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**The fungal diversity associated with maize from emerging farms in
the Eastern Cape, South Africa**

By

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Declaration of Originality
University of Pretoria

I, **Jenna-Lee Price** declare that the dissertation, which I hereby submit for the degree Magister Scientae at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:

A handwritten signature in black ink, appearing to be 'JLP', with a long horizontal stroke extending to the right.

DATE: 4 November 2022

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Preface

Maize is an important crop planted across the world. In South Africa, the Eastern Cape contributes less than 1% to the overall production. The farming community in the Eastern Cape is largely comprised of small scale and emerging maize farms. Historically, maize production in the Eastern Cape were limited due to pests and diseases, including mycotoxigenic fungi and their toxic metabolites. Recently the Eastern Cape was identified as a region to be developed into a large maize producer. However, there is a knowledge gap on the fungal species associated with and impacting maize production in the province. This prompted the present study in which we explored the fungal diversity and mycotoxins associated with maize from emerging maize farms in the Eastern Cape.

The first chapter of the dissertation provides an in-depth literature review on how maize became such an important crop within a South African context, how maize ear rots and associated mycotoxins threaten its production, and how mycotoxin management and more importantly prevention strategies can be introduced to safeguard future maize production in the area.

The second chapter examines the fungal diversity associated with post-harvest maize collected from the Eastern Cape. This chapter closes a knowledge gap where past fungal surveys identified strains using morphology. We took a modern approach and used DNA sequence data to identify strains to species.

The third chapter of this study aimed to identify the fungal species and mycotoxins associated with maize ear rots collected from maize farms in the Eastern Cape. Current knowledge surrounding the type of ear rot present, the species causing it and the potential mycotoxins that are subsequently produced is lacking. We thus aimed to determine the distribution and severity of maize ear rots across the Eastern Cape, isolate fungal strains from collected ear rot samples, identify these strains to species using DNA sequence data, and determine the mycotoxins present.

List of Abbreviations

AcCN: acetonitrile

AER: Aspergillus Ear Rot

AFs: aflatoxins

AFB₁: aflatoxin B₁

AFB₂: aflatoxin B₂

AFG₁: aflatoxin G₁

AFG₂: aflatoxin G₂

AFM₁: aflatoxin M₁

ALT: altenuene

AN: Alfred Nzo

AME: alternariol monomethyl ether

AOH: alternariol

ATXs: altertoxins

BEA: beauvericin

BenA: beta-tubulin

BLAST: basic local alignment search tool

Bt: *Bacillus thuringensis*

CaM: calmodulin

CER: Cladosporium Ear Rot

CH: Chris Hani

CUL: culmorin

DAS: diacetoxyscirpenol

DER: Diplodia Ear Rot

DG18: Dichloran-18%-Glycerol agar

DIP: diplodiatoxin

DON: deoxynivalenol

DON-3G: deoxynivalenol-3-glucoside

3-ADON: 3-acetyldeoxynivalenol

15-ADON: 15-acetyldeoxynivalenol

EC: Eastern Cape

EFSA: European Food and Safety Authority

ENN: enniatins

FABI: Forestry and Agricultural Biotechnology Institute

FAO: Food and Agricultural Organization

FCSC: *Fusarium chlamydosporum* species complex

FDA: Food and Drug Administration

FER: Fusarium Ear Rot

FFSC: *Fusarium fujikuroi* species complex

FHB: Fusarium Head Blight

FIESC: *Fusarium incarnatum-equiseti* species complex

FOSC: *Fusarium oxysporum* species complex

FS: Free State

FSAMSC: *Fusarium sambucinum* species complex

FSM: Fusarium-selective-media

FSSC: *Fusarium solani* species complex

FUMs: fumonisins

FUMB₁: fumonisin B₁

FUMB₂: fumonisin B₂

FUMB₃: fumonisin B₃

FUS: fusaproliferin

GAPDH: glyceraldehyde 3-phosphate dehydrogenase

GER: Gibberella Ear Rot

GMOs: genetically modified organisms

GP: Gauteng

HPLC: high-performance liquid chromatography

HPLC-MS: high-performance liquid chromatography mass spectrometry

HT2: HT-2 toxin

ITS: internal transcribed spacer region

JG: Joe Gqabi

KZN: Kwa-Zulu Natal

LCMS-MS: liquid-chromatography mass spectrometry- mass spectrometry

LSU: large subunit of nuclear ribosomal RNA

MeOH: methanol

MON: moniliformin

MP: Mpumalanga

NCBI: National Centre for Biotechnology Information

NEO: neosolaniol

NIV: nivalenol

NW: North West

ORT: OR Tambo

OTA: ochratoxin A

PDA: Potato Dextrose Agar

PER: Penicillium Ear Rot

qPCR: quantitative polymerase chain reaction

SADC: South African Development Community

SAGL: South African Grain Laboratory

TeA: tenuazonic acid

TEN: tentoxins

TEF: translation elongation factor 1- α

TRIs: trichothecenes

T2: T-2 toxin

USA: United States of America

WHO: World Health Organisation

ZEA: zearalenone

ZEA-14G: zearalenone-14-glucoside

ZEA-16G: zearalenone-16-glucoside

ZEA-14S: zearalenone-14-sulfate

CHAPTER 1: Literature Review

Maize and mycotoxigenic fungi: Do we really know it all?

Introduction

Maize is one of the top four commodities produced across 33 countries in the world (CIMMYT 2022). Eighteen of these are from Africa, with South Africa being the largest commercial producer. Maize production in South Africa occurs across a large farming area and is consumed locally in many different forms (Beukes et al. 2017). This highly sought-after commodity is negatively affected by fungal pathogens causing various diseases, while mycotoxigenic fungi and their mycotoxins may cause further losses. This association not only occurs within South Africa, but on a global scale. The issue of contamination by fungi and / or their mycotoxins is of great concern and impacts humans on several factors.

When fungi and their mycotoxins contaminate maize, it may result in it being rejected for export as there are many health risks associated with the consumption of mycotoxins above determined threshold levels. Mycotoxins are toxic secondary metabolites (extrolites) produced by filamentous fungi typically classified in the phylum Ascomycota that can cause death or disease in humans or animals (Pitt 2000, Richard 2008). Mycotoxin contamination can occur throughout the production chain during pre- and post-harvest stages. *Aspergillus*, *Fusarium*, *Penicillium* and *Stenocarpella* are the most important mycotoxigenic fungal genera, with *Aspergillus*, *Fusarium* and *Penicillium* producing the so-called 'big five' mycotoxins on food and feed (Embaby et al. 2013, Sweeney and Dobson 1998). These include aflatoxins (AFs), deoxynivalenol (DON), fumonisins (FUMs), ochratoxin A (OTA), and zearalenone (ZEA).

Countries around the world regulate mycotoxins so to not expose humans and animals above threshold levels that were determined to be safe. There is a difference between regulation and prevention, however, and it is the latter that is arguably more difficult to control. Understanding why, how and when fungi produce mycotoxins is crucial in our fight to produce higher quality food and address the United Nations Sustainable Development Goal 2 of Zero Hunger. This literature review is focused on: (1) how maize became such an important crop, especially within a South African context, (2) maize ear rots and mycotoxins threatening its production, and (3) how mycotoxin management and more importantly prevention strategies can be introduced to safeguard future maize production.

Maize

Origin

The exact origin of maize (*Zea mays* L.) is still being debated, ranging from Paraguay to Colombia in South America, or Guatemala and Mexico in Mesoamerica (Goodman and Galinat 1988). Currently, Mexico is most widely considered the origin, mainly due to the fact that the closest relative of maize (Balsas teosinte (*Zea mays parviglumis*)) is restricted to this region and archaeological evidence gathered from the above-mentioned sites is older in Mexico than in South America.

Maize is most likely a descendant from the Mexican wild grass known as 'teosinte' (Fig. 1), which looks very different from the maize varieties we know and use today (Fig. 2) (Goodman and Galinat 1988). Three hypotheses explain the origin of maize and include the "Common origin" or "Divergent evolution" hypothesis which suggests that maize and its closest relatives, teosintes and *Tripsacum*, all diverged from a common ancestor (Randolph 1976, Weatherwax 1935). The "Descent from Teosinte" hypothesis suggests that maize was derived directly and only from teosinte due to human selection (Beadle 1980, Galinat 1971). The "Maize as an Ancestor of Teosinte" hypothesis suggested the teosintes of Mexico descended from maize or a maize x perennial teosintes cross (Mangelsdorf 1983). The "Descent from Teosinte" is currently the more widely accepted hypothesis.

Following the discovery of maize from caves in the Tehuacàn region located in south-central Mexico (Miracle 1965), maize was moved southwards into South America and Brazil, and northwards into the United States of America (USA) and Canada (Doebley et al. 1988). Europeans discovered maize in Cuba upon their discovery of the New World and introduced it into Europe via Spain in 1493 (Doebley 1990, Goodman and Galinat 1988).

Maize was thought to have been introduced into Western Africa by Portuguese sailors in 1502 as a result of the Spanish and Portuguese slave trade routes (McCann 2001, Miracle 1965). Reports of maize movement across the African continent include Ethiopia in 1623; Uganda in 1860 and Western Kenya in 1917, where it was used as a supporting vegetable in garden agriculture (McCann 2001, McCann 1995). By the 18th century, maize was considered a principal within many

regions in East and West Africa. Jan van Riebeeck did not report seeing maize in southern Africa upon his arrival in 1652 and requested maize seed to be sent where it was used as a 'support' to garden vegetables (McCann 2001). By the mid-19th century, maize was established as part of a cropping system in Malawi. The first genetically modified maize (Bt (*Bacillus thuringensis*) maize) was introduced in 1998. Since its discovery, there have been several breeding programmes developed across the world with the goal of adapting maize for various purposes.

Production regions and types of maize

The production and consumption of cereal grains like maize are not equal across the world, and is highly dependent on the climate of the production regions (Ranum et al. 2014). For instance, in developed countries, maize-based food products are preferred for consumption, whereas in developing countries maize is considered a staple food. Maize and maize-based foods are consumed in large quantities in Central America, Southern and Eastern Africa and Mexico. Preferences for maize and maize-based foods differ across Africa. For instance, in Sub-Saharan Africa maize is considered one of the major staple crops which is in many forms including infant foods, snacks and main dishes (mielie meal) (Ekpa et al. 2018).

General requirements to grow maize includes sufficient water, the correct climate and soil quality (Du Plessis 2003, SAGL 2020). Maize requires between 450–600 mm of water per season, which is obtained through irrigation fed or dryland (rain-fed) farming practices. Irrigation fed methods are preferred to obtain higher and more consistent yields. However, within a South African context, most maize farms are drylands. Maize is a warm weather crop that requires a mean daily temperature above 19 °C for growth and a minimum temperature of 10 °C for successful germination. Temperatures above 32 °C have been found to be detrimental to the maize yield (Belfield and Brown 2008, Du Plessis 2003). The preferred soil for maize production is one that has good internal drainage; balanced with enough plant nutrients like nitrogen, phosphorus, potassium and zinc; and a favourable depth (Department of Agriculture 2008, Du Plessis 2003).

Planting dates are dependent on the climate of a specific region as well as the maize cultivar used.

Maize is produced across South Africa, however, commercial production is concentrated in the North West (NW), Free State (FS), Mpumalanga (MP) and Kwa-Zulu Natal (KZN) provinces (SAGL 2020). The production areas within South Africa are divided into four main regions. Each region is defined by their climatic conditions which has an impact on the planting and harvesting times, as well as favouring specific maize varieties (Fig. 3). These regions are the Warm Western region (western parts of the FS and a large part of the NW provinces), the Temperate Eastern region (Gauteng and central parts of the FS), the Cold Eastern region (MP highveld and eastern parts of FS); and the Kwa-Zulu Natal region (western/upland and central/mainland parts of KZN). These regions experience varying summer rainfall between November and March. For instance, the Warm Western regions experience high heat and precipitation levels between 550–650 mm which is especially favourable for white maize production (Gravelet-Blondin 2013). In the central and eastern areas, precipitation levels are between 650–800 mm and there is a low heat which is favourable for yellow maize production. The Eastern Cape (EC) is divided into two regions namely the EC East Region and the EC West Region (Hamann and Tuinder 2012). These regions differ in their precipitation levels, with the East region experiencing summer rainfall between 600–800 mm (similar to the central and eastern areas), where as the West Region is semi-arid and experiences precipitation levels between 200–500 mm (Gravelet-Blondin 2013).

Maize kernels consist of three main parts, the pericarp, endosperm and embryo (Fig. 4) (Belfield and Brown 2008). Maize is divided into varieties based on a number of properties including, the environment in which it is grown, endosperm and kernel composition, colour and its use. Several different maize varieties are grown across the globe and each of these varies in colour and consumer preference (Ranum et al. 2014). The colour of maize ranges anywhere between white (or cream), yellow, red and black. In the USA, yellow maize is the preferred maize variety and is consumed directly from the cob, as well as through second cycle produce that is maize-based. In regions such as Southern USA, Africa and

Central America white maize is preferred and is consumed mainly in the form of flour and mielie meal.

There are five main varieties produced in South Africa including dent corn, flint corn, popcorn, sweet corn and waxy corn (Suleiman et al. 2013). In Africa, both dent and flint corn are used for the production of food products; however, the type of varietal used is dependent on grain colour followed by grain texture and food properties (Ekpa et al. 2018). Dent corn is the most common variety planted and is used for animal food and silage; whereas flint corn or “green maize” produces a high-quality flour and is often consumed as whole kernels (typically having more nutritional benefits) either in a roasted or boiled form (Iken and Amusa 2004). Waxy corn looks similar to dent corn but has a waxy appearance when cut or damaged, and is often used in food processing as thickening agents and remoistening adhesives (Brown et al. 1986). Sweet corn (also referred to as the standard sugary corn) is harvested and consumed at the immature milk stage of development and is eaten directly from the cob across the globe. Yellow sweet corn is preferred for human consumption due to its high levels of vitamins A and C (Ranum et al. 2014). Popcorn is considered a minor crop compared to dent and sweet corn production and is mainly used for human consumption as freshly popped popcorn or popcorn-based snacks (Ranum et al. 2014).

South African maize is produced for three main purposes namely human and animal food, and industrial use (Department of Agriculture 2008). The required end-product in each sector determines the form of maize processing. For human consumption, white maize is a staple used for direct consumption in the form of grain, meal, or green mielies; and is obtained by using dry milling. Maize is also processed and consumed as a snack or cereal. For animal consumption, yellow maize is predominantly used as feed for the meat and dairy sectors (Department of Agriculture 2008). The industrial sector uses maize for the production of bioethanol (especially in developed countries) and alcoholic beverages (e.g., home-brewed beers in South Africa) (Badu-Apraku and Fakorede 2017). The production of bioethanol is not often explored by developing countries due to the increased risk it poses to food security. Other industrial uses of maize in Southern Africa include the production of adhesives, laundry starch, and textile and paper

sizing. The animal and industrial sectors predominantly make use of wet milling to obtain their required end-products.

Maize trade

Many animals and humans rely on maize as a source of nutrition, making it one of the most widely traded agricultural commodities in the world (Wu and Guclu 2013). Some countries can produce sufficient maize to supply the needs of its population; but many must rely on imports or donations. The international trade of agricultural products has more than doubled from 1995 (USD 680 billion) to 2018 (USD 1.5 trillion) (FAO 2020, FAO 2021a). There are five main drivers of global agricultural trade, including a countries gross domestic product (GDP), population growth, demographic changes, technological progress and trade policies (FAO 2020, Rakotoarisoa et al. 2011). The income or financial potential of a country is often the determining factor in developing countries as the payment of import bills redirects funds from important development agendas and further delays food security.

There are a large number of developing countries, especially within Africa, that are able to export agricultural products, however many have become net importers of cereals (Rakotoarisoa et al. 2011). Africa has vast agricultural potential, but has seen a steady increase in basic food imports since the 1970s (Wandile 2016). This has been attributed to rapidly increasing population growth, poor infrastructure, and a difference in trade policies between developed and developing countries. In addition, there are mineral rich and more industrialized countries that import these commodities rather than produce them locally.

The USA is the largest maize producer as well as a net exporter of maize globally, having produced 347 048 thousand tonnes in 2021 (FAO 2021b). They are followed by Argentina (56 961 thousand tonnes), China (260 958 thousand tonnes), Brazil (101 139 thousand tonnes) and Ukraine (35 880 thousand tonnes). South Africa is considered a small contributor to the global maize market, having produced 11 276 thousand tonnes in 2021. It is however the largest of the three net maize exporters within the Southern African Development Community (SADC) region, followed by Tanzania (2500 thousand tonnes) and Zimbabwe (1650 thousand tonnes) (Cutts and Hassan 2003, Wandile 2016). Almost all other

countries within the SADC region are net importers of maize. The SADC region relies heavily on South Africa and Zimbabwe in order to be deemed food-secure in terms of maize as these countries have a consistent net positive maize trade (Cutts and Hassan 2003).

South African maize exports can vary between seasons. For example in 2021 our trade partners were Botswana (20 935 tonnes), Eswatini (9 015 tonnes), Italy (39 447 tonnes), Japan (143 368 tonnes), South Korea (55 346 tonnes), Lesotho (5 888 tonnes), Mozambique (5 134 tonnes), Namibia (4 432 tonnes) and Taiwan (57 589 tonnes) (Dhlamini 2021). In 2022, our trade partners changed, where most of our maize was exported to Botswana (239 784 tonnes), Italy (201 659 tonnes), Japan (792 364 tonnes), South Korea (415 145 tonnes), Taiwan (815 318 tonnes) and Vietnam (432 111 tonnes) (GrainSA 2022). In times of drought, South Africa typically imported maize from Argentina, Botswana, Brazil, Ukraine, USA and Zambia (Wandile 2016). During the South African 2018/19 marketing season, 8 774 784 and 7 291 275 tonnes of white and yellow maize were produced respectively (SASDE 2019). Of the white maize produced, 4 600 000 tonnes were used for human consumption, 1 700 000 tonnes for animal and industrial use, 608 000 tonnes were exported; and there was an estimated surplus of 3 500 tonnes. For yellow maize, 540 000 tonnes were used for human consumption, 3 650 000 tonnes for the animal and industrial use, 1 665 000 tonnes were exported and there was an estimated surplus of 12 000 tonnes. In the 2019/20 marketing season, a total of 6 904 124 tonnes of white and 6 484 445 tonnes of yellow maize is expected to be produced (SASDE 2019). Maluleke (2019) predicts that international maize production for the 2018/2019 season is going to increase by 2,3% because of increased production in Argentina, Brazil and the USA, while its utilization will increase by 1,7% during the 2019/2020 growing season.

Maize ear diseases and their impacts

Maize is prone to a number of ear, foliar and root diseases that are caused by bacteria, fungi and / or viruses. During optimal environmental conditions, these diseases can result in reduced / severe yields and at times complete failure of the crop. Many fungal diseases affect maize production and can occur in or on

different parts of the plant. For the purpose of this review, only maize ear diseases will be discussed in depth.

Ear rots occur around the world wherever maize is grown, and some can be identified in the field during various stages of maize development (Bigirwa 2007). They are of economic importance and result in a reduced yield and often mycotoxin contamination in the infected tissues, provided that there are optimal environmental conditions that favour disease onset and spread. This poses a serious health risk to the consumer (Oldenburg et al. 2017). Ear rots of maize are caused by several fungi and include Aspergillus ear rot (caused by *Aspergillus* spp.), Cladosporium ear rot (caused by *Cladosporium* spp.), Diplodia ear rot (caused by *Stenocarpella* spp.), Fusarium ear rot (caused by *Fusarium* spp.), Gibberella ear rot (caused by *Fusarium* spp.), Penicillium ear rot (caused by *Penicillium* spp.) and Corn smut ear rot (caused by *Mycosarcoma* (= *Ustilago* spp.) (Fig. 5A–F). *Aspergillus*, *Fusarium*, *Penicillium* and *Stenocarpella* species can be mycotoxigenic and contamination of maize by these genera are thus a cause of concern. The co-occurrence of ear rot pathogens, especially of *Fusarium* is well documented and is influenced by climatic conditions (Oldenburg et al. 2017). This creates additional complications with regards to its prevention and it can also result in a cocktail of mycotoxins present in maize that can have antagonistic health impacts on the consumer (Logrieco et al. 2007, Oldenburg et al. 2017, Robertson-Hoyt et al. 2007). In cases where more than one ear rot has been recorded, it has been found that the pathogens compete which has an impact on the type of mycotoxins produced (Marín et al. 1999, Munkvold 2003b).

There are numerous ways in which these fungi gain access to the maize ear, which vary depending on the species and geographical distribution (Munkvold 2003b, Oldenburg et al. 2017). The first is from exposed silks through the silk channel to the ear tip; however, undamaged husks are effective at preventing most fungal infections. The second is physical injury to the maize ear caused by hail, insects, etc. (Duncan and Howard 2010). Finally, direct penetration of the developing or mature kernels by fungi. This is the process whereby fungi gain access to maize kernels by entering through a healthy pericarp (without damage for assistance). This entry mechanism is rare due to the plant-producing biochemicals within the pericarp that hinder fungal infection; as well as the

physical properties of the pericarp which is thick and has a wax layer (Sampietro et al. 2009).

Gibberella ear rot (GER, Fig. 5A) — also referred to as red ear rot, is most commonly caused by *Fusarium* species such as *F. graminearum* and *F. culmorum* (from the *Fusarium sambucinum* species complex (FSAMSC)) (Bottalico 1998, Munkvold 2017). In Europe, several *Fusarium* species from other species complexes are associated with this disease such as *F. culmorum* (FSAMSC), *F. cerealis* (FSAMSC), *F. equiseti* (*Fusarium incarnatum-equiseti* species complex (FIESC)) and *F. poae* (FSAMSC) (Logrieco et al. 2021, Logrieco et al. 2002, Munkvold 2017). These species, except *F. cerealis*, can produce the regulated mycotoxins DON and ZEA (Logrieco et al. 2002, Munkvold 2017). They typically infect maize in cooler and humid climatic regions with frequent precipitation throughout the growing season (Bottalico 1998, Munkvold 2017). The disease is identified by a pink or red coloured mould that covers a large proportion of the maize ear (Munkvold 2003b). Infection typically starts at the tip of the maize ear after gaining entry via the maize silks. Visible signs of infection are only observed several weeks following an initial infection (Oldenburg and Ellner 2015). Mycotoxin (e.g., DON and ZEA) contamination often correlates with the infection progress and the development of the ear rot symptoms. Should mycotoxins be produced; they mainly accumulate in the visibly infected parts of the maize ears (Oldenburg et al. 2017).

Fusarium ear rot (FER, Fig. 5B) —also referred to as pink ear rot, is common in warm and dry climatic regions (Koehler 1959, Oldenburg et al. 2017). FER is caused by *Fusarium* species most commonly classified in the *Fusarium fujikuroi* species complex (FFSC). *Fusarium verticillioides* (FFSC) is the most common species associated with the disease (Munkvold 2003b, Nelson 1992), but *F. annulatum* (= *proliferatum* (Yilmaz et al. 2021)), *F. subglutinans* and *F. temperatum* have also been implicated (Boutigny et al. 2017, Scaufaire et al. 2011b). This disease is most commonly associated with maize in the USA (Koehler 1959) and more recently in southern and central Europe (Boutigny et al. 2011a, Logrieco et al. 2003, Oldenburg et al. 2017, Scaufaire et al. 2011b). These fungi predominantly gain entry through wounds as a result of weather or insect damage and can be identified by a white to light pink mould that develops

on kernels (Bottalico 1998, Munkvold 2003b). *Fusarium verticillioides* has been isolated from asymptomatic maize kernels; and it is suggested that this species displays endophytic behaviour which changes to pathogenic when subjected to different environmental conditions (Bacon and Hinton 1996). *Fusarium verticillioides* is capable of producing FUMs which are regulated (Frisvad et al. 2006, Munkvold 2017), while FUMs production in *F. subglutinans* and *F. temperatum* is still under investigation (Fumero et al. 2020, Munkvold 2017, Munkvold et al. 2021).

Aspergillus ear rot (AER, Fig. 5C) —this ear rot is mostly caused by *Aspergillus* section *Flavi* species, commonly *A. flavus* and *A. parasiticus* (Woloshuk and Wise 2011). These fungi obtain access to the plant through any portal of entry and interferes with the integrity of the seed coat allowing it to proliferate in the living tissue of the host, including the ears and kernels of developing grain (Janse van Rensburg 2012, Richard 2007). The most prominent method of infection is via erect maize ears that collect water. It thus mostly becomes problematic when rains are experienced towards the end of the season under high humidity conditions. AER is identified by masses of yellow-green dry conidia on kernels (Janse van Rensburg 2012). Maize that has more severe and older infections show olive-green masses of conidia (Woloshuk and Wise 2011). The symptoms of new fungal infections are typically observed at the tip of the maize ear; however, under severe fungal infection, the mould can be found spanning the maize ear from the tip to the base. *Aspergillus flavus* and *A. parasiticus* are potent AFs producers, but sixteen others from the section also produce this mycotoxin (Frisvad et al. 2019).

Diplodia ear rot (DER, Fig. 5D) —caused by *Stenocarpella maydis* (= *Diplodia maydis* and *D. zeae*) or *S. macrospora* (= *D. macrospora*) and are also known to produce diplodiatoxin (DIP) (Earle 1897, Masango et al. 2015, Wicklow et al. 2011). *Stenocarpella maydis* is more prevalent in South Africa and *S. macrospora* being more commonly associated with low altitude maize production areas in KZN (Flett and Wehner 1991, Wicklow et al. 2011). *Stenocarpella maydis* is most commonly reported from humid regions, while *S. macrospora* is reported to be prevalent in humid and subtropical regions (Wicklow et al. 2011). Fungal infection mostly occur through the base of the ears and progress towards the tip, although

it can also develop from the tip (Masango et al. 2015, Woloshuk and Wise 2008). *Stenocarpella maydis* has been shown to enter the maize tissues through the formation of appressoria as well as enzymatic digestion (Bensch et al. 1992). Infection can be identified by the husk colour change from bright green to a pale yellow-green (Burrill and Barrett 1909). As the infection advances, the husks become darker in colour to a sooty black appearance. Underneath the husks, less severe fungal infections can be identified by the presence of a white-greyish mould on and between the kernels on part of the maize ear. More severe infections on the other hand can be identified on maize cobs that are completely rotted with visible greyish to greyish brown mould on and between the kernels. In addition to the mould, the maize ear is noticeably lightweight. The presence of pycnidia, which appear as black specks on and within cobs are also an indication of DER and useful for in-field diagnoses (Koehler 1959). DER epidemics have been recorded in South Africa from 1986–1989 planting seasons and are often favoured by early season droughts and heavy, late season rain (Rheeder 1993).

Penicillium ear rot (PER, Fig. 5E) — also referred to as blue-eye, is commonly caused by *Penicillium oxalicum* (Currie and Thom 1915), however *P. chrysogenum*, *P. cyclopium* and *P. glaucum* have also been implicated (Shurtleff 1980). Fungal infection typically occurs in maize ears injured as a result of insect, bird and hail damage; and in ears with a high moisture contents between 15–20%. The infection typically occurs late in the growth season and is common at ear tips; however, it can spread to other parts (Koehler 1959). Indication of a fungal infection is the presence of a greyish blue green mould on the kernels.

Corn smut (Fig. 5F) — also known as Common smut or Boil smut, is a maize ear rot caused by *Mycosarcoma maydis* (also known as *Ustilago maydis*, *U. zeae* and *U. zeae mays*). Immature maize silks are susceptible to infection which is visible by gall formation (Banuett 1995). The infection and proliferation of *M. maydis* is favoured in hot, dry weather followed by a strong rainy period (Rehman et al. 2021). The spores are more easily spread through high winds and heavy rains. Whilst this fungus is considered a pathogen across the world, it is considered a delicacy in Central America, Mexico and the USA (du Toit and Pataky 1999, du Toit and Pataky 1999b). In these regions, farmers intentionally infect maize in order to obtain ear galls (also referred to as maize mushrooms) which are

subsequently consumed. Corn smut has not been implicated in the production of mycotoxins (Shurtleff 1980).

Storage of maize

Maize cultivars are often bred and adapted specifically for a region. The harvest time is determined by the maturity of the maize, indicated by leaf dieback which starts from the lower leaves and moves upwards (Department of Agriculture 2008). The grain drying period is progressively monitored by the disappearance of the milk-line, also referred to as the black line, at the tip of the kernel. Maize is typically harvested once a moisture level between 15-20% has been reached in the kernels; after which it is either used for immediate consumption or it is placed in storage until processed (Thamaga-Chitja et al. 2004).

Govender et al. (2008) describe proper and safe storage conditions as those that maintain seed quality for at least three years. However, there is an increased need to extend postharvest storage periods to cater for frequent droughts and other erratic weather patterns that are experienced in many parts of Africa that affect production. In addition to securing food, good postharvest storage practices also enable farmers to increase their annual income by holding on to stocks until more favourable market conditions are present (Tefera et al. 2011). On many emerging farms, the stored grain is utilized directly at a farm-level as poultry feed, production of cooked foods for sale and traditional beer production to name a few (Thamaga-Chitja et al. 2004). It is often during storage that especially *Aspergillus* and / or *Fusarium* can become problematic and contaminate maize with their mycotoxins (Tsedaley and Aduugna 2016).

Several storage practices are used on both commercial and emerging farms in South Africa, which can be classified as either hermetic (airtight) or non-hermetic (not airtight) (Fig. 6). Hermetic storage practices are commonly used on commercial farms, while non-hermetic storage practices are more common on small scale or emerging farms. The type of storage method used on latter farms is often determined by its size, total produce and income available (Phokane et al. 2019). Both storage methods aim to control moisture levels and prevent insect pests from damaging grains.

Hermetic storage

Hermetic storage containers have complete and airtight seals and create a modified atmosphere by reducing oxygen and increasing carbon dioxide concentrations (Gough 1985, Smith et al. 1990). The increase in carbon dioxide is typically caused by the respiration of living organisms that are present in the sealed storage system. The sealed structures also act as a moisture barrier preventing moisture mitigation during storage, as well as preventing and reducing mould growth (Chigoverah and Mvumi 2016, Gough 1985, Lane et al. 2018).

There are three main hermetic storage methods used for both commercial and emerging farms, namely hermetic storage bags like Super GrainBags (GrainPro, United States), silos (metal and concrete) or bunkers. Hermetic storage bags, like Super GrainBags (Fig. 6A), mostly consist of a single high-density polyethylene plastic liner with low oxygen permeability which is housed inside a polypropylene bag to provide extra protection (Chigoverah and Mvumi 2016). These are economically more appealing especially to emerging farmers when compared to the initial financial outlay needed for building silos. However, due to the differences in farming practices, farmers need to be made aware of the different types of bags available and how they are used most effectively to reduce postharvest losses. Whilst this storage practice sounds appealing and more financially bearable in developing countries, farmers need to practice caution when purchasing the bags as counterfeit bags are making their way into the market which may ultimately put their harvest at risk (Coulibaly et al. 2012).

Silos (Fig. 6B) are cylindrical structures constructed either from galvanised iron sheets or concrete and are hermetically sealed, creating an airtight environment (Tefera et al. 2011). This is the preferred storage method of maize across the globe as to some degree it offers standardised environmental conditions and a longer storage period when compared to other standard storage practices. However, this is only if they are maintained properly. The overall field performance of this storage practice is also highly dependent on the workmanship. Poor workmanship will result in air leaks which impacts the moisture content of the stored grain (Chigoverah and Mvumi 2016). This can be overcome by making use

of experienced artisans, as well as by conducting silo fabrication and airtightness tests.

Bunkers for storing maize have been used since prehistoric times and was designed for the storage of large quantities of grain for prolonged periods of time (Currid and Navon 1989, Navarro et al. 1992). This storage method is only effective provided that there is an effective moisture barrier between the floor of the bunker and the grain, while the walls and the roof of the structure must be impermeable to the rain.

Non-hermetic storage

Non-hermetic storage methods allow for a dynamic interaction between stored grain and environmental conditions. These storage conditions are widely used in emerging farming communities due to their affordability, accessibility and feasibility (Phokane et al. 2019). These storage methods include maize sacks, cribs, roof storage, metal drums, or traditional silos.

Maize sacks (Fig. 6C) are used to store maize on the cob and are often reused. These sacks are made from polyethylene, polypropylene or cotton (Thamaga-Chitja et al. 2004). A crib (Fig. 6D) is a structure constructed annually just before the maize is harvested (Markham 1981, Mijinyawa et al. 2006). The walls of the crib can be constructed using basketwork, matting or slats. The maize cobs are stacked at regular intervals inside these structures (Markham 1981). Any of these construction materials allow for the grain to be ventilated which will further dry the grain and thus prevents mould development.

For roof storage (Fig. 6E), maize cobs are hung from the roof (typically a thatch roof) above the cooking area, preferably over an open wood fire (Modi 2002). Many emerging farmers make use of a thatch roof which allows the smoke to act as a fumigant for the seed preventing any insect damage. Another storage method is to use metal or plastic drums (Fig. 6F) which can either be ventilated (non-hermetic) or sealed (hermetic) through the absence / presences of a lid respectively (Mijinyawa et al. 2006). Emerging farmers in sub-Saharan Africa make use of traditional silos (*inqolobane*) which are typically constructed from mud and twigs (Govender et al. 2008). Maize is often stored directly on the floor in these structures. These structures are often erected close to homesteads, and are

relatively inexpensive; however, their construction is very labour intensive (Phokane et al. 2019).

Emerging farms in South Africa often rely on traditional (non-hermetic) storage practices. This is due to a lack of suitable and affordable storage structures, as well as a lack of suitable management technologies that are unique to the South African climate and resource availability. This often results in yield loss, reduced seed quality and ultimately poor seed performance. Gaël et al. (2020) have found that the use of traditional storage structures for maize can result in postharvest losses of up to 60%. It is encouraged that appropriate and affordable storage methods are used to prevent yield loss or mycotoxin contamination. Hermetic storage methods are the preferred methods; however, farmers need to be informed about the best options available based on the time period over which they intend to store their produce (Baoua et al. 2013, Baoua et al. 2014, De Groote et al. 2013).

Mycotoxigenic fungi and their mycotoxins associated with maize

Mycotoxins are toxic secondary metabolites (extrolites) produced by filamentous fungi that can cause disease or death in humans or animals (Pitt 2000, Richard 2008). *Aspergillus*, *Fusarium* and *Penicillium* are some of the most common mycotoxigenic genera and they produce some of the most important mycotoxins on food and feed (Embaby et al. 2013, Sweeney and Dobson 1998). *Aspergillus* and *Penicillium* are osmotolerant and many are xerophilic, which allows for these species to be found on stored grains, cereals and other preserved foods with a low water activity (often due to high sugar or salt content) (Pitt and Hocking 2009, Visagie et al. 2017). On the other hand, *Fusarium* is not xerophilic like *Aspergillus* and *Penicillium*, and can be found throughout the production line; however, it is most commonly found during pre-harvest.

Consumption of seeds and grains that are contaminated with mycotoxins can have various effects on consumer health, especially on individuals with elevated allergies (Benndorf et al. 2008, Eskola et al. 2020). The severity of the effect is dependent on the quantity, frequency of intake; and toxicity of the compound. Mycotoxicosis are diseases caused by mycotoxins and can either be classed as acute or chronic (Zain 2011). Acute mycotoxicosis has a rapid onset and an

obvious toxic response; and occur as a result of consuming a high amount of contaminated food (or high concentration) over a short time period and can often result in death. Chronic toxicosis is a result of a low-dose exposure over a long period of time and generally results in irreversible effects like cancers. The health impacts of mycotoxin consumption are of major concern in developing countries due to the majority of the population being malnourished which predisposes their immune systems to the side effects (Al-Seeni 2012). These individuals are also chronically exposed to high mycotoxin levels due to the lack of resources, technology, infrastructure, food monitoring systems and toxin control (Wu and Guclu 2012). According to Sibanda et al. (1997), reports of mycotoxicosis in developing countries is not widespread when compared to the reports in developed countries. This is due to limited knowledge in terms of disease diagnosis, occurrence of mycotoxins, finances of governmental institutions and their policies, as well as the acute and chronic effects of mycotoxins.

Due to their negative impacts, different regulatory bodies such as the Food and Drug Administration (FDA (Association 2011)) and the European Food and Safety Authority (EFSA (Commission 2006)), have set their own standards referred to as the maximum tolerable daily intake; and these are set based on food consumption patterns (Alshannaq and Yu 2017, Chain et al. 2017, Matumba et al. 2017). However, there is a global operator in mycotoxin standard setting known as the Codex Alimentarius Commission of the Food, which is comprised of different governing bodies from the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) (Eskola et al. 2020). Regulations are implemented to protect the consumers from mycotoxicosis, particularly from imported grains and cereals. When mycotoxin limits are established, scientific and socio-economic factors need to be considered (Lu et al. 2020). These factors include toxicological and exposure data, the availability of analytical facilities, food sufficiency and the mycotoxin legislation in other countries with which a country has a trade agreement with. Developing countries tend to export their best produce as they have a lower mycotoxin level than what they keep for domestic use. The produce destined for export is still at risk of a reduced price, complete rejection or they must be destroyed due to mycotoxin contamination not in line with the accepting country (Wu 2004, Wu and Guclu 2012). It is suggested that

regulatory limits should be harmonized across the globe in order to facilitate international trade (Matumba et al. 2017). This will not be widely accepted as it will result in a significant economic impact on both developed and developing countries. There will be many countries that will have to spend more money on attempts to reduce mycotoxin levels in food for trade, and even more that cannot afford this and will as a result be barred from trade altogether.

Mycotoxins have economic impacts on different countries, and these impacts can vary. Costs associated with mycotoxin contamination can affect each stage of the production line. These range from control and surveillance at a farming level, handling and testing costs, losses in the livestock industry from decreased efficiency / productivity; and losses from the export market and crop insurance pay-outs from governments (Mitchell et al. 2016). It is often assumed that the direct economic impact of mycotoxins is mainly due to the enforcement of the regulations by regulatory bodies, however; indirect costs are also incurred due to the consumption of mycotoxins by humans and animals and the associated medical costs. It can be difficult to accurately determine the indirect economic impact of mycotoxins (Conte et al. 2020, Mitchell et al. 2016). For example, in developed countries such as the USA, aflatoxin-related losses are linked to trade rather than consumer impact (Mitchell et al. 2016). However, in developing countries, aflatoxin-related losses are mainly linked to consumer impact as well as to trade (Udomkun et al. 2017). Additional cost may be incurred for all medical related costs and product recalls that occur due to contamination. This is not only in having to either refund or replace already used products, but also customers lost due to distrust.

Fungal identification

Fungal communities play a vital role in the quality and safety of maize and other cereal grains. Characterising these populations and detecting fungal species associated with cereal grains is thus important. There are several countries such as South Africa that generally have diverse fungal communities, however knowledge on the species and their habitats is lacking (Crous et al. 2006).

Traditionally, a morphological species concept was the standard in fungal taxonomy, and thus used for identifications. The drawbacks of using morphology

for identifications include certain morphological characters being influenced by different environmental factors, variations that are often difficult to interpret even for experienced taxonomists (Visagie et al. 2014). In addition, morphology does not allow for the differentiation between cryptic species (species that look alike but are different genetically). Previous surveys have been largely based on morphological identifications, and as a result may have plenty of misidentified strains.

Most taxonomists currently employ a phylogenetic or polyphasic species concept for fungi. This modern approach led to species identifications that rely on DNA sequence data, typically internal transcribed spacer region (ITS) data, but this is only possible where complete reference sequence databases are available. These databases were created using taxonomic revisions and often resulted in ITS not being reliable in resolving between species within a genus. This resulted in the proposal of several alternative gene sequences for identification, with these sequences resolving better between the genera. The gene sequences proposed include calmodulin (*CaM*) for *Aspergillus* (Samson et al. 2014), beta-tubulin (*BenA*) for *Penicillium* (Visagie et al. 2014), translation elongation factor 1-alpha (*TEF*) for *Fusarium* (O'Donnell et al. 2000) and ITS for unknown species (Schoch et al. 2012). Whilst many fungal culture-based diversity studies have been conducted, they have made use of ITS DNA sequence data for identifications (Rahman et al. 2020). As a result, many of these species may be misidentified. Using alternative gene sequences for identification of fungal species, modern surveys can be conducted to better understand the fungal communities associated with important crops and how they may affect each other, for example promote or prevent mycotoxin production.

Mycotoxins

Mycotoxins can be classed into three broad categories, namely regulated, emerging and masked (Gareis et al. 1990, Jestoi 2008, Rychlik et al. 2014, Vaclavikova et al. 2013). Worldwide, the five most important regulated mycotoxins are AFs, DON, FUMs, OTA and ZEA. Another important and regulated group of mycotoxins is the trichothecenes (TRIs) which is the largest and one of the most economically important group of *Fusarium* mycotoxins (Munkvold et al. 2021).

DON is a type B trichothecene, other important and regulated type A trichothecenes associated with maize include T-2 (T2) and HT-2 (HT2) toxin. These are regulated due to the known severity of their potential health impacts on consumers.

Emerging mycotoxins of concern include beauvericin (BEA), culmorin (CUL), enniatins (ENN), fusaproliferin (FUS), moniliformin (MON) and many others (Biomin 2018, Biomin 2019, Biomin 2021). Two of the regulated mycotoxins, DON and ZEA, have been shown to be the main parent molecules for the formation masked mycotoxins like ZEA-14-sulfate (ZEA-14S) and DON-3-glucoside (DON-3G). Currently, the gold standard used for the simultaneous screening, identifying, and measuring mycotoxins is high-performance liquid chromatography mass spectrometry (HPLC-MS/MS) (Bueno et al. 2015, Eskola et al. 2020, Meyer et al. 2019). This not only allows for the detection of regulated mycotoxins at low concentrations, but also of masked mycotoxins that are conjugated to polar compounds, provided that the commercial standards are available (Eskola et al. 2020, Weaver et al. 2020). The use of highly sensitive detection tools allows for more accurate and increased sensitivity in determining the quantity of mycotoxins present, and also allows for the detection of multiple mycotoxins from a single commodity (Park et al. 1999). This may give the perception that mycotoxin levels are increasing in contaminated food around the world (from the original 25% predicted by the FAO) (Eskola et al. 2020).

Regulated mycotoxins and their producers relevant to maize

Aflatoxins

Aflatoxins (AFs) were discovered and characterised following the death of approximately 1000 turkeys in Great Britain in 1960 after consuming contaminated peanut meal (Blount 1961). This outbreak was named Turkey “X” Disease because the exact cause of the disease remained unknown. The first major human aflatoxicosis outbreak was recorded in India in the 1970s, which resulted in 106 deaths following the consumption of contaminated maize (Krishnamachari et al. 1975).

Aflatoxicosis is predominantly reported in rural areas that rely on maize as a staple food. The biggest human aflatoxicosis outbreak was recorded in Kenya in

2004 with over 300 cases reported and approximately 200 deaths that occurred (Azziz-Baumgartner et al. 2005). In 2012, canine aflatoxicosis occurred in the Gauteng province, South Africa, due to contaminated dog food products (Arnot et al. 2012). Aflatoxicosis outbreaks associated with maize consumption have also been recorded in Tanzania in 2016 and 2017 which resulted in 67 and 8 cases being reported respectively (Kamala et al. 2018, News 2017). A more recent outbreak recorded in South Africa involved Montego, who recalled large batches of contaminated dog food produced over a period of a month due to contamination of a 'yellow mould' most probably representing *Aspergillus flavus* (Crouth 2020, Focus 2019, Smit 2019). While these products did not contain high levels of AFs, consumer pressure led to the recall which cost the company millions of rands.

AFs are produced by the genus *Aspergillus*, most importantly by *A. flavus* and *A. parasiticus* (Miller 1995), but also other species classified in sections *Flavi* (16 species), *Nidulantes* (3 species) and *Ochraceorosei* (2 species) (Frisvad 2015b, Frisvad et al. 2019, Frisvad and Larsen 2015a). There are four major AFs namely, AFB₁, AFB₂, AFG₁ and AFG₂. It was previously reported that *A. flavus* only produces AFB₁ and AFB₂, whereas *A. parasiticus* produces all four variants (Abbas et al. 2009, Hocking et al. 2006). However, Frisvad et al. (2019) reported that *A. flavus* strains studied from South Korea produced all four types. AFs can be visually distinguished from each other when they are placed under a UV light with AFB₁ and AFB₂ having a blue fluorescence, while AFG₁ and AFG₂ have a green fluorescence (Hocking et al. 2006). AFB₁ is the most toxic and naturally occurring of the four variants (Miller 1995). When AFB₁ is metabolized by dairy cattle, the AFM₁ toxin is produced and can be detected in milk (Van Egmond 1989).

AFs contamination can occur at any time during the production line. Contamination that occurs during preharvest is most common following high temperature, prolonged drought periods, late seasonal rain and in some cases increased insect activity; whereas postharvest contamination is most common at warm temperatures and high humidity (Atanda et al. 2011). AFs are typically associated with cottonseed, maize, nuts, oilseeds, sorghum and wheat (Pitt 2000, Shephard 2008). In South Africa, *A. flavus* and *A. parasiticus* have been found sporadically throughout commercial and subsistence maize farms; however, they

have not been implicated in ear rots or aflatoxin contamination under our local environmental conditions (Shephard 2003).

Interestingly, *A. oryzae* and *A. sojae* represent domesticated forms of *A. flavus* and *A. parasiticus* respectively and do not produce mycotoxins. Strains of these species have long been used in Asia during koji fermentations (e.g., in Japan such as sake, vinegar, soy-sauce and miso) or enzyme production (Houbraken et al. 2014, Terabayashi et al. 2010). In Japan, *A. oryzae* is the national microorganism (Kitamoto 2002), and *A. oryzae*, *A. sojae* and *A. tamarii* are also referred to as yellow-koji moulds. *Aspergillus oryzae* and *A. sojae* cannot be distinguished from *A. flavus* and *A. parasiticus*, respectively, when using conventional identification protocols, even though deletions and genetic factors have seemingly silenced the aflatoxin-producing pathway (Takahashi et al. 2002).

Acute aflatoxicosis often results in death, whereas chronic aflatoxicosis results in “slow” pathological conditions such as cancer and immune suppression (Zain 2011). The primary target for AFs is the liver which can result in the onset of jaundice or in liver cancer. The effect of AFs varies between different animal species (Table 1). Aflatoxicosis cases in individuals suffering with hepatitis B have been shown to cause late stage liver cancers and in many cases death (Krishnamachari et al. 1975). Regions that have an existing high hepatitis B prevalence are China, South East Asia and Africa (Wu and Guclu 2012). In children, chronic exposure to AFs at weaning stage has been associated with stunting of growth and immune suppression (Gong et al. 2002, Gong et al. 2016, Owaga et al. 2011, Turner et al. 2007, Turner et al. 2003). The immune-suppression is caused by the reactivity of the AFs with the host T-cells which results in decreased vitamin K activities, as well as a decrease in the phagocytic activity within macrophages (Wagacha and Muthomi 2008).

A world mycotoxin survey was conducted by Biomin (2019) which found that samples obtained from Africa (7%), Asia (31%), the Middle East and North Africa (37%) and South and Central America (21%) contained AFs levels that were above the regulated threshold limits. In the 2020 survey, Biomin (2021) revealed a decrease in the AFs concentration detected in samples across all regions.

Fumonisin

Fumonisin (FUMs) were first discovered in South Africa in the 1980s when Bezuidenhout et al. (1988) explored the cause of oesophageal cancer in the Transkei region. FUMs were also linked to an equine leuko-encephalomalacia outbreak in the Transkei which causes the necrosis of the cerebral hemispheres in horses resulting in abnormal movements, aimless circling, and lameness (Kellerman et al. 1972, Marasas et al. 1988b, Marasas et al. 1976).

Fumonisin B comprises of FUMB₁, FUMB₂ and FUMB₃, with FUMB₁ occurring more frequently than the others (Miller 1995, Rheeder et al. 2002). FUMs producers are predominantly from the FFSC with the major producers comprising of *F. annulatum* (= *F. proliferatum* (Yilmaz et al. 2021)) and *F. verticillioides* (= *F. moniliforme*) (Marasas et al. 1976, Miller 1995, Munkvold et al. 2021, Seifert et al. 2003). Some *F. fujikuroi* strains have also been implicated in FUMs production, however this has only been associated with sorghum and wild grasses (Proctor et al. 2004). Other FUMs producers include *F. nygamai* and *F. ramigenum* (Marasas et al. 1991, Munkvold et al. 2021). *Fusarium thapsinum* has been reported to produce FUMs, however there are variations in the production ability between different strains (Munkvold et al. 2021). Some *Aspergillus* section *Nigri* species are also capable of producing FUMs, most notably *A. niger* that can produce FUMB₂ and FUMB₃ (Frisvad and Larsen 2015a).

Fusarium annulatum (= *F. proliferatum* (Yilmaz et al. 2021)) and *F. verticillioides* are predominantly found in humid and sub-humid regions and are not very common in cooler temperate regions (Logrieco et al. 2002). FUMs have been found in a number of food commodities such as black tea, cereals, figs, wheat, maize, maize based products and feeds, millet, rice, soybean and sorghum (Bennett and Klich 2003, Shephard 2008).

FUMB₁ consumption has been associated with mycotoxicosis (Table 1) such as porcine pulmonary edema, equine leukoencephalomalacia in animals; and neural tube birth defects in humans (Constable et al. 2003, Marasas et al. 2004, Smith et al. 2002). Neural tube defects are a result of the reduced uptake of folate in various cell lines. The consumption of FUMs has been linked to increased incidences of human oesophageal cancer in Asia, Central America and parts of

Africa (Marasas et al. 1991, Marasas et al. 1981, Marasas et al. 1988a, Miller 1995). While consumption of FUMs by animals leads to respiratory difficulties, it is also nephrotoxic and hepatotoxic damaging the kidneys and liver respectively (Thiel et al. 1992).

The presence of high FUMs levels on finished feed, cereal samples and maize is a global problem. A study conducted by Biomin (2019) found samples analysed from Africa (78%), Asia (100%), Europe (73%), the Middle East and North Africa (47%), North America (78%); South and Central America (90%) and were contaminated with FUMs levels over the threshold limits. A follow up study conducted in 2020, observed a decrease in the number of samples contaminated from the respective regions, except in the Middle East and North Africa where an increase was observed (77%) (Biomin 2021).

Deoxynivalenol

Deoxynivalenol (DON) was first identified in 1970 in Japan following the consumption of mouldy maize by locals. *Fusarium sambucinum* (= *F. roseum*) was identified as the species producing this mycotoxin, which is also referred to as 'vomitoxin' due to its strong emetic effects (Yazar and Omurtag 2008). According to the WHO (1993), DON is considered to be the most widely distributed mycotoxin implicated in food and feed contamination.

DON is predominantly produced by species from the FSAMSC. Species within this complex have been subdivided into three chemotype groups (Munkvold et al. 2021). These are 15-acetyldeoxynivalenol (15-ADON) chemotype, which produces DON and 15-ADON; the 3-acetyldeoxynivalenol (3-ADON) chemotype, which produces DON and 3-ADON; and the nivalenol (NIV) chemotype (Mirocha et al. 1989, Seo et al. 1996). In South Africa, the 15-ADON chemotype is commonly found (Munkvold et al. 2021).

DON is commonly found in cereals and is typically produced by *F. culmorum* and *F. graminearum* (Miller 1991, Munkvold et al. 2021). *Fusarium asiaticum*, *F. boothii* and *F. pseudograminearum* have also been found to produce DON in lower amounts (Hocking et al. 2006, Munkvold 2017, Munkvold et al. 2021, Zain 2011). *Fusarium pseudograminearum* is normally found in warmer climates, whereas *F. graminearum* is found in cooler regions (Hocking et al. 2006,

Munkvold et al. 2021). *Fusarium pseudograminearum* has been reported in Canada, South Africa and the USA, and has been implicated as a pathogen under very wet conditions (Munkvold et al. 2021). However, this species does not generally infect grains, so it is not considered a toxicological risk. *Fusarium boothii* were reported to have a host preference for maize (Munkvold 2017). Typically DON production is favoured by low temperatures and high humidity (Pestka and Smolinski 2005).

DON is associated with cereals such as barley, cereal by-products, maize, soybeans, sunflower seeds and wheat; while it can also be present on bananas and peanuts (Bennett and Klich 2003, Hocking et al. 2006, Shephard 2008). DON and ZEA mycotoxins are typically found to co-occur (Miller 1995). Mycotoxicosis caused by DON consumption include red mould disease in Japan, feed refusal in swine and estrogenic syndrome (Table 1) (Munkvold et al. 2021). DON is highly toxic to humans and animals and can penetrate the lipid bi-layers of cell membranes (Miller and Marasas 2002). This is undesirable as the toxin can react with DNA, RNA and cellular organelles directly, having even greater health impacts. In many animals, DON has the potential to inhibit their reproductive performance, reduce milk production in cows, restricts growth and results in feed refusal (Pestka 2007, Prelusky 1994). The health impacts of DON in humans is not completely understood, but it is known to cause nausea, diarrhoea and fever (Miller 1995).

The presence of DON across the world is also a major concern. A survey conducted by Biomin (2019) found that maize samples from Africa (83%), Asia (88%), Europe (83%), Middle East and North Africa (87%), North America (85%); and South and Central America (56%), were contaminated with DON at levels over threshold limits. In a follow up survey conducted in 2020, a decrease in the number of samples contaminated by DON was observed from all the regions (Biomin 2021).

Zearalenone

Zearalenone (ZEA) was first isolated from *Fusarium* infected maize following reports of genital defects in animals fed from mould-spoiled grain. The producing fungus was later identified as *Fusarium graminearum* (Stob et al. 1962). Together with *F. culmorum*, they are the major ZEA producers (Miller 1991). Other known producers are also found within the FSAMSC, as well as in the FIESC (Munkvold et al. 2021). Species from the FSAMSC that are known to produce ZEA include *F. asiaticum*, *F. pseudograminearum* and *F. sporotrichioides*; and species from the FIESC that are known to produce ZEA include *F. equiseti*. ZEA is associated with a number of food commodities such as barley, maize, oats, rye sorghum and wheat (Miller 1995, Shephard 2008).

ZEA has an impact on both human and animal health, predominantly affecting reproductive health in both (Table 1) (Munkvold 2017, Munkvold et al. 2019). As a result, ZEA is considered one of the most economically important mycotoxins. In pigs exposure can cause genital and urinary problems with hypoestrogenism which results in reproduction problems, decreased litter size, as well as weak and still-born piglets (Miller 1991). In humans, ZEA consumption is of particular concern in children where it has been linked as a possible carcinogen (WHO 1993). The exact impact of ZEA on human health is not completely known, however, it is suggested that humans share similar sensitivity towards the mycotoxin as pigs (Munkvold et al. 2019). Some data shows that ZEA causes changes as well as lesions in the female reproductive system (WHO 1993).

In a survey conducted by Biomin (2019), ZEA contamination was found in samples obtained from Africa (56%), Asia (75%), Europe (58%), the Middle East and North Africa (67%), North America (55%); and South and Central America (27%). In a follow up study conducted in 2020, it was found that like DON, a decrease in the number of contaminated samples was observed across all regions except samples obtained from South and Central America, where an increase in the number of contaminated samples was observed (44%) (Biomin 2021).

Masked mycotoxins in maize

Masked mycotoxins are chemical derivatives that are structurally related to the parent molecule but have been biologically modified due to plant or food metabolism through conjugation with more polar compounds (EFSA 2014, Galaverna et al. 2009, Gareis et al. 1990). These are not detected using conventional detection methods, due to a lack of commercial standards. This results in underestimation of the total mycotoxin content in contaminated foods.

The toxicity of these molecules can either increase or decrease depending on the parent molecule or its modifications, however they are typically less toxic than the parent molecule (Munkvold et al. 2021). During digestion and food processing however, they can be hydrolysed or bio-transformed to their parental forms (Berthiller et al. 2013, EFSA 2014). Masked mycotoxins are a cause for concern as they can accumulate as well as co-occur with parental and non-parental forms in the edible parts of the plant (Berthiller et al. 2013, Lu et al. 2020). The combinatory effect as well as the toxicity of the modified mycotoxins remains unknown. More research is required in terms of the impacts of masked mycotoxins on humans and animals following consumption.

DON and ZEA are common parent molecules for masked mycotoxins in maize. DON has three main modified forms, D3G; 3-ADON and 15-ADON. The acetylated forms (3-ADON and 15-ADON) are of fungal origin and are intermediates of fungal DON biosynthesis (Bretz et al. 2005, Maul et al. 2012), while the glycosylated (D3G) form is a product of plant metabolism (Sewald et al. 1992). ZEA also has three main modified forms, ZEA-14-glucoside (ZEA-14G), ZEA-16-glucoside (ZEA-16G), which are modified forms of plant origin; and ZEA-14S which is a derivative of fungal origin (Binder et al. 2017, Michlmayr et al. 2017).

Emerging mycotoxins in maize

Emerging mycotoxins are not routinely tested for since they are not legislatively regulated; however, research on these shows that they are very common on crops (Gruber-Dorninger et al. 2016, Vaclavikova et al. 2013). These are not regulated

due to the lack of complete toxicological data as well as poor bioavailability studies (Escrivá et al. 2017).

Emerging mycotoxins are mostly produced by *Alternaria*, *Aspergillus*, *Penicillium*, and *Fusarium* species (Braun et al. 2021). According to the Biomin reports, aurofusarin (produced mainly by species in the FSAMSC and some in the *Fusarium tricinctum* species complex) (Munkvold 2017, Munkvold et al. 2021); and moniliformin (MON, produced predominantly by species in the FFSC and some isolates in the *Fusarium oxysporum* species complex (FOSC)) (Munkvold 2017, Munkvold et al. 2021) are the emerging mycotoxins most frequently detected from raw maize commodities across the globe (Biomin 2019, Biomin 2020). Other emerging mycotoxins of concern are alternariol (AOH) and alternariol monomethyl ether (AME) (predominantly produced by *Alternaria alternata* (Ostry 2008, Pinto and Patriarca 2017)), beauvericin (BEA, predominantly produced by species if the FFSC and some species in the FSAMSC), enniatins (ENN, predominantly produced by species in the FSAMSC) (Jestoi 2008, Munkvold 2017, Munkvold et al. 2021) and sterigmatocystin (produced by *Aspergillus*, *Bipolaris*, *Chaetomium*, *Emericella*, *Humicola* and one *Penicillium* species (Gruber-Dorninger et al. 2016)). As we experience fluctuations in temperature and weather patterns, we can expect to observe fluctuations in the number and types of emerging mycotoxins found in food and feed. Due to a change in climate conditions, more data needs to be collected on the toxicity of emerging mycotoxins in order to implement the correct legislations and regulations.

Mycotoxin prevention and management

Fungal spoilage and subsequent mycotoxin contamination can occur pre- or postharvest. Different control strategies throughout the processing stages have been explored. Emerging control strategies from developed countries have not been widely adopted in third world countries as they are commonly inaccessible, require a large financial outlay; and no single mitigation measure has proven robust enough for wide scale adoption (Kagot et al. 2019). Strategies are divided into three classes, namely physical, chemical and biological (Loi et al. 2017). Physical strategies make use of the mechanical removal of contaminated grains from the raw food and feed material (Vanhoutte et al. 2016). Chemical methods

make use of treatments such as acid treatments (Aiko et al. 2016), bisulphites (Altug et al. 1990), peroxidation (Fouler et al. 1994) and ozonation (Maeba et al. 1988). Chemical methods are not commonly applied due to the side effects on the quality of the raw materials, potential toxicity to the consumer, poor efficacy and large financial outlay (Loi et al. 2017). Finally, biological strategies make use of enzymes or microorganisms that can form less toxic, stable and in some cases harmless compounds through metabolism, or complete destruction or deactivation of the parent molecule (Loi et al. 2017). Application of biological strategies does not have negative impacts on the food quality or consumer health. When these management strategies (physical, chemical or biological) are implemented individually, they have limited success, but in conjunction with others, controlling mycotoxin contamination becomes much more successful.

Pre-harvest

Pre-harvest control strategies include resistant maize cultivars against specific fungi, insect control, fungicide use, biocontrol agents and removal of plant debris following harvest. Resistant seed varieties are developed using conventional and transgenic technologies (Duvick 2001, Kagot et al. 2019, Munkvold 2003a). Resistant traits are conferred by multiple genes, which is why there is currently no maize line available that offers resistance to multiple fungal species. In addition, the development and use of Genetically Modified Organisms (GMOs) is not widely accepted across the world.

The use of insecticides and / or fungicides are often used to control insect activity and fungal growth respectively. However, this practice is not often used on emerging farms (also referred to as emerging smallholders, farms that are market orientated produce sellers with the goal to commercialise their produce) (Alberts et al. 2019). In these cases, farmers are encouraged to plant Bt-hybrids which provides protection against *Lepidoptera* pests like stalk borers, fall army worm and others. Fungicides are typically effective at preventing fungal growth on commodities and is applied to seeds prior planting or sprayed at various points during the growing season.

Abbas et al. (2006) and Dorner and Cole (2002) suggested the use of a biocontrol agent such as nontoxigenic fungal strains. Under suboptimal environmental

conditions where fungal growth occurs, the nontoxigenic strain (preferably native to respective regions) allows for competition for the nutrients and space and results in the reduction in mycotoxin contamination. Examples of such products include Aflasafe (IITA BiP; Ibadan, Nigeria), Afla-Guard (Syngenta, United States) and AF36 (ACRCP, United States) (Amaike and Keller 2011, Kagot et al. 2019). The possibility of the toxigenic strains horizontally transferring their mycotoxin gene clusters to atoxigenic strains is unknown, but it is the suggested mechanism by which the OTA gene cluster in *P. nordicum* was supposedly inherited from *A. steynii* (Gil-Serna et al. 2018, Landan et al. 1990).

Following harvest, the removal and destruction of the debris will remove major sources of inoculum that can overwinter and contribute to any future onsite contamination (Alberts et al. 2019). Other factors that will aid in reducing fungal contamination include crop rotation, maintaining the same planting dates, irrigation and the use of fertilizers (Peng et al. 2018).

Postharvest

Maize is exposed to a number of possible contaminants during the harvesting process, these include microorganisms present on insects, in the air, on equipment or agronomic practices (Magan and Aldred 2007, Magan et al. 2004). However, postharvest fungal and mycotoxin contamination is mainly due to improper storage (Magan et al. 2004). It is thus important for feed handlers, grain mill operators and silo managers to ensure storage conditions are kept at conditions that prevents fungal growth (Abbas et al. 2009, Zain 2011). There are several methods used to prevent postharvest mycotoxin contamination. These conditions include the minimisation of time between harvesting and drying, effective and optimal cleaning of maize prior to storage, a moisture content of less than 14% achieved through drying, storage facilities that are clean, dry and largely oxygen free, and in some cases chemical treatment of seeds (Magan and Aldred 2007).

While there are prevention strategies that have been developed, they are not always successful, especially when used alone and not in conjunction with multiple methods. Maize that is contaminated with high levels of mycotoxins cannot be consumed or used for trade. There are some management strategies

that have been developed to reduce the mycotoxin levels, these include dilution, dry and wet sorting and rapid drying of maize.

Dilution is the mixture of heavily contaminated commodities with non-contaminated commodities until an “acceptable” mycotoxin level is detected for storage (Wagacha and Muthomi 2008). This is not accepted by regulatory bodies as small doses of the mycotoxin is still consumed and can have an impact on the consumers health (Peng et al. 2018). Dry sorting is the removal of visibly infected maize or maize with abnormalities before storage (Kabak et al. 2006). Depending on the production scale, this can be done manually or mechanically. Wet sorting is achieved based on the difference in densities between fungal infected and non-infected grains, with infected grains being less dense (Huff and Hagler 1985). Rapid drying of the seeds provide an unfavourable environment for fungal growth and proliferation, provided that it is done in the correct manner (Lakshmi Prasanna and Panda 1997, Peng et al. 2018). This method is used by a number of emerging farms in South Africa where they leave the maize to dry in the field for anywhere up to three months prior to harvesting (Munkvold 2003a).

Enzymatic detoxification of mycotoxins, also referred to as biotransformation, is a biological strategy that makes use of enzymes produced by bacteria or fungi for the degradation of mycotoxins into non-toxic metabolites (Boudergue et al. 2009). This strategy is promising, however when identifying a potential enzyme, it needs to be ensured that the detoxified product is not more toxic than the parent molecule. In addition, identification of a suitable enzyme is challenging and time consuming (Loi et al. 2017). Enzymes currently available include Biomin ® BBSH 797 and Biomin ® MTV, FUMzyme ® and Mycofix ® (Biomin Holding GmbH, Getzersdorf, Austria).

Mycotoxin binders are a physical technique used for mycotoxin decontamination of feed (Jans et al. 2014). Mycotoxin decontamination occurs when the binders are added to feed and they strongly bind the mycotoxins to them, thus preventing any toxic interactions or absorption of the mycotoxin in the digestive tract of the consumer (Whitlow 2006). The use of binders in human food has been tested in Africa, namely in Ghana (Phillips et al. 2008) and Kenya (Kagot et al. 2019). Whilst the use of binders shows promising results, concerns have been raised

surrounding the possibility of adsorption of essential minerals and nutrients required for normal, and healthy bodily functioning (Kagot et al. 2019).

Conclusions

Since its discovery, maize has become one of the world's most important crops. In South Africa, both the human and animal sector rely heavily on maize and its by-products as the main food source. The production of this crop is often threatened by fungi and subsequent mycotoxin contamination during cultivation and storage.

There are prevention and management strategies that have been developed to control fungal growth. Although they sound appealing, it should be noted that the strategies utilized in developed countries cannot be readily used in developing countries (Wagacha and Muthomi 2008). This is due to the difference in the characteristics of the food systems, the available technological infrastructure, and most importantly the financial outlay required. There are many populations in developing countries that rely heavily on diets that are susceptible to mycotoxin contamination. Due to this, developing countries, especially those in Africa, should be assisted and encouraged to develop prevention and management strategies that are feasible and applicable to their environment and available materials.

In order to develop prevention and management strategies unique to South Africa, baseline knowledge of which fungal species are present in the community is required. Studies conducted in the past have either mainly used morphology or molecular methods targeting the ITS gene region for fungal identification. Within a South African context, there is a lack of diversity studies that have been conducted, especially within the Eastern Cape province. This knowledge gap creates the opportunity for future studies to be conducted in this region. Once we have identified the species present, with emphasis on the mycotoxin-producing species, we can further explore abiotic and biotic variables that control mycotoxin production.

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Tables

Table 1: Summary of human and animal health risks associated with mycotoxins

Mycotoxin	Animal health risk	Human health risk	Reference
Aflatoxins	Poultry and swine particularly at risk- lethargy, ataxia; reduced reproduction & feed conversion	Growth stunting, loss of weight & kwashiorkor, death when associated with hepatitis B, hepatotoxic; immuno-suppressive	Bennett and Klich 2003, Miller 1995
Fumonisin	Hepatotoxic and nephrotoxic, respiratory difficulties (pulmonary edema) in swine, leukoencephalomalacia	Increased incidence of oesophageal cancer, immunosuppressive, neural tube defects	Miller 1995, Thiel, et al. 1992
Deoxynivalenol	Inhibition in reproductive performance in many animals, reduced milk production in cows & vomiting in swine, slowed growth, feed refusal, suppression of immune response	Affects not completely understood & known, toxicosis, nausea, diarrhoea, fever	Miller 1995, Pestka 2007, Prelusky 1994
Zearalenone	Pigs particularly at risk- genital & urinary problems with hypoestrogenism, alterations in reproductive tract, reduced milk production, possible infertility	Children most at risk, possible human carcinogen; changes & lesions in female reproductive system	Miller 1995, WHO 1993

Figures

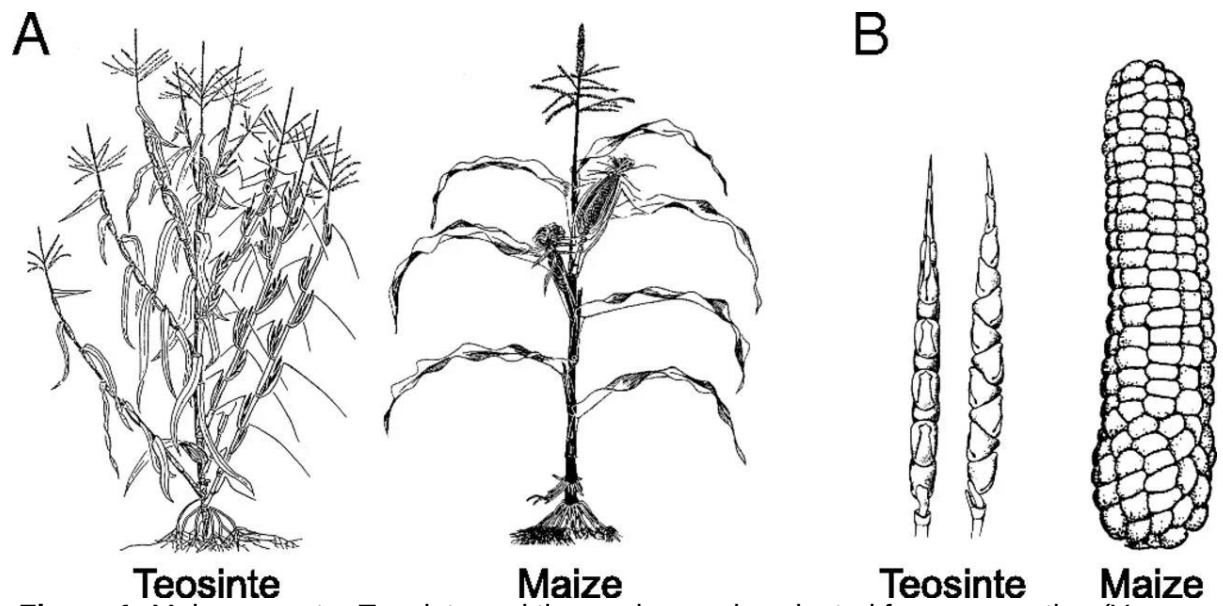


Figure 1: Maize ancestor Teosinte and the modern maize planted for consumption (Yang et al. 2019).

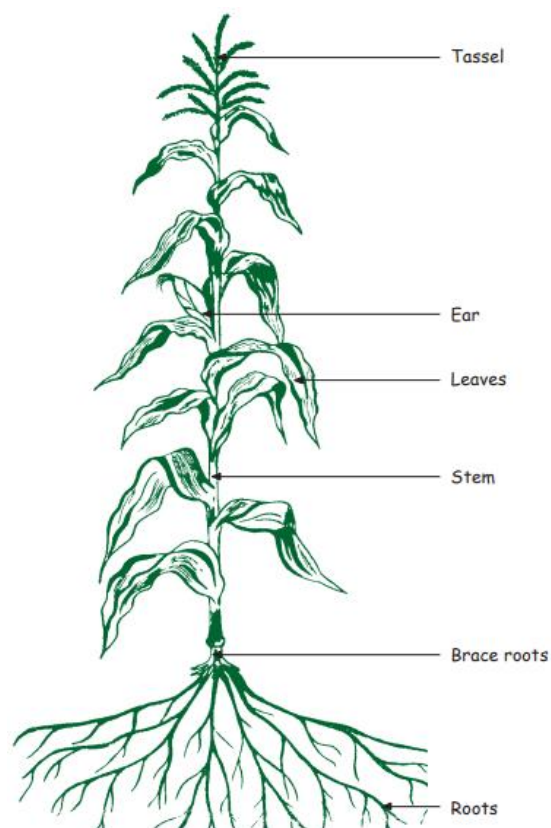


Figure 2: The structure of a mature maize plant (DAFF 2003).

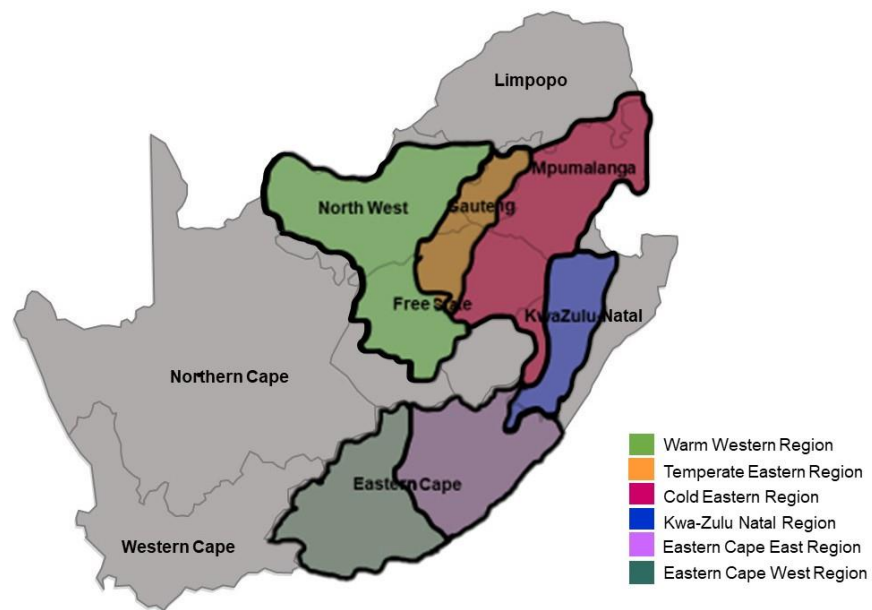


Figure 3: Different climatic regions of maize planting areas in South Africa (adapted from Sihlobo 2019).

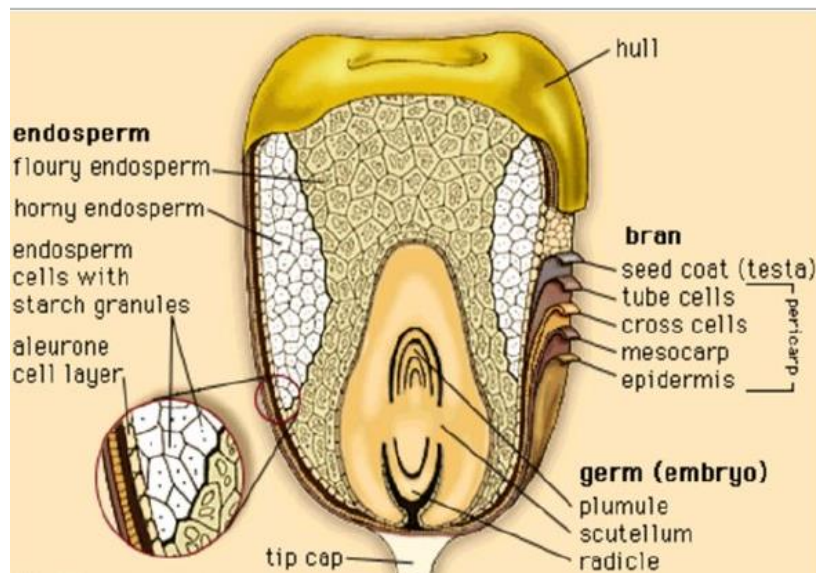


Figure 4: Maize kernel structure (Suleiman et al. 2013).



Figure 5: Maize ear rots. *Gibberella* ear rot (A) (Pioneer 2021), *Fusarium* ear rot (B) (Team 2017), *Aspergillus* ear rot (C) (Bradley 2012), *Diplodia* ear rot (D) (Reese 2012), *Penicillium* ear rot (E) (JACKSON-Ziems and Rees 2016) and Corn smut (F) (Unknown).



Figure 6: Maize storage methods. Hermetic storage can be observed: SuperGrain bags (A) (GrainPro) and metal silo (B) (FDSP). Non-hermetic storage: Maize sacks (C) (Agri-worxx 2020), crib storage (D) (IFPRI 2016), roof storage (E) (Commons 2014) and metal drum storage (F) (Ncube et al. 2021).

CHAPTER 2:

The fungal diversity associated with post-harvest maize from the Eastern
Cape

Abstract:

Maize is one of the top four food commodities produced across the world. Its production in South Africa occurs across a large farming area and is consumed locally in many different forms. Many fungi are associated with maize, and some can negatively impact production causing diseases or contamination with mycotoxins. *Aspergillus*, *Fusarium* and *Penicillium* are the most important mycotoxigenic genera and produce the 'big five' globally regulated mycotoxins. Knowledge of the fungal species associated with maize is lacking, especially in the Eastern Cape which represents a historically neglected production region. This study thus explored the fungal diversity associated with post-harvest maize from emerging maize farms in the Eastern Cape. A total of 481 fungal strains were isolated that belonged to *Alternaria* (n=10), *Aspergillus* (n=21), *Cladosporium* (n=58), *Fusarium* (n=203), *Penicillium* (n=90) and 15 other genera. Representative strains were sequenced using the genus-specific identification markers glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*; for *Alternaria* and other *Pleosporales*), calmodulin (*CaM*; for *Aspergillus*), translation elongation factor 1- α (*TEF*; for *Fusarium* and *Cladosporium*), β -tubulin (*BenA*; for *Penicillium*) while the nuclear ribosomal internal transcribed spacer (ITS) rDNA region was sequenced for strains that could not be identified to a genus using morphology. Communities were found to be relatively diverse with strains identified to 84 species. Amongst these were several known mycotoxin producers. The most common species identified were *Fusarium boothii*, *F. poae*, *F. temperatum*, and *Cladosporium cladosporioides*. This survey is the first reporting a diverse range of fungal species from Eastern Cape produced maize that employed DNA sequences to make identifications creating important baseline knowledge for future surveys that will focus on the impact fungal communities may have on the production of high-quality maize in the region, the diseases they may cause, and the mycotoxins they may produce.

Introduction

Maize is a summer rainfall crop and is produced worldwide. It is one of the top four food commodities with rice, sugar cane and wheat (FAO 2021). Within South Africa, maize is considered one of the major staple crops and is produced for animal, human and industrial consumption (Department of Agriculture 2008, Ekwomadu et al. 2018). White maize is predominantly used for human consumption either as mielie meal or maize-based foods such as infant foods and snacks (Ekpa et al. 2018). Yellow maize is predominantly produced for animal consumption as food and feed; and recently for industrial use where the use of maize as raw material for biofuel production is being explored.

South Africa is the largest commercial maize producer in Africa with production mostly concentrated in the central provinces such as the Free State (FS), Kwa-Zulu Natal (KZN), Mpumalanga (MP) and the North West (NW) (Beukes et al. 2017, Cutts and Hassan 2003, Sihlobo 2014). The largest contributor to commercial maize production is the FS followed by MP (SAGL 2020). In the 2020/21 growing season, South Africa produced 15 778 million tonnes, consisting of white (8 957 million tonnes) and yellow (6 821 million tonnes) maize (Department of Agriculture 2021). The Eastern Cape (EC) currently has a high proportion of subsistence, emerging and small-scale maize farmers who contributes to less than 1% of the total maize produced across South Africa.

Globally, maize production can be impacted by fungal pathogens causing devastating diseases. Further losses can be experienced due to contamination by mycotoxigenic fungi and their mycotoxins. Mycotoxins are toxic secondary metabolites produced by fungi that can cause disease or death in humans or animals (Pitt 2000, Richard 2008). The health implications associated with the consumption of foods contaminated with mycotoxins resulted in strict regulations that aims to limit intake by consumers (Eskola et al. 2020). This in turn results in cost implications to lower mycotoxin levels in produce. Due to these regulations and an increasing awareness of food security and safety, the continuous monitoring of mycotoxins and fungal contamination in our food sources is considered important.

South African maize is routinely monitored for mycotoxins (Meyer et al. 2019), but modern surveys identifying fungi associated with this important crop are lacking. Traditionally, fungal identifications were made using morphological characters, which often resulted in misidentifications (Frisvad 2011, Houbraken et al. 2021, Visagie et al. 2014). This was largely due to inter- and infraspecies variations in strains that make morphological differences difficult to interpret, while cryptic species (species that are morphologically identical but differ genetically) further complicated this. Few surveys have adopted DNA sequences to characterise the fungal communities associated with South African maize (Aveling et al. 2020, Boutigny et al. 2012, Boutigny et al. 2011a, Ekwomadu et al. 2018, Phoku et al. 2012), and a gap in our knowledge of what species occur on our maize thus exists.

EC maize producers have a history of experiencing problems with *Fusarium* and their mycotoxins occurring at high levels (Marasas et al. 1988a, Marasas et al. 1988b, Marasas et al. 1979). This was subsequently linked to an increase in the oesophageal cancer rate in the region (Marasas et al. 1981). Since the abovementioned studies, little data has been published regarding fungal communities associated with maize in the region. It was, however, identified as one to be further developed to make larger future contributions and fulfil its production potential. Producers have indicated that they believe pests and diseases are limiting their production. It is thus our goal to explore the fungal diversity associated with maize in the EC and create the baseline knowledge needed to start addressing these problems. For our survey, we thus isolated, preserved and identified fungal strains using modern taxonomic approaches which encompass the use of both morphology and DNA sequence data for species identification, with an emphasis on mycotoxigenic genera.

Materials and Methods

Sampling and isolations

A total of 36 samples were collected during July 2020 from four EC districts, namely Alfred Nzo (AN), Chris Hani (CH), Joe Gqabi (JG) and OR Tambo (ORT) (Fig. 1). Of these samples, fifteen were collected from storage sites in the CH and JG districts and 21 samples were collected from recently harvested maize fields in all four districts. The number of farms surveyed, and samples collected per farm is summarised in Table 1.

For fungal isolations, maize kernels were randomly selected from the top, middle and bottom of the cobs. Both surface disinfested and non-surface disinfested kernels were plated onto three growth media in triplicate. Kernels were disinfested as follows: 3 min in 2% bleach, 30 s in 70% ethanol and 10 s in sterilized water. Isolation media included Potato Dextrose Agar (PDA) supplemented with chloramphenicol (100 mg/ 1L), Fusarium-selective-media (FSM) supplemented with streptomycin (1 g/ 1L) and Dichloran-18%-Glycerol agar (DG18). The plates were incubated for ~4 d at room temperature ($\pm 23^{\circ}\text{C}$).

Fungi of interest were selected by observing growth with a stereomicroscope and transferring culture explants onto $\frac{1}{4}$ PDA or DG18 in order to obtain pure colonies. For *Fusarium* isolates, single spore cultures were prepared following Leslie and Summerell (2006). Pure cultures were preserved in 10% glycerol and stored at -80°C ; and accessioned into the CN culture collection; and representative strains were accessioned into the CMW culture collection, working collections housed at the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from 7 d old cultures grown on $\frac{1}{4}$ PDA or DG18 incubated at room temperature. For *Fusarium*, DNA extractions were carried out using PrepMan® Ultra (Applied Biosystems, USA) following the manufacturer's instructions. For *Aspergillus*, *Penicillium*, *Cladosporium*, *Talaromyces* and other pigmented fungi, DNA extractions were carried out using the Quick-DNA™

Fungal/Bacterial Miniprep kit (Zymo Research, USA) following the manufacturer's instructions.

Subsequent PCR amplifications were made using a 25 µL PCR master mix consisting of 5 µL BioLine 5X MyTaq Reaction Buffer (Meridian BioScience, USA); 0.50 µL of each primer (10 µM); 17.85 µL demineralized sterile water and 0.15 µL BioLine MyTaq DNA Polymerase (Meridian BioScience, USA). Different loci were targeted for amplification based on genus level identifications. These were translation elongation factor 1- α (*TEF*) for *Fusarium* (O'Donnell et al. 1998) and *Cladosporium* (Carbone and Kohn 1999), beta-tubulin (*BenA*) for *Penicillium* (Glass and Donaldson 1995, Visagie et al. 2014), calmodulin (*CaM*) for *Aspergillus* (Hong et al. 2006, Samson et al. 2014), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) for *Alternaria* and other *Pleosporales* (Berbee et al. 1999). The nuclear ribosomal internal transcribed spacer (ITS) rDNA region was used for strains that could not be identified to genus using morphology (De Hoog and van den Ende 1998, Masclaux et al. 1995, Schoch et al. 2012). Additional gene regions were sequenced where necessary. PCR conditions and primers used for these are included in Table 2.

Amplified fragments were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (ThermoFischer Scientific, Massachusetts, USA) and sequenced in both directions using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) with the same primers used for PCR. Sequencing reactions were run on the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Contigs were assembled and edited in Geneious Prime v. 2019.2.1 (BioMatters Ltd., Auckland, New Zealand).

Newly generated sequences were compared to mostly curated databases assembled from taxonomic studies using the local Basic Local Alignment Search Tool (BLAST) searches. Comparisons, especially for ITS (O'Leary et al. 2015, Schoch et al. 2012), were made using BLAST against the National Centre for Biotechnology Information (NCBI's) nucleotide database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Newly generated sequences were submitted to GenBank, listed in Table 3.

Results

Isolations resulted in 481 strains accessioned into the CN working culture collection housed at FABI (Table 3). These were identified into 20 genera (Fig. 2) and 83 species, including *Acremonium* (1 species), *Alternaria* (3 species), *Aspergillus* (13 species), *Aureobasidium* (1 species), *Cladosporium* (3 species), *Colletotrichum* (1 species), *Dendrostoma* (1 species), *Didymella* (1 species), *Epicoccum* (6 species), *Fusarium* (15 species), *Lecanicillium* (1 species), *Leptosphaerulina* (1 species), *Penicillium* (26 species), *Periconia* (1 species), *Pseudopithomyces* (1 species), *Sordariomycetes* (1 species), *Sarocladium* (1 species), *Talaromyces* (2 species), *Trichoderma* (3 species) and *Xylariales* (1 species). The most diverse and frequently isolated genera were *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium* and *Trichoderma*. The species distribution for each district is summarised in the following figures: AN (Fig. 4), CH (Fig. 5), JG (Fig. 6) and ORT (Fig. 7).

The distribution of the genera (Fig. 3) and species (Fig. 4–7), differed between the districts but a core community was identified, with *Alternaria*, *Aspergillus*, *Epicoccum*, *Fusarium*, *Penicillium* and *Talaromyces* present in all producing regions. *Fusarium* was isolated most commonly, followed by *Penicillium*, *Aspergillus*, *Talaromyces* and *Alternaria*. At a species level, *Fusarium temperatum* was the most common species, followed by *F. boothii*, *F. poae*, *Penicillium brevicompactum*, *P. rubens*; and *Aspergillus cibarius* and *A. pseudoglaucus*. Genera that were unique to a single district included *Aureobasidium* and *Lecanicillium* (AN), *Colletotrichum*, *Leptosphaerulina* and *Periconia* (CH), *Dendrostoma* (JG), and *Sarocladium* (ORT).

Fusarium species identified belongs to the *Fusarium chlamydosporum* (FCSC), *Fusarium fujikuroi* (FFSC), *Fusarium incarnatum-equiseti* (FIESC) and *Fusarium sambucinum* (FSAMSC) species complexes. *Fusarium boothii* (FSAMSC; n=53) and *F. temperatum* (FFSC; n=92) were the main species isolated from all four districts. *Fusarium poae* (FSAMSC; n=35) was isolated from all districts except the ORT district; and *F. verticillioides* (FFSC; n=6) was isolated from all districts except the JG district. Other species found in multiple districts include *F. atrovinosum* and *F. chlamydosporum* (FCSC; n=2 respectively) from the CH and

ORT districts, *F. konzum* (FFSC; n=3) from the JG and ORT districts; and *F. oxysporum* (FOSC; n=2) from the CH and JG districts. In the AN district, *F. sporodochiale* (FCSC; n=2) was isolated, while *F. aethiopicum* (FSAMSC; n=1) and *F. equiseti* (FIESC; n=1) were isolated from the CH district. Other species isolated in the JG district include *F. babinda* (FFSC; n=1) and *F. graminearum* (FSAMSC; n=1); and in the ORT district *F. acaciae-mearnsii* (FSAMSC; n=1) and *F. pseudonygamai* (FFSC; n=1) were isolated.

Penicillium was the second most commonly isolated mycotoxigenic genus. The majority of samples obtained from the CH and JG districts were stored maize samples in which we expect to find high numbers of *Penicillium* as it is a known storage mold. *Penicillium brevicompactum* (section *Brevicompacta*; n=16) was found in all four districts, *P. citrinum* (section *Citrina*; n=5) and *P. glabrum* (section *Aspergillioides*; n=6) were found in all districts except the JG district; *P. manginii* (section *Citrina*; n=9) was found in all three districts except AN; and *P. aurantioviolaceum* (section *Aspergillioides*; n=6), *P. crustosum* (section *Fasciculata*; n=6) and *P. rubens* (section *Chrysogena*; n=16) was found in all three districts except the ORT district. Other strains found in multiple districts include *P. chrysogenum* and *P. flavigenum* (section *Chrysogena*; n=3 respectively), both from the CH and JG districts; *P. crocicola* (section *Aspergillioides*; n=2) from the AN and ORT districts and *P. sajarovii* (section *Ramosa*; n=2) from the AN and CH districts. In the AN district, *P. armarii* (section *Sclerotiora*; n=1), *P. corylophilum* (section *Cinnamopurpurea*; n=1), *P. olsonii* (section *Brevicompacta*; n=1), *P. paradoxum* (section *Paradoxa*; n=1), *P. radiatolobatum* (section *Canescentia*; n=1) and *P. salamii* (section *Brevicompacta* n=1) were isolated. In the CH district, *P. lemhiiflumine* (section *Cinnamopurpurea*; n=1) and *P. rudallense* (section *Aspergillioides*; n=1) were isolated; and in the JG district *P. oxalicum* (section *Lanata-Divaricata*; n=1), *P. polonicum* (section *Ramosa*; n=1), *P. ubiquetum* (section *Citrina*; n=1) and *P. vanluykii* (section *Chrysogena*; n=2) were isolated. Other species isolated from the ORT district include *P. admetzioides* (section *Sclerotiorum*; n=1), *P. decaturense* (section *Citrina*; n=1) and *P. raistricki* (section *Ramosa*; n=1).

Aspergillus was the third most commonly isolated mycotoxigenic genus in this study. Many samples obtained from the CH and JG districts were stored maize

samples in which we expect to find high numbers of *Aspergillus* due to its implication as a storage mold. *Aspergillus pseudoglaucus* (section *Aspergillus*; n=4) was found in all four districts; *A. cibarius* (section *Aspergillus*; n=5) and *A. uvarum* (section *Nigri*; n=2) were isolated from JG and ORT districts; and *A. flavus* (section *Flavi*; n=3, aflatoxins (AFs) producer) was isolated from CH and JG districts. Other species isolated from the respective districts include *A. welwitschiae* (section *Nigri*; n=1) from the AN district; and *A. tamarii* (section *Flavi*; n=1) from the CH district. The JG district was found to be more diverse in the different species isolated, others isolated include *A. montevidensis* (section *Aspergillus*; n=1), *A. nomiae* (section *Flavi*; n=1), *A. ostianus* (section *Circumdati*; n=1, some strains weak ochratoxin A (OTA) producers), *A. parasiticus* (section *Flavi*, n=1; AFs producer), *A. protuberus* (section *Nidulantes*; n=1) and *A. tubingensis* (section *Nigri*; n=1). Finally, *A. purpureocrustaceus* (section *Nidulantes*; n=1) was isolated from the ORT district.

Alternaria species that are known to produce mycotoxins were also isolated in this study. *Alternaria alternata* (section *Alternaria*; n=7) was found in all three districts except for the ORT district, *A. destruens* (section *Alternaria*; n=2) was isolated from the CH and ORT districts; and *A. infectoria* (section *Infectoria*; n=1) was isolated from the CH district.

Talaromyces was the least isolated mycotoxigenic genus from all districts. Two species of *Talaromyces* were isolated in this study, namely *T. amestolkiae* (n=27) and *T. stollii* (n=4) both from section *Talaromyces*. *Talaromyces amestolkiae* was isolated from all four districts, while *T. stollii* was only isolated from the JG district.

In addition to the mycotoxigenic genera isolated in this study, *Cladosporium*, *Didymella*, *Epicoccum*, and *Trichoderma* were also identified (Fig. 2a). *Cladosporium* was isolated from all districts except ORT. *Cladosporium cladosporioides* (n=29) and *C. pseudocladosporioides* (n=27) were found to be equally distributed between the three regions. In addition to the above-mentioned species, *C. anthropophilum* (n=2) was also isolated.

Epicoccum was also common, with *E. sorghinum* (n=11) isolated from all districts, *E. italicum* (n=13) and *E. nigrum* (n=9) isolated from all districts except ORT, *E. thailandicum* (n=4) isolated from the CH and JG districts, *E. dendrobii* in the AN

district and *E. plurivorum* from the CH district (n=1 respectively). *Didymella* was only isolated from two of the four districts, namely AN and CH. *Didymella subherbarum* was isolated from these districts.

The distribution of *Trichoderma* isolated in this study varied between the districts. No *Trichoderma* was isolated from the AN district, while *T. gamsii* (n=2) was the only species shared between the CH and ORT districts. All other *Trichoderma* species were found in specific districts. In the JG district, *T. arenarium* (n=1) and *T. koningiopsis* (n=2) were isolated.

Other species isolated less frequently include *Acremonium* sp. (n=3) from the AN, CH and ORT districts, *Pseudopithomyces sacchari* (n=2) from the AN and CH districts, *Sordariomycetes* sp. (n=2) from the AN and ORT districts; and *Xylariales* sp. (n=3) from the AN (n=1) and JQ (n=2) districts. Species that were unique to a single district included *Aureobasidium melanogenum* (n=2) and *Lecanicillium dimorphum* (n=1) (AN), *Colletotrichum graminicola* (n=1), *Leptosphaerulina medicaginicola* (n=1) and *Periconia* sp. (n=1) (CH), *Dendrostoma castaneum* (n=1) (JG), and *Sarocladium* sp. (n=1) (ORT)

Discussion

This study completed the first fungal survey associated with maize from emerging maize farms in the Eastern Cape that identified strains based on DNA sequence data from alternative gene sequences. Our results showed that the fungal diversity of maize analysed was diverse with 20 genera and 83 species identified. This is in contrast to previous studies which have been conducted in South Africa which identified between two and six genera using ITS DNA sequence data (Aveling et al. 2020, Ekwomadu et al. 2018), or species-specific quantitative PCR (qPCR) (Janse van Rensburg 2012, Phokane 2018); or using morphology (Chilaka 2012, Dawal 2010, Ncube 2008, Rheeder et al. 1995, Rheeder et al. 2016, Shabangu 2009, Shephard et al. 2013); while Nji et al. (2022) made use of *BenA*, *CaM* and ITS sequence data. International studies conducted in countries such as Ecuador (Pacin et al. 2003), Lesotho (Mohale et al. 2013) and Pakistan (Saleemi et al. 2012) identified an average of four genera. Similar to these studies, we found the mycotoxigenic genera *Fusarium*, *Penicillium* and *Aspergillus* to dominate communities. At the species level, the frequency at which they are identified is different from the results obtained in this study. For example, in this study, *Fusarium temperatum*, followed by *F. boothii*, *F. poae*, *Penicillium brevicompactum*, *P. rubens*, *Aspergillus cibarius* and *A. pseudoglaucus* were the dominant species isolated. However, other studies conducted in South Africa found *F. verticillioides*, *F. graminearum*, *A. flavus*, *A. parasiticus* and *P. digitatum* to dominate the communities. whereas internationally *F. verticillioides*, *F. annulatum* (= *F. proliferatum* (Yilmaz et al. 2021)) and *A. parasiticus* were found to dominate the communities (Aveling et al. 2020, Chilaka 2012, Dawal 2010, Ekwomadu et al. 2018, Janse van Rensburg 2012, Ncube 2008, Nji et al. 2022, Phokane 2018, Rheeder et al. 1995, Rheeder et al. 2016, Shabangu 2009, Shephard et al. 2013).

Fusarium contains many species that can be plant pathogens and produce mycotoxins on for example maize (Munkvold 2017). These species typically belong to the FFSC, FSAMSC, FOSC and *Neocosmospora* (= *Fusarium solani* species complex (FSSC)), all of which pose a threat to a broad range of economically important crops such as maize and wheat (O'Donnell et al. 2018, Summerell 2019). This study isolated species predominantly from the FFSC and FSAMSC.

The FFSC contains several important plant pathogens that cause Fusarium ear (FER) and stalk rot, as well as seedling disease of maize (Munkvold 2017). Our survey most commonly isolated *F. temperatum* (previously reported in South Africa as *F. subglutinans* Group 1) followed by *F. verticillioides* (Desjardins et al. 2006, Desjardins et al. 2000, Munkvold et al. 2009, Steenkamp et al. 2001, Steenkamp et al. 2002). In comparison, Marasas et al. (1988a) found *F. verticillioides* most commonly followed by *F. subglutinans*. A similar trend was also observed by Aveling et al. (2020), Dawal (2010), Ekwomadu et al. (2018), Janse van Rensburg (2012), Rheeder et al. (2016) and Shephard et al. (2013) from maize farms across South Africa. In contrast, Ncube et al. (2011) found *F. subglutinans* to be more common than *F. verticillioides* or *F. annulatum* (= *F. proliferatum* (Yilmaz et al. 2021)) from the EC, KZN and MP, which they attributed to the cooler climatic conditions of the region. Many of the above-mentioned studies made use of morphology for fungal identification. This methodology often results in misidentifications, especially of cryptic species such as the sister species *F. subglutinans* and *F. temperatum*. These species were initially recognised as two separate groups (Group 1 and 2 for *F. temperatum* and *F. subglutinans* respectively) within *F. subglutinans*, and were later identified as two distinct species using DNA sequence data (Scauflaire et al. 2011, Steenkamp et al. 2002). As a result, the true mycotoxin production of these species is also misidentified. Species in the FFSC have a wide mycotoxin production potential. *Fusarium subglutinans* and *F. temperatum* can produce fusaproliferin (FUS) and fusaric acid (Munkvold 2017, Munkvold et al. 2021). In addition to the above-mentioned mycotoxins, *F. subglutinans* can produce moniliformin (MON), while *F. temperatum* produces beauvericin (BEA) (Fumero et al. 2020, Jestoi 2008, Moretti et al. 2008, Munkvold et al. 2021). It was previously reported that *F. subglutinans*, *F. temperatum* and *F. verticillioides* can all produce fumonisins (FUMs); however, FUMs production in *F. subglutinans* and *F. temperatum* is still under review as these species are reported to lack the FUM gene cluster (Fumero et al. 2020, Munkvold 2017, Munkvold et al. 2021). *Fusarium verticillioides* is considered a globally distributed maize pathogen capable of infecting all parts of the plant and is a known FUMs producer (Marasas et al. 1988a, Munkvold and Desjardins 1997).

Species belonging to FSAMSC colonise a variety of grain crops around the world and are often the causal agents for Gibberella Ear Rot (GER) of maize or Fusarium Head Blight (FHB) of wheat (Bennett and Klich 2003). *Fusarium boothii* was isolated in high numbers from all four districts during this study. This species has been found almost exclusively on maize ears in South Africa, especially from GER (Boutigny et al. 2011a, Munkvold 2017). Several other countries including Argentina, China, Guatemala, Hungary, South Korea, Mexico, and Nepal have reported primarily isolating *F. boothii* from maize (Backhouse 2014, Boutigny et al. 2011a, Desjardins and Proctor 2011, O'Donnell et al. 2004, Sampietro et al. 2011, Tóth et al. 2005). A single isolate of *F. graminearum* was found during this study which correlates with the results of Marasas et al. (1988a), and Ncube et al. (2011) which did not find this species at all. It has been frequently reported from countries such as Canada and the United States of America (van der Lee et al. 2015). *Fusarium poae* was isolated frequently during this study, which is similar to Marasas et al. (1981) and Phoku et al. (2012) who also isolated this species. However, the results of this study contrasts with Chilaka et al. (2012), Dawal (2010) and Ekwomadu et al. (2018) who found few to no isolates. This species is considered a weak pathogen compared to *F. graminearum*, and has been associated with FHB of wheat, barley and sorghum in South Africa (Beukes et al. 2017, Boutigny et al. 2011a, Boutigny et al. 2011b, Stenglein 2009). *Fusarium poae* species has been associated with GER in North and central Europe, and North and Central America, but less frequently than *F. graminearum* (Logrieco et al. 2021, Logrieco et al. 2002, Munkvold 2017). *Fusarium boothii* and *F. graminearum* have the potential to produce deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-ADON); while *F. poae* is known to varies in its ability to produce a wide range of the emerging mycotoxins such as diacetoxyscirpenol (DAS) and nivalenol (NIV) (Munkvold 2017).

Penicillium species are commonly found on a wide variety of substrates and are of particular importance as post-harvest pathogens or food spoilers. This is due to their xerophilic or osmotolerant nature while many species are psychrotolerant too (Pitt and Hocking 2009, Visagie et al. 2017). *Penicillium* species produce a wide range of extrolites (Frisvad et al. 2004), but only three namely, *P. verrucosum*, *P. nordicum* and *P. thymicola* (classified in series *Verrucosa*) produces the commonly regulated OTA, mostly when associated with barley, sorghum, wheat, and peanuts (Frisvad

2015b, Frisvad and Samson 2004, Visagie et al. 2014). *Penicillium* species isolated from maize in the EC do not produce regulated mycotoxins. *Penicillium citrinum* and *P. manginii* are known to produce citrinin, a nephrotoxic compound to animals and has been associated with grains like maize (Chain 2012, Frisvad et al. 2006), and *P. crustosum* is known to produce an unregulated mycotoxin penitrem A which causes animal mycotoxicosis and tremors in humans (Botha et al. 2019). In South Africa, *Penicillium* has been one of the major genera to be associated with commercial maize, but there is no report of mycotoxin production (Chilaka et al. 2012, Jimenez et al. 1985).

Aspergillus was the third most frequently isolated genus in this study. Species of concern include section *Flavi* members, most notably *A. flavus* and *A. parasiticus* as these are known AFs producers (Frisvad 2018, Frisvad et al. 2019, Frisvad et al. 2006). These species and their mycotoxins are known to occur sporadically in both commercial and home-grown maize producing areas in South Africa at a low incidence (Biomin 2019, Biomin 2020, Biomin 2021, Shephard 2008, Shephard et al. 2013). Although these recent studies indicate that South Africa typically does not experience AFs contamination, the producing species are present and improper harvest and storage practices can give rise to increased fungal growth and AFs contamination as a result (Meyer et al. 2019). Other species isolated in this study that have mycotoxigenic potential include, *A. nomiae* (AFs), and *A. tubingensis* and *A. uvarum* (FUMs and OTA) (Chen et al. 2017, Frisvad and Larsen 2015a). Species in section *Aspergillus* are xerophiles and are not considered a threat to human health (Chen et al. 2017, Greco et al. 2015), but some produce extrolites like echinulin, flavoglaucons and physcion shown to be toxic to animals (Ali et al. 1989, Bachmann et al. 1979, Nazar et al. 1984). *Aspergillus purpureocrustaceus* was identified from our study, a species recently described from South Africa based on only two strains (Visagie and Houbraken 2020). This is an example of how diversity studies such as this contribute to growing culture collections for future work.

Alternaria is ubiquitous in nature and has various lifestyles ranging from saprophytic to endophytes and as pathogens to plants and animals (Chen et al. 2021, Woudenberg et al. 2013). It has been implicated as important pre- and postharvest pathogens where they are associated with spoilage of food in unfavourable conditions (Coates and Johnson 1997, Woudenberg et al. 2013). This study isolated

A. alternata which has been shown to produce several non-regulated mycotoxins such as alternariol (AOH), alternariol monomethyl ether (AME), altertoxins (ATXs), altenuene (ALT), tenuazonic acid (TeA) and tentoxins (TEN) (Chain 2011). This is in line with the results obtained by Ekwomadu et al. (2018), Pacin et al. (2003) and Saleemi et al. (2012). Studies conducted in South Africa have not yet recorded the isolation and identification of *Alternaria* from maize. Although there is an increasing concern surrounding this genus and its mycotoxins, there is no legislation that has been established by any food safety authorities across the world.

The results of the study indicate that there is a large fungal diversity associated with maize, with many species having mycotoxigenic potential. Whilst some of these species are not known to produce regulated mycotoxins, many of them have the potential to produce non-regulated, emerging or masked mycotoxins. It should be noted that previous studies conducted in South Africa have made use of morphology or ITS DNA sequence data for identification of fungi associated with maize. This is problematic as neither of these approaches work well to distinguish closely related species in genera like *Aspergillus*, *Fusarium* or *Penicillium*. This study is the first of its kind to make use of alternative gene sequences for fungal identification. It has also resulted in the identification and preservation of species into culture collections. In addition to expanding culture collections, studies such as this generate additional sequence data which is crucial for future studies. For example, strains and sequences generated can be used for taxonomic revisions. Sequences generated in this study have been submitted to GenBank, which results in a better database for accurate identifications. Sequence data generated in this study has led to identification of the following *Fusarium* species: *F. acacia-mearnsii*, *F. aethiopicum*, *F. atrovinosum*, *F. babinda*, *F. konzum*, *F. pseudonygamai*, and *F. sporodochiale*; amongst other genera (*Aspergillus*, *Penicillium*) typically identified from maize in South Africa. These species have been identified for the first time based on published data to date. In addition, the strains isolated in this study will be used for biological studies such as the identification of the abiotic as well as biotic triggers of mycotoxin production in each species. Studies such as these are only possible once the baseline knowledge is developed.

This study provides baseline knowledge on the fungal diversity associated with maize from emerging maize farms in the EC, South Africa. Using this information, we

can monitor how fungal communities change over time and determine the presence of emerging pathogens in the area. The next step is to obtain a baseline knowledge on what ear rots occur on maize in the EC, and the risk of mycotoxins produced.

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Tables

Table 1: Samples collected during this study

Substrate	District	Farm number	Field/Storage
Maize on cob	Alfred Nzo	1	Field 1
Maize on cob	Alfred Nzo	2	Field 2
Maize on cob	Alfred Nzo	2	Field 3
Maize on cob	Alfred Nzo	2	Field 4
Maize on cob	Alfred Nzo	3	Field 5
Maize on cob	Alfred Nzo	3	Field 6
Maize on cob	Alfred Nzo	4	Field 7
Maize on cob	Alfred Nzo	5	Field 8
Stored maize	Chris Hani	6	Storage bags
Maize on cob	Chris Hani	7	Field 1
Maize on cob	Chris Hani	7	Field 2
Maize on cob	Chris Hani	7	Field 3
Maize on cob	Chris Hani	7	Field 4
Stored maize	Joe Gqabi	8	Storage tank
Maize on cob	Joe Gqabi	8	Field 1
Maize on cob	Joe Gqabi	8	Field 2
Maize on cob	Joe Gqabi	9	Field 4
Maize on cob	OR Tambo	10	Field 1
Maize on cob	OR Tambo	10	Field 2
Maize on cob	OR Tambo	10	Field 3
Maize on cob	OR Tambo	11	Field 4
Maize on cob	OR Tambo	12	Field 5

Table 2: PCR amplification conditions

Locus	Primer	Primer sequence 5'–3'	PCR amplification protocol	Genus	Reference
<i>BenA</i>	T10	ACGATAGGTTACCTCCAGAC	94°C 5 min; 35 cycles of 94°C 45s, 56°C 45s, 72°C 60s; 72°C 7min; 10°C ∞	<i>Penicillium</i>	O'Donnell and Cigelnik (1997)
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC			Glass and Donaldson (1995)
	Btub2Fd	GTBCACCTYCARACCGGYCARTG	94°C 5 min; 35 cycles of 94°C 30s, 52°C 30s, 72°C 30s; 72°C 7min; 10°C ∞	<i>Epicoccum / Phoma</i>	Woudenberg et al. (2009)
	Btub4Rd	CCRGAYTGRCCRAARACRAAGTTGTC			
	T1	AACATGCGTGAGATTGTAAGT	95 °C 5 min; 35 cycles of 95 °C 45 s, 52 °C 45 s, 72 °C 90 s; 72 °C 8 min; 10 °C ∞	<i>Fusarium</i>	O'Donnell and Cigelnik (1997)
	T2	TAGTGACCCTTGGCCCAGTTG			
<i>CaM</i>	cmd5	CCGAGTACAAGGARGCCTTC	94°C 5 min; 35 cycles of 94°C 45s, 56°C 45s, 72°C 60s; 72°C 7min; 10°C ∞	<i>Aspergillus</i>	Hong et al. (2006)
	cmd6	CCGATRGAGGTCATRACGTGG			
	CL1	GARTWCAAGGAGGCCTTCTC	94 °C 90 s; 35 cycles of 94 °C 45 s, 50 °C 45 s, 72 °C 1 min; 72 °C 10 min; 10 °C ∞	<i>Fusarium</i>	O'Donnell et al. (2000)
	CL2A	TTTTTGTCATCATGAGTTGGAC			
<i>GAPDH</i>	gdp1	CAACGGCTTCGGTCGCATTG	94°C 5min; 30 cycles of 94°C 45s, 52°C 60s, 72°C 60s; 72°C 7min; 10°C soak	<i>Alternaria</i>	Berbee et al. (1999)
	gdp2	GCCAAGCAGTTGGTTGTGC			
ITS	V9G	TTACGTCCCTGCCCTTTGTA	94°C 5 min; 35 cycles of 94°C 45s, 55°C 45s, 72°C 60s; 72°C 7min; 10°C ∞	Unknown	De Hoog and van den Ende (1998)
	LS266	GCATTCCCAAACAACCTCGACTC			Masclaux et al. (1995)
<i>TEF</i>	728F	CATCGAGAAGTTCGAGAAGG	94°C 5 min; 35 cycles of 94°C 45s, 52°C 45s, 72°C 60s; 72°C 7min; 10°C ∞	<i>Cladosporium</i>	Carbone and Kohn (1999)
	986R	TACTTGAAGGAACCTTACC			
	EF1	ATGGGTAAGGARGACAAGAC	94°C 5 min; 35 cycles of 94°C 45s, 53°C 45s, 72°C 60s; 72°C 7min; 10°C ∞	<i>Fusarium</i>	O'Donnell et al. (1998)
	EF2	GGARGTACCAGTSATCATG			
<i>ACT</i>	512F	ATGTGCAAGGCCGGTTTCGC	94 °C 5 min; 40 cycles of 94 °C 45s, 52 °C 30s, 72 °C 90s; 72 °C 6min; 10 °C ∞	<i>Cladosporium / Epicoccum</i>	Carbone and Kohn (1999)
	783R	TACGAGTCCTTCTGGCCCAT			
<i>RPB2</i>	5F2	GGGGWGAYCAGAAGAAGGC	95 °C 5 min; 40 cycles of 94 °C 30 s, 51 °C 90 s, 68 °C 2 min; 68 °C 5 min; 10 °C ∞	<i>Epicoccum</i>	Reeb et al. (2004)
	7Cr	CCCATRGCTTGYTTRCCCAT			Liu et al. (1999)
LSU	LR5	ATCCTGAGGGAACTTC	94 °C 5 min; 40 cycles of 94 °C 45s, 48 °C 30s, 72 °C 90s; 72 °C 6min; 10 °C ∞	<i>Epicoccum / Phoma</i>	Vilgalys and Hester (1990)
	LROR	GTACCCGCTGAACTTAAGC			Rehner and Samuels (1994)

Table 3: Fungal species isolated during this study

Species	Strain number	District	Substrate	Sequences
<i>Acremonium sp.</i>	CN053B3_CMW58071	Chris Hani	Maize on cob	ITS = ON207822
	CN081B1	Alfred Nzo	Maize on cob	ITS = ON229447
	CN082C6	OR Tambo	Maize on cob	ITS = ON209606
<i>Alternaria alternata</i>	CN059E5_CMW58068	Alfred Nzo	Maize on cob	<i>GAPDH</i> = ON601595
	CN059E6	Alfred Nzo	Maize on cob	<i>GAPDH</i> = ON601596
	CN059E8	Alfred Nzo	Maize on cob	<i>GAPDH</i> = ON601597
	CN059E9	Alfred Nzo	Maize on cob	<i>GAPDH</i> = ON601598
	CN074A3	Joe Gqabi	Maize on cob	ITS = ON209591, <i>GAPDH</i> = ON601599
	CN080B7	Alfred Nzo	Maize on cob	<i>GAPDH</i> = ON601600
	CN080B8	Chris Hani	Maize on cob	<i>GAPDH</i> = ON601601
<i>A. destruens</i>	CN046D9_CMW58069	Chris Hani	Maize on cob	ITS = ON204074
	CN073I2	OR Tambo	Maize on cob	ITS = ON229425
<i>A. infectoria</i>	CN081B5_CMW58070	Chris Hani	Maize on cob	ITS = ON229453
<i>Aspergillus cibarius</i>	CN073A9_CMW58055	Joe Gqabi	Maize on cob	<i>CaM</i> = ON601552
	CN073B1	Joe Gqabi	Maize on cob	<i>CaM</i> = ON601553
<i>A. cibarius</i>	CN073B7	OR Tambo	Maize on cob	<i>CaM</i> = ON601554
	CN073B8	OR Tambo	Maize on cob	<i>CaM</i> = ON601555
	CN073B9	Joe Gqabi	Maize on cob	<i>CaM</i> = ON601556
<i>A. flavus</i>	CN045C1_CMW58056	Chris Hani	Stored maize	<i>BenA</i> = ON601602
	CN045H8	Joe Gqabi	Stored maize	<i>BenA</i> = ON601603
	CN073B4	Joe Gqabi	Maize on cob	<i>CaM</i> = ON601557
<i>A. montevicensis</i>	CN046F9_CMW58067	Joe Gqabi	Stored maize	<i>CaM</i> = ON601558
<i>A. nomiae</i>	CN045B3_CMW58058	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601604, <i>CaM</i> = ON601559
<i>A. ostianus</i>	CN047A3_CMW58059	Joe Gqabi	Stored maize	ITS = ON207625, <i>CaM</i> = ON601594

Species	Strain number	District	Substrate	Sequences
<i>A. parasiticus</i>	CN073A8_CMW58060	Joe Gqabi	Maize on cob	CaM = OP198580
<i>A. protuberus</i>	CN073B2_CMW58061	Joe Gqabi	Maize on cob	CaM = ON601560
<i>A. pseudoglaucus</i>	CN073A7_CMW58062	OR Tambo	Maize on cob	CaM = ON601561
	CN073B5	Joe Gqabi	Maize on cob	CaM = ON601562
	CN080B2	Chris Hani	Maize on cob	ITS = ON209595
	CN081C6	Alfred Nzo	Maize on cob	ITS = ON229450, CaM = ON601563
<i>A. purpureocrustaceus</i>	CN080A5_CMW58063	OR Tambo	Maize on cob	ITS = ON209594, BenA = ON601605, CaM = ON601564
<i>A. tubingensis</i>	CN073B3_CMW58065	Joe Gqabi	Maize on cob	CaM = ON601566
<i>A. tamarii</i>	CN080A6_CMW58064	Chris Hani	Maize on cob	CaM = ON601565
<i>A. uvarum</i>	CN080A7_CMW58066	Alfred Nzo	Maize on cob	CaM = ON601567
	CN080A9	OR Tambo	Maize on cob	CaM = ON601568
<i>A. welwitschiae</i>	CN051F8_CMW58067	Alfred Nzo	Maize on cob	CaM = ON601569
<i>Aureobasidium melanogenum</i>	CN059F5_CMW58072	Alfred Nzo	Maize on cob	ITS = ON207836
	CN080B9	Alfred Nzo	Maize on cob	ITS = ON209596
<i>Cladosporium anthropophilum</i>	CN051H5_CMW58073	Chris Hani	Maize on cob	ITS = ON207628, TEF = OP245178
	CN051H8	Chris Hani	Maize on cob	ITS = ON207630, TEF = OP245179
<i>C. cladosporioides</i>	CN045I9_CMW58074	Joe Gqabi	Stored maize	ITS = ON204058, TEF = OP198581
	CN046A4	Chris Hani	Maize on cob	ITS = ON204062, TEF = OP198582
	CN051G9	Alfred Nzo	Maize on cob	ITS = ON207627, TEF = OP198583
	CN051H6	Chris Hani	Maize on cob	ITS = ON207629, TEF = OP198584
	CN052B1	Chris Hani	Maize on cob	ITS = ON207633, TEF = OP198585
	CN052B5	Chris Hani	Maize on cob	ITS = ON207635, TEF = OP198586
	CN052B6	Chris Hani	Maize on cob	ITS = ON207636, TEF = OP198587
	CN052B8	Chris Hani	Maize on cob	ITS = ON207637, TEF = OP198588
	CN052C2	Chris Hani	Maize on cob	ITS = ON207638

Species	Strain number	District	Substrate	Sequences
	CN058I9	Alfred Nzo	Maize on cob	ITS = ON207832, <i>TEF</i> = OP198589
	CN059A4	Alfred Nzo	Maize on cob	ITS = ON229458, <i>TEF</i> = OP198590
	CN059A6	Alfred Nzo	Maize on cob	<i>TEF</i> = OP198591
	CN059B3	Alfred Nzo	Maize on cob	ITS = ON229460, <i>TEF</i> = OP245295
	CN059B4	Alfred Nzo	Maize on cob	ITS = ON229461, <i>TEF</i> = OP245296
	CN059B6	Alfred Nzo	Maize on cob	<i>TEF</i> = OP198592
	CN059C4	Alfred Nzo	Maize on cob	<i>TEF</i> = OP198593
	CN073C3	Joe Gqabi	Maize on cob	ITS = ON229433, <i>TEF</i> = OP245300
	CN073C4	Joe Gqabi	Maize on cob	ITS = ON229434, <i>TEF</i> = OP198594
	CN073C5	Joe Gqabi	Maize on cob	ITS = ON229435, <i>TEF</i> = OP198595
	CN073C7	Joe Gqabi	Maize on cob	ITS = ON229437, <i>TEF</i> = OP198596
	CN073C8	Joe Gqabi	Maize on cob	ITS = ON209580, <i>TEF</i> = OP198597
	CN073C9	Joe Gqabi	Maize on cob	ITS = ON209581, <i>TEF</i> = OP198598
	CN073D1	Joe Gqabi	Maize on cob	ITS = ON209582, <i>TEF</i> = OP198599
	CN080C7	Alfred Nzo	Maize on cob	ITS = ON209598, <i>TEF</i> = OP198600
	CN080C8	Alfred Nzo	Maize on cob	ITS = ON209599, <i>TEF</i> = OP198601
	CN080D1	Alfred Nzo	Maize on cob	ITS = ON229438, <i>TEF</i> = OP198602
	CN080E2	Chris Hani	Maize on cob	ITS = ON229440, <i>TEF</i> = OP198603
	CN080E5	Chris Hani	Maize on cob	ITS = ON229442, <i>TEF</i> = OP198604
	CN081B4	Chris Hani	Maize on cob	ITS = ON229449
<i>C. pseudocladosporioides</i>	CN046B8	Joe Gqabi	Stored maize	ITS = ON204064, <i>TEF</i> = OP245279
	CN046C4_CMW58076	Chris Hani	Maize on cob	ITS = ON204065, <i>TEF</i> = OP245280
	CN046E5	Chris Hani	Maize on cob	ITS = ON209695, <i>TEF</i> = OP245281
	CN046F2	Joe Gqabi	Stored maize	ITS = ON207617
	CN051H9	Chris Hani	Maize on cob	ITS = ON207631, <i>TEF</i> = OP245282

Species	Strain number	District	Substrate	Sequences
	CN051I2	Chris Hani	Maize on cob	<i>TEF</i> = OP245283
	CN051I8	Alfred Nzo	Maize on cob	ITS = ON207632, <i>TEF</i> = OP245284
	CN052A6	Chris Hani	Maize on cob	<i>TEF</i> = OP245285
	CN052B2	Chris Hani	Maize on cob	ITS = ON207634, <i>TEF</i> = OP245286
	CN052C3	Chris Hani	Maize on cob	ITS = ON207639
	CN052C8	Alfred Nzo	Maize on cob	ITS = ON207640, <i>TEF</i> = OP245287
	CN052G7	Chris Hani	Maize on cob	ITS = ON207646
	CN053A5	Chris Hani	Maize on cob	ITS = ON209696, <i>TEF</i> = OP245288
	CN053A6	Alfred Nzo	Maize on cob	ITS = ON207653, <i>TEF</i> = OP245289
	CN058H7	Alfred Nzo	Maize on cob	ITS = ON207829, <i>TEF</i> = OP245290
	CN058H8	Alfred Nzo	Maize on cob	ITS = ON207830, <i>TEF</i> = OP245291
	CN058H9	Alfred Nzo	Maize on cob	ITS = ON207831, <i>TEF</i> = OP245292
	CN058I3	Alfred Nzo	Maize on cob	<i>TEF</i> = OP245293
	CN059A9	Alfred Nzo	Maize on cob	ITS = ON229459, <i>TEF</i> = OP245294
	CN059C1	Alfred Nzo	Maize on cob	ITS = ON229462, <i>TEF</i> = OP245297
	CN059F6	Alfred Nzo	Maize on cob	ITS = ON207837, <i>TEF</i> = OP245298
	CN073C2	Joe Gqabi	Maize on cob	<i>TEF</i> = OP245299
	CN073C6	Joe Gqabi	Maize on cob	ITS = ON229436, <i>TEF</i> = OP245301
	CN080C2	Alfred Nzo	Maize on cob	<i>TEF</i> = OP245302
	CN080C3	Alfred Nzo	Maize on cob	<i>TEF</i> = OP245303
	CN080E3	Alfred Nzo	Maize on cob	ITS = ON229441, <i>TEF</i> = OP245304
	CN080E4	Alfred Nzo	Maize on cob	<i>TEF</i> = OP245305
<i>Colletotrichum graminicola</i>	CN046G9_CMW58081	Chris Hani	Maize on cob	ITS = ON207619
<i>Dendrostoma castaneum</i>	CN046I3_CMW58082	Joe Gqabi	Stored maize	ITS = ON207624
<i>Didymella subherbarum</i>	CN046F1_CMW58084	Chris Hani	Stored maize	ITS = ON207616

Species	Strain number	District	Substrate	Sequences
	CN053A9	Chris Hani	Maize on cob	ITS = ON207820
	CN081C1	Chris Hani	Maize on cob	ITS = ON229457
	CN081D4	Alfred Nzo	Maize on cob	ITS = ON229451
<i>Epicoccum dendrobii</i>	CN063A3_CMW58085	Alfred Nzo	Maize on cob	ITS = ON229423
<i>E. italicum</i>	CN046C6_CMW58086	Chris Hani	Maize on cob	ITS = ON204066, <i>BenA</i> = ON601606, <i>RPB2</i> = ON602020
	CN046C7	Chris Hani	Maize on cob	ITS = ON204067, <i>BenA</i> = ON601607, <i>RPB2</i> = ON602021
	CN046C9	Joe Gqabi	Stored maize	ITS = ON204069
	CN046D1	Joe Gqabi	Stored maize	ITS = ON204070
	CN046D4	Joe Gqabi	Stored maize	ITS = ON204071
	CN052D5	Alfred Nzo	Maize on cob	ITS = ON204072, <i>ACT</i> = OP245180
	CN052E1	Chris Hani	Maize on cob	ITS = ON207642
	CN058G5	Alfred Nzo	Maize on cob	ITS = ON207825, <i>BenA</i> = ON601608, <i>RPB2</i> = ON602022
	CN058G6	Alfred Nzo	Maize on cob	ITS = ON207826, <i>BenA</i> = ON601609, <i>RPB2</i> = ON602023
	CN058H1	Alfred Nzo	Maize on cob	ITS = ON207828, <i>BenA</i> = ON601610, <i>RPB2</i> = ON602024
	CN073D3	Joe Gqabi	Maize on cob	ITS = ON209584
	CN080E7	Alfred Nzo	Maize on cob	ITS = ON229443, <i>RPB2</i> = ON602025
	CN080F1	Alfred Nzo	Maize on cob	ITS = ON229445
<i>E. nigrum</i>	CN046C8	Chris Hani	Maize on cob	ITS = ON204068
	CN046D5	Joe Gqabi	Stored maize	ITS = ON204072
	CN058G7	Alfred Nzo	Maize on cob	ITS = ON207827, <i>BenA</i> = ON601611, <i>CaM</i> = OP245189, <i>RPB2</i> = ON602026
	CN073D2_CMW58087	Joe Gqabi	Maize on cob	ITS = ON209583
	CN073D4	Joe Gqabi	Maize on cob	ITS = ON209585
	CN073D6	Joe Gqabi	Maize on cob	ITS = ON209586, <i>RPB2</i> = ON602027
	CN073I7	Joe Gqabi	Maize on cob	ITS = ON229428
	CN073I9	Joe Gqabi	Maize on cob	ITS = ON229430
	CN080E9	Alfred Nzo	Maize on cob	ITS = ON229444

Species	Strain number	District	Substrate	Sequences
<i>E. plurivorum</i>	CN081B8_CMW58088	Chris Hani	Maize on cob	ITS = ON229455
<i>E. sorghinum</i>	CN046E7	Chris Hani	Maize on cob	ITS = ON204075, <i>BenA</i> = ON601612, <i>RPB2</i> = OP198605, LSU = OP219655
	CN046F4	Joe Gqabi	Stored maize	ITS = ON207618, LSU = OP219656, <i>ACT</i> = ON602018
	CN046G5	Chris Hani	Maize on cob	<i>BenA</i> = ON601613, LSU = OP219657
	CN051G7	Chris Hani	Maize on cob	ITS = ON207626, LSU = OP219658, <i>ACT</i> = ON602019
	CN052I5	Chris Hani	Maize on cob	ITS = ON207651
	CN053B2	Chris Hani	Maize on cob	ITS = ON207821, <i>BenA</i> = ON601614, <i>RPB2</i> = OP198606, LSU = OP219659
	CN073I3	Joe Gqabi	Maize on cob	ITS = ON229426, <i>BenA</i> = ON601615, <i>RPB2</i> = OP198607, LSU = OP219660
	CN073I4	Joe Gqabi	Maize on cob	ITS = ON209587, LSU = OP219661
	CN073I5_CMW58089	OR Tambo	Maize on cob	ITS = ON229427, <i>BenA</i> = ON601616, LSU = OP219662
	CN073I8	Joe Gqabi	Maize on cob	ITS = ON229429, <i>BenA</i> = ON601617, LSU = OP219663
	CN080C1	Alfred Nzo	Maize on cob	ITS = ON209597
<i>E. thailandicum</i>	CN046H4	Chris Hani	Maize on cob	ITS = ON207621, <i>BenA</i> = OP245183, LSU = OP219664, <i>ACT</i> = OP245181
	CN046I1_CMW58091	Chris Hani	Maize on cob	ITS = ON207623, <i>BenA</i> = OP245184, LSU = OP219665, <i>ACT</i> = OP245182
	CN074A1	Joe Gqabi	Maize on cob	ITS = ON209589, <i>BenA</i> = OP245185, <i>TEF</i> = ON601955
	CN074A2	Joe Gqabi	Maize on cob	ITS = ON209590, <i>BenA</i> = OP245186, <i>RPB2</i> = OP245200, LSU = OP219666
<i>Fusarium acaciae-mearnsii</i>	CN082F5_CMW58092	OR Tambo	Maize on cob	ITS = ON209619, <i>CaM</i> = OP245190, <i>TEF</i> = OP245201
<i>F. aethiopicum</i>	CN062A3_CMW58093	Chris Hani	Maize on cob	ITS = ON207917, <i>BenA</i> = ON601619, <i>CaM</i> = OP245193, <i>TEF</i> = ON601768
<i>F. atrovinosum</i>	CN062E1	Chris Hani	Stored maize	<i>TEF</i> = ON601769
	CN082F7_CMW58094	OR Tambo	Maize on cob	<i>TEF</i> = ON601770
<i>F. babinda</i>	CN061F5_CMW58095	Joe Gqabi	Maize on cob	<i>TEF</i> = OP245202
<i>F. boothii</i>	CN061I5	Joe Gqabi	Stored maize	ITS = ON207915, <i>BenA</i> = ON601618, <i>CaM</i> = OP245192, <i>TEF</i> = ON601869
	CN061I6	Joe Gqabi	Stored maize	<i>TEF</i> = ON601870
	CN061I7	Joe Gqabi	Stored maize	<i>TEF</i> = ON601871
	CN061I8	Joe Gqabi	Stored maize	<i>TEF</i> = ON601872

Species	Strain number	District	Substrate	Sequences
	CN061I9	Joe Gqabi	Stored maize	ITS = ON207916, <i>TEF</i> = ON601873
	CN062A1	Joe Gqabi	Stored maize	<i>TEF</i> = ON601874
	CN062A2	Chris Hani	Stored maize	<i>TEF</i> = ON601875
	CN062A4	Chris Hani	Maize on cob	<i>TEF</i> = ON601876
	CN062A5	Chris Hani	Maize on cob	<i>TEF</i> = ON601877
	CN062A6	Joe Gqabi	Stored maize	ITS = ON207918, <i>TEF</i> = ON601878
	CN062A7	Joe Gqabi	Stored maize	<i>TEF</i> = ON601879
	CN062A8_CMW58096	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601880
	CN062A9	Chris Hani	Maize on cob	<i>TEF</i> = ON601881
	CN062B1	Chris Hani	Maize on cob	<i>TEF</i> = ON601882
	CN062B2	Joe Gqabi	Stored maize	<i>TEF</i> = ON601883
	CN062B4	Joe Gqabi	Stored maize	<i>TEF</i> = ON601884
	CN062B5	Chris Hani	Stored maize	<i>TEF</i> = ON601885
	CN062B6	Chris Hani	Stored maize	<i>TEF</i> = ON601886
	CN062B7	Chris Hani	Stored maize	<i>TEF</i> = ON601887
	CN062B8	Chris Hani	Stored maize	ITS = ON207920, <i>BenA</i> = ON601620, <i>TEF</i> = ON601888
	CN062B9	Joe Gqabi	Maize on cob	ITS = ON207921, <i>BenA</i> = ON601621, <i>CaM</i> = OP245194, <i>TEF</i> = ON601889
	CN062C1	Joe Gqabi	Stored maize	<i>TEF</i> = ON601890
	CN062C2	Joe Gqabi	Stored maize	<i>TEF</i> = ON601891
	CN062C3	Joe Gqabi	Stored maize	<i>TEF</i> = ON601892
	CN062C4	Joe Gqabi	Stored maize	ITS = ON207922, <i>TEF</i> = ON601893
	CN062C5	Joe Gqabi	Stored maize	<i>TEF</i> = ON601894
	CN062C6	Joe Gqabi	Stored maize	<i>TEF</i> = ON601895
	CN062C7	Chris Hani	Stored maize	<i>TEF</i> = ON601896
	CN062C9	Joe Gqabi	Stored maize	<i>TEF</i> = ON601897
	CN062D1	Chris Hani	Maize on cob	<i>TEF</i> = ON601898

Species	Strain number	District	Substrate	Sequences
	CN062D2	Chris Hani	Maize on cob	<i>TEF</i> = ON601899
	CN062D3	Joe Gqabi	Stored maize	<i>TEF</i> = ON601900
	CN062D4	Chris Hani	Stored maize	ITS = ON207924, <i>TEF</i> = ON601901
	CN062D5	Joe Gqabi	Stored maize	<i>TEF</i> = ON601902
	CN062D7	Joe Gqabi	Stored maize	<i>TEF</i> = ON601903
	CN062E2	Joe Gqabi	Stored maize	ITS = ON207927, <i>CaM</i> = OP245195, <i>TEF</i> = ON601904
	CN062E3	Joe Gqabi	Stored maize	<i>TEF</i> = ON601905
	CN062E4	Chris Hani	Stored maize	<i>TEF</i> = ON601906
	CN062G7	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601907
	CN062G8	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601908
	CN062G9	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601909
	CN062H1	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601910
	CN062H2	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601911
	CN062H3	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601912
	CN062H4	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601913
	CN082E7	OR Tambo	Maize on cob	<i>TEF</i> = ON601914
	CN082E8	Chris Hani	Maize on cob	<i>TEF</i> = ON601915
	CN082F2	Chris Hani	Maize on cob	<i>TEF</i> = ON601916
	CN082F3	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601917
	CN082F4	Joe Gqabi	Maize on cob	ITS = ON209618, <i>BenA</i> = ON601622, <i>CaM</i> = OP245196, <i>TEF</i> = ON601918
	CN082F6	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601919
	CN082F9	OR Tambo	Maize on cob	ITS = ON209621, <i>BenA</i> = ON601623, <i>CaM</i> = OP245197, <i>TEF</i> = ON601920
	CN082G1	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601921
<i>F. chlamydosporum</i>	CN062E5	Chris Hani	Stored maize	ITS = ON207928
	CN082F8_CMW58098	OR Tambo	Maize on cob	ITS = ON209620
<i>F. equiseti</i>	CN061A6_CMW58099	Chris Hani	Stored maize	<i>TEF</i> = OP198576

Species	Strain number	District	Substrate	Sequences
<i>F. graminearum</i>	CN046H5_CMW58100	Joe Gqabi	Stored maize	ITS = ON207622
<i>F. konzum</i>	CN061D8	Joe Gqabi	Stored maize	<i>TEF</i> = ON601771
	CN061E2	Joe Gqabi	Stored maize	<i>TEF</i> = ON601772
	CN082C7_CMW58103	OR Tambo	Maize on cob	ITS = ON209607, <i>BenA</i> = ON601624, <i>CaM</i> = ON601570, <i>TEF</i> = ON601773
<i>F. oxysporum</i>	CN046G8	Joe Gqabi	Stored maize	ITS = ON204059
	CN062F8_CMW58104	Chris Hani	Stored maize	<i>TEF</i> = ON601774
<i>F. poae</i>	CN062D8	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601922
	CN062D9	Chris Hani	Maize on cob	ITS = ON207926, <i>TEF</i> = ON601923
	CN062E6	Joe Gqabi	Maize on cob	ITS = ON207929, <i>TEF</i> = ON601924
	CN062E7	Joe Gqabi	Stored maize	<i>TEF</i> = ON601925
	CN062E8	Joe Gqabi	Stored maize	<i>TEF</i> = ON601926
	CN062E9	Joe Gqabi	Stored maize	ITS = ON207930, <i>TEF</i> = ON601927
	CN062F1	Joe Gqabi	Stored maize	ITS = ON207931, <i>TEF</i> = ON601928
	CN062G3	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601929
	CN062G4	Alfred Nzo	Maize on cob	ITS = ON229418, <i>TEF</i> = ON601930
	CN062G5_CMW58106	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601931
	CN062G6	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601932
	CN062I2	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601933
	CN062I3	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601934
	CN062I4	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601935
	CN062I5	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601936
	CN062I6	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601937
	CN062I7	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601938
	CN062I8	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601939
	CN062I9	Alfred Nzo	Maize on cob	ITS = ON229421, <i>TEF</i> = ON601940
	CN063A1	Alfred Nzo	Maize on cob	ITS = ON229422, <i>TEF</i> = ON601941

Species	Strain number	District	Substrate	Sequences
	CN063A2	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601942
	CN082C8	Alfred Nzo	Maize on cob	ITS = ON209608, <i>TEF</i> = ON601943
	CN082C9	Alfred Nzo	Maize on cob	ITS = ON209609, <i>TEF</i> = ON601944
	CN082D2	Alfred Nzo	Maize on cob	ITS = ON209611, <i>TEF</i> = ON601945
	CN082D3	Alfred Nzo	Maize on cob	ITS = ON209612, <i>TEF</i> = ON601946
	CN082D4	Alfred Nzo	Maize on cob	ITS = ON209613
	CN082D5	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601947
	CN082D6	Alfred Nzo	Maize on cob	ITS = ON209614, <i>TEF</i> = ON601948
	CN082D7	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601949
	CN082E1	Joe Gqabi	Maize on cob	ITS = ON209615, <i>TEF</i> = ON601950
	CN082E2	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601951
	CN082E3	Alfred Nzo	Maize on cob	ITS = ON209616, <i>TEF</i> = ON601952
	CN082E4	Joe Gqabi	Maize on cob	ITS = ON209617
	CN082E5	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601953
	CN082E9	Alfred Nzo	Maize on cob	<i>TEF</i> = Yes
<i>F. pseudonygamai</i>	CN082I6_CMW58108	OR Tambo	Maize on cob	ITS = ON209694, <i>BenA</i> = OP245187, <i>CaM</i> = OP245198, <i>TEF</i> = OP198577
<i>F. sporodochiale</i>	CN082E6_CMW58109	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601775
	CN082F1	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601776
<i>F. temperatum</i>	CN061A7	Joe Gqabi	Stored maize	<i>TEF</i> = ON601777
	CN061A8	Joe Gqabi	Stored maize	<i>TEF</i> = ON601778
	CN061A9	Joe Gqabi	Maize on cob	ITS = ON229464, <i>CaM</i> = ON601571, <i>TEF</i> = ON601779
	CN061B1	Joe Gqabi	Stored maize	<i>TEF</i> = ON601780
	CN061B2	Joe Gqabi	Stored maize	ITS = ON229465, <i>BenA</i> = ON601625, <i>CaM</i> = ON601572
	CN061B4	Joe Gqabi	Stored maize	<i>TEF</i> = ON601781
	CN061B5	Chris Hani	Maize on cob	<i>TEF</i> = ON601782
	CN061B6	Chris Hani	Maize on cob	<i>TEF</i> = ON601783

Species	Strain number	District	Substrate	Sequences
	CN061B7	Chris Hani	Maize on cob	<i>TEF</i> = ON601784
	CN061B8	Chris Hani	Maize on cob	<i>TEF</i> = ON601785
	CN061B9	Chris Hani	Maize on cob	<i>TEF</i> = ON601786
	CN061C1	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601787
	CN061C2	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601788
	CN061C3	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601789
	CN061C4	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601790
	CN061C5	Chris Hani	Stored maize	<i>TEF</i> = ON601791
	CN061C6	Alfred Nzo	Maize on cob	ITS = ON229467, <i>BenA</i> = ON601626, <i>CaM</i> = ON601573, <i>TEF</i> = ON601792
	CN061C7	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601793
	CN061C8	Joe Gqabi	Stored maize	<i>TEF</i> = ON601794
	CN061C9	Joe Gqabi	Stored maize	<i>TEF</i> = ON601795
	CN061D1	Joe Gqabi	Stored maize	<i>TEF</i> = ON601796
	CN061D2	Chris Hani	Stored maize	<i>TEF</i> = ON601797
	CN061D3	Chris Hani	Stored maize	<i>TEF</i> = ON601798
	CN061D4	Joe Gqabi	Stored maize	ITS = ON229468, <i>BenA</i> = ON601627, <i>CaM</i> = ON601574, <i>TEF</i> = ON601799
	CN061D5	Joe Gqabi	Stored maize	ITS = ON229469, <i>BenA</i> = ON601628, <i>CaM</i> = ON601575, <i>TEF</i> = ON601800
	CN061D6	Alfred Nzo	Maize on cob	ITS = ON229470, <i>BenA</i> = ON601629, <i>CaM</i> = ON601576, <i>TEF</i> = ON601801
	CN061D7	Chris Hani	Maize on cob	<i>TEF</i> = ON601802
	CN061E3	Chris Hani	Stored maize	ITS = ON229473, <i>BenA</i> = ON601630, <i>CaM</i> = ON601577, <i>TEF</i> = ON601803
	CN061E4	Chris Hani	Stored maize	<i>TEF</i> = ON601804
	CN061E6	Chris Hani	Maize on cob	<i>TEF</i> = ON601805
	CN061E7	Chris Hani	Maize on cob	<i>TEF</i> = ON601806
	CN061E8_CMW58112	Chris Hani	Maize on cob	<i>TEF</i> = ON601807
	CN061E9	Chris Hani	Maize on cob	<i>TEF</i> = ON601808
	CN061F1	Chris Hani	Maize on cob	<i>TEF</i> = ON601809

Species	Strain number	District	Substrate	Sequences
	CN061F2	Chris Hani	Maize on cob	<i>TEF</i> = ON601810
	CN061F3	Chris Hani	Maize on cob	<i>TEF</i> = ON601811
	CN061F4	Chris Hani	Maize on cob	<i>TEF</i> = ON601812
	CN061F7	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601813
	CN061F8	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601814
	CN061F9	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601815
	CN061G1	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601816
	CN061G2	Joe Gqabi	Stored maize	<i>TEF</i> = ON601817
	CN061G3	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601818
	CN061G4	Joe Gqabi	Maize on cob	<i>ITS</i> = ON229476, <i>BenA</i> = ON601631, <i>CaM</i> = ON601578, <i>TEF</i> = ON601819
	CN061G5	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601820
	CN061G6	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601821
	CN061G7	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601822
	CN061G8	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601823
	CN061G9	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601824
	CN061H1	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601825
	CN061H2	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601826
	CN061H3	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601827
	CN061H4	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601828
	CN061H5	Chris Hani	Stored maize	<i>TEF</i> = ON601829
	CN061H6	Joe Gqabi	Stored maize	<i>TEF</i> = ON601830
	CN061H7	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601831
	CN061H8	Chris Hani	Maize on cob	<i>TEF</i> = ON601832
	CN061H9	Chris Hani	Maize on cob	<i>TEF</i> = ON601833
	CN061I1	Chris Hani	Maize on cob	<i>ITS</i> = ON229477, <i>BenA</i> = ON601632, <i>CaM</i> = ON601579, <i>TEF</i> = ON601834
	CN061I2	Joe Gqabi	Stored maize	<i>TEF</i> = ON601835

Species	Strain number	District	Substrate	Sequences
	CN061I3	Joe Gqabi	Stored maize	<i>TEF</i> = ON601836
	CN061I4	Chris Hani	Maize on cob	<i>TEF</i> = ON601837
	CN062F2	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601838
	CN062F3	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601839
	CN062F4	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601840
	CN062F5	Chris Hani	Stored maize	<i>TEF</i> = ON601841
	CN062F6	Alfred Nzo	Maize on cob	ITS = ON207932, <i>BenA</i> = ON601633, <i>CaM</i> = ON601580, <i>TEF</i> = ON601842
	CN062F7	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601843
	CN062F9	Alfred Nzo	Maize on cob	ITS = ON207933, <i>BenA</i> = ON601634, <i>CaM</i> = ON601581, <i>TEF</i> = ON601844
	CN062G1	Alfred Nzo	Maize on cob	ITS = ON207934, <i>BenA</i> = ON601635, <i>CaM</i> = ON601582, <i>TEF</i> = ON601845
	CN062G2	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601846
	CN062H5	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601847
	CN062H6	Alfred Nzo	Maize on cob	ITS = ON229419, <i>BenA</i> = ON601636, <i>CaM</i> = ON601583, <i>TEF</i> = ON601848
	CN062H7	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601849
	CN062H8	Alfred Nzo	Maize on cob	ITS = ON229420, <i>BenA</i> = ON601637, <i>CaM</i> = ON601637, <i>TEF</i> = ON601850
	CN062H9	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601851
	CN062I1	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601852
	CN082G5	OR Tambo	Maize on cob	<i>TEF</i> = ON601853
	CN082G6	Alfred Nzo	Maize on cob	ITS = ON209623, <i>BenA</i> = ON601638, <i>CaM</i> = ON601584, <i>TEF</i> = ON601854
	CN082G7	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601855
	CN082G8	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601856
	CN082G9	Alfred Nzo	Maize on cob	ITS = ON209624, <i>BenA</i> = ON601639, <i>CaM</i> = ON601585, <i>TEF</i> = ON601857
	CN082H1	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601858
	CN082H3	Chris Hani	Maize on cob	ITS = ON209625, <i>BenA</i> = ON601640, <i>CaM</i> = ON601586, <i>TEF</i> = ON601860
	CN082H4	Alfred Nzo	Maize on cob	ITS = ON209689, <i>TEF</i> = ON601861
	CN082H6	Alfred Nzo	Maize on cob	<i>TEF</i> = ON601862

Species	Strain number	District	Substrate	Sequences
	CN082H7	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601863
	CN082H8	OR Tambo	Maize on cob	ITS = ON209691, <i>BenA</i> = ON601641, <i>TEF</i> = ON601864
	CN082I3	Joe Gqabi	Maize on cob	<i>TEF</i> = ON601865
	CN082I4	OR Tambo	Maize on cob	<i>TEF</i> = ON601866
	CN082I5	Joe Gqabi	Maize on cob	ITS = ON209693, <i>BenA</i> = ON601642, <i>CaM</i> = ON601587, <i>TEF</i> = ON601867
	CN082I7	OR Tambo	Maize on cob	<i>TEF</i> = ON601868
<i>F. verticillioides</i>	CN061D9	Alfred Nzo	Maize on cob	ITS = ON229471, <i>BenA</i> = ON601760, <i>TEF</i> = ON601957
	CN061E1	Chris Hani	Stored maize	ITS = ON229472, <i>BenA</i> = ON601761, <i>TEF</i> = ON601958
	CN061E5	Chris Hani	Stored maize	ITS = ON229474, <i>CaM</i> = ON601592, <i>TEF</i> = ON601959
	CN082H5_CMW58114	OR Tambo	Maize on cob	ITS = ON209690, <i>BenA</i> = ON601762, <i>CaM</i> = ON601593, <i>TEF</i> = ON601960
	CN082H9	OR Tambo	Maize on cob	<i>TEF</i> = ON601961
	CN082I1	OR Tambo	Maize on cob	ITS = ON209692, <i>TEF</i> = ON601962
<i>Lecanicillium dimorphum</i>	CN059F2_CMW58117	Alfred Nzo	Maize on cob	ITS = ON207834
<i>Leptosphaerulina medicaginicola</i>	CN046I2_CMW58116	Chris Hani	Maize on cob	ITS = ON204060
<i>Penicillium adametzioides</i>	CN073E2_CMW58119	OR Tambo	Maize on cob	<i>BenA</i> = ON601643
<i>P. armarii</i>	CN059D4_CMW58120	Alfred Nzo	Maize on cob	ITS = ON229463, <i>BenA</i> = ON601644, <i>CaM</i> = ON601588
<i>P. aurantioviolaceum</i>	CN052F2_CMW58121	Chris Hani	Maize on cob	<i>BenA</i> = ON601645
	CN052F4	Chris Hani	Maize on cob	<i>BenA</i> = ON601646
	CN052F6	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601647
	CN052F7	Chris Hani	Maize on cob	<i>BenA</i> = ON601648
	CN052F8	Chris Hani	Maize on cob	<i>BenA</i> = ON601649
	CN073F6	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601650
<i>P. brevicompactum</i>	CN045I3	Joe Gqabi	Stored maize	<i>BenA</i> = ON601651
	CN052E8	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601652
	CN052G3	Chris Hani	Maize on cob	<i>BenA</i> = ON601653

Species	Strain number	District	Substrate	Sequences
	CN059D7	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601654
	CN073E3_CMW58122	OR Tambo	Maize on cob	<i>BenA</i> = ON601655
	CN073E4	OR Tambo	Maize on cob	<i>BenA</i> = ON601656
	CN073E5	OR Tambo	Maize on cob	<i>BenA</i> = ON601657
	CN073E7	OR Tambo	Maize on cob	<i>BenA</i> = ON601658
	CN073E8	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601659
	CN073E9	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601660
	CN073F1	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601661
	CN073F7	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601662
	CN073G8	OR Tambo	Maize on cob	<i>BenA</i> = ON601663
	CN073G9	OR Tambo	Maize on cob	<i>BenA</i> = ON601664
	CN080H9	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601665
	CN081A3	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601666
<i>P. chrysogenum</i>	CN045E8_CMW58123	Joe Gqabi	Stored maize	<i>BenA</i> = ON601763
	CN052G4	Chris Hani	Maize on cob	<i>BenA</i> = ON601764
	CN073F8	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601765
<i>P. citrinum</i>	CN073F9_CMW58124	OR Tambo	Maize on cob	<i>BenA</i> = ON601667
	CN073G1	OR Tambo	Maize on cob	<i>BenA</i> = ON601668
	CN080H4	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601669
	CN080H8	Chris Hani	Maize on cob	<i>BenA</i> = ON601670
	CN081A5	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601671
<i>P. corylophilum</i>	CN059D5_CMW58125	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601672
<i>P. crocicola</i>	CN080I9	Alfred Nzo	Maize on cob	ITS = ON229446, <i>BenA</i> = ON601766
	CN081A6_CMW58126	OR Tambo	Maize on cob	ITS = ON229452, <i>BenA</i> = ON601767
<i>P. crustosum</i>	CN045B2	Chris Hani	Stored maize	<i>BenA</i> = ON601673
	CN045B6	Chris Hani	Stored maize	<i>BenA</i> = ON601674

Species	Strain number	District	Substrate	Sequences
	CN045B9	Chris Hani	Stored maize	ITS = ON204056, <i>BenA</i> = ON601675, <i>CaM</i> = ON601589
	CN045C3	Chris Hani	Stored maize	<i>BenA</i> = ON601676
	CN045I4	Joe Gqabi	Stored maize	<i>BenA</i> = ON601678
	CN080G7_CMW58127	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601680
<i>P. decaturense</i>	CN080G5_CMW58128	OR Tambo	Maize on cob	<i>BenA</i> = ON601682
<i>P. flavigenum</i>	CN052F9_CMW58129	Chris Hani	Maize on cob	<i>BenA</i> = ON601683
	CN052G1	Chris Hani	Maize on cob	ITS = ON207643, <i>BenA</i> = ON601684, <i>CaM</i> = ON601591
	CN073E1	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601685
<i>P. glabrum</i>	CN059D6	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601686
	CN073F5	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601687
	CN080H3	Chris Hani	Maize on cob	<i>BenA</i> = ON601688
	CN080I4_CMW58130	OR Tambo	Maize on cob	<i>BenA</i> = ON601689
	CN080I5	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601690
	CN081A8	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601691
<i>P. lemhiiflumine</i>	CN052G2_CMW58131	Chris Hani	Maize on cob	ITS = ON207644, <i>BenA</i> = OP245188, <i>CaM</i> = OP245199
<i>P. manginii</i>	CN052I7	Chris Hani	Maize on cob	<i>BenA</i> = ON601692
	CN073E6	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601693
	CN073H3	OR Tambo	Maize on cob	<i>BenA</i> = ON601694
	CN073H4_CMW58132	OR Tambo	Maize on cob	<i>BenA</i> = ON601695
	CN073H5	OR Tambo	Maize on cob	<i>BenA</i> = ON601696
	CN073H6	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601697
	CN073H7	OR Tambo	Maize on cob	<i>BenA</i> = ON601698
	CN073H8	OR Tambo	Maize on cob	<i>BenA</i> = ON601699
	CN073H9	OR Tambo	Maize on cob	<i>BenA</i> = ON601700
<i>P. olsonii</i>	CN080G9_CMW58133	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601701
<i>P. oxalicum</i>	CN073G7_CMW58134	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601702

Species	Strain number	District	Substrate	Sequences
<i>P. paradoxum</i>	CN059F1_CMW58135	Alfred Nzo	Maize on cob	ITS = ON207833, <i>BenA</i> = ON601703
<i>P. polonicum</i>	CN045I1_CMW58136	Joe Gqabi	Stored maize	<i>BenA</i> = ON601704
<i>P. radiatolobatum</i>	CN052E5_CMW58138	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601705
<i>P. raistricki</i>	CN081A2_CMW58137	OR Tambo	Maize on cob	<i>BenA</i> = ON601706
<i>P. rubens</i>	CN045D5_CMW58139	Joe Gqabi	Stored maize	<i>BenA</i> = ON601707
	CN045E7	Joe Gqabi	Stored maize	<i>BenA</i> = ON601708
	CN045E9	Joe Gqabi	Stored maize	<i>BenA</i> = ON601709
	CN045F1	Joe Gqabi	Stored maize	<i>BenA</i> = ON601710
	CN045F5	Joe Gqabi	Stored maize	<i>BenA</i> = ON601711
	CN045F8	Joe Gqabi	Stored maize	<i>BenA</i> = ON601712
	CN045G4	Joe Gqabi	Stored maize	<i>BenA</i> = ON601713
	CN045H2	Joe Gqabi	Stored maize	<i>BenA</i> = ON601714
	CN045H4	Joe Gqabi	Stored maize	<i>BenA</i> = ON601715
	CN052E9	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601716
	CN052F1	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601717
	CN052G5	Chris Hani	Maize on cob	<i>BenA</i> = ON601718
	CN059D3	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601719
	CN080G2	Chris Hani	Maize on cob	<i>BenA</i> = ON601720
	CN080G4	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601721
	CN080H6	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601722
<i>P. rudallense</i>	CN080H2_CMW58140	Chris Hani	Maize on cob	<i>BenA</i> = ON601723
<i>P. sajarovii</i>	CN059D8_CMW58142	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601724
	CN074A4	Joe Gqabi	Maize on cob	ITS = ON209592
<i>P. salamii</i>	CN059E4_CMW58141	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601725
<i>P. ubiquetum</i>	CN045I6_CMW58143	Joe Gqabi	Stored maize	<i>BenA</i> = ON601726
<i>P. vanluykii</i>	CN045F6_CMW58144	Joe Gqabi	Stored maize	<i>BenA</i> = ON601727

Species	Strain number	District	Substrate	Sequences
	CN045G1	Joe Gqabi	Stored maize	<i>BenA</i> = ON601728
<i>Periconia sp.</i>	CN053A3_CMW58145	Chris Hani	Maize on cob	ITS = ON207652
<i>Pseudopithomyces sacchari</i>	CN053A8	Chris Hani	Maize on cob	ITS = ON207654
	CN059F7_CMW58146	Alfred Nzo	Maize on cob	ITS = ON207838
<i>Sordariomycetes sp.</i>	CN059F8	Alfred Nzo	Maize on cob	ITS = ON207839
	CN074A5_CMW58148	OR Tambo	Maize on cob	ITS = ON209593
<i>Sarocladium sp.</i>	CN082D1_CMW58149	OR Tambo	Maize on cob	ITS = ON209610
<i>Talaromyces amestolkiae</i>	CN045C6	Chris Hani	Stored maize	<i>BenA</i> = ON601729
	CN045D1	Joe Gqabi	Stored maize	<i>BenA</i> = ON601730
	CN045D2	Joe Gqabi	Stored maize	<i>BenA</i> = ON601731
	CN045D3	Joe Gqabi	Stored maize	<i>BenA</i> = ON601732
	CN045E2	Joe Gqabi	Stored maize	<i>BenA</i> = ON601733
	CN045E3	Joe Gqabi	Stored maize	<i>BenA</i> = ON601734
	CN045E4	Joe Gqabi	Stored maize	<i>BenA</i> = ON601735
	CN045E5	Chris Hani	Maize on cob	<i>BenA</i> = ON601736
	CN045E6	Chris Hani	Maize on cob	<i>BenA</i> = ON601737
	CN045F2	Joe Gqabi	Stored maize	<i>BenA</i> = ON601738
	CN045H7	Joe Gqabi	Stored maize	<i>BenA</i> = ON601739
	CN045H9	Joe Gqabi	Stored maize	<i>BenA</i> = ON601740
	CN059C9	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601741
	CN059D1	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601742
	CN059D2	Alfred Nzo	Maize on cob	<i>BenA</i> = ON601743
	CN073D7_CMW58153	OR Tambo	Maize on cob	<i>BenA</i> = ON601744
	CN073F3	OR Tambo	Maize on cob	<i>BenA</i> = ON601745
	CN073F4	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601746

Species	Strain number	District	Substrate	Sequences
	CN073G2	OR Tambo	Maize on cob	<i>BenA</i> = ON601747
	CN073G3	OR Tambo	Maize on cob	<i>BenA</i> = ON601748
	CN073G4	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601749
	CN073H1	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601750
	CN073H2	OR Tambo	Maize on cob	<i>BenA</i> = ON601751
	CN080F7	Chris Hani	Maize on cob	<i>BenA</i> = ON601752
	CN080F9	Chris Hani	Maize on cob	<i>BenA</i> = ON601753
	CN080G1	Chris Hani	Maize on cob	<i>BenA</i> = ON601754
	CN080I8	Chris Hani	Maize on cob	<i>BenA</i> = ON601755
<i>T. stollii</i>	CN045C5_CMW58154	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601756
	CN045C7	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601757
	CN045C8	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601758
	CN045C9	Joe Gqabi	Maize on cob	<i>BenA</i> = ON601759
<i>Trichoderma arenarium</i>	CN073I1_CMW58156	Joe Gqabi	Maize on cob	<i>TEF</i> = OP198579
<i>T. gamsii</i>	CN052D3_CMW58158	Chris Hani	Maize on cob	<i>TEF</i> = ON601954
	CN080D3	OR Tambo	Maize on cob	<i>TEF</i> = ON601956
<i>T. koningiopsis</i>	CN046F7_CMW58160	Joe Gqabi	Stored maize	<i>TEF</i> = ON602016
	CN046F8	Joe Gqabi	Stored maize	<i>TEF</i> = ON602017
<i>Xylariales sp.</i>	CN061B3	Joe Gqabi	Stored maize	ITS = ON229466
	CN063A4_CMW58161	Alfred Nzo	Maize on cob	ITS = ON229424
	CN073I6	Joe Gqabi	Maize on cob	ITS = ON209588

Figures

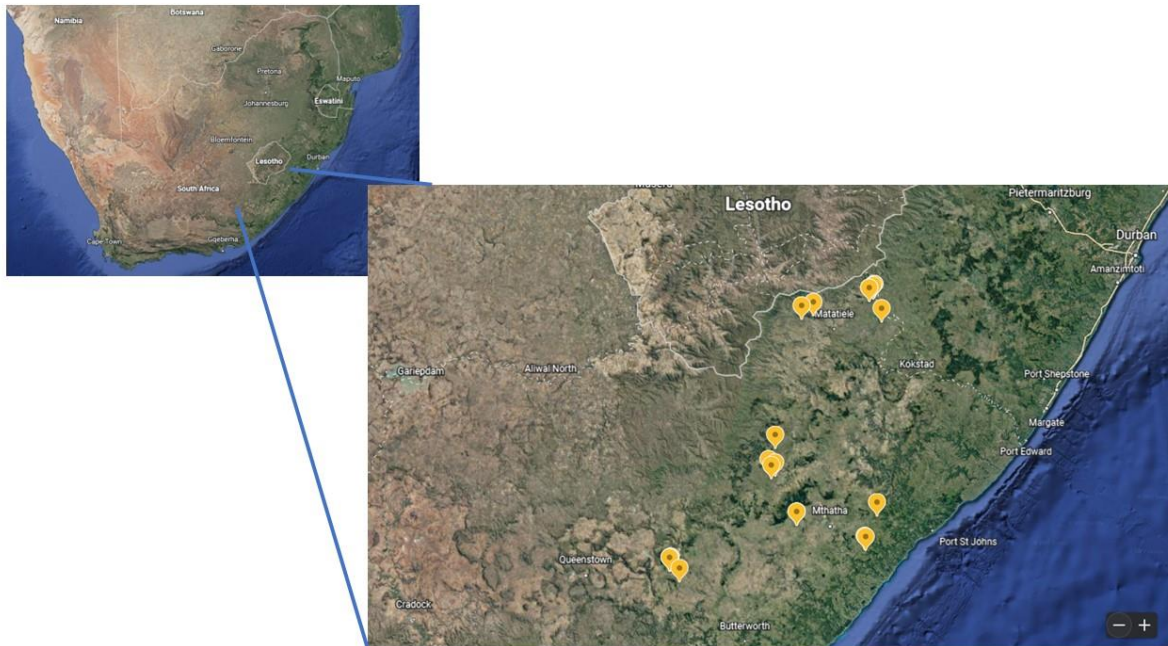


Figure 1: Sampling sites in the Eastern Cape, South Africa.

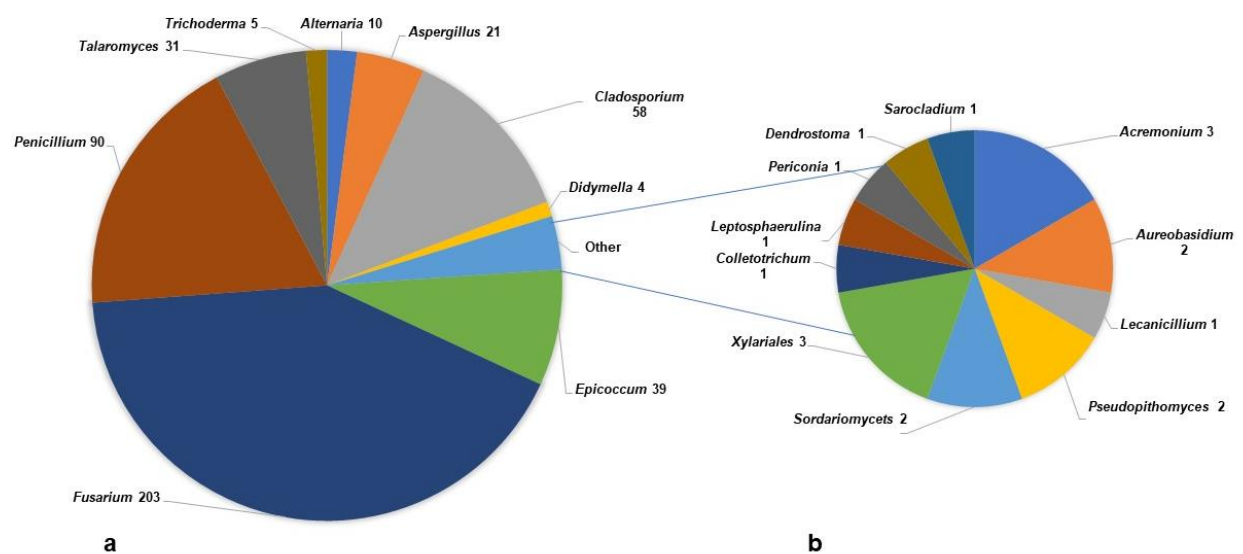


Figure 2: Genera isolated from stored and field maize samples in the Eastern Cape (a) Main genera, including mycotoxigenic genera, isolated frequently (b) Other genera isolated less frequently. Numbers represent total numbers of strains.

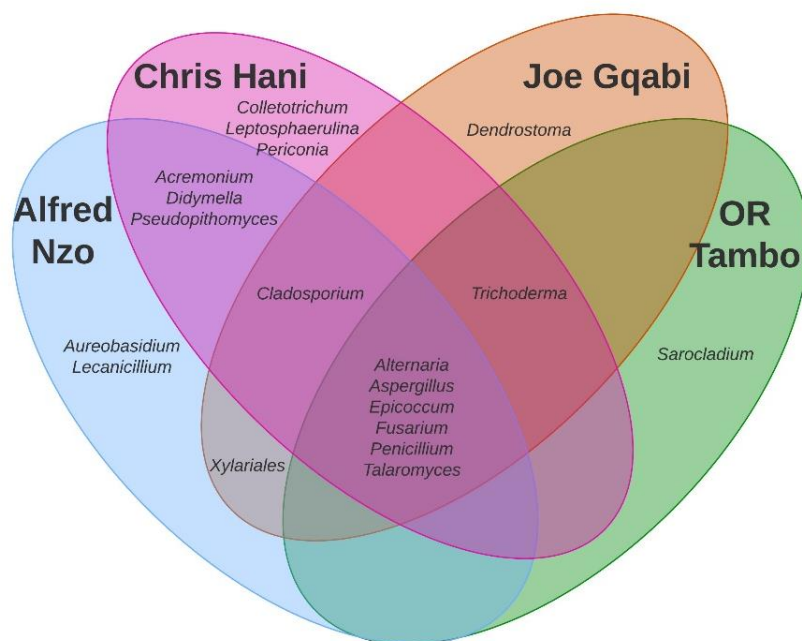


Figure 3: Distribution of isolated genera between the Alfred Nzo (blue), Chris Hani (purple), Joe Gqabi (orange) and OR Tambo (yellow) districts.

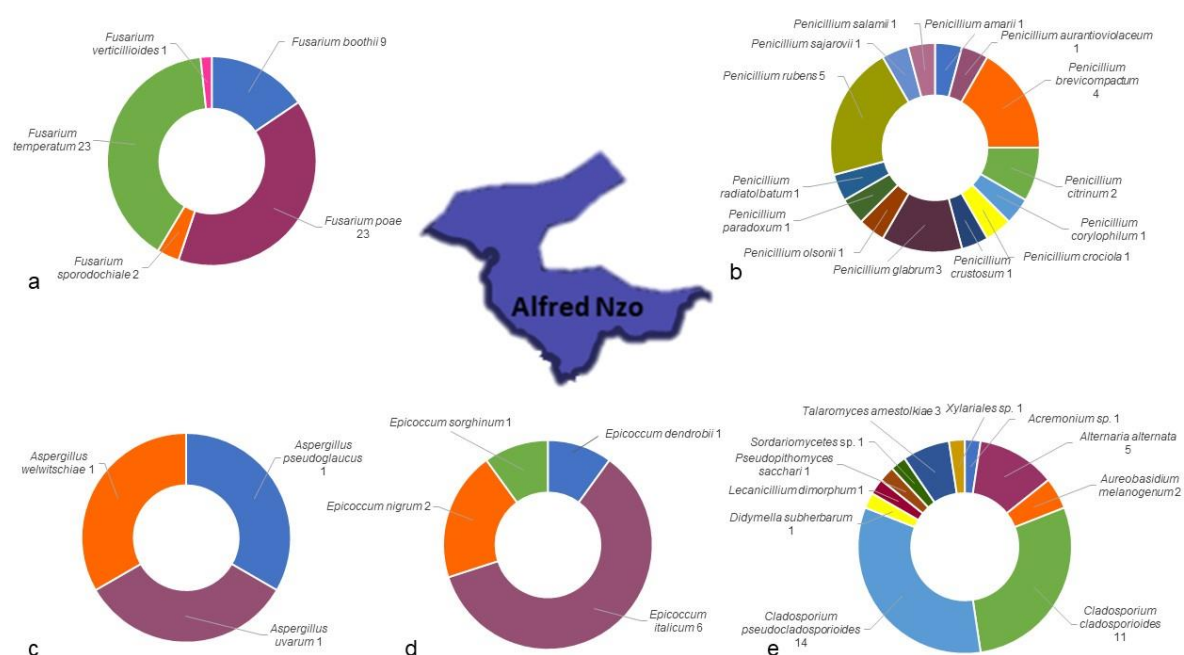


Figure 4: Distribution of species isolated in Alfred Nzo, Eastern Cape. (a) *Fusarium* species isolated (b) *Penicillium* species isolated, (c) *Aspergillus* species isolated, (d) *Epicoecum* species isolated and (e) other species isolated. Other species isolated (e) is based on less than 3 species isolated in a genus. Numbers represent total numbers of strains.

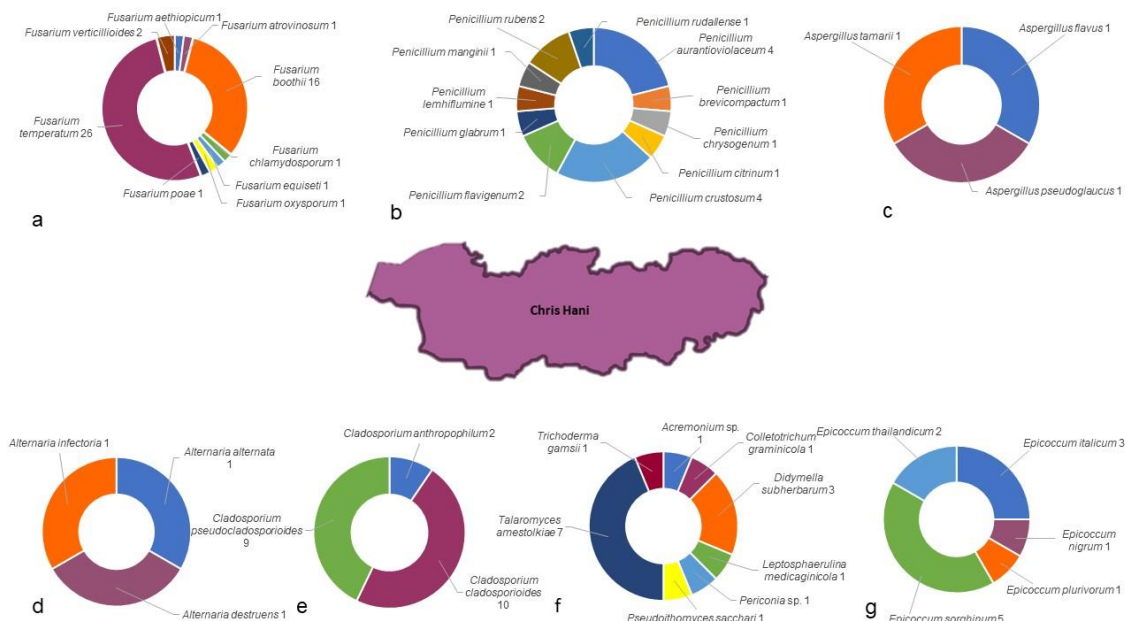


Figure 5: Distribution of species isolated in Chris Hani, Eastern Cape. (a) *Fusarium* species isolated (b) *Penicillium* species isolated, (c) *Aspergillus* species isolated, (d) *Alternaria* species isolated, (e) *Cladosporium* species isolated, (f) other species isolated, and (g) *Epicoccum* species isolated. Other species isolated (f) is based on less than 3 species isolated in a genus. Numbers represent total numbers of strains.

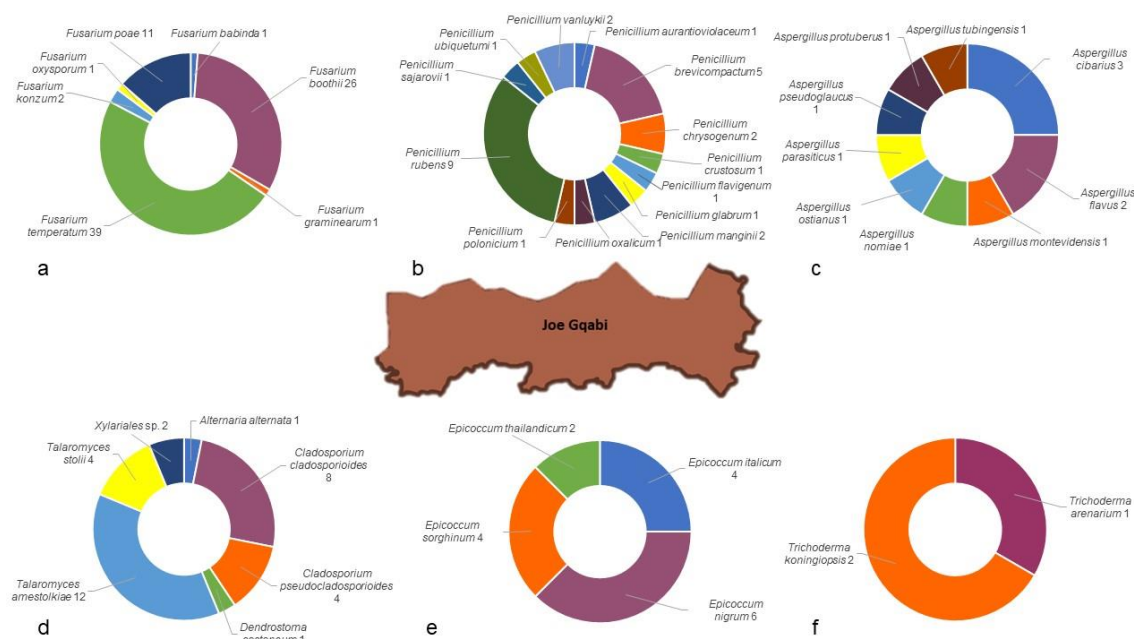


Figure 6: Distribution of species isolated in Joe Gqabi, Eastern Cape. (a) *Fusarium* species isolated (b) *Penicillium* species isolated, (c) *Aspergillus* species isolated, (d) other species isolated. Other species isolated (d) is based on less than 3 species isolated in a genus. Numbers represent total numbers of strains.

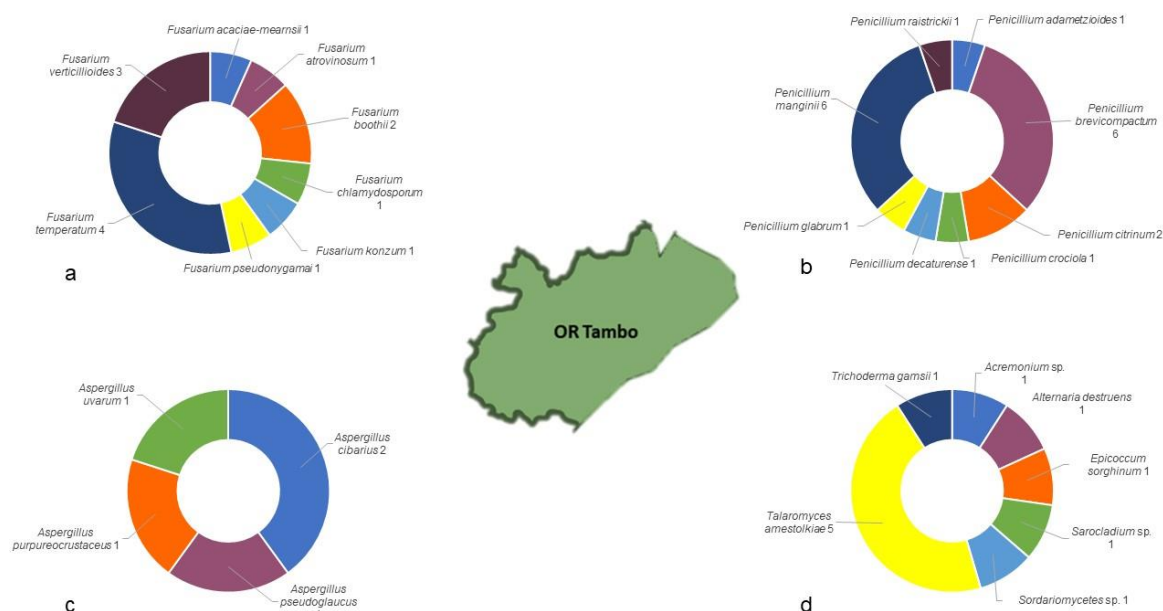


Figure 7: Distribution of species isolated in OR Tambo, Eastern Cape. (a) *Fusarium* species isolated (b) *Penicillium* species isolated, (c) *Aspergillus* species isolated, (d) other species isolated. Other species isolated (d) is based on less than 3 species isolated in a genus. Numbers represent total numbers of strains.

CHAPTER 3:

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

Abstract:

Maize is an important commodity produced across the world. In South Africa, much of its production is focused in the central provinces. The Eastern Cape (EC) contributes less than 1% of the gross total but is steadily increasing its production and has been identified as a priority region for future growth. During its production, maize is prone to several ear, foliar and root diseases. These are of economic importance as they result in reduced yields, while ear rots may contaminate kernels with mycotoxins. In this study, we surveyed ear rots from emerging maize farms in the Eastern Cape and determined mycotoxins present in collected samples. Fungal isolations were made from mouldy ears and species identified using morphology and DNA sequences generated for the internal transcribed spacer region (ITS), the large subunit (LSU) of the nuclear ribosomal RNA (rRNA) and the translation elongation factor 1- α (*TEF*) gene regions. From nineteen farms surveyed, we observed *Cladosporium* (CER, n=5), *Diplodia* (DER, n=6), *Fusarium* (FER, n=10) and *Gibberella* (GER, n=5) ear rots across twelve farms, while no ear rots were observed from seven farms. We collected 78 ear rot samples and recovered 84 fungal strains. *Stenocarpella macrospora* and *S. maydis* were identified from DER samples, *Fusarium clavus*, *F. oxysporum*, *F. scirpi*, *F. sporodochiale*, *F. subglutinans* and *F. temperatum* from FER samples, and *F. boothii*, *F. graminearum* and *F. poae* from GER samples. Other species identified included *Cladosporium cladosporioides* and *C. pseudocladosporioides* from CER; and *Epicoccum sorghinum*, *Mucor irregularis*, and *Trichoderma gamsii* from DER. Collected ear rots were pooled on a per farm basis and LC-MS/MS multi-mycotoxin analysis subsequently performed. Analyses revealed that 50% of samples contained multiple mycotoxins- deoxynivalenol (DON, >2000 $\mu\text{g/kg}$), 15-acetyl-deoxynivalenol (15-ADON, not regulated), diplodiatoxin (DIP, not regulated) and zearalenone (ZEA, <75 $\mu\text{g/kg}$); 33% contained a single mycotoxin and 17% contained no mycotoxins. Samples containing DON concentrations typically had 15-ADON present too. *Fusarium verticillioides* or fumonisins were not detected from ear rots collected in this study. This study revealed that ear rots are potentially caused by a wide range of species. It has also highlighted a shift in the dominant species and subsequent mycotoxins associated with ear rots in the EC. Strains collected during this study will be used as reference material for future

studies that will examine the biotic and abiotic triggers of mycotoxin production, thus contributing to ensuring food security.

Introduction:

Maize is a staple crop produced by commercial, small scale and subsistence farmers in many African countries, especially in sub-Saharan Africa (Chukwudi et al. 2021). In South Africa, commercial maize production is concentrated in the central regions of the country that includes the Free State (FS), North West (NW), Gauteng (GP) and Mpumalanga (MP) provinces (Gravelet-Blondin 2013). The Eastern Cape (EC) contributes about 1% of South Africa's annual maize produced, with many small scale and emerging farms across the province (Aveling et al. 2020).

Maize production in Africa continuously faces challenges like pests and diseases preventing many regions from reaching their full production potential (Logrieco et al. 2021). Maize ears are prone to fungal infection often resulting in ear rots and subsequent mycotoxin contamination of kernels. Economically, it is one of the most important diseases (Boutigny et al. 2012), either directly associated with reduced trade revenue due to contamination, or with adverse health effects (Chukwudi et al. 2021). Globally there are several fungal ear rots of maize, including *Aspergillus* (AER), *Cladosporium* (CER), *Diplodia* (DER), *Fusarium* (FER), *Gibberella* (GER), *Penicillium* (PER) and Corn smut ear rots (Munkvold 2017). The distribution of ear rots is dependent on climatic and geographical conditions; however, DER, FER and GER ear rots are most common to South Africa (Boutigny et al. 2012, Flett 2016, Mabuza 2017). DER is caused by *Stenocarpella maydis* (= *Diplodia maydis*), whereas FER and GER are caused by several species within the *Fusarium fujikuroi* (FFSC) and *Fusarium sambucinum* (FSAMSC) species complexes respectively (Fandohan et al. 2003, Munkvold 2017, Munkvold et al. 2021, Reid et al. 1999). *Fusarium verticillioides* (= *F. moniliforme*) is most commonly linked with FER, while *F. graminearum* is most commonly linked with GER. However, several other species from both the FFSC and FSAMC can also cause ear rots and differ in their abilities to produce mycotoxins. These secondary metabolites, or extrolites, can cause disease or death in animals or humans if consumed above threshold levels (Pitt 2000). Mycotoxin contamination of maize can occur across the entire value chain, from the field, during transportation or during storage, and is affected by many biotic and abiotic factors (Eskola et al. 2020, Lillehoj et al. 1976, Shotwell 1975,

Sweeney and Dobson 1998). Mycotoxin levels in South African maize is monitored annually by the South African Grain Laboratory (SAGL), but this usually does not include EC maize.

For EC maize production, it is important to know what ear rots are common in the area, which species are causing them and what potential mycotoxins they may produce. Previously, the occurrence of maize ear rots in South Africa has been examined (Flett et al. 1998, Flett et al. 2001, Flett and Wehner 1991, Mabuza 2017, Ncube et al. 2018, Phokane 2018, Rheeder et al. 1990, Rheeder et al. 2016). These studies identified the species associated with the ear rots either using morphology or species-specific primers for quantitative PCR (qPCR) targeted species identification. Fungal morphology often results in misidentifications, especially of cryptic species (species that are morphologically identical, but differ genetically) (Visagie et al. 2014). Identification using species-specific qPCR does not consider the possibility of the presence of other potential ear rot causal agents and known mycotoxin producers.

Even though mycotoxin contamination of maize in South Africa has also been studied, the correlation between the ear rots and species associated with it has not been linked especially using molecular identification (Ekwomadu et al. 2021, Ekwomadu et al. 2020, Gruber-Dorninger et al. 2018, Janse van Rensburg et al. 2015, Meyer et al. 2019). For this study, we thus completed a maize ear rot survey of EC farms, with the goals to 1) determine their distribution and severity, 2) identify the fungal species from specific ear rots and 3) determine the mycotoxins present on collected ear rot samples.

Materials and Methods:

Ear rot survey and Sample collections

Nineteen emerging maize farms across the EC were surveyed for maize ear rots during May 2021, approximately two weeks before harvesting. Our survey was carried out in the Alfred Nzo (AN), Chris Hani (CH), Joe Gqabi (JG) and OR Tambo (ORT) districts. Disease severity was determined using a disease rating scale of one to nine, where one is resistant and nine is very susceptible (Czembor et al. 2019). Disease distribution was calculated based on the proportion of disease scored maize cobs to the number of maize cobs surveyed. Whole symptomatic maize ears were removed from the plant and placed in individual paper bags labelled with the respective sample number. Samples were stored at 4 °C until processing.

Mycotoxin analysis

Ear rot samples were processed at SAGL (ISO17025 accredited) and mycotoxin levels determined following the methodology published by Meyer et al. (2019). Maize kernels were removed from the cob and milled with a 1 mm sieve (Retsch ZM 200 mill). Milled samples were mechanically mixed for 90 mins. Subsamples (10.00 g $\pm$ 0.05 g) were weighed out and 40 mL extraction solvent, comprising of 50:25:25 ultra-pure water (<18.2 M Ω .cm): methanol (MeOH, for high performance liquid chromatography (HPLC), >99.9%): acetonitrile (AcCN, ACS/HPLC grade, Burdick and Jackson) was added. The samples were blended using an overhead stirrer for 1 min and were subsequently transferred to 50 mL polypropylene centrifuge tube. Centrifuge tubes were incubated on a mechanical shaker in the horizontal position for 15 min at 260 rpm and were subsequently centrifuged for 10 min at 3000 rpm. A volume of 5 mL of the supernatant was aliquoted into a volumetric flask, and 5 mL of the dilution solution (25% MeOH in H₂O) was added. Sample extracts were filtered into HPLC amber vials using 13 mm, 0.22 μ m syringe filters.

We monitored for fourteen mycotoxins, including aflatoxins (AFs; including B₁ B₂ G₁ and G₂), deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), diplodiatoxin (DIP), fumonisins (FUMs; including B₁ B₂ and B₃), HT-2 toxin (HT2),

ochratoxin A (OTA), T-2 toxin (T2), and zearalenone (ZEA). A standard (10 µg/mL) was prepared for AFs, DON, 15-ADON, DIP, FUMB₁, OTA, T2, ZEA; the HT2 toxin calibrant solution (100 µg/mL) (Romner Labs Diagnostic GmbH; Tulln, Austria); and FUMs (B₂ and B₃) (Cape Peninsula University of Technology; Cape Town, South Africa) respectively. Recoveries were prepared using maize samples not contaminated with the analysed mycotoxins. Additionally, blank samples (negative controls) were prepared using only the extraction solvent, and spiked samples (positive controls) were prepared at a concentration of 5 and 50 µg/kg respectively in 40 mL of the extraction solvent.

Liquid chromatography mass spectrometry-mass spectrometry (LC-MS/MS) analysis was carried out on an ultra-performance liquid chromatograph (Waters Acquity UPLC) with a C₁₈ column (Acquity BEH) at 30 °C in a Waters tandem quadrupole mass spectrometer (Acquity TQD). A programmed gradient elution buffer comprising of mobile phase A (0.5 mM ammonium acetate (Purity ≥ 98% Sigma-Aldrich/Merck) and 0.1% formic acid (98–100% Suprapur, Merck)), and mobile phase B (AcCN with 0.1% formic acid) was used for the separation of the fourteen mycotoxins. Samples were analyzed in duplicate, and the mean values are reported.

Fungal isolations

Symptomatic maize ears were observed under a stereomicroscope and fungal material transferred using a sterile needle onto ¼ Potato Dextrose Agar (PDA) supplemented with chloramphenicol (100 mg/L). The plates are incubated for ~7 d at room temperature (± 23°C). Single spore cultures were prepared following Leslie and Summerell (2006). Pure cultures were preserved in 10% glycerol and stored at -80 °C. The cultures were accessioned into the CN working collection and representative cultures for each species into the CMW culture collection, both housed at the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from 7 d old cultures grown on ¼ PDA. *Fusarium* and *Stenocarpella* DNA extractions were carried out using the PrepMan® Ultra

(Applied Biosystems, USA) following the manufacturer's instructions. For pigmented fungi such as *Cladosporium*, *Epicoccum* and *Trichoderma*, DNA extractions were carried out using the Quick-DNA™ Fungal/Bacterial Miniprep kit (Zymo Research, USA) following the manufacturer's instructions.

PCR amplifications were made in 25 µL PCR master mixes consisting of 5 µL BioLine 5X MyTaq Reaction Buffer (Meridian BioScience, USA); 0.50 µL of each primer (10 µM); 17.85 µL demineralized sterile water and 0.15 µL BioLine MyTaq DNA Polymerase (Meridian BioScience, USA). The internal transcribed spacer region (ITS), the large subunit (LSU) of the nuclear ribosomal RNA (rRNA) and the translation elongation factor 1-alpha (*TEF*) gene regions were amplified. PCR conditions and primers used for these are included in Table 1.

Amplified fragments were purified using Exo-SAP-IT PCR Product Cleanup Reagent (ThermoFischer Scientific, Massachusetts, USA) and sequenced in both directions using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) using the same primers used for amplification. Sequencing reactions were run on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Contigs were assembled and edited using Geneious Prime v. 2019.2.1 (BioMatters Ltd., Auckland, New Zealand).

Sequences generated in this study were compared to locally curated databases, mainly compiled from taxonomic studies, and obtained from the National Centre for Biotechnology Information's (NCBI) nucleotide database (<http://www.ncbi.nlm.nih.gov/nucleotide/>). Newly generated sequences were submitted to GenBank.

Results:

Ear rot survey

We observed ear rots at 12 of the 19 farms (Table 2, Fig. 1). Ear rots observed include CER (5 farms, Fig. 1A–C), DER (6 farms, Fig. 1D–F), FER (10 farms; Fig. 1G–I), and GER (5 farms; Fig. 1J–L). Generally, ear rot distribution (0.8–14 %) and severity (an average score of 2 recorded) was low. The exception was farm 5 where 90% of the field had severe DER, attributed to a hailstorm that damaged cobs a month earlier. From the farms where ears rots were observed, a single ear rot was observed at four farms including farm 3 (FER), farm 8 (CER), farm 9 (CER) and farm 11 (FER). Farms where two types of ear rots were observed include farm 2 (DER and FER), farm 4 (CER and FER), farm 6 (DER and FER), farm 7 (FER and GER) and farm 10 (DER and FER). Three types of ear rots were observed on farm 1 (CER, DER and FER) and farm 5 (DER, FER and GER); while four types of ear rots were observed on farm 12 (CER, DER, FER and GER).

Mycotoxin analysis

AFs, FUMs, HT2, OTA and T2 were not detected at any farm. We did, however, detect DON, 15-ADON, DIP and ZEA (summarized in Table 2). All four of these mycotoxins were detected on farms 5 and 12. Multiple mycotoxins were detected from six of the twelve farms, including farm 2 (DON, 15-ADON and DIP), farm 4 (DON and 15-ADON), farm 6 (DON and DIP) and farm 7 (DON, 15-ADON and ZEA). A single mycotoxin was detected at farm 1 (DIP), farm 8 (DON), farm 9 (DIP) and farm 10 (DIP), while no mycotoxins were detected from farms 3 and 11 even though FER were detected at both.

DON was detected from seven farms. It was detected from four farms where GER was collected, including farm 5 (5678 µg/kg), farm 6 (254 µg/kg), farm 7 (18306 µg/kg) and farm 12 (7273 µg/kg). It was also detected from three farms (farm 2 (879 µg/kg), farm 4 (3143 µg/kg) and farm 8 (89 µg/kg)) where GER was not observed and no FSAMSC species were isolated.

The DON derivative, 15-ADON, was detected from all farms where DON was detected, except for farms 6 and 8. It was detected from three farms where GER

samples were collected including farm 5 (257 µg/kg), farm 7 (1263 µg/kg) and farm 12 (736 µg/kg). It was also detected from farms where GER was not observed and no FSAMSC species were isolated, including farm 2 (174 µg/kg) and farm 4 (301 µg/kg). The levels of 15-ADON detected were far less than DON.

DIP was detected from seven farms. It was detected from six farms where DER was collected including farm 1 (726 µg/kg), farm 2 (122 µg/kg), farm 5 (33808 µg/kg), farm 6 (2648 µg/kg), farm 10 (1268 µg/kg) and farm 12 (23004 µg/kg), but also from farm 9 (25 µg/kg) where no DER or producing species was observed. Lastly, ZEA was detected from three farms (farm 5 (23 µg/kg), farm 7 (639 µg/kg) and farm 12 (67 µg/kg)) where GER was collected.

Fungal identifications

Isolations from 78 ear rot samples resulted in 84 strains cultured. These were identified into six genera and sixteen species, including *Cladosporium* (2 species), *Epicoccum* (1 species), *Fusarium* (9 species), *Mucor* (1 species), *Stenocarpella* (2 species), and *Trichoderma* (1 species). The *Epicoccum* (n=1), *Mucor* (n=1) and *Trichoderma* (n=1) species isolated were not associated with any ear rot symptoms. Table 3 summarises the strains isolated, species identified, and gene sequences generated with GenBank numbers for them.

From FER samples, we isolated and identified six *Fusarium* species (Fig. 2A), including *F. clavus* (n=1, *Fusarium incarnatum-equiseti* species complex (FIESC)), *F. oxysporum* (n=2, *Fusarium oxysporum* species complex (FOSC)), *F. scirpi* (n=1, FIESC), *F. sporodochiale* (n=1; *Fusarium chlamydosporum* species complex (FCSC)), *F. subglutinans* (n=3, FFSC); and *F. temperatum* (n=35, FFSC). *Fusarium temperatum* was isolated from all farms where FER was observed; and this was the only species isolated from FER on farm 3. Three *Fusarium* species were identified from GER, and all belong to the FSAMSC (Fig. 2B). These include *F. boothii* (n=7), *F. graminearum* (n=4) and *F. poae* (n=3).

Stenocarpella maydis (n=17) and *S. macrospora* (n=1) were isolated from DER samples (Fig. 2C). *Stenocarpella maydis* was isolated from all farms where DER was identified, with a single isolate of *S. macrospora* isolated from a DER sample together with *S. maydis*. *Cladosporium cladosporioides* (n=4) and *C. pseudocladosporioides* (n=2) was isolated from CER (Fig. 2D).

Discussion

This is the first survey conducted in the EC to make use of DNA sequences to accurately identify all species associated with maize ear rots. Nineteen farms were surveyed with no ear rots observed at seven farms, while ear rots were observed at twelve farms, including CER (5/12), DER (6/12), FER (10/12) and GER (5/12). Fungal isolations from these, resulted in the identification of six genera and seventeen species, most notably *Cladosporium*, *Fusarium* and *Stenocarpella*. Four mycotoxins (DON, 15-ADON, DIP and ZEA) were detected from collected maize ear rots from 10/12 farms surveyed in this study.

FER has previously been reported in South Africa (Flett 2016, Flett et al. 1998, Flett and Wehner 1991, Rheeder 1993, Rheeder et al. 1990) and globally (Czembor et al. 2014, Fumero et al. 2015, Logrieco et al. 2002, Njeru et al. 2020, Pfordt et al. 2020, Robertson et al. 2011, Stępień et al. 2019, Zhang et al. 2016), with *F. verticillioides* being the dominant species identified, followed by *F. subglutinans* and *F. temperatum*. During this study, no *F. verticillioides* or FUMs were detected from FER samples. This differs from previous studies where *F. verticillioides* was the dominant species isolated from FER in South Africa (Flett 2016, Flett et al. 1998, Flett and Wehner 1991, Rheeder 1993, Rheeder et al. 1990). The absence of *F. verticillioides* and FUMs was surprising considering *F. verticillioides* and its mycotoxin, FUMB₁, have been frequently detected from smallholder produced maize in the EC (Marasas et al. 1981, Marasas et al. 1988a, Marasas et al. 1976, Rheeder et al. 2016). During this study, *F. subglutinans* and *F. temperatum* were frequently isolated from FER. These are cryptic sister species that are reproductively isolated and were previously reported as distinct groups (1 and 2) of *F. subglutinans* (O'Donnell et al. 1998, Steenkamp et al. 2002). The two groups were later described as two distinct species, *F. subglutinans* (previously Group 2) and *F. temperatum* (previously Group 1) (Scauflaire et al. 2011). *Fusarium subglutinans* is prevalent in cooler maize production areas such as the northern United States of America and Canada (Munkvold et al. 2019, Vigier et al. 1997) and central and northern Europe (Bottalico 1998). Recently, *F. temperatum* has been found to dominate maize production areas in North America, Argentina and Europe (Munkvold 2017). It was previously reported that both *F. subglutinans* and *F. temperatum* species can

produce FUMs (Munkvold et al. 2021, Nelson et al. 1992, Scaufaire et al. 2011). However, recent research has indicated that the former species lack the biosynthetic gene clusters required for FUMS production (Fumero et al. 2015, Fumero et al. 2020). Whilst these species are sister species, their mycotoxin production potential differs, and they are known to produce emerging mycotoxins (Munkvold et al. 2021). *Fusarium subglutinans* can produce moniliformin (MON), which is cytotoxic and has been implicated in protein synthesis inhibition, chromosome damage; intestinal haemorrhaging, coma and death in poultry (Fumero et al. 2015, Jestoi 2008, Munkvold 2017); while *F. temperatum* can produce beauvercin (BEA) which has been implicated in induction of apoptosis and enzyme inhibition (Fumero et al. 2020, Kouri et al. 2003, Munkvold et al. 2021, Scaufaire et al. 2011). Neither of which were tested for in this study.

GER has also previously been reported in South Africa (Boutigny et al. 2011a, Flett 2016, Flett et al. 1998, Flett and Wehner 1991, Rheeder et al. 1990) and globally (Logrieco et al. 2002, Pfordt et al. 2020, Robertson et al. 2011, Zhang et al. 2016), with *F. graminearum* being the most common species reported. During this study, known GER causal agents *F. boothii*, *F. graminearum* and *F. poae* were isolated and identified (Laraba et al. 2021). GER is found to predominate in maize production areas that experience cooler temperatures and high precipitation (Bottalico 1998, Logrieco et al. 2002). *Fusarium graminearum* is the most economically important species within the FSAMSC and is the dominant causal agent frequently identified from GER across the world (Bottalico 1998, Munkvold et al. 2019, Vigier et al. 1997). *Fusarium poae* is a pathogen that dominates small grain cereals and is typically associated with Fusarium Head Blight (FHB) of wheat in South Africa (Beukes et al. 2017, Boutigny et al. 2011a, Boutigny et al. 2011b). It has previously been isolated from asymptomatic maize in South Africa (Marasas et al. 1981, Phoku et al. 2012), however this is a first report of it being isolated from GER in the country. *Fusarium boothii* and *F. graminearum* are producers of DON (a regulated mycotoxin, < 2000 µg/kg for human consumption) and its derivative 15-ADON (an unregulated mycotoxin) (Hocking et al. 2006, Miller 1991). In addition to producing these mycotoxins, *F. graminearum* produces ZEA (a regulated mycotoxin, < 75 µg/kg for human consumption) (Stob et al. 1962). *Fusarium poae* is a known producer of

diacetoxyscirpenol (DAS), fusarenone X, neosolaniol (NEO) and nivalenol (NIV) (emerging mycotoxins) (Munkvold et al. 2021). DON, 15-ADON and ZEA were detected in this study and have also been detected from other maize producing regions in South Africa (Biomin 2019, Biomin 2020, Biomin 2021, Ekwomadu et al. 2021, Ekwomadu et al. 2020, Gruber-Dorninger et al. 2018, Janse van Rensburg et al. 2015, Meyer et al. 2019). Biomin (2019), Biomin (2021) and Meyer et al. (2019) conduct annual surveys on the mycotoxins detected in a number of agricultural produce, including maize. The authors have observed a shift in the dominant mycotoxin detected from all agricultural produce in South Africa. Previously, reports indicated FUMB₁ being the dominant mycotoxin, especially associated with maize. This study, and the above-mentioned studies have observed that FUMB₁ is no longer the dominant mycotoxin, and that DON, 15-ADON, and ZEA are becoming more prominent. Health implications following consumption of DON include feed refusal by swine and altered immune function, while consumption of ZEA results in estrogenic activity of mammals (Logrieco et al. 2002, Miller 1991, Miller 1995, Munkvold et al. 2019).

Like FER and GER, DER has also been previously reported in South Africa (Flett et al. 1998, Flett et al. 2001, Flett and Wehner 1991, Rheeder 1993, Rheeder et al. 1990), with *S. maydis* being the main causal agent, while *S. macrospora* has also been shown to be associated with DER (Flett and Wehner 1991, Lamprecht et al. 2011, Wicklow et al. 2011). DER is often found in maize production regions that experience dry weather early in the season, followed by wet weather conditions prior to and after silking conditions (Lamprecht et al. 2011, Shurtleff 1980). Outbreaks of DER typically occur in the late winter months (July - September) (Rheeder 1993). This study isolated *S. maydis* from all DER samples, with only a single isolate of *S. macrospora* found together with the former. *Stenocarpella maydis* is known to produce DIP and chaetoglobosins K and L, however the production potential has been found to differ between strains (Kellerman et al. 1991, Steyn et al. 1972, Wicklow et al. 2011). DIP is an unregulated neuromycotoxin affecting mainly cattle and sheep and has been linked to diplodiosis outbreaks in South Africa, Argentina, and Brazil (Kellerman et al. 1991, Odriozola et al. 2005, Riet-Correa et al. 2009). Symptoms of diplodiosis include ataxia and paralysis, however toxicity studies conducted have not

definitively identified DIP as the metabolite responsible for this mycotoxicosis (Zaccaron et al. 2017). This is the first report of DIP detected from maize in the EC.

The type of ear rot; distribution and prevalence of the species, and the mycotoxins associated are influenced by environmental conditions (Logrieco et al. 2002). In general, the EC experiences moderate to warm temperatures during the day, and cooler temperatures at night (Fig. 3) (Anonymous 2022, Price et al. 2022). However, there are variations in climatic conditions between the districts within the EC. For example, the AN district will experience cooler climatic conditions as it is found at a higher elevation close to a mountainous region (Affairs 2020a). However, the ORT district experiences more humid conditions, and moderate to higher rainfall along its sub-tropical coast; but it also has pockets of mountainous areas (Affairs 2020b). Suboptimal environmental conditions can contribute to the lack of detection of mycotoxins from two farms in this study, even though the producing species were identified. Variations in the climatic conditions not only impacts the distribution of the ear rots observed, but also the species and the mycotoxins that are associated with them. This should be studied further in order to gain a better understanding of the influence of the climatic conditions on the ear rots and the associated species observed.

Ear rot infection and subsequent mycotoxin contamination can be mitigated following the application of good agricultural practices. This study observed a difference in the dominant species (from *F. verticillioides* to *F. temperatum*) and mycotoxins (from FUMB1 to multiple mycotoxins) previously reported to be associated with maize in the EC and South Africa (Biomin 2019, Biomin 2020, Biomin 2021, Marasas et al. 1981, Marasas et al. 1988a, Marasas et al. 1988b, Marasas et al. 1979, Meyer et al. 2019). It can be hypothesized that a shift in the dominant species is occurring, however in order to confirm this more extensive sampling over a longer period of time should be carried out. In addition, this study has also highlighted the growing concern of multiple species and the multiple mycotoxins associated with them in EC maize. Due to the production potential of the EC, continuous maize ear rot and mycotoxin monitoring should be conducted over multiple seasons in order to study the ear rots and mycotoxins typical for the area. This will ensure continuous safe food production with the goal to minimize

the financial loss directly associated with yield loss, as well as indirectly associated with consumer related health impacts. Isolates obtained during this study have been accessioned into a culture collection and can be used in future studies that focus on further understanding the abiotic as well as biotic factors that trigger mycotoxin production. This will not only contribute to the development of prevention and management strategies that are unique to the EC microclimate, but also sustainable food security.

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Tables

Table 1: PCR conditions used for amplification

Locus	Primer	Primer sequence 5'–3'	PCR amplification protocol	Genus	Reference
ITS	V9G	TTACGTCCCTGCCCTTTGTA	94°C 5 min; 35 cycles of 94°C 45s, 55°C 45s, 72°C 60s; 72°C 7min; 10°C ∞	<i>Stenocarpella</i> / Unknown	De Hoog and van den Ende (1998)
	LS266	GCATTCCCAAACAACCTCGACTC			Masclaux et al. (1995)
<i>TEF</i>	728F	CATCGAGAAGTTCGAGAAGG	94°C 5 min; 35 cycles of 94°C 45s, 52°C 45s, 72°C 60s; 72°C 7min; 10°C ∞	<i>Cladosporium</i> / <i>Stenocarpella</i>	Carbone and Kohn (1999)
	986R	TACTTGAAGGAACCCTTACC			
	EF1	ATGGGTAAGGARGACAAGAC	94°C 5 min; 35 cycles of 94°C 45s, 53°C 45s, 72°C 60s; 72°C 7min; 10°C ∞	<i>Fusarium</i>	O'Donnell et al. (1998)
	EF2	GGARGTACCAGTSATCATG			
LSU	LR5	ATCCTGAGGGAAACTTC	94 °C 5 min; 40 cycles of 94 °C 45s, 48 °C 30s, 72 °C 90s; 72 °C 6min; 10 °C ∞	<i>Epicoccum</i> / <i>Phoma</i>	Vilgalys and Hester (1990)
	LROR	GTACCCGCTGAACTTAAGC			Rehner and Samuels (1994)

Table 2: Mycotoxins detected and the species isolated from ear rots from the respective farms in this study

Farm number	Ear rot	Species detected	District	General disease		Mycotoxins detected (Average µg/kg)			
				Distribution (%)	Severity (average)	DON	15-ADON	DIP	ZEA
Farm 1	CER	<i>Cladosporium pseudocladosporioides</i>	Alfred Nzo	14	1	—	—	726	—
	DER	<i>Stenocarpella maydis</i>							
	FER	<i>Fusarium temperatum</i>							
Farm 2	DER	<i>Stenocarpella maydis</i>	Alfred Nzo	6	1	879	174	122	—
	FER	<i>Fusarium temperatum</i>							
Farm 3	FER	<i>Fusarium temperatum</i>	Alfred Nzo	2	2	—	—	—	—
Farm 4	CER	<i>Cladosporium cladosporioides</i> , <i>C. pseudocladosporioides</i>	Chris Hani	3	2	3143	301	—	—
	FER	<i>Fusarium temperatum</i> , <i>F. scirpi</i>							
Farm 5	DER	<i>Stenocarpella maydis</i>	Chris Hani	35	7	5678	257	33808	23
	FER	<i>Fusarium oxysporum</i> , <i>F. temperatum</i>							
	GER	<i>Fusarium boothii</i> , <i>F. poae</i>							
Farm 6	DER	<i>Stenocarpella maydis</i>	Chris Hani	5	2	254	—	2648	—
	FER	<i>Fusarium clavus</i> , <i>F. oxysporum</i> , <i>F. subglutinans</i> , <i>F. temperatum</i>							
Farm 7	FER	<i>Fusarium oxysporum</i> , <i>F. temperatum</i>	Joe Gqabi	3	2	18306	1263	—	639
	GER	<i>Fusarium boothii</i> , <i>F. graminearum</i> , <i>F. poae</i>							
Farm 8	CER	<i>Cladosporium</i> sp.	Joe Gqabi	6	1	89	—	—	—
Farm 9	CER	<i>Cladosporium cladosporioides</i>	Joe Gqabi	11	1	—	—	25	—

Farm number	Ear rot	Species detected	District	General disease		Mycotoxins detected (Average µg/kg)			
				Distribution (%)	Severity (average)	DON	15-ADON	DIP	ZEA
Farm 10	DER	<i>Stenocarpella macrospora</i> , <i>S. maydis</i>	OR Tambo	2	2	—	—	1268	—
	FER	<i>Fusarium temperatum</i>							
Farm 11	FER	<i>Fusarium subglutinans</i> , <i>F. sporodochiale</i>	OR Tambo	1	2	—	—	—	—
Farm 12	CER	<i>Cladosporium cladosporioides</i>	OR Tambo	0.8	1	7273	736	23004	67
	DER	<i>Stenocarpella maydis</i>							
	FER	<i>Fusarium temperatum</i>							
	GER	<i>Fusarium boothii</i>							

Table 3: Fungal species identified during this study

Species	Strain	District	Sequences
<i>Cladosporium cladosporioides</i>	CN122B3	Chris Hani	TEF = OP245275
	CN122B5	Joe Gqabi	TEF = OP245276
	CN124G9_CMW58075	OR Tambo	TEF = OP245277
	CN130C3	Joe Gqabi	TEF = OP245278
<i>C. pseudocladosporioides</i>	CN122B6_CMW58077	Alfred Nzo	TEF = ON601963
	CN130C4	Joe Gqabi	TEF = ON601964
<i>Epicoccum sorghinum</i>	CN124H2_CMW58090	Chris Hani	ITS = ON209718
<i>Fusarium boothii</i> (FSAMSC)	CN122E1	Joe Gqabi	TEF = OP245204
	CN122E7	Joe Gqabi	TEF = OP245205
	CN122E8	Joe Gqabi	TEF = ON601965
	CN122F5_CMW58097	OR Tambo	TEF = ON601966
	CN124H7	OR Tambo	TEF = ON601967
	CN130A6	OR Tambo	TEF = OP245206
	CN135H4	Chris Hani	TEF = OP245207
<i>F. clavus</i> (FIESC)	CN122C3	Chris Hani	ITS = ON209707, TEF = OP198608
<i>F. graminearum</i> (FSAMSC)	CN122E6_CMW58101	Joe Gqabi	TEF = ON601975
	CN122E9	Joe Gqabi	TEF = ON601976
	CN124I2	Joe Gqabi	TEF = ON601977
	CN135H7	Joe Gqabi	TEF = OP198609
<i>F. scirpi</i> (FIESC)	CN124H1_CMW58102	Chris Hani	TEF = OP198612
<i>F. oxysporum</i> (FOSC)	CN122F1_CMW58105	Joe Gqabi	TEF = ON601978
	CN124H8	OR Tambo	TEF = ON601979
<i>F. poae</i> (FSAMSC)	CN122E4_CMW58107	Joe Gqabi	TEF = ON601980
	CN135H5	Chris Hani	TEF = OP198610
	CN135H6	Chris Hani	TEF = OP198611
<i>F. sporodochiale</i> (FCSC)	CN122F4_CMW58110	OR Tambo	TEF = ON601981
<i>F. subglutinans</i> (FFSC)	CN122F3	OR Tambo	TEF = ON601982
	CN124H9_CMW58111	Chris Hani	TEF = ON601983
	CN124I1	Chris Hani	TEF = ON601984

Species	Strain	District	Sequences
<i>F. temperatum</i> (FFSC)	CN124I4	OR Tambo	TEF = ON601985
	CN122D5	Chris Hani	TEF = OP198613
	CN122D6	Chris Hani	TEF = ON601986
	CN122D7	Chris Hani	TEF = ON601987
	CN122D8_CMW58113	Chris Hani	TEF = ON601988
	CN122D9	Chris Hani	TEF = ON601989
	CN122E2	Joe Gqabi	TEF = ON601990
	CN122E5	Joe Gqabi	TEF = ON601991
	CN122F2	OR Tambo	TEF = ON601992
	CN122F6	Alfred Nzo	TEF = ON601993
	CN122F7	Alfred Nzo	TEF = ON601994
	CN122F8	Alfred Nzo	TEF = ON601995
	CN122F9	Alfred Nzo	TEF = ON601996
	CN122G2	Alfred Nzo	TEF = ON601997
	CN122G3	Alfred Nzo	TEF = ON601998
	CN122G4	Alfred Nzo	TEF = OP198614
	CN122G5	Alfred Nzo	TEF = ON601999
	CN122G6	Alfred Nzo	TEF = ON602000
	CN122G7	Alfred Nzo	TEF = ON602001
	CN122G8	Alfred Nzo	TEF = ON602002
	CN122G9	Alfred Nzo	TEF = OP198615
	CN122H1	Alfred Nzo	TEF = ON602003
	CN122H2	Alfred Nzo	TEF = ON602004
	CN122H3	Alfred Nzo	TEF = ON602005
	CN122H4	Alfred Nzo	TEF = ON602006
	CN122H5	Alfred Nzo	TEF = ON602007
	CN124I3	Joe Gqabi	TEF = ON602008
	CN124I5	OR Tambo	TEF = ON602009
	CN124I6	Alfred Nzo	TEF = ON602010
	CN124I7	Alfred Nzo	TEF = OP198616
	CN124I8	Alfred Nzo	TEF = ON602011
	CN130A7	Alfred Nzo	TEF = ON602012

Species	Strain	District	Sequences
<i>F. temperatum</i> (FFSC)	CN130A8	Alfred Nzo	<i>TEF</i> = ON602013
	CN130C2	OR Tambo	<i>TEF</i> = Yes
	CN135H3	Joe Gqabi	<i>TEF</i> = OP198617
<i>Mucor irregularis</i>	CN122D1_CMW58118	Alfred Nzo	ITS = ON209714
<i>Stenocarpella macrospora</i>	CN122C8_CMW58150	OR Tambo	ITS = ON209712, <i>TEF</i> = OP198618
<i>S. maydis</i>	CN122B7_CMW58151	Chris Hani	ITS = ON209702, <i>TEF</i> = OP198619, LSU = OP219667
	CN122B8	Chris Hani	ITS = ON209703, <i>TEF</i> = OP198620, LSU = OP219668
	CN122B9	Chris Hani	ITS = ON209704, <i>TEF</i> = OP198621
	CN122C1	Chris Hani	ITS = ON209705, <i>TEF</i> = OP198622
	CN122C2	Chris Hani	ITS = ON209706, <i>TEF</i> = OP198623
	CN122C4	Chris Hani	ITS = ON209708, <i>TEF</i> = OP198624, LSU = OP219669
	CN122C5	Chris Hani	ITS = ON209709, <i>TEF</i> = OP198625, LSU = OP219670
	CN122C6	Chris Hani	ITS = ON209710, <i>TEF</i> = OP198626, LSU = OP219671
	CN122C7	Chris Hani	ITS = ON209711, <i>TEF</i> = OP198627
	CN122C9	OR Tambo	ITS = ON209713, <i>TEF</i> = OP198628
	CN122D2_CMW58152	Alfred Nzo	ITS = ON209715, <i>TEF</i> = OP198629, LSU = OP219672
	CN122D3	Alfred Nzo	ITS = ON209716, <i>TEF</i> = OP198630
	CN122D4	Alfred Nzo	ITS = ON209717, <i>TEF</i> = OP198631, LSU = OP219673
	CN124H3	Chris Hani	ITS = ON209719, <i>TEF</i> = OP198632
	CN124H4	Chris Hani	ITS = ON209720, <i>TEF</i> = OP198633
	CN124H5	Chris Hani	ITS = ON209721, <i>TEF</i> = OP198634
	CN124H6	OR Tambo	ITS = ON209722, <i>TEF</i> = OP198635
<i>Trichoderma gamsii</i>	CN125A4_CMW58159	Alfred Nzo	<i>TEF</i> = ON602015

Figures



Figure 1: Ear rots collected across the Eastern Cape. (A–C) *Cladosporium* ear rot, (D–F) *Diplodia* ear rot, (G–I) *Fusarium* ear rot and (J–L) *Gibberella* ear rot.

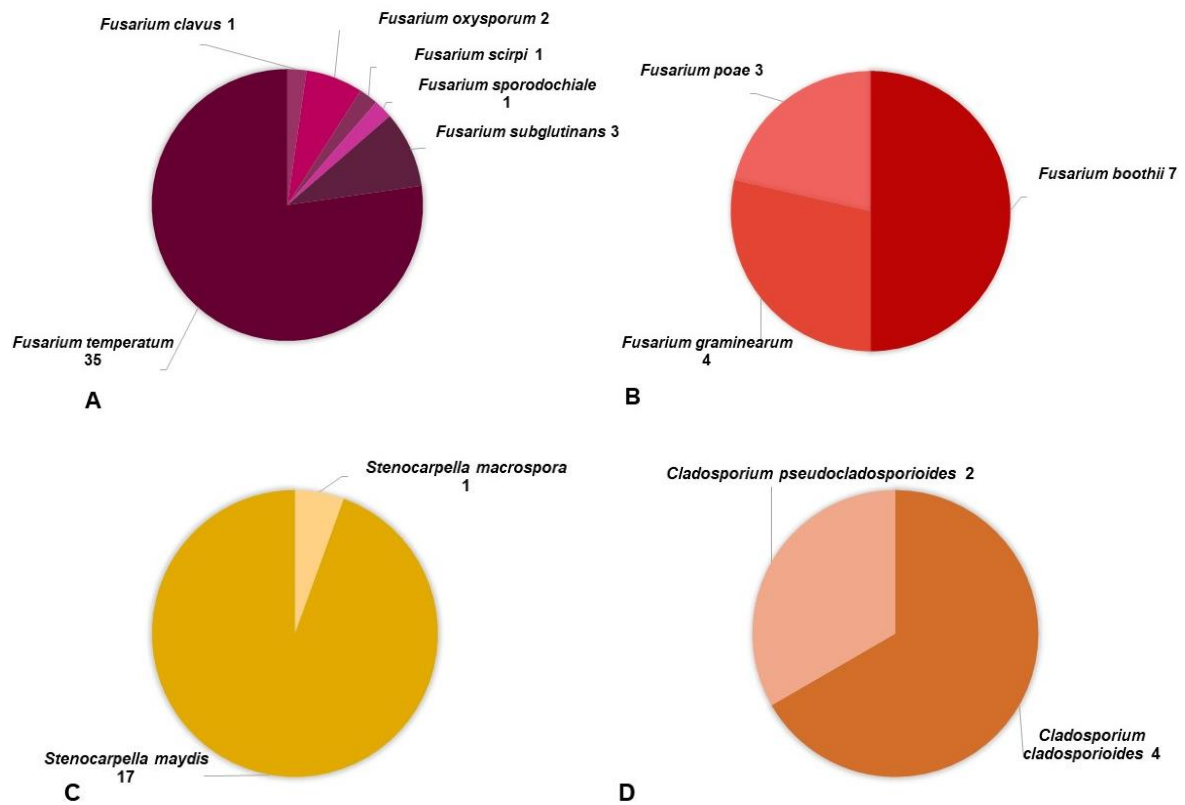


Figure 2: Species isolated from different ear rots in the Eastern Cape. (A) *Fusarium* species isolated from FER, (B) *Fusarium* species isolated from GER, (C) *Stenocarpella* species isolated from DER; and (D) *Cladosporium* species isolated from CER. Numbers represent the number of strains isolated.

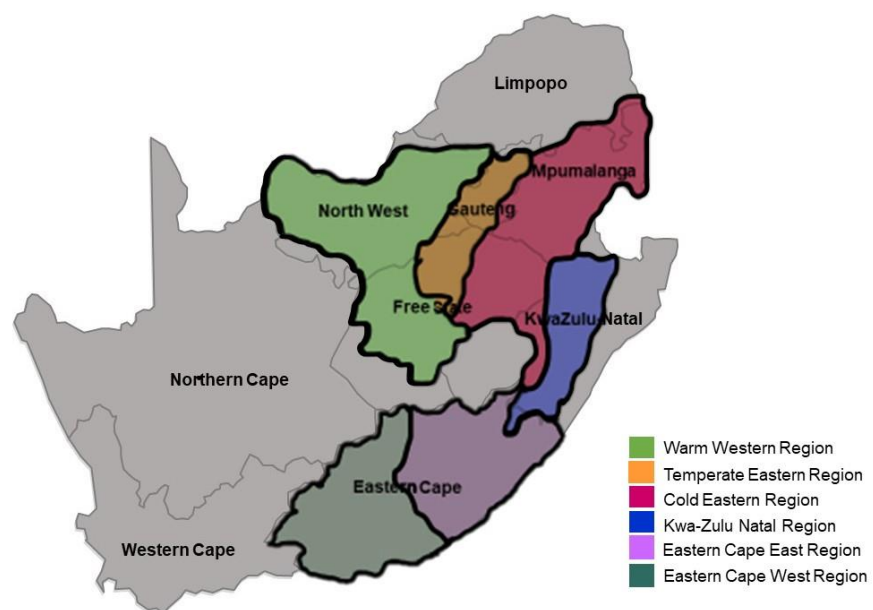


Figure 3: Different climatic regions of maize planting areas in South Africa (adapted from Sihlobo 2019).

Summary

Maize is a staple food crop cultivated globally. In South Africa, the Eastern Cape contributes less than 1% to the overall production. The farming community in the Eastern Cape is largely comprised of small scale and emerging maize farms. Maize production in the province has previously been associated with mycotoxigenic fungi and their toxic metabolites. The Eastern Cape has recently been identified as a region to be developed into a large maize producer. However, there is a gap in the knowledge on the fungi associated with maize in the province. This prompted the present study in which we explored the fungal diversity and mycotoxins associated with maize from emerging maize farms in the Eastern Cape.

The first chapter of this study provides a comprehensive literature review on the importance of maize in South Africa, and maize ear rots and the associated mycotoxins that threaten its production. Management strategies are also discussed.

The second chapter examined the fungal diversity associated with post-harvest maize collected from the Eastern Cape. This study resulted in the isolation of 481 fungal strains belonging to *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and 15 other genera. Communities were found to be relatively diverse with strains identified to 84 species. Amongst these were several known mycotoxin producers. The most common species identified were *Fusarium boothii*, *F. poae*, *F. temperatum*, and *Cladosporium cladosporioides*. This survey is the first reporting a diverse range of fungal species from Eastern Cape produced maize that employed DNA sequences to make identifications.

The third chapter of this study aimed to identify the fungal species and mycotoxins associated with maize ear rots collected from maize farms in the Eastern Cape. We observed *Cladosporium*, *Diplodia*, *Fusarium* and *Gibberella* ear rots. We noted that ear rots are caused by a more diverse range of species than we previously thought. The co-occurrence of mycotoxins associated with these species was also recorded. This is the first study conducted in the Eastern Cape to consider all of these aspects together.

This study creates an important baseline knowledge for future surveys that will focus on the impact fungal communities may have on the production of high-quality maize in the region, the diseases they may cause, and the mycotoxins they may produce. Future studies should focus on monitoring how the fungal communities, ear rots and subsequently mycotoxin distribution are changing between the planting seasons. Additional studies should be conducted examining the variation in the ear rots, fungi and mycotoxins present between the different growing districts within the EC as each of these experience different climatic conditions. This data is especially important as we experience fluctuations in climate conditions. Seasonal data will serve as an early warning system for new and / or emerging pathogens in the province, allowing for sufficient time for intervention and prevention. In addition, strains collected during this study will be used as reference material for future studies that will examine the biotic and abiotic triggers of mycotoxin production. The above-mentioned approaches enable us to gain a better understanding of how the fungal communities are changing, as well as how these fungi regulate mycotoxin production. As a result, prevention and management strategies that are unique to the South African climate can be developed, thus contributing to ensuring food security.