND	ND	ND	340	118	<LOQ	458	131	ND	ND	ND	<i>A. flavus</i> , <i>F. sporodochiale</i>
Northern Cape: Marydale	(18/19) MO 17	Y	ND	ND	ND	ND	1365	257	81	1703	ND	ND	ND	ND	<i>F. annulatum</i> , <i>F. verticillioides</i>

^a W = white maize, Y = yellow maize.

^b Limit of quantitation (LOQ) means the lowest concentration level that can be quantified with acceptable precision and accuracy by the mass spectrometer. In this table of results a concentration measured below the LOQ was reported as < LOQ.

^c A. = *Aspergillus*; F. = *Fusarium*.

maize associated with stalk rot in China (Crous et al., 2019; Han et al., 2023). *Fusarium mirum* was described from *Sorghum bicolor* in Egypt and Cameroon (Costa et al., 2022). Whether *F. awaxy* or *F. mirum* produce mycotoxins is unknown and requires further investigation.

Deoxynivalenol was detected in 37 samples, with concentrations ranging from 100 to 3604 µg/kg. As a member of the type B-trichothecene group, DON is one of the most common contaminants in food and feed and is frequently detected in South African maize (Boutigny et al., 2011; Meyer et al., 2019). Research has repeatedly shown that DON can cause significant acute toxic effects in both humans and other animals, with potential long-term consequences such as cytotoxicity, immunotoxicity and teratogenicity (Knutsen et al., 2018; Urbanek et al., 2018). In our study, we observed the co-occurrence of 15-ADON, a derivative of DON, in nine samples with concentrations of 103–593 µg/kg, a similar pattern as reported by Meyer et al. (2019). These mycotoxins are commonly produced by FSAMSC species, including *F. boothii* and *F. graminearum* (Laraba et al., 2021; Munkvold et al., 2019). In South Africa, *F. boothii* is commonly identified as responsible for Gibberella ear rot (Boutigny et al., 2011; Price et al., 2024). *Fusarium sambucinum* species complex species were isolated from only eight samples, while no other DON-producing species were identified in most of the maize samples. It is not clear why DON was present in these samples, but the producing species were not. Similar results were observed by Czembor et al. (2015), who isolated *F. verticillioides* and *F. temperatum* as the predominant species. Although *F. graminearum* was present in smaller quantities, DON was detected in 66.67% of samples. Many factors can influence the composition of fungal communities and mycotoxin levels in maize, but climatic conditions are thought to play a particularly important role. For example, *F. verticillioides* tends to thrive in dry and hot conditions, while *F. graminearum* favours a cooler and more humid environment (Bottalico, 1998; Logrieco et al., 2002; Munkvold, 2003; Munkvold et al., 2019). *Fusarium verticillioides* can also germinate and grow across a wider spectrum of temperatures and water activities compared to *F. graminearum* (Reid et al., 1999). It will, therefore, be interesting to investigate in the future how the fungal communities change during a growing season. In South Africa, maize is usually grown between the end of November and the beginning of June. Climatic conditions vary in different growing regions, but in general it is cooler and more humid at the beginning of the growing season before becoming hotter and drier mid-season, then becoming drier and cooler. Could FSAMSC species like *F. graminearum* dominate the fungal community at the beginning of the season, and eventually be replaced by FFSC species like *F. verticillioides* as the season progress? This may explain why DON is present in maize, although FSAMSC species were only isolated from eight samples.

Zearalenone was detected in six samples with concentrations ranging from 26 to 957 µg/kg. *Fusarium* species within the FSAMSC and the FIESC are known producers. Although we isolated species from these complexes in our study, their occurrence was relatively low. Overall, ZEA appears to be a minor contaminant in South African maize as previously reported (Meyer et al., 2019; Rheeder et al., 1995; Shephard et al., 2013).

This current study revealed that the fungal communities in maize are more diverse than previously reported. This applies in particular to the frequently isolated mycotoxigenic genera *Fusarium*, *Aspergillus* and *Penicillium*. These fungi can readily adapt to low-moisture conditions by producing compatible solutes such as glycerol that facilitate physiological processes at very low water activity (Stevenson et al., 2017b) and trehalose that facilitates survival of desiccation-rehydration events (Ribeiro et al., 2024). Furthermore, they are incredibly diverse and taxonomically complicated, making species identification notoriously difficult (Crous et al., 2021; O'Donnell et al., 2015; Samson et al., 2014; Visagie et al., 2014). A morphological approach results in time-consuming identifications that, even when done by taxonomic experts, can lead to misidentifications. As DNA sequencing has become readily available around the world and incorporated into species

Table 3

Summary of mycotoxin contamination in white and yellow maize samples collected (post-harvest, pre-storage) in the 2018/19 maize production season in South Africa.

Mycotoxins	White maize					Yellow maize				
	Number of positive samples	Positive %	Range (µg/kg)	Mean ^a (µg/kg)	Median ^b (µg/kg)	Number of positive samples	Positive %	Range (µg/kg)	Mean ^a (µg/kg)	Median ^b (µg/kg)
AFLA B ₁	1	4.0	48 ^c	–	–	0	0.0	–	–	–
AFLA G ₁	1	4.0	95 ^c	–	–	0	0.0	–	–	–
FUM Total	12	48.0	22–1303	282	127	20	64.5	23–1703	472	248
DON	19	76.0	100–3604	736	509	18	58.1	112–1137	346	310
15-ADON	8	32.0	103–593	245	167	1	3.2	101 ^d	–	–
ZEA	2	8.0	31–70	51	51	4	12.9	26–957	272	52

^a Mean of the positive samples.

^b Median of the positive samples.

^c Only one white maize sample (MO401) contained AFLA B₁ and AFLA G₁ (See Table 2).

^d Only one yellow maize sample (MO552) contained 15-ADON (See Table 2).

concepts in modern taxonomies, identifications are now mostly based on DNA sequence data. Accurate identifications are the crucial first step in understanding which species are present in a given crop and unlocks a body of information related to the name (e.g. what risk they may pose to production or food safety). The modes-of-action of many mycotoxins are not yet elucidated and, whereas there is an assumption that they exert specific toxicity, it is possible that they exert their potent activities via chaotropicity-mediated hydrophobic effects on biomacromolecules that are therefore stress-mediated rather than toxic *per se* (McCammick et al., 2010; Noel et al., 2023).

Our study provides baseline knowledge of which fungi are present in South African maize, but further surveys in more sampling areas and taking into account yearly crops will be required. This will be crucial to understand how climate change might affect communities in the future. Furthermore, it is known that some *Fusarium* has an endophytic life cycle in maize, while *Aspergillus*, *Cladosporium*, *Penicillium* and *Talaromyces* are easily spread by wind. At what point these fungi enter the value chain or from which source they originate is still unknown. A culture-dependent approach, as we have used here, is impractical and too costly for more extensive surveys. The development of high-throughput sequencing tools that can identify communities at the species level will therefore be important to address some of these questions. However, the strains obtained in this study using a culture-dependent approach are important as future reference strains. For the mycotoxigenic species, we are sequencing the genomes and transcriptomes of selected strains to investigate, for example, the biotic and abiotic factors that trigger their mycotoxin production.

CRedit authorship contribution statement

Cobus M. Visagie: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hannalién Meyer:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Neriman Yilmaz:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

In reference to the submitted manuscript ‘Exploring the fungal diversity and mycotoxins in South African pre-stored maize’, I confirm that we have no conflict of interest or other declarations to be made.

Acknowledgements

We dedicate this paper to our friend and colleague Professor Naresh Magan, who sadly passed away in 2023. His passion for fungi and understanding of the conditions under which they grow and produce

mycotoxins was inspiring (Hallsworth et al., 2023a). We acknowledge the Future Leaders - African Independent Research fellowship program (FLAIR, FLR\R1\201831), which funded this work. The FLAIR fellowship program is a partnership between the African Academy of Sciences and the Royal Society funded by the UK Government’s Global Challenges Research Fund. We also acknowledge Alice Mthembu, Claudette Dewing, Jenna-Lee Price, Nicole van Vuuren and Nombulelo Qikani for the support they provided in the laboratory.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.funbio.2024.03.009>.

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