The fungal and oomycete diversity associated with commercial maize farm soils of South Africa

by

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Submitted in partial fulfilment of the requirements for the degree

Master of Science
(Magister Scientiae)

In the Faculty of Natural & Agricultural Sciences
Department of Biochemistry, Genetics, and Microbiology

University of Pretoria, Pretoria, South Africa

November 2022

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Declaration of originality

I, Nombulelo Qikani declare that the thesis/dissertation, which I hereby submit for the degree Master of Science 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: .....

DATE: November 2022

Acknowledgements

I would like to thank my incredible supervisors Prof Cobus Visagie, Dr Neriman Yilmaz and Prof Emma Steenkamp for guiding me through this journey. Thank you for your advice, support, and patience.

I would also like thank the National Research Foundation (NRF), the Tree Protection Cooperative Programme (TPCP), GrainSA, and the Grain Research programme funding my work.

Special thanks to Dr David Nsibo for assisting me with the laboratory work and the mycology group for the laughter and words of encouragement.

I owe a profound gratitude to Mr. Athenkosi Makebe for his constant encouragement and support.

Finally, thanks to my parents, my siblings and my friends who have supported me throughout the years.

Preface

Microbial diversity plays a crucial role in soil and plant health. Studies have suggested agricultural soils with higher diversity are generally healthier compared to soils with less diversity. Maize is one of South Africa's most important crops, however, little is known about the fungal and oomycete communities residing in maize rhizosphere soils. The work presented in this dissertation aims to provide this baseline knowledge.

Chapter one provides a literature review on soilborne pathogens that are associated with maize plants and the estimated impacts that climate change can have on these pathogens in the future. It also summarizes information about species identification using traditional and molecular methods. Particular attention is given to the gene regions that are currently being used for molecular species identification in order to follow trends in fungal taxonomy. The chapter summarizes the pros and cons of using the ITS region for fungal species identification and compares it to secondary identification markers that have recently gained popularity.

Chapter two explores fungal diversity in maize rhizosphere soils that were collected from the Free State and North West provinces of South Africa. A culture-dependent approach was used to isolate fungi from soil samples using various growth media. Cultures that were obtained were identified to genus level based on morphology and accessioned into the CN working collection housed at FABI prior to extracting their DNA. Different regions were amplified and sequenced depending on the genus that a strain belonged to. Identifications were made by comparing the obtained sequences to reference data previously deposited in the RefSeq Target Loci and in GenBank databases of the National Centre for Biotechnology Information (NCBI) using “Nucleotide BLAST” search and to the locally curated databases available for *Fusarium*, *Penicillium*, and *Trichoderma*.

Chapter three investigates the oomycete diversity on the same rhizosphere soils. Oomycetes were baited by floating healthy *Rosa alba* petals and *Hedera canariensis* leaves in a soil solution. Baits that developed lesions were cultured onto two different media and the outgrowing hyphal tips were sub-cultured on carrot agar. The cultures were accessioned into internal working collection of the Applied Mycology Group for Oomycetes (CN-Oom) collection prior to the DNA extractions. The ITS region of the

strains was amplified and sequenced. Identifications were made by comparing the obtained sequences to reference data previously deposited in the RefSeq Target Loci and GenBank databases of the National Centre for Biotechnology Information (NCBI) using “Nucleotide BLAST” search.

The information in this dissertation created baseline knowledge in our quest of understanding the fungi and oomycetes that are associated with maize soil. The sequence data that was generated will be made available in GenBank and will serve as reference data for future identifications. The strains were deposited into the CN working culture collection housed at FABI and will be available for future studies.

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Chapter 1: Soil-borne Fungal and Oomycete diseases associated with maize

Abstract

Maize is widely cultivated and a staple crop in many African countries including South Africa. It is also a susceptible host to a wide range of phytopathogens classified as fungi and oomycetes. *Aspergillus*, *Fusarium* and *Penicillium* are generally the most important fungal genera as they are considered major producers of mycotoxins along with other less common genera. Species from these genera can cause devastating diseases and are producers of FUMs, DON, Aflatoxins, ZEN, Ochratoxins and other mycotoxins. These mycotoxins are poisonous to humans and animals.

Traditional identifications were based on morphological characters. However, fungal identification based on morphology is difficult, time-consuming and requires many years of experience. Because of this, literature often contains misidentifications and since a name links the user to a wealth of information about the species, this can have serious implications from a disease diagnostic standpoint. DNA barcoding has become the standard approach for fungal identifications as it is less time-consuming, provides more robust precise identifications and does not require as much experience as the morphological route. However, this is only true if DNA reference sequences are available for comparisons. Nevertheless, DNA based methods will give an insight on what pathogens are truly associated with crops, in this case, maize. This knowledge will be an important baseline for communities to know what species are present and thus set priorities for research looking at disease management.

Introduction

Maize (*Zea mays* L.) is the third most important crop in the world (Mwatuni *et al.*, 2020), after wheat and rice. The world's maize production was 1,148 million tonnes in 2019 (Knoema, 2020). Maize is a staple food in Sub-Saharan Africa, and is grown mostly for consumption and animal feed (Udomkun *et al.*, 2017). Approximately 65% of maize produced around the world is estimated to be used in animal feed, 15% for human consumption, and 20% is processed (Abbassian, 2006).

In South Africa, maize is one of the most produced cereal crops. There are two types of maize grown in South Africa, white maize which is mostly grown for human consumption and yellow maize for animal feed. In 2019/2020, South African maize production was 15 408,180 tons covering an area of 2 610,8 hectares (GrainSA, 2020). A production of 8 666.310 tons and 6 741. 870 tons of white and yellow maize respectively were recorded (GrainSA, 2020). Major maize-producing provinces in South Africa include the Free State, North West, and Mpumalanga (Figure 1).

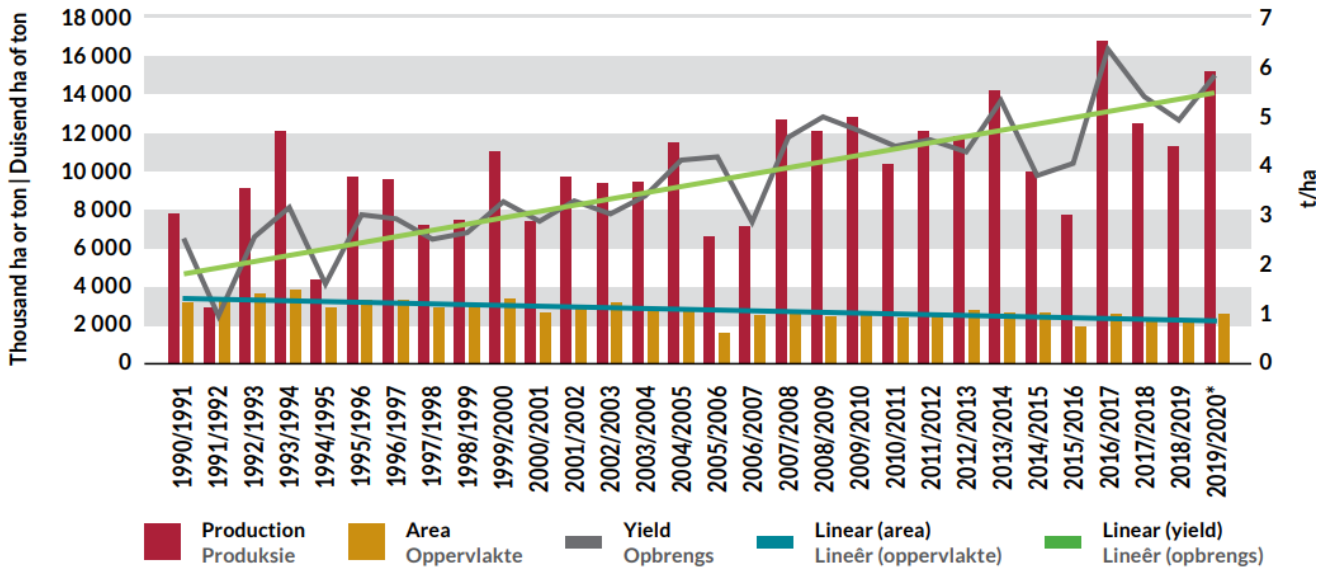

Figure 1: Maize total area, production, and yield from 1990/1991 to 2019/2020 (GrainSA, 2020).

Sustainable Development Goal 2 (SDG 2- Zero Hunger) was set to seek ways to end hunger and malnutrition by 2030. However, the number of hungry people around the world is increasing. With the increase in the world's human population, there is a need for increased food production to achieve food security. Currently, more than 820

million people around the world are estimated to regularly go to bed hungry and about 135 million are malnourished (Mishra & Rampal, 2020). It is estimated that the world's population by 2050 will reach 9 billion. To sustain the population growth, agricultural production will need to increase by 70 - 100% (Reid & Greene, 2012).

Fungi can invade host plants in any part if there are suitable entry points. For example, in grains, fungi can invade the stem and produce enough mycelia in the phloem and xylem and that can reduce or inhibit the movement of water and nutrients to other parts of the plant (Jouany, 2007). Some fungal species produce mycotoxins which are harmful to humans and livestock (Kostic *et al.*, 2019). These organisms thus represent great risk to our current and future food security.

The concept of 'disease triangle' linked with climate change predictions

The concept of "disease triangle" from plant pathology states that there should be an interaction between three components, a susceptible host plant, virulent pathogen, and conducive environment for a successful infection or for a disease to occur. The interaction of these three components can be represented in a "disease triangle'' (Figure 2) with the sides representing each component. A change in one of the components affects the disease severity on the pathosystem and if any of the components is absent, there will be no disease (Francl, 2001). If the environmental conditions are conducive to a virulent pathogen, susceptible host plants can be infected and that can lead to severe disease development. This has enabled the development of management strategies such as cultural, chemical, and resistant host plant cultivars to prevent, eliminate or control diseases.

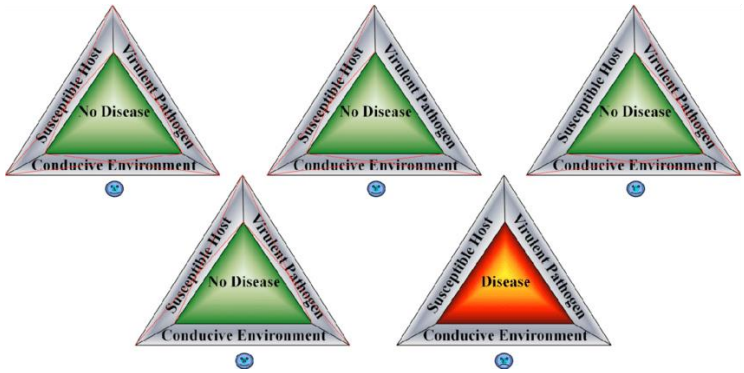

Figure 2: The disease triangle represents three components (susceptible host, virulent pathogen, and conducive environment) required for a disease to occur (Munir, 2018).

Climate change is predicted to negatively impact the agricultural industry, including a potential impact in emerging plant disease epidemics. This is also true for maize, where the industry faces challenges regarding yield loses, with estimated global losses ranging between 20 and 40% coming from pathogens (Deshapande *et al.*, 2019). Small-scale farmers in Sub-Saharan Africa mostly depend on rain for their agricultural productivity (Rosegrant *et al.*, 2002), with approximately 90% of cereal production from rainfed agriculture. It has been predicted that global temperatures will increase in the future and this will result in changes in water availability (Pachauri *et al.*, 2014). Lack of water induces drought stress in plants. Temperatures in Southern Africa are increasing quicker compared to the world's average (Collier *et al.*, 2008). The virulence of many fungal plant pathogens also increase under warm and humid temperatures (Clarkson *et al.*, 2014). Farmers, especially small-scale farmers might face yield losses due to drought stress and increased species pathogenicity. For example, in 2013, there was a reported outbreak of charcoal stalk rot in the Free State province of South Africa (Coleman, 2013) and the disease incidence was increasing in warmer regions such as in the Mpumalanga, Free State and North West (Jordaan & van der Waals, 2016).

Another consequence of climate change is an increase in atmospheric carbon dioxide (CO_2) concentrations, which has already been recorded (Velásquez *et al.*, 2018) and is estimated to reach 550 ppm by 2050. South Africa was ranked as a 14th largest emitter of CO_2 (Mcsweeney, 2018). Elevated CO_2 levels tend to increase photosynthesis, leaf area, shoot, and roots of the plants. Increased levels of CO_2 may favour a crop growth, however, increased leaf area and wide stomata radius of the plant might be advantageous to a pathogen as will have more space for infections. Long roots might also reach the buried (through tillage) pathogen's inoculum. Fungal pathogens like *Fusarium graminearum* whose strain virulence can be increased by elevated CO_2 levels (Váry *et al.*, 2015) could result in severe disease development when they encounter susceptible hosts.

A small change in one of three components responsible for a disease development can cause substantial changes in a pathosystem dynamics. An avirulent pathogen now might be virulent in the future when environmental conditions become conducive. If a pathogen can evolve to overcome host resistance genes, it might also evolve in

response to climate change. With incomplete knowledge on how climate change will affect the performance of plant pathogens, molecular identifications of pathogenic fungi and oomycetes in agricultural soils are crucial as they can serve as reference data in future studies.

Diseases caused by soil-borne fungal and oomycetes pathogens on maize

When there is a susceptible host and conditions are favorable, several fungal and oomycete plant pathogens can cause deterioration in the quality and quantity of the produce. Soil-borne pathogens such as *Fusarium* species, *Pythium* spp., *Phytophthora* spp., *Rhizoctonia* spp., *Sclerotinia* spp., and *Verticillium* spp. are estimated to cause 50-75% of production loss in many crops (Panth *et al.*, 2020). Some fungal pathogens also produce mycotoxins, which are toxic secondary metabolites produced by certain fungi and are harmful when ingested by humans or livestock (Richard, 2007). In Sub-Saharan Africa, mycotoxin contamination was severely at risk with all samples contaminated with at least one *Fusarium* mycotoxin (Biomin, 2021). Deoxynivalenol (DON) was the most abundant mycotoxin in the South African maize sample followed by fumonisins (FUM) and zearalenone (ZEN) (Biomin, 2021). However, there are no reports linking diseases caused by soil-borne fungi with mycotoxin contaminations.

Diseases such as stalk rots, root, and crown rots are generally associated with a complex of soil-borne pathogens. Root and stalk rot disease commonly occurs at the same time. Whitney & Mortimore (1961) found maize root rots do not always coincide with stalk rots, however, stalk rots always occur with root rots.

Oomycetes (also known as 'water moulds') are a group of filamentous microorganisms with some being one the most global food security threat as the can cause devastating diseases in various crops. They were once classified as fungi due to their filamentous growth and feeding from decaying organic matter (Kamoun, 2003). However, it was later observed that oomycetes are different from fungi because their cell wall is made up of cellulose compounds and glycan (Thines, 2014). They have two flagella in their zoospores instead of one as is the case in some basal fungi (Strange & Scott, 2005).

Oomycetes are more phylogenetically related to diatoms and brown algae in the kingdom *Straminopila* (Thines & Kamoun, 2010), and unrelated to filamentous fungi. The group that currently have the largest number of oomycetes species are the plant pathogens that fall under the white blister rusts (*Albuginaceae*) and the downy mildews (*Peronosporaceae* p.p.) with more that 60% of species parasitic to the plants (Thines & Kamoun, 2010).

Soil-borne pathogens of maize are listed in Table 1 with their associated diseases. Charcoal, Diplodia, Gibberella and Fusarium rot have been reported as the most common fungal stalk rots in South Africa (Flett & van Rensburg, 2021). *Pythium* species are also linked to South African maize root rots (Lamprecht, 2013, Schoeman & Craven, 2016). Thus, this review will focus on them. The review will also cover Anthracnose stalk rot as it has been reported as one of the most common soilborne diseases of maize (Ma *et al.*, 2022).

Table 1: A list of soil-borne fungal and oomycetes pathogens associated with maize diseases

Pathogen	**Disease**	**References**
Cephalosporium acremonium	Black bundle disease	(Veerabhadraswamy & Garampalli, 2011)
Colletotrichum graminicola (Ces.) G.W. Wils.	Leaf blight and seedling blight Anthracnose stalk rot	(Bergstrom & Nicholson, 1999)
Exserohilum pedicellatum (Henry) K.J. Leonard & E.G. Suggs	Root rot, seed rot, seedling blight, ear-rot	(Isakeit *et al.*, 2007) (Chambers, 1987)
Fusarium chlamydosporum (Wollenw. & Reiking)	Root, crown, and stalk rot	(Sangalang *et al.*, 1995)
Fusarium equiseti (Corda) Sacc	Minor root rots, crown, and stalk rot	(Smit & Rijkenberg, 1997).
Fusarium graminearum (Schwabe)	Gibberella ear and stalk rot, seed rot and seedling blight	(Leyva-Madrigal *et al.*, 2017)

Fusarium oxysporum (Schlectend)	Minor root rots, wilts, and minor stalk rots	(Butt *et al.*, 2019)
Fusarium vercitilliodes	Fusarium ear and stalk rot	(Gai *et al.*, 2018)
Macrophomina phaseolina (Tassi) Goid	Charcoal rot, seed rot, seedling blight, root and stem rot	(Pal *et al.*, 2001)
Pythium arrhenomanes Drechs	Pythium root rot	(Deep & Lipps, 1996)
Pythium graminicola Subramanian	Seedling blight	(Kageyama *et al.*, 2005)
Pythium aphanidermatum (Edson) Fitzp.; *Pythium butleri* Subramanian	Pythium stalk rot	(Elliott, 1943) (Sadik *et al.*, 1982)
Phoma terrestris, Pythium irregulare	Red root rot	(Deb *et al.*, 2020) (Mao *et al.*, 1998)
Rhizoctonia solani (Kühn)	Leaf and sheath spot, stalk rot, seed rot (failure to germinate)	(Sumner & Bell, 1982)
Stenocarpella maydis (Berk.) Sutton,	Diplodia (Stenocarpella) Stalk Rot	(Lamprecht *et al.*, 2013)
Trichoderma species	Trichoderma ear rot	(Pfordt *et al.*, 2020)

Fusarium stalk rot

Fusarium stalk rot of maize is caused by several *Fusarium* species that belong to the *Fusarium fujikuroi* species complex (FFSC) including *Fusarium verticillioides* (Sacc.) Nirenberg, *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, and *F. subglutinans* (Wollenw. & Reinking) P.E. Nelson, Toussoun & Marasa, and *F. temperatum*. *Fusarium verticilliodes* was earlier known as a predominant stalk rot pathogen in Nigeria (Bankole *et al.*, 2003), and is the most important and predominant pathogen responsible for Fusarium stalk rot. The pathogen is also responsible for causing Fusarium ear rot of maize and production of toxins. Fusarium stalk rot of maize usually occurs just before crops reach physiological maturity. The plants either die

prematurely and produce poorly filled kernels or lodge due to the rotting stalk, thereby, making harvesting hard.

Symptoms. Maize plants affected by Fusarium stalk rot display a pinkish-white pith (Figure 3). Fusarium stalk rot symptoms usually manifest in warm and dry regions. The disease symptoms become clearer during the plant's senescence stage and more severe during grain filling. Other symptoms include premature death and lodging.

Figure 3: Shredded pith with white-pinkish colour caused by *F. verticillioides* (Robertson, 2011).

Disease cycle. *Fusarium verticillioides* overwinters as thick mycelia or conidia on soil and plant debris. Under favourable conditions, the mycelia infect the roots and stalk of the crop plant. The pathogen can enter the plant naturally through wounds caused by insects or mechanical damages and direct penetration (Yu *et al.*, 2017). The European corn borer and western corn rootworm have been reported to create wounds as they feed on maize stalk, thus, creating entries for the pathogen (Gilbertson *et al.*, Sobek & Munkvold, 1999). Unlike *F. graminearum*, *F. verticillioides* can also be transmitted systemically through seeds (Munkvold & Carlton, 1997).

Management strategies. Management of *Fusarium* spp. is difficult as a complex of species can influence the plant at different growth stages and under different environmental conditions. Removal of plant debris, crop rotation, and planting of disease-free seeds reduces the disease incidence and disease severity.

Gibberella stalk rot

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch) is a pathogen of crops that can cause ear and stalk rot in maize. It can lead to reduced yield due to plant lodging and crop premature death. In South Africa, the disease is mostly common in irrigated maize fields (Flett & van Rensburg, 2018). Plant lodging can cause up to 80% of yield reduction, along with poor grain quality (Tams *et al.*, 2004). However, it is difficult to estimate yield losses caused by this disease as it commonly co-occurs with other physiological factors such as water stress.

Symptoms. Gibberella stalk rot and Fusarium stalk rot symptoms are almost similar. They are both associated with the premature death of maize plants as they interfere with nutrient and water uptake from the roots to above-ground maize parts. The pink/red discolouration of the maize pith is a major distinctive symptom of Gibberella stalk rot (Figure 4). Similar symptoms include wilting of the leaves, and disintegration of the internal pith, and root rots. Gibberella stalk rot usually occurs in cool, moist regions (Shin *et al.*, 2014).

Figure 4: Pink pith caused by *F. graminearum* (Robertson, 2011).

Disease cycle. *Fusarium* species produces spores that are dispersed by wind, splashing water, and insects. Fungi overwinter in soil, where they can infect maize crops directly through the roots. Plant residues and alternate host in the fields can increase soil contamination levels. They can also invade maize crops via wounds created by insects, direct penetration through root or stalk and wounds caused by

mechanical damage. The next crop becomes infected during pre-harvest by the conidia and ascospores from the contaminated soil. Ascospores plays an important role maize infection by *F. graminearum* as they are released by fungal perithecia from the organic matter on the soil. These spores are disseminated in short distances; thus, crop residues are important in the infection cycle of *F. graminearum* as they are the source of primary inoculum (Munkvold, 2003).

Management Strategies. Removal of plant debris might reduce fusarium infections as they are the major source primary inoculum. Crop rotation with quickly decomposing non-host crops of members of *F. graminearum* species complex might reduce the disease incidence. High yield is usually the most desirable trait on breeding programmes. Maize crops produce high yield due to large cobs. Large cobs could mean the crop transport large amounts of carbohydrates to the ears and less to the lower parts, making the stalk and roots liable to rots. Therefore, a balanced breeding for both resistance and yield could control the severity of the disease (Flett & van Rensburg, 2018). Reduction of plant stress can also reduce the disease incidence. Currently, there is no registered fungicides specific to Fusarium and Gibberella stalk rot, however application of other chemicals that reduces plant stress reduces the disease severity.

Diplodia stalk rot

Diplodia stalk rot is caused by *Stenocarpella maydis* (Berk.) Sutton, a fungus that is also responsible for Diplodia ear rot of maize. Disease development occurs when *St. maydis* infect maize plants through roots, crown or/and mesocotyl (White, 1999). This pathogen can also be transmitted to seeds (Sutton & Waterston, 1966). Annual yield losses caused by Diplodia stalk rot and lodging in South Africa range from 5% to 20% (Flett & van Rensburg, 2021).

Symptoms. A diseased plant commonly show symptoms shortly after silking (Sutton & Waterston, 1966). Diplodia stalk rot becomes intensive a few weeks after pollination (The Andersons, 2015). An affected plant becomes dry and develop dark brown or black spots (reproductive structures called pycnidia) in the rind of the lower stalk (Figure 5) (White, 1999). Like most stalk diseases, Diplodia stalk rot can weaken the

stalk, thus increasing its susceptibility to lodging caused by wind or other environmental stress (Steckel, 2003).

Figure 5: *Stenocarpella maydis* pycnidia embedded on a maize stalk (Grau & Robertson, 2019).

Disease cycle. The fungus overwinters as mycelia in maize stubbles buried in or on the soil. Under warm, moist conditions, spores are released by the fully developed pycnidia. The spores are spread by wind and rain throughout the field. Crown and roots are the main entry points and are where primary infections of maize mostly occur. Early infections have been reported in seed embryos and may later develop into crown and stalk rot infections (Bensch, 1995). Diplodia stem rot is likely to be more severe during drier conditions after wet early seasons (Flett & van Rensburg, 2021).

Management Strategies. Crop rotation with suitable alternative crops such as soybeans, groundnuts, wheat, and dry beans reduces *S. maydis* inoculum as maize stubbles acquire time to break down before the next plantation (Flett & van Rensburg, 2021). There are no registered fungicides specifically for the control of Diplodia stalk rot (Flett & van Rensburg, 2021).

Anthracnose stalk rot

Anthracnose stalk rot is a disease caused by the fungus *Colletotrichum graminicola* (Ces.) G.W. Wils. The disease is favoured by hot humid conditions and long periods of wet weather (Jirak-Peterson & Esker, 2011). A temperature range for successful infestation is 25 to 30 ℃. Monoculture, crop rotation with a different host plant and

zero tillage would facilitate the pathogen's infestation between growing seasons (Jirak-Peterson & Esker, 2011).

Symptoms. Infected plants senesce prematurely. Black shiny spots or streaks appear on the lower internodes of the stalk (Figure 6). When infestation is severe, the whole stalk becomes black. The plant becomes loose and thus easily falls or split when pushed or squeezed. The pith appears discoloured and rotten. Crop losses are often a result of lodging caused by systemic stalk infections, which are most likely originated from infected roots.

Figure 6: Black spots on maize stalk and discoloured pith caused by *C. graminicola* (Bergstrom & Nicholson, 1999).

Disease cycle. *Colletotrichum graminicola* overwinters in crop residues left in the field. Under warm rainy conditions, the fungus produces spores that can infect foliage or roots of maize seedlings. Conidial spores can be disseminated through raindrops, wind, and splashing (Jirak-Peterson & Esker, 2011). Primary infections are caused by rain-splashed fungal spores. When infection occurs below the soil surface, the fungus colonizes the roots and pith, and it is difficult to observe the disease at the early stages of development.

Management Strategies. Crop rotation is the most effective control method for *C. graminicola*. A study in 2009 showed that maize plants grown in fields that were used for maize last season show severe symptoms compared to fields that were used to grow soybeans (Jirak-Peterson & Esker, 2011). Other cultural methods such as deep

ploughing and also use of resistant hybrids to disrupt the pathogen's primary inoculum may be practiced (Jirak-Peterson & Esker, 2011).

Charcoal rot

Charcoal stalk rot is caused by *Macrophomina phaseolina* (Tassi) Goid, a fungal plant pathogen responsible for damping-off, charcoal rot, seedling blight, stalk rot, root rot, etc. in several different plant species (Babu *et al.*, 2007). *Macrophomina phaseolina* has a wide host range and can infect hundreds of plant species from different families. Its hosts include wheat, maize, chickpea, tomato, soybean, sunflower, sorghum, and more (ur Rehman *et al.*, 2021). The fungus is favoured by hot dry conditions.

Charcoal rot in maize usually occurs when the plant undergoes severe drought stress. Up to 70% yield losses due to Charcoal rot in Africa have been recorded and the Free State and North West provinces of South Africa had high incidences of this disease in the season 2008/2009 (van Rensburg & Flett, 2012). In 2013, Free State farmers lost 30 % yield due to the disease (van Rensburg & Flett, 2012).

Symptoms. The roots of the infected maize plant develops brown, watery soaked lesions that later turn black. The fungus spread to the lower internodes of the stalk where it causes premature ripening and later shredded pith. The affected plant develops black sclerotinia in the vascular tissues of the stalk (Figure 7).

Figure 7: *Macrophomina phaseolina* black sclerotinia in the vascular tissues of maize stalk (Munkvold, 2021).

Disease cycle. *Macrophomina phaseolina* overwinters in soil or/and maize residues as black structures called sclerotia. Sclerotia are dormant spores that can survive harsh environmental conditions for a long period of time. During dry hot periods, the sclerotinia germinate and penetrate maize roots. The fungus moves into the vascular tissues and start colonisation, thus disrupting the transportation of water and nutrients to the upper parts of the crop.

Management Strategies. Fungicides that include carbendazim, fluquinconazole, iprodione, metalaxyl, pyraclostrobin, tolyfluanid, penflufen + trifloxystrobin, and thiram can be used to stop the mycelial development of *M. phaseolina* (Tonin *et al.*, 2013). Soil solarization and crop rotation manages the pathogen effectively, also soil solarization has been proved to be as efficient as fungicides (Rehman *et al.*, 2021).

Trichoderma ear rot

Trichoderma species are among the most frequently encountered fungi in different plant roots and soil ecosystems. Most species are antagonists to phytopathogenic fungi, and as a result their strains are used to make commercial products that can be utilized as biocontrols of several fungal diseases (Vinale *et al.*, 2008). *Trichoderma* strains that are commonly used as biological control agents are those of *T. asperellum*, *T. atroviride*, *T. hamatum,* and *T. harzianum* (Sharma *et al.*, 2011). *Trichoderma* products that are commonly used in South Africa include Bio-Tricho, Eco-T, Eco-77, Tricho Plus, Romulus, and they mostly contain different *Trichoderma harzianum* strains as active agents (Woo *et al.*, 2014). Mechanisms deployed by *Trichoderma* against phytopathogenic fungi include competition for nutrients or/and niche, direct parasitism, antibiosis, and even induce systematic resistance in plant hosts (Benítez *et al.*, 2004).

A few *Trichoderma* species including *T. aggressivum* and *T. viride* are causal agents of green mould on button mushrooms (Samuels *et al.*, 2002) and dieback disease on *Pinus nigra* seedlings (Li Destri Nicosia *et al.*, 2015). Recent studies have reported a new maize ear rot disease caused by *T. afroharzianum* (Pfordt *et al.*, 2020, Sanna *et al.*, 2022). However, there is still a limited information about this disease.

Maize stalk rots caused by *Pythium* species

Most *Pythium* species are soilborne pathogens that are responsible for pre- or post-emergence damping-off, seedling blight and root rots (Parveen & Sharma, 2015). Some plants in favourable condition can continue growing despite the root infections (Dodd & White, 1999). However, the infections may lead to reduced and stunted plant roots, thus resulting reduced yield quality and quantity. *Pythium* infestations of maize seeds and seedling commonly occur in the early season when soil is moist and cool (Rao *et al.*, 1978).

Symptoms. A severely infected maize plant has discoloured root system with visible lesions (Figure 8A) (Farr *et al.*, 1989). Maize rind and pith develop soft, brown, and water-soaked lesions (Figure 8B). The root cortex appears discoloured and rotten (Farr *et al.*, 1989, Martin & Loper, 1999). An infected maize plant with no obvious necrotic symptoms yields low quality produce and in this case diagnosis can be done by observing oospores present in the tissues (Martin & Loper, 1999). Infestation of a seed or seedling prior to emergence from the soil results in seedling blight (Farr *et al.*, 1989) and stunted growth (Figure 8C) (Martin & Loper, 1999).

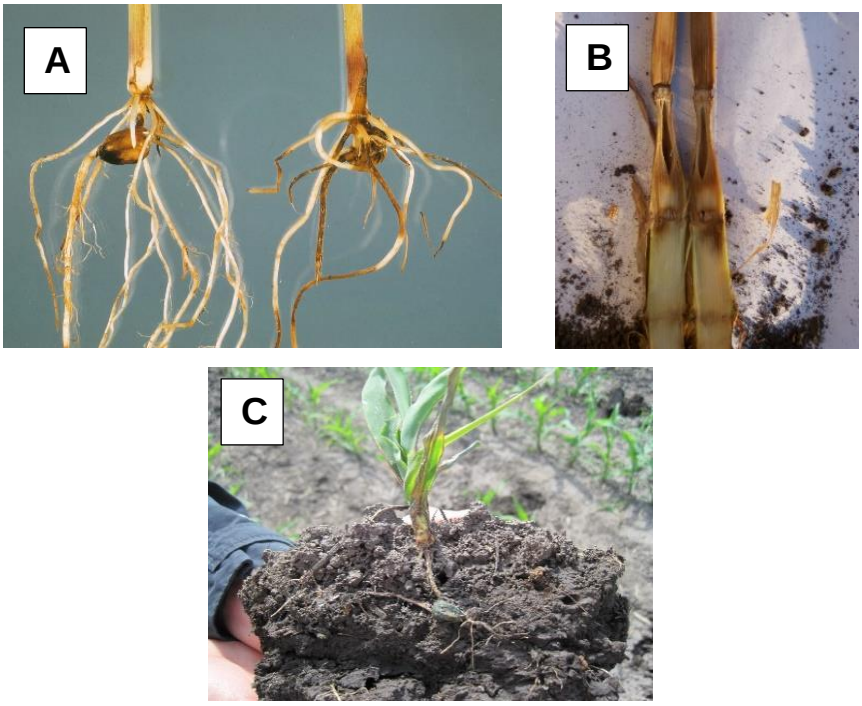

Figure 8: *Pythium* symptoms on maize. A- Lesions on the roots. B- Soft, brown, and water-soaked maize pith rind and pith. C- Chlorosis and stunted growth (Grau & Robertson, 2019).

Disease cycle. *Pythium* species overwinters as oospores in plant debris and in soil. Under favourable conditions, the oospores germinate and produce mycelium or sporangia. The mycelia and zoospores produced in sporangia infect seedling roots, mostly after rain as they become more active in high soil moisture (Hendrix & Campbell, 1983).

Management Strategies. *Pythium* infections are favoured by moist and cool conditions, therefore soil drainage or planting when soil temperatures are high might reduce disease incidence. Soil and seed treatment using metalaxyl and other systemic fungicides controls infections, however, the treatment becomes less effective as the plant continues to grow (Agrios, 2005). Crop rotation has been shown to be less effective in managing *Pythium* spp.

Morphological identification of fungi

In the past mycologists identified fungi using morphological characters, such as colony diameter, texture, colour of spores, mycelia, and conidiophore structures (Visagie *et al.*, 2014). They collected macroscopic reproductive structures visible on or used pure cultures of the isolates obtained directly from the soil to examine soil fungal communities (Rossman, 1998). However, collection of fruiting bodies is limited because some species fruiting bodies are present for a short time (Straatsma *et al.*, 2001). Furthermore, other species that are found underground can be poorly or not represented by fruiting bodies above-ground (Gardes & Bruns, 1996). Several books and monographs used morphology alone to divide families, genera, and species (Hyde *et al.*, 2010).

Morphological identification plays a vital role in fungal identification, especially when a new species is believed to be discovered, however, it requires a well-trained and experienced mycologist as it can be challenging and sometimes misleading. For example, identification of ascomata for sexual states based only on morphology can be difficult as they are not often produced in culture (Raja *et al.*, 2017). Identifying fungi based on morphological characteristics might fail when working with cryptic species (Damm *et al.*, 2010). Molecular identification has provided an alternative way for fungal identification, thus improving fungal diversity research (Tanabe & Toju, 2013).

DNA barcoding

Use of molecular data for fungal identifications started in the 1990s and have become a strong tool for mycologists as it has improved fungal identifications. DNA barcoding was first proposed by Paul Hebert in 2003. The concept was to generate a unique barcode for a species based on a short DNA fragment. DNA barcoding involves identification of species using information of one or more gene regions. It involves the process of generating PCR amplicons from a particular region and sequencing them. The sequences are compared to reference sequences in databases that have been curated by nomenclatural experts.

DNA regions that are can be used for fungal identifications include large subunit (28S), small subunit (18S) and Internal transcribed spacer (ITS) region (ITS1,5.8S, ITS2) of the ribosomal RNA cistron (Stockinger *et al.*, 2010). However, observations showed these regions evolve at different rates thus resulting in different degrees of genetic variation (Slowest evolution exhibit less variation) (White *et al.*, 1990). With this information, one could know exactly which region to sequence depending to the study to be conducted. Briefly, if a researcher is interested in identifying fungi at higher taxonomic level, 18S can be amplified and sequenced as it evolves the slowest (White *et al.*, 1990). For fungal identification at intermediate taxonomic level, one can sequence 28S. ITS evolves the fastest and can be used to identify fungi to a species level. ITS region was chosen as an official barcode for fungi amongst six other gene regions as its probability of successfully identifying a wider range of fungi is high (Bruns *et al.*, 1991). However, the ITS region sometimes fails to precisely identify fungi in some genera such as *Fusarium*, *Penicillium*, *Aspergillus*, *Trichoderma*, and *Cladosporium* as it is not consistent in distinguishing all closely related species (Rico-Munoz *et al.*, 2019).

The inconsistency of ITS region in identifying the isolates of the above stated genera to species level has led to use of secondary identification markers (protein-coding genes) instead. These secondary markers have intron regions that often evolve faster. A selected secondary identification marker should be easy to amplify, be able to differentiate among closely related species and have a complete reference data (Visagie *et al.*, 2014). Secondary identification markers such as the genes encoding beta-tubulin (*tub2*/*BenA*), calmodulin (*CaM*), the largest (*RPB1*) and second largest

(*RPB2*) subunits of RNA polymerase, and translation elongation factor 1-alpha (*tef1*) have been commonly used for barcoding and phylogenetic identifications (Table 2). A secondary barcode marker that is recommended for *Penicillium* species is *BenA* (Visagie *et al.*, 2014). For *Aspergillus*, *CaM* is recommended as barcode maker (Samson *et al.*, 2014). *Tef1* is a recommended barcode marker for *Fusarium* (O'Donnell *et al.*, 2015), *Cladosporium* (Bensch *et al.*, 2015), and *Trichoderma*. *RPB2* region can also be used for *Trichoderma* identifications using phylogenetic approaches.

Available databases for fungal Identification

The unknown sequences that are obtained from DNA barcoding are compared against sequence databases (Table 3) for identification. A long list of databases that can be used for identification is provided by Robert *et al.* (2015) and Singh & Gupta (2017). GenBank BLAST search on National Centre for Biotechnology Information (NCBI) is the most popular approach that is used to identify unknown strains. The user "BLASTs" an unknown ITS sequence on the GenBank and identify strain based on similarity between an unknown strain and reference strain.

However, the NCBI contains many misidentified sequences (Federhen, 2015) where some sequences are unnamed or partially named (Robert *et al.*, 2015). About 27% of ITS sequences on GenBank were deposited with insufficient taxonomic identification (Nilsson *et al.*, 2006) and several described fungal species have not been sequenced (Rossman & Palm-Hernández, 2008). Other sequences deposited in GenBank are unpublished (Rossman & Palm-Hernández, 2008). Sequences on RefSeq Targeted Loci (RTL) database were reannotated and verified as an attempt to resolve the above-mentioned issues. However, RefSeq only covers the ITS region and does not include secondary identification markers (Visagie *et al.*, 2014).

UNITE is another popular database that identifies unknown ITS sequences based on similarity hypotheses and has been curated (Nilsson *et al.*, 2015). FUSARIUM-ID is a curated database used for identification of *Fusarium* species using gene regions listed on Table 4.2. ICTT International Commission on Trichoderma Taxonomy (ICTT) TrichoKey and TrichoBLAST database can be used to identify *Trichoderma* species. Fungal MLST database Q-Bank is another curated database that can be used to

identify fungi belonging in different genera (Bonants *et al.*, 2013). Other researchers use local databases for fungal identifications that are sometimes private.

Conclusions

The purpose of this review has been to provide a summary on morphological and modern fungal identification approaches, and the most common soilborne pathogens of maize in South Africa. About 148,000 fungal species worldwide have been described (Cheek *et al.*, 2020) with over 8 000 species confirmed as plant pathogens (Jermy, 2017). Approximately 171 500 fungal species are estimated to be found in South Africa (Crous *et al.*, 2006). Most fungal species have been identified based on morphology. Some sequences of the described species are either misidentified or missing their types. For genera such as *Fusarium*, *Aspergillus*, *Trichoderma* that requires additional genes for identification, only ITS region sequences are available. These misidentifications can have serious implications from a disease diagnostic and management standpoint.

With DNA barcoding approach, fungi can be identified in less time with more precise identifications using the available curated DNA reference sequences for comparison. This will give an insight on what fungal community and/ or pathogens are truly associated with maize. This knowledge will be an important baseline for communities to know what species are present and thus set priorities for research looking at disease management.

Table 2: Barcode region for fungal identification (Adapted from (Visagie *et al.*, 2014) with some modifications)

Genus	Region	Primer	Direction	Primer Sequence (5'⟶ 3')	References
Unidentified genus	Internal transcribed spacer region (ITS)	ITS1	Forward	TCCGTAGGTGAACCTGCGG	(White *et al.*, 1990)
		ITS4	Reverse	TCCTCCGCTTATTGATATGC	(White *et al.*, 1990)
		V9G	Forward	TTACGTCCCTGCCCTTTGTA	(De Hoog & Van den Ended, 1998)
		LS266	Reverse	GCATTCCCAAACAACTCGACTC	(Masclaux *et al.*, 1995)
Aspergillus	Calmodulin (*CaM*)	CMD5	Forward	CCG AGT ACA AGG ARG CCT TC	(Hong *et al.*, 2006)
		CMD6	Reverse	CCG ATR GAG GTC ATR ACG TGG	(Hong *et al.*, 2006)
Alternaria	Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)	gpd1	Forward	CAACGGCTTCGGTCGCATTG	(Berbee *et al.*, 1999)
		gpd2	Reverse	GCCAAGCAGTTGGTTGTGC	(Berbee *et al.*, 1999)
Penicillium	Beta-tubulin (*tub2*)	Bt_2a	Forward	GGTAACCAAATCGGTGCTGCTTTC	(Glass & Donaldson, 1995)
		Bt_2b	Reverse	ACCCTCAGTGTAGTGACCCTTGGC	(Glass & Donaldson, 1995)
Cladosporium *Trichoderma*	Translation elongation factor 1-alpha (*tef1*)	EF1-728F	Forward	CATCGAGAAGTTCGAGAAGG	(Carbone & Kohn, 1999)
		EF2	Reverse	GGARGTACCAGTSATCATGTT	(O'Donnell *et al.*, 1998)
Fusarium	Translation elongation factor 1-alpha (*tef1*)	EF1	Forward	ATGGGTAAGGARGACAAGAC	(O'Donnell *et al.*, 1998)
		EF2	Reverse	GGARGTACCAGTSATCATGTT	(O'Donnell *et al.*, 1998)
Phoma	RNA polymerase II subunit (*RPB2*)	5Feur	Forward	GAYGAYCGKGAYCAYTTCGG	(Houbraken *et al.*, 2012)
		7CReur	Reverse	CCCATRGCYTGYTTRCCCAT	(Houbraken *et al.*, 2012)

Table 3: Available databases for fungal identifications (Adapted from (Yahr *et al.*, 2016))

Database	Website address	Region
RefSeq Target Loci	http://www.ncbi.nlm.nih.gov/refseq/targetedloci/	ITS, 18S, 28S
UNITE, User-friendly Nordic ITS Ectomycorrhiza Database	https://unite.ut.ee/	ITS
FUSARIUM-ID	http://www.fungalbarcoding.org https://www.fusarium.org	ITS, *tef1*, *RPB1*, *RPB2*, *BenA*
Fungal MLST database Q-Bank	http://www.q-bank.eu/Fungi/	partial actin, *BenA*, *RPB1, RPB2*, *tef1*
International Subcommision on Hypocrea and Trichoderma (ISHT) TrichoKey and TrichoBLAST	http://www.isth.info/tools/blast/	ITS and *tef1*, *RPB2*
Centraalbureau voor Schimmelcultures BioloMICS Databases (CBS-KNAW)	http://www.cbs.knaw.nl/Collections/BioloMICSSequences.aspx	ITS
Naiv̈ e Bayesian Classifier	http://rdp.cme.msu.edu/classifier/classifier.jsp	ITS

References

Abbassian A (2006) Maize: international market profile. *Food and Agriculture Organization of the United Nations* 1-37.

Agrios GN (2005) Plant Pathology, 5th edn.Lodon. *Academic Press*: 903.

Babu BK, Saxena AK, Srivastava AK & Arora DK (2007) Identification and detection of *Macrophomina phaseolina* by using species-specific oligonucleotide primers and probe. *Mycologia* **99**: 797-803.

Bankole S, Mabekoje O & Enikuomehin O (2003) *Fusarium* spp. and fumonisin B1 in stored maize from Ogun State, Nigeria. *Tropical Science* **43**: 76-79.

Benítez T, Rincón AM, Limón MC & Codon AC (2004) Biocontrol mechanisms of *Trichoderma* strains. *International Microbiology* **7**: 249-260.

Bensch K, Groenewald J, Braun U, Dijksterhuis J, de Jesús Yáñez-Morales M & Crous PW (2015) Common but different: The expanding realm of *Cladosporium*. *Studies in Mycology* **82**: 23-74.

Bensch M (1995) Stenocarpella maydis (Berk.) Sutton colonization of maize ears. *Journal of Phytopathology* **143**: 597-599.

Berbee M, Pirseyedi M & Hubbard S (1999) Cochliobolus phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* **91**: 964-977.

Bergstrom GC & Nicholson RL (1999) The biology of corn anthracnose: knowledge to exploit for improved management. *Plant Disease* **83**: 596-608.

Biomin (2021) BIOMIN Mycotoxin Survey Q3 2021 Results [Online]. Available: https://www.biomin.net/science-hub/biomin-mycotoxin-survey-q3-2021-results/ [Accessed 08 October 2021]

Bonants P, Edema M & Robert V (2013) Q-bank, a database with information for identification of plant quarantine plant pest and diseases. *European and Mediterranean Plant Protection Organization Bulletin* **43**: 211-215.

Bruns TD, White TJ & Taylor JW (1991) Fungal molecular systematics. *Annual Review of Ecology and Systematics* **22**: 525-564.

Butt UR, Naz R, Nosheen A, Yasmin H, Keyani R, Hussain I & Hassan MN (2019) Changes in pathogenesis-related gene expression in response to bioformulations in the apoplast of maize leaves against *Fusarium oxysporum*. *Journal of Plant Interactions* **14**: 61-72.

Carbone I & Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553-556.

Chambers K (1987) Epidemiology of maize root rot in South Africa. *Journal of Phytopathology* **118**: 84-93.

Cheek M, Nic Lughadha E, Kirk P, Lindon H, Carretero J, Looney B, Douglas B, Haelewaters D, Gaya E & Llewellyn T (2020) New discoveries: plants and fungi. *Plants, People, Planet* **2**: 371-399.

Clarkson JP, Fawcett L, Anthony SG & Young C (2014) A model for *Sclerotinia sclerotiorum* infection and disease development in lettuce, based on the effects of temperature, relative humidity and ascospore density. *PLoS One* **9**: e94049.

Coleman A (2013) Maize harvest affected by charcoal rot outbreaks: Widespread outbreaks of charcoal rot (*Macrophomina phaseolina*) in maize have been reported in the Free State, in Wesselsbron, Hoopstad, Viljoenskroon and Bultfontein, according to Pannar's agronomist in the region, AK Geldenhuys [Online] Available: https://www.farmersweekly.co.za/agri-news/south-africa/maize-harvest-affected-by-charcoal-rot-outbreaks/ [Accessed 07 June 2022]

Collier P, Conway G & Venables T (2008) Climate change and Africa. *Oxford Review of Economic Policy* **24**: 337-353.

Crous PW, Rong IH, Wood A, Lee S, Glen H, Botha W, Slippers B, de Beer WZ, Wingfield MJ & Hawksworth DL (2006) How many species of fungi are there at the tip of Africa? *Studies in Mycology* **55**: 13-33.

Damm S, Schierwater B & Hadrys H (2010) An integrative approach to species discovery in odonates: from character-based DNA barcoding to ecology. *Molecular Ecology* **19**: 3881-3893.

De Hoog G & Van den Ended A (1998) Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses-Berlin* **41**: 183-190.

Deb D, Khan A & Dey N (2020) Phoma diseases: Epidemiology and control. *Plant Pathology* **69**: 1203-1217.

Deep I & Lipps P (1996) Recovery of *Pythium arrhenomanes* and its virulence to corn. *Crop Protection* **15**: 85-90.

Deshapande AS, Giraddi SG, Karibasappa K & Desai SD (2019) Fungal disease detection in maize leaves using haar wavelet features. *Information and Communication Technology for Intelligent Systems* 275-286.

Dodd J & White D (1999) Seed rot, seedling blight, and damping-off. *Compendium of Corn Diseases DG White, ed American Phytopathological Society, St Paul, MN* 10-11.

Elliott C (1943) A Pythium stalk rot of corn. *Journal of Agricultural Research* **66**.

Farr DF, Bills GF, Chamuris GP & Rossman AY (1989) *Fungi on plants and plant products in the United States*. APS press.

Federhen S (2015) Type material in the NCBI Taxonomy Database. *Nucleic acids research* **43**: D1086-D1098.

Flett B & van Rensburg BJ (2018) A close look at Gibberella root, crown and stalk rots [Online]. Available: https://www.grainsa.co.za/a-closer-look-at-gibberella-root-crown-

and-stalk rots#:~:text=Symptoms%20of%20Gibberella%20root%2C%20crown,lower%20stalks%20become%20straw%20coloured. [Accessed 07 June 2022]

Flett B & van Rensburg BJ (2021) Maize stalk rots: unseen yield assassins [Online]. Available: https://sagrainmag.co.za/2021/07/12/maize-stalk-rots-unseen-yield-assassins/ [Accessed 28 February 2021]

Francl LJ (2001) The disease triangle: a plant pathological paradigm revisited. *The Plant Health Instructor* **10**.

Gai X, Dong H, Wang S, Liu B, Zhang Z, Li X & Gao Z (2018) Infection cycle of maize stalk rot and ear rot caused by *Fusarium verticillioides*. *PLoS One* **13**: e0201588.

Gardes M & Bruns T (1996) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above-and below-ground views. *Canadian Journal of Botany* **74**: 1572-1583.

Gilbertson R, Brown Jr W, Ruppel E & Capinera J (1986) Association of Corn Stalk Rot *Fusarium spp.* and Western Corn Rootworm Beetles. *Phytopathology* **76**:1309-1314

Glass NL & Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* **61**: 1323-1330.

GrainSA (2020) GrainSA annual report [Online]. Available: https://www.grainsa.co.za/annualreport_2020/Grain%20SA%20Annual%20Report%202020/files/assets/common/downloads/publication.pdf [Accessed 19 November 2021]

Grau C & Robertson A (2019) Root rots of corn. Crop protection network [Online]. Available: https://cropprotectionnetwork.org/resources/articles/diseases/root-rots-of-corn [Accessed 02 July 2022]

Hendrix, F. F. Jr. and Campbell, W. A. 1983. Some pythiaceous fungi, new roles for old organisms. Chen. W. [Ed]. Compendium of corn diseases, 3rd edn. Minnesota. *The American Phytopathology Society*.

Hong S-B, Cho H-S, Shin H-D, Frisvad JC & Samson RA (2006) Novel *Neosartorya* species isolated from soil in Korea. *International Journal of Systematic and Evolutionary Microbiology* **56**: 477-486.

Houbraken J, Spierenburg H & Frisvad JC (2012) *Rasamsonia*, a new genus comprising thermotolerant and thermophilic *Talaromyces* and *Geosmithia* species. *Antonie van Leeuwenhoek* **101**: 403-421.

Hyde KD, Abd-Elsalam K & Cai L (2010) Morphology: still essential in a molecular world. *Mycotaxon* **114**: 439-451.

Isakeit T, Gao X & Kolomiets M (2007) *Exserohilum pedicellatum* Root Rot of Corn in Texas. *Plant Disease* **91(5):** 634.doi: 10.1094/PDIS-91-5-0634C.

Jermy A (2017) Stop neglecting fungi. *Nature Microbiology* **2**: 17120.

Jirak-Peterson JC & Esker PD (2011) Tillage, crop rotation, and hybrid effects on residue and corn anthracnose occurrence in Wisconsin. *Plant Disease* **95**: 601-610.

Jordaan E & van der Waals J (2016) Charcoal rot: a threat to staple food crops in South Africa [Online]. Available: https://theconversation.com/charcoal-rot-a-threat-to-staple-food-crops-in-south-africa-64385 [Accessed 07 June 2022]

Jouany JP (2007) Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. *Animal Feed Science and Technology* **137**: 342-362.

Kageyama K, Nakashima A, Kajihara Y, Suga H & Nelson EB (2005) Phylogenetic and morphological analyses of *Pythium graminicola* and related species. *Journal of General Plant Pathology* **71**: 174-182.

Kamoun S (2003) Molecular genetics of pathogenic oomycetes. *Eukaryotic cell* **2**: 191-199.

Knoema (2020) World - Maize production quantity [Online]. Available: https://knoema.com/atlas/World/topics/Agriculture/Crops-Production-Quantity-tonnes/Maize-production [Accessed 12 August 2020]

Kostic AŽ, Milincic DD, Petrovic TS, Krnjaja VS, Stanojevic SP, Barac MB, Tešic ŽL & Pešic MB (2019) Mycotoxins and Mycotoxin Producing Fungi in Pollen. *TOXINS* **11**.

Lamprecht S (2013) Fungal pathogens associated with crown and root rot of no-till maize in KwaZulu-Natal [Online]. Available: https://www.grainsa.co.za/fungal-pathogens-associated-with-crown-and-root-rot-of-no-till-maize-in-kwazulu-natal [Accessed 07 June 2022]

Lamprecht SC, Crous PW, Groenewald JZ, Tewoldemedhin YT & Marasas WF (2011) *Diaporthaceae* associated with root and crown rot of maize. *The International Mycological Association Fungus* **2**: 13-24.

Leyva-Madrigal KY, Sandoval-Castro E, Calderón-Vázquez CL, Larralde-Corona CP & Maldonado-Mendoza IE (2017) Pathogenic and genetic variability of *Fusarium verticillioides* from maize in northern Mexico. *Canadian Journal of Plant Pathology* **39**: 486-496.

Li Destri Nicosia M, Mosca S, Mercurio R & Schena L (2015) Dieback of *Pinus nigra* Seedlings Caused by a Strain of Trichoderma viride. *Plant Disease* **99**: 44-49.

Ma W, Gao X, Han T, Mohammed MT, Yang J, Ding J, Zhao W, Peng Y-L & Bhadauria V (2022) Molecular Genetics of Anthracnose Resistance in Maize. *Journal of Fungi* **8**: 540.

Mao W, Carroll R & Whittington D (1998) Association of *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* in causing red root rot of corn. *Plant Disease* **82**: 337-342.

Martin FN & Loper JE (1999) Soilborne plant diseases caused by *Pythium* spp.: ecology, epidemiology, and prospects for biological control. *Critical Reviews in Plant sciences* **18**: 111-181.

Masclaux F, Guého E, De Hoog G & Christen R (1995) Phylogenetic relationships of human-pathogenic *Cladosporium* (*Xylohypha*) species inferred from partial LS rRNA sequences. *Journal of Medical and Veterinary Mycology* **33**: 327-338.

Mcsweeney R (2018) In the fourth article in a series on how key emitters are responding to climate change, Carbon Brief looks at South Africa's heavy dependence on coal and expanding effort to develop renewables [Online]. Available: https://www.carbonbrief.org/the-carbon-brief-profile-south-africa/ [Accessed 07 June 2022]

Mishra K & Rampal J (2020) The COVID-19 pandemic and food insecurity: A viewpoint on India. *World Development* **135**: 105068.

Munir M (2018) Plant disease epidemiology: disease triangle and forecasting mechanisms in highlights. *Hosts Virus* **5**: 7-11.

Munkvold G (2021) Charcoal rot of corn. Crop protection network [Online]. Available: https://cropprotectionnetwork.org/resources/articles/diseases/charcoal-rot-of-corn [Accessed 07 June 2022]

Munkvold G & Carlton W (1997) Influence of inoculation method on systemic F*usarium moniliforme* infection of maize plants grown from infected seeds. *Plant Disease* **81**: 211-216.

Munkvold GP (2003) Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *European Journal of Plant Pathology* **109**: 705-713.

Mwatuni FM, Nyende AB, Njuguna J, Zhonguo X, Machuka E & Stomeo F (2020) Occurrence, genetic diversity, and recombination of maize lethal necrosis disease-causing viruses in Kenya. *Virus Research* **286**: 198081.

Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson K-H & Kõljalg U (2006) Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. *PloS one* **1**: e59.

Nilsson RH, Tedersoo L, Ryberg M, Kristiansson E, Hartmann M, Unterseher M, Porter TM, Bengtsson-Palme J, Walker DM & De Sousa F (2015) A comprehensive, automatically updated fungal ITS sequence dataset for reference-based chimera control in environmental sequencing efforts. *Microbes and Environments* **30**:145-150.

O'Donnell K, Kistler HC, Cigelnik E & Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences* **95**: 2044-2049.

O'Donnell K, Ward TJ, Robert VA, Crous PW, Geiser DM & Kang S (2015) DNA sequence-based identification of *Fusarium*: current status and future directions. *Phytoparasitica* **43**: 583-595.

Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe Q & Dasgupta P (2014) *Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change*. ISBN: 978-92-9169-143-2.

Pal K, Tilak K, Saxcna A, Dey R & Singh C (2001) Suppression of maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. *Microbiological Research* **156**: 209-223.

Panth M, Hassler SC & Baysal-Gurel F (2020) Methods for Management of Soilborne Diseases in Crop Production. *Agriculture* **10**: 16.

Parveen T & Sharma K (2015) *Pythium* diseases, control and management strategies: a review. *International Journal of Plant, Animal and Environmental Sciences* **5**: 244-257.

Pfordt A, Schiwek S, Karlovsky P & Von Tiedemann A (2020) *Trichoderma Afroharzianum* Ear Rot–A New Disease on Maize in Europe. *Frontiers in Agronomy* **2**: 11.

Raja HA, Miller AN, Pearce CJ & Oberlies NH (2017) Fungal identification using molecular tools: a primer for the natural products research community. *Journal of Natural Products* **80**: 756-770.

Rao B, Schmitthenner A, Caldwell R & Ellett C (1978) Prevalence and virulence of *Pythium* species associated with root rot of corn in poorly drained soil. *Phytopathology* **68**: 1557-1563.

Rehman F, Adnan M, Kalsoom M, Naz N, Husnain MG, Ilahi H, Ilyas MA, Yousaf G, Tahir R & Ahmad U (2021) Seed-borne fungal diseases of Maize (*Zea mays* L.): A review. *Agrinula: Jurnal Agroteknologi dan Perkebunan* **4**: 43-60.

Reid A & Greene SE (2012) How Microbes Can Help Feed the World [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK559436/ [Accessed 19 August 202]

Rico-Munoz E, Samson RA & Houbraken J (2019) Mould spoilage of foods and beverages: Using the right methodology. *Food Microbiology* **81**: 51-62.

Robert V, Cardinali G, Stielow B, Vu T, dos Santos FB, Meyer W & Schoch C (2015) Fungal DNA barcoding. *Molecular Biology of Food and Water Borne Mycotoxigenic and Mycotic Fungi* 37-56.

Rosegrant MW, Cai X & Cline SA (2002) World water and food to 2025: dealing with scarcity.United States. *International Food Policy Research Institute*: 322.

Rossman AY (1998) Protocols for an all taxa biodiversity inventory of fungi in a Costa Rican conservation area. United States. *Parkway Publishers*: 213.

Rossman AY & Palm-Hernández ME (2008) Systematics of plant pathogenic fungi: why it matters. *Plant Disease* **92**: 1376-1386.

Sadik E, Mehta S, Mehta S, Payak M & Srinivasan M (1982) Isolation and partial characterization of an extracellular phytotoxin produced by Pythium aphanidermatum, a stalk rot pathogen of maize. *Journal of Plant Diseases and Protection* **89:** 266-275.

Samson RA, Visagie CM, Houbraken J, Hong S-B, Hubka V, Klaassen CH, Perrone G, Seifert KA, Susca A & Tanney JB (2014) Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Studies in mycology* **78**: 141-173.

Samuels GJ, Dodd SL, Gams W, Castlebury LA & Petrini O (2002) *Trichoderma* species associated with the green mold epidemic of commercially grown Agaricus bisporus. *Mycologia* **94**: 146-170.

Sangalang A, Burgess L, Backhouse D, Duff J & Wurst M (1995) Mycogeography of *Fusarium* species in soils from tropical, arid and Mediterranean regions of Australia. *Mycological Research* **99**: 523-528.

Sanna M, Pugliese M, Gullino ML & Mezzalama M (2022) First report of Trichoderma afroharzianum causing seed rot on maize in Italy. *Plant Disease* **106**: 1982.

Schoeman A & Craven M (2016) Research evolution on soilborne diseases of maize [Online]. Available: https://www.grainsa.co.za/research-evolution-on-soilborne-diseases-of maize#:~:text=These%20fungal%20isolates%20include%20Pythium,.%2C%20Fusarium%20chlamydosporum%2C%20F [Accessed 07 June 2022]

Sharma P, Kumar V, Ramesh R, Saravanan K, Deep S, Sharma M, Mahesh S & Dinesh S (2011) Biocontrol genes from *Trichoderma* species: a review. *African Journal of Biotechnology* **10**: 19898-19907.

Shin J-H, Han J-H, Lee JK & Kim KS (2014) Characterization of the maize stalk rot pathogens *Fusarium subglutinans* and *F. temperatum* and the effect of fungicides on their mycelial growth and colony formation. *The Plant Pathology Journal* **30**: 397.

Singh BP & Gupta VK (2017) Molecular markers in mycology. Switzerland. S*pringer International Publishing*: 369.

Smit E, Van Rensburg, GDJ* & Rijkenberg F (1997) Number of isolates of maize root fungi in different crop rotation systems. *South African Journal of Plant and Soil* **14**: 127-130.

Sobek E & Munkvold G (1999) European corn borer (*Lepidoptera*: *Pyralidae*) larvae as vectors of *Fusarium moniliforme*, causing kernel rot and symptomless infection of maize kernels. *Journal of Economic Entomology* **92**: 503-509.

Steckel S (2003) Biology and management of Diplodia (*Stenocarpella maydis*) ear and stalk rot. *Journal of Natural Resources and Life Sciences Education* **32**: 5.

Stockinger H, Krüger M & Schüßler A (2010) DNA barcoding of arbuscular mycorrhizal fungi. *New Phytologist* **187**: 461-474.

Straatsma G, Ayer F & Egli S (2001) Species richness, abundance, and phenology of fungal fruit bodies over 21 years in a Swiss forest plot. *Mycological Research* **105**: 515-523.

Strange RN & Scott PR (2005) Plant disease: a threat to global food security. *Annual Review of Phytopathology* **43:** 83-116.

Sumner DR & Bell D (1982) Root Diseases Induced in Corn by *Rhizoctonia solani* and *Rhizoctonia zeae*. *Phytopathology* **72:** 86-91.

Sutton B & Waterston J (1966) *Diplodia macrospora*.[Descriptions of Fungi and Bacteria]. *CAB International* **9**. doi.org/10.1079/DFB/20056400083.

Tams A, Mooney S & Berry P (2004) The effect of lodging in cereals on morphological properties of the root-soil complex. *Proceedings of the SuperSoil*.

Tanabe AS & Toju H (2013) Two new computational methods for universal DNA barcoding: a benchmark using barcode sequences of bacteria, archaea, animals, fungi, and land plants. *PloS one* **8**: e76910.

The Andersons (2015) Stalk rots in corn [Online]. Available: https://andersonscanada.com/2015/09/03/stalk-rots-corn/ [Accessed 22 October 2020]

Thines M (2014) Phylogeny and evolution of plant pathogenic oomycetes—a global overview. *European Journal of Plant Pathology* **138**: 431-447.

Thines M & Kamoun S (2010) Oomycete–plant coevolution: recent advances and future prospects. *Current Opinion in Plant Biology* **13**: 427-433.

Tonin RFB, Avozani A, Danelli ALD, Reis EM, Zoldan SM & Garcés-Fiallos FR (2013) In vitro mycelial sensitivity of *Macrophomina phaseolina* to fungicides. *Pesquisa Agropecuária Tropical* **43**: 460-466.

Udomkun P, Wiredu AN, Nagle M, Bandyopadhyay R, Müller J & Vanlauwe B (2017) Mycotoxins in Sub-Saharan Africa: Present situation, socio-economic impact, awareness, and outlook. *Food Control* **72**: 110-122.

van Rensburg BJ & Flett B (2012) Maize stalk rots: of importance in the current season [Online]. Available: https://www.grainsa.co.za/maize-stalk-rots:-of-importance-in-the-current-season [Accessed 15 September 2020]

Váry Z, Mullins E, McElwain JC & Doohan FM (2015) The severity of wheat diseases increases when plants and pathogens are acclimatized to elevated carbon dioxide. *Global Change Biology* **21**: 2661-2669.

Veerabhadraswamy A & Garampalli RH (2011) Effect of arbuscular mycorrhizal fungi in the management of black bundle disease of maize caused by *Cephalosporium acremonium*. *Science Research Reporter* **1**: 96-100.

Velásquez AC, Castroverde CDM & He SY (2018) Plant–pathogen warfare under changing climate conditions. *Current Biology* **28**: R619-R634.

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL & Lorito M (2008) *Trichoderma*–plant–pathogen interactions. *Soil Biology and Biochemistry* **40**: 1-10.

Visagie C, Houbraken J, Frisvad JC, Hong S-B, Klaassen C, Perrone G, Seifert K, Varga J, Yaguchi T & Samson R (2014) Identification and nomenclature of the genus *Penicillium*. *Studies in Mycology* **78**: 343-371.

White D (1999) Compendium of corn diseases, 3rd edn. St. Paul, Minnesota. *APS Press, American Phytopathological Society.*

White TJ, Bruns T, Lee S & Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *Academic Press* 315-322

Whitney N & Mortimore C (1961) Root and stalk rot of field corn in Southwestern Ontario: ii. Development of the disease and isolation of organisms. *Canadian Journal of Plant Science* **41**: 854-861.

Woo SL, Ruocco M, Vinale F, Nigro M, Marra R, Lombardi N, Pascale A, Lanzuise S, Manganiello G & Lorito M (2014) *Trichoderma*-based products and their widespread use in agriculture. *The Open Mycology Journal* **8**.

Yahr R, Schoch CL & Dentinger BT (2016) Scaling up discovery of hidden diversity in fungi: impacts of barcoding approaches. *Philosophical Transactions of the Royal Society B: Biological Sciences* **371**: 20150336.

Yu C, Saravanakumar K, Xia H, Gao J, Fu K, Sun J, Dou K & Chen J (2017) Occurrence and virulence of *Fusarium* spp. associated with stalk rot of maize in North-East China. *Physiological and Molecular Plant Pathology* **98**: 1-8.

Chapter 2: Fungal diversity associated with maize agricultural soils collected in the Free State and North West provinces of South Africa

Abstract

Maize is a staple food in South Africa and our biggest and most important crop produced. Studies characterising microbial communities associated with maize rhizosphere soil are essential for understanding their contribution to producing healthy maize. The aim of our study was therefore to complete a survey and identify and characterize fungi from maize rhizosphere soils collected from farms in the Free State and North-West provinces. Fungal strains were isolated from soil using various growth media and identified to genus level using morphology. Subsequent species identifications were done using appropriate DNA sequences of informative genes (*CaM, tef1*, *tub2*) for each genus and ITS for fungi that could not be identified morphologically. Isolations resulted in 460 strains that were classified into 28 genera and 80 species. *Fusarium*, *Neocosmospora*, *Penicillium* and *Trichoderma* were found to dominate rhizosphere communities, with *F. tardicrescens*, *N. solani*, *P. raperi*, and *T. afroharzianum* the most common and consistently isolated species across farms. Other genera identified include *Aspergillus*, *Chaetomium*, *Cladosporium*, *Metarhizium*, *Talaromyces*, and *Umbelopsis*. Notably, *Fusarium chlamydosporum*, *F. oxysporum*, *F. subglutinans, F. temperatum*, *Neocosmospora falciformis* and *Setophoma terrestris* were isolated from soils and have previously been reported to cause stalk, root, and /or crown rots of maize plants. Potentially beneficial species commonly used as biological control agents (such as *Beauveria bassiana*, *Clonostachys rosea*, *Metarhizium pinghaense*, *Trichoderma hamatum*, and *T. gamsii*) were also isolated. The findings presented here provide the baseline knowledge needed to start understanding fungal community compositions of maize rhizosphere soils and give us an insight into what soilborne pathogens may be associated with maize. This is the first survey to use modern taxonomic approaches to identify and characterise fungal communities from maize farms in South Africa.

Keywords: Sequence based identifications, Soil fungi, *Zea mays*

Introduction

Maize is one of the most important crops planted worldwide, ranking third after wheat and rice (Adnan & Bilal, 2020). In South Africa, maize is a staple crop with 16 211 265 tons recorded in 2020/21 (GrainSA, 2021). It is consumed directly as food, used to produce food products like maize meal, breakfast cereals, and snacks, or used for animal feed. The Free State (45,1 %), Mpumalanga (20,9 %), and North-West (18,0 %) provinces produce the most maize in the country, followed by KwaZulu-Natal (4,8 %), Northern Cape (4,4 %) and Gauteng (4,1 %). The Limpopo (1,5 %), Eastern Cape (1.0 %), and Western Cape (0,2 %) provinces contribute a small percentage of the country's total maize produced (GrainSA, 2020).

To sustain the growing human population and feed livestock, maize crop yields are typically increased by the use of chemical fertilizers (Fixen & West, 2002). However, fertilizers are expensive (Musokwa *et al.*, 2019) and they also negatively interfere with the structure of the soil ecosystem (Tripathi *et al.*, 2020) including fungal communities present in the soil. Microbial diversity is an essential component for maintaining healthy agricultural soils (Jeffries *et al.*, 2003, Izquierdo *et al.*, 2005). For example, mycorrhizal fungi (e.g. *Glomus mosseae*, *G. etunicatum,* and *G. clarum*) are known to help maize plants grow and develop by facilitating nutrient absorption, especially phosphorus (Bai *et al.*, 2008, Evelin *et al.*, 2009). On the other hand, some soil fungi are plant pathogens and can negatively impact maize plants when they cause diseases leading to quality and yield reduction.

The fungal kingdom is extremely diverse, ranging from unicellular yeast to large multicellular macro- and micro fungi. They can be found in almost all substrates that contain carbon and life without them would not be possible. Generally, substrates with a high fungal diversity are thought to be healthier than substrates that lack diversity (Bell *et al.*, 2009), especially where the soil is concerned. The primary role of soil fungi in the ecosystem is to decompose organic matter. Also, the hyphae of filamentous fungi growing through the soil matrix intertwine soil particles, thus forming soil aggregates (Lehmann *et al.*, 2017) that can limit soil erosion (Hagn *et al.*, 2003). However, fungal diversity is typically affected by environmental factors, such as soil type, pH, temperature, above ground vegetations and many more (Angst *et al.*, 2021). Land management strategies such as crop rotation or tillage also play a role in

determining fungal communities in agricultural soils (Acosta-Martinez *et al.*, 2014, Classen *et al.*, 2015).

Studies of microbial communities associated with maize rhizosphere soils are essential for understanding the potential ecological role and interrelationship between a plant and microbes in this ecosystem. Microorganisms that have a positive impact in agricultural soils are important for their potential use in selection of management strategies, i.e., understanding the fungal diversity and their roles in maize agricultural ecosystems is essential. Fungal genera that are commonly associated with maize related soils include *Aspergillus*, *Fusarium*, *Penicillium*, and *Trichoderma* (Nesci et al., 2006, Swer et al., 2014, Khonglah et al., 2015, Nyongesa et al., 2015, Zhao et al., 2022). Traditionally, fungal surveys identified fungi based on only morphology which is complicated to do and often led to misidentifications or underreporting of diversity. Past identifications do not necessarily follow more recent taxonomies based on phylogenetic species concepts (Eicker, 1970, Marasas *et al.*, 1981, Roux & Van Warmelo, 1997, Cavaglieri *et al.*, 2009, Küçük & Kıvanç, 2011, Swer *et al.*, 2014, Nyongesa *et al.*, 2015, Widiati *et al.*, 2015, Adeyemi *et al.*, 2019). The use of DNA sequences allows mycologists the opportunity to identify community members more accurately and robustly as long as reliable reference data are available. Modern surveys that identify fungi using DNA sequence markers instead of morphology are thus needed to obtain a better baseline knowledge on the fungi that are associated with maize rhizosphere soils in South Africa.

This study aimed to survey maize rhizosphere soils collected from commercial farms in the Free State and North West provinces and determined their fungal community composition. The main objectives were to isolate, preserve and identify fungal strains to the species level using a DNA sequencing approach and advance our knowledge of the fungal species diversity from South Africa.

Materials and methods

Soil sampling and fungal isolation

Representative soil samples were collected from 19 farms in the Free State (17 farms) and North West (2 farms) provinces in March 2020. Soil was sampled

around roots of maize plants, 10 cm below the surface using a hand shovel. Six soil samples were randomly collected across each farm and stored in 4 ºC until processed for isolations.

From each sample, 10 g of soil was suspended in 100 ml of sterile distilled water. Serial dilutions were prepared down to $1x10^{-3}$ and 1 ml from each dilution was spread plated in triplicate onto Difco™ potato dextrose agar (Becton, Dickinso and Company, Sparks, USA). For *Fusarium* isolations, a small amount of soil was sprinkled directly onto *Fusarium* selective media (Difco agar amended with peptone powder, KH2PO4, MgSO4.7H2O and pentachloronitrobenzene). All media were amended with 2 ml of chloramphenicol (50 ppm) and streptomycin (100 ppm) to prevent bacterial growth. Plates were incubated at ±21 °C for 5–7 days. Isolations were subsequently made by transferring spores/mycelia to small plates containing low-strength PDA (¼ PDA). Single spore cultures were prepared by suspending mycelia into 100 µl double sterilized distilled water in an eppendorf tube. The mixture was mixed, spread plated onto ¼ PDA plates, and plates incubated face down overnight at ±21 °C. Plates were observed using a stereo microscope and single germinating spores were transferred to new ¼ PDA plates. Isolates were grouped and identified to genus level using morphology and then accessioned into the CN working collection housed at FABI. Spore suspensions of the strains were prepared in vials containing 10% glycerol and stored at -80 °C.

DNA extractions

Fungal DNA of pigmented strains (e.g. *Penicillium*, *Trichoderma,* etc.) was extracted from 7–10 day old cultures as per the manufacturer's protocol using the ZR Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, CA, USA). PrepMan™ Ultra (Thermo Fisher Scientific, Warrington, UK) was used for extracting DNA from isolates with hyaline conidia. Briefly, the mycelium of each isolate was suspended into 70 µl PrepMan™ Ultra in an eppendorf tube, vortexed for 30 sec, and heated for 10 min. The eppendorf tubes were then centrifuged at 20800 g for 7 min. The supernatant containing DNA was transferred to a new

eppendorf tube. All DNA extracts were accessioned into the CN-DNA collection and stored at -20 °C.

Polymerase Chain Reaction (PCR) and Sequencing

Four identification markers were used to make species identifications. They included beta-tubulin (*tub2*), translation elongation factor 1-alpha (*tef1*), and calmodulin (*CaM*), as well as the internal transcribed spacer rDNA region (ITS). They were amplified and sequenced, depending on the genus a strain belonged to (Table 1). PCR amplification was done in 25 µl reaction mixtures consisting of 17.5 µl MilliQ water; 5.0 µl BioLine 5X MyTaq Reaction Buffer (Bioline, England, UK), 0.50 µl of each primer (concentration), 0.50 µl BioLine MyTaq DNA polymerase and 1 µl of the extracted DNA. Thermal cycles used for each gene region are listed in Table 2. The relative concentrations and sizes of PCR products were estimated using electrophoresis on a 1% agarose gel stained with GelRed and 100 bp ladder (Thermo Fisher Scientific, Massachusetts, USA).

PCR products were purified using the ExoSAP-IT PCR Product Clean-up Reagent (Thermo Fisher Scientific, Massachusetts, USA) to remove primer dimers and unincorporated nucleotides. Briefly, a reaction of 2 µl ExoSAP-IT and 5 µl of PCR product was placed in a thermal cycle of: 37 °C for 15 min, and 85 °C for 15 min. Purified PCR amplicons were sequenced in both directions, separately using the same primers used for PCR and the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems). Reactions were analysed at the DNA Sanger Sequencing Facility, Faculty of Natural and Agricultural Sciences, University of Pretoria.

Contigs were assembled and base calls were made where needed using Geneious Prime v. 2019. To make identifications, sequences were compared to reference data previously deposited in RefSeq Target Loci and in GenBank of the National Center for Biotechnology Information (NCBI) using “Nucleotide BLAST” search, while *Aspergillus*, *Penicillium,* and *Fusarium* sequences were compared with locally curated databases. The *Aspergillus* and *Penicillium* databases are largely based on Houbraken *et al.* (2020), while *Fusarium* and

Trichoderma were largely based on Crous *et al.* (2021) and Bissett *et al.* (2015) respectively.

Results

Fungal isolations and identifications

A total of 114 soil samples (102 from the Free State, 12 from North West) were collected from 19 farms. Isolations resulted in 460 fungal strains representing 20 families (Figure 1) with *Aspergillaceae* (n = 156) and *Nectriaceae* (n = 145) dominating communities, followed by *Hypocreaceae* (n = 74), *Cladosporiaceae* (n = 21), *Didymosphaeriaceae* (n = 9), *Mortierellaceae* (n = 5), *Bionectriaceae* (n = 3), *Clavicipitaceae* (n = 3), *Phaeosphaeriaceae* (n = 3), *Chaetomiaceae* (n = 2), *Aureobasidiaceae* (n = 1), *Cordycipitaceae* (n = 1), *Cunninghamellaceae* (n = 1), *Myrmecridiaceae* (n = 1), *Ophiocordycipitaceae* (n = 1), *Pleosporaceae* (n = 1), and *Stachybotryaceae* (n = 1).

Aspergillaceae was the most commonly isolated group, represented by two genera, *Aspergillus* and *Penicillium*. In total, 27 species were identified, five *Aspergillus* as *A. calidoustus* (n = 1), *A. dimorphicus* (n = 1), *A. pseudodeflectus* (n = 4), *A. terreus* (n = 2), and *A. tubingensis* (n = 1), and 22 *Penicillium* species as *P. raperi* (n = 75), *P. arabicum* (n = 16), *P. flavigenum* (n = 13), *P. chalabudae* (n = 5), *P. bilaiae* (n = 4),*P. ortum* (n = 4), *P. crustosum* (n = 3), *P. rubens* (n = 3), *P. citrinum* (n = 2), *P. cluniae* (n = 2), *P. raistrickii* (n = 2), and single strains recovered for *P. atrovenetum*, *P. brevicompactum*, *P. desertorum*, *P. frequentans*, *P. griseofulvum*, *P. murcianum*, *P. olsonii*, *P. sacculum*, *P. sizovae*, *P. striatisporum*, and *P. yunnanense* (Figure 3). *Penicillium raperi* was the most commonly isolated species and was recovered from all farms. Three putative new *Penicillium* species were also isolated.

Nectriaceae were the second most commonly isolated group. Strains represented two genera, *Fusarium* and *Neocosmospora* (formerly the *Fusarium solani* species complex). *Fusarium* species identified belonged to five species complexes (Figure 4), namely, the *Fusarium chlamydosporum* species complex (FCSC; n = 7), *Fusarium fujikuroi* species complex (FFSC; n = 8), *Fusarium incarnatum-equiseti* species complex (FIESC; n = 2), *Fusarium oxysporum* species complex (FOSC; n = 94), and

Fusarium sambucinum species complex (FSAMSC; n = 8). *Fusarium* communities were dominated by strains (n = 94) from FOSC. A total of 15 *Fusarium* species were identified. From FCSC *F. atrovinosum* (n = 1), *F. chlamydosporum* (n = 1) and *F. sporodochiale* (n = 5) were identified; from FFSC *F. subglutinans* (n = 6) and *F. temperatum* (n = 2) were identified; from FIESC *F. clavus* (n = 1) and *F. equiseti* (n = 1) were identified; from FOSC *Fusarium callistephi* (n = 20), *F. carminascens* (n = 1), *F. glycines* (n = 11), *F. grosmichelii* (n = 4), *F. nirenbergiae* (n = 4) and *F. tardicrescens* (n = 38) were identified; and from FSAMSC *F. boothii* (n = 1) and *F. transvaalense* (n = 7) were identified. A putative new species, *Fusarium* sp. nov. 1, (n = 16) that belong to FOSC (Figure 5) was also isolated. *Fusarium tardicrescens* was found in many soil samples (11 out of 19 farms). The most commonly isolated *Neocosmospora* species was *N. solani* (n = 24), but *N. falciformis* (n = 2) was also recovered.

Trichoderma (classified in *Hypocreaceae*) was also a commonly isolated genus that represented eight species (Figure 6). *Trichoderma afroharzianum* (n = 23) was the most commonly isolated species, followed by *T. gamsii* (n = 16), *T. hamatum* (n = 16), *T. rifaii* (n = 8), *T. arundinaceum* (n = 6), *T. saturnisporum,* (n = 3), *T. spirale* (n = 1) and *T. virens* (n = 1).

A total of 121 strains identified as 27 species belonged to less commonly isolated genera that included *Albifimbria, Alternaria, Aureobasidium, Beauveria, Chaetomium, Cladosporium, Clonostachys, Didymella, Gamsiella, Gongronella, Leptosphaerulina, Metarhizium, Mortierella, Myrmecridium, Paraconiothyrium, Paraphaeosphaeria, Pseudopithomyces, Pseudothielavia*, *Purpureocillium, Sarocladium, Setophoma, Talaromyces,* and *Umbelopsis* (Table 3).

Discussion

This study aimed to complete a fungal survey on maize rhizosphere soils by identifying strains based on DNA sequence data. This survey was conducted across 17 Free State and two North West farms, and it resulted in 460 isolates identified as 80 species from 28 genera and 20 families. *Fusarium*, *Neocosmospora*, *Penicillium,* and *Trichoderma* were found to dominate the examined communities, with *Fusarium*

tardicrescens, *Neocosmospora solani*, *Penicillium raperi*, and *Trichoderma afroharzianum* being the most common species isolated across the farms.

Fungal communities mostly consisted of *Penicillium* (*Aspergillaceae*), *Fusarium* (*Nectriaceae*), and *Trichoderma* (*Hypocreaceae*). These results partially agree with those of Nesci *et al.* (2006) who reported *Cladosporium*, *Fusarium*, *Penicillium*, and *Trichoderma* as common from maize soils that underwent conventional tillage in Argentina. They are also similar to those of Chávez-Díaz *et al.* (2021) who identified *Aspergillus*, *Fusarium*, *Metarhizium, Penicillium*, *Talaromyces*, and *Trichoderma* species present in Mexican maize landraces when they surveyed for maize rhizosphere fungi that exhibit potential biocontrol properties against *Fusarium* strains from four different *Fusarium* species complexes. Beyers (2019) who used real-time PCR (qPCR) to identify and quantify soilborne pathogens associated with maize roots in the Free State, South Africa reported high abundance *Phoma* spp., *F. oxysporum* and *F. chlamydosporum*. Gaddeyya *et al.* (2012) reported *Aspergillus terreus*, *Penicillium frequentans*, *Trichoderma viride*, *Fusarium oxysporum*, *Neocosmospora solani* (as *F. solani*) in cultivable fields, which are the species that were also found from maize fields in the current study. Zhao *et al.* (2021) surveyed rhizosphere microbial community in continuous mono-maize seed production using high-throughput sequencing approaches, and reported much more diverse communities, detecting the genera *Mortierella*, *Chrysosporium*, *Pseudeurotium*, *Fusarium*, *Podospora*, *Chaetomium*, *Exophiala*, *Bipolaris*, *Acremonium*, *Cladosporium*, *Microascus*, *Cladorrhinum*, *Guehomyces*, *Scopulariopsis*, *Plectosphaerella*, *Wardomyces*, *Tetracladium*, *Inocybe*, *Holtermanniella*, and *Bionectria*.

Penicillium was the most commonly isolated genus, with strains that represented 22 species. *Penicillium raperi* was the most prevalent species. *Penicillium raperi* belongs to section Lanata-Divaricata which is a very common section in soil, and this might explain the high abundance *P. raperi*. The results from this study are different (except for *P. brevicompactum* and *P. sacculum*) from those of Khonglah *et al.* (2015) who reported *P. canescens*, *P. daleae*, *P. fellutanum*, *P. janthinellum*, *P. jensenii*, *P. lanosum*, *P. restrictum*, *P. simplicissimum*, and *P. spinulosum* on the rhizospheric soils of maize plants. *Penicillium* species are generally considered saprophytes that break down complex organic substrates and make these compounds available for

plants to use. Others are known to fulfill important ecosystem functions where they can solubilize phosphates and promote plant growth. *Penicillium bilaiae, P. citrinum*, and *P. griseofulvum* which have also been isolated from this survey were shown to have phosphate solubilization (Yadav *et al.*, 2011, Gómez-Muñoz *et al.*, 2018) and plant growth promotion activities (Khan *et al.*, 2008). Wakelin *et al.* (2004) reported efficient phosphate solubilization by *P. bilaiae* and *P. griseofulvum* when investigating the occurrence and levels of phosphate solubilization activity of *Penicillium* species associated with the wheat rhizosphere in Australia. To this end, *P. bilaiae* is currently registered as a plant growth-promoting microorganism (PGPM) in Canada. Leggett *et al.* (2015) also reported a yield increase in maize plants inoculated with *P. bilaiae*. With the high abundance of *P. raperi* (75 strains) from all fields, we suggest studies investigating the ecological roles of *P. raperi* on maize agricultural fields and the relationship between this species and maize plants need to be conducted. For example, it would be interesting to explore if *P. raperi* can solubilize phosphate from the soil and make it available for plant intake.

Fusarium was the second most abundant genus represented by 16 species, most of which belonged to the FOSC, followed by FSAMSC, FFSC, and FCSC. FOSC species can be found almost in every soil around the world and the species that were identified in the current study included *F. callistephi*, *F. carminascens*, *F. glycines*, *F. grosmichelii*, *F. nirenbergiae*, and *F. tardicrescens*. Although most of the *Fusarium* species isolated from this study were from FOSC, none of them belonged to the *Fusarium oxysporum sensu* stricto (Lombard *et al.*, 2019). All the identified species belonged to the *Fusarium oxysporum sensu lato* with *F. tardicrescens* which has been recently described by Maryani *et al.* (2018) being most common. Cobo-Díaz *et al.* (2019) who investigated *Fusarium* composition in maize soils and residues using MiSeq metabarcoding sequencing reported a high abundance of *F. oxysporum* in soil samples. However, Ge *et al.* (2018) reported high abundance of *F. verticillioides*, *F. equiseti*, and *F. graminearum* in maize rhizosphere soil in China. Other *Fusarium* species that were isolated included *F. atrovinosum*, *F. boothii*, *F. chlamydosporum*, *F. clavus, F. equiseti*, *F. subglutinans*, *F. sporodochiale*, *F. temperatum*, and *F. transvaalense*. These results partially agree with those of Chehri (2011) who reported *F. chlamydosporum*, *F. equiseti*, *F. subglutinans* along with other species on maize rhizosphere soil when surveying *Fusarium* associated with economically important

agricultural crops in Iran. Rheeder & Marasas (1998) also reported *F. chlamydosporum*, *F. equiseti*, *F. oxysporum*, *F. subglutinans*, *N. solani* (F. solani) and other 11 species from plant debris in soil samples collected from cultivated maize fields in the Eastern Cape, South Africa. There is also a putative new species, *Fusarium* sp. nov.1 isolated, but that needs a further study for confirmation.

Several species that were isolated from this survey have been reported to cause diseases in maize. Species from FOSC are reported to infect more than a wide range of host plants (Michielse & Rep, 2009), however, not all of them are pathogens. *Fusarium oxysporum* typically causes wilt and stalk rot in many crops and was included in the top ten fungal pathogens known (Dean *et al.*, 2012). Several species from FSAMSC and FFSC have been reported as plant pathogens and are producers of important mycotoxins (Aoki *et al.*, 2014, Niehaus *et al.*, 2016, Laraba *et al.*, 2021, Yilmaz *et al.*, 2021). *Fusarium boothii*, *F. subglutinans*, and *F. temperatum* were among the species that were isolated in this study, and they are known to cause maize ear rots (Sampietro *et al.*, 2011, Czembor *et al.*, 2014). *Fusarium chlamydosporum* from FCSC has been isolated with other *Fusarium* species in diseased maize kernels (Morales-Rodríguez *et al.*, 2007, Fu *et al.*, 2015). These pathogens can overwinter in the soil and on maize residues, and later their spores are disseminated by air to infect the above-ground maize plant tissues including maize ears. *Fusarium subglutinans* and *F. temperatum* are also known to cause stalk, root, and crown rots in maize. *Fusarium equiseti* from FIESC was recently reported as a causal agent of maize stalk rot in India (Swamy *et al.*, 2020). Two other species, *Neocosmospora falciformis* and *Setophoma terrestris* were present in the soil and they have been reported to cause maize stalk rot and red root rot, respectively (Mao *et al.*, 1998, Koenning *et al.*, 2007, Douriet-Angulo *et al.*, 2019). The presence of potential pathogens may be of concern, but it is worth noting that their presence does not necessarily mean that they will cause diseases. It is our opinion that many of the species detected in our survey are common and widely occurring soil-borne species and that future studies should look into the biotic and abiotic factors that will make these strains/species pathogenic.

Trichoderma was the third most commonly found genus with a total of eight species identified. *Trichoderma* species are generally considered avirulent plant symbionts in soil (Harman *et al.*, 2004) and several species have been applied as biocontrol agents

against numerous plant pathogens (Samuels, 1996). *Trichoderma afroharzianum* was the most commonly isolated species. This species is able to promote plant growth (Juan *et al.*, 2021) by upregulating functional genes related to plant growth. *Trichoderma afroharzianum* is also one of the more commonly used biocontrol agents against several plant pathogens (Zhang *et al.*, 2015, Mokhtari *et al.*, 2017, Stummer *et al.*, 2020). However, recent studies reported that *T. afroharzianum* may cause maize ear rot (Pfordt *et al.*, 2020, Sanna *et al.*, 2022). Therefore, it is vital not to overrule the pathogenetic effect of *T. afroharzianum* when applying it as biological control agent. *Trichoderma gamsii* and *T. hamatum* were also commonly isolated, followed by *T. rifaii*, *T. arundinaceum*, *T. saturnisporum*, *T. spirale* and *T. virens*. All these species except for *T. rifaii* and *T. saturnisporum* have been previously tested as biological control agents with promising results against a wide range of pathogens of many crops (Chet *et al.*, 1981, Howell *et al.*, 2000, Malmierca *et al.*, 2013, Chen *et al.*, 2016, Baiyee *et al.*, 2019). Other potentially beneficially fungi that were identified included *Beauveria bassiana*, *Clonostachys rosea, Metarhizium anisopliae*, and *M. pinghaense*. *Beauveria bassiana* and *M. anisopliae* are entomopathogens and have been explored for their potential control of agricultural destructive insects (Cherry *et al.*, 2004, Ravindran *et al.*, 2015). *Clonostachys rosea* is a mycoparasite of other fungi and nematodes. Its properties were tested and proved to be effective in the management of fungal and nematodes pathogens (Cota *et al.*, 2008, Zhang *et al.*, 2008, Rodriguez *et al.*, 2011, Iqbal *et al.*, 2018).

This is the first survey to use modern taxonomic approaches to identify and characterise fungal communities from maize farms in South Africa. Due to a lack of modern tools for fungal identifications, previous studies surveying maize rhizosphere fungal diversity identified strains solely based on morphology, while more recent surveys identified fungi based on ITS sequencing. However, morphological identifications are notoriously difficult for even experienced taxonomists, while ITS cannot differentiate between closely related species of common genera like *Fusarium*, *Penicillium*, and *Trichoderma* (Raja *et al.*, 2017). As a result, we felt it necessary to complete a survey based on modern taxonomic approaches in order to obtain a better understanding of the fungi present in maize rhizosphere soils. In the process, this survey expanded biological resources with strains preserved in cultures collections that will serve as important reference material for future studies, while the 460 new

DNA sequences generated help capture infraspecies variation that will make future identifications easier. We also discovered several putative new *Penicillium* and *Fusarium* species, that will be described elsewhere.

Conclusion

Fungal diversity was found to be relatively high for agricultural soils. Fungal communities were dominated by *Fusarium*, *Penicillium*, and *Trichoderma*. Among isolated *Fusarium* species, *F. subglutinans*, and *F. temperatum* can cause stalk rot, and root and crown rot in maize. However, there are no reports of *Penicillium* species causing soilborne diseases in maize and we speculate that they may, in fact, be beneficial to plants in soils (e.g. what role does *P. raperi* play). Other potential plant pathogenic species of maize identified, included *Neocosmospora falciformis* and *Setophoma terrestris*. *Beauveria bassiana*, *Clonostachys rosea*, *Metarhizium anisopliae*, *Trichoderma hamatum*, and *T. gamsii* have been previously reported as potential biological control agents. These findings provide the baseline knowledge needed to start understanding fungal community compositions of maize rhizosphere soils and give us the chance to speculate into the potential impact that these communities may have. Future studies that use both culture-dependent and independent approaches should be conducted in order to survey farms more broadly and determine how and why communities may change between growing seasons.

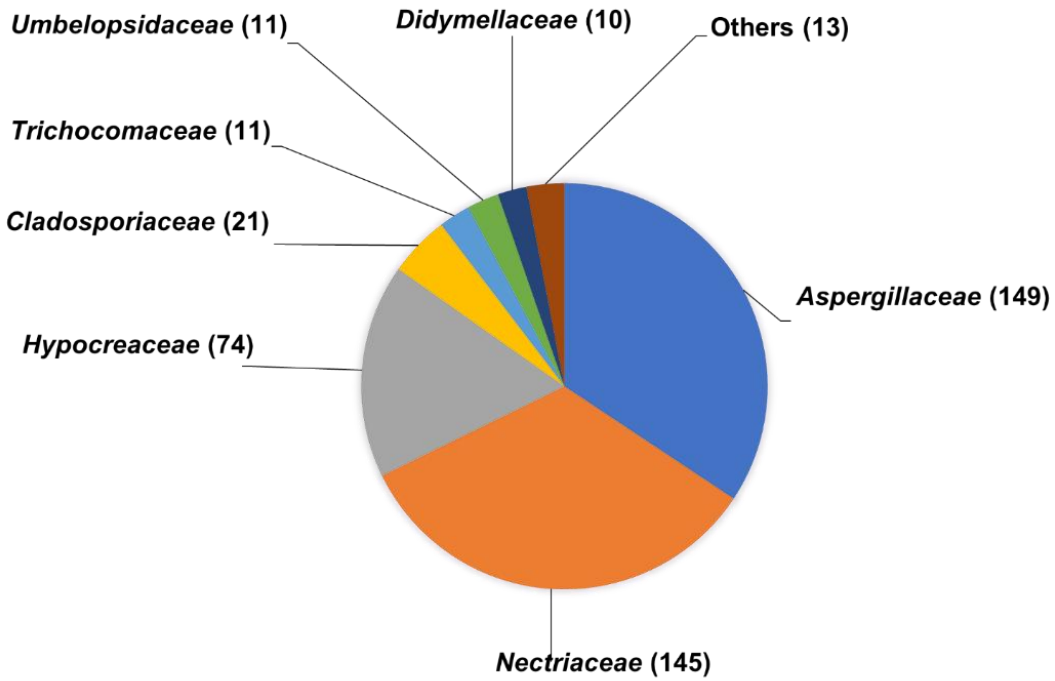

Figure 1: The proportion of fungal strains in maize soils identified to family-level. "Others" include families with less than ten fungal strains (*Aureobasidiaceae*, *Bionectriaceae*, *Chaetomiaceae*, *Clavicipitaceae*, *Cordycipitaceae*, *Cunninghamellaceae*, *Didymosphaeriaceae*, *Mortierellaceae*, *Myrmecridiaceae*, *Ophiocordycipitaceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Stachybotryaceae*). Numbers between brackets represent the number of strains obtained for each family.

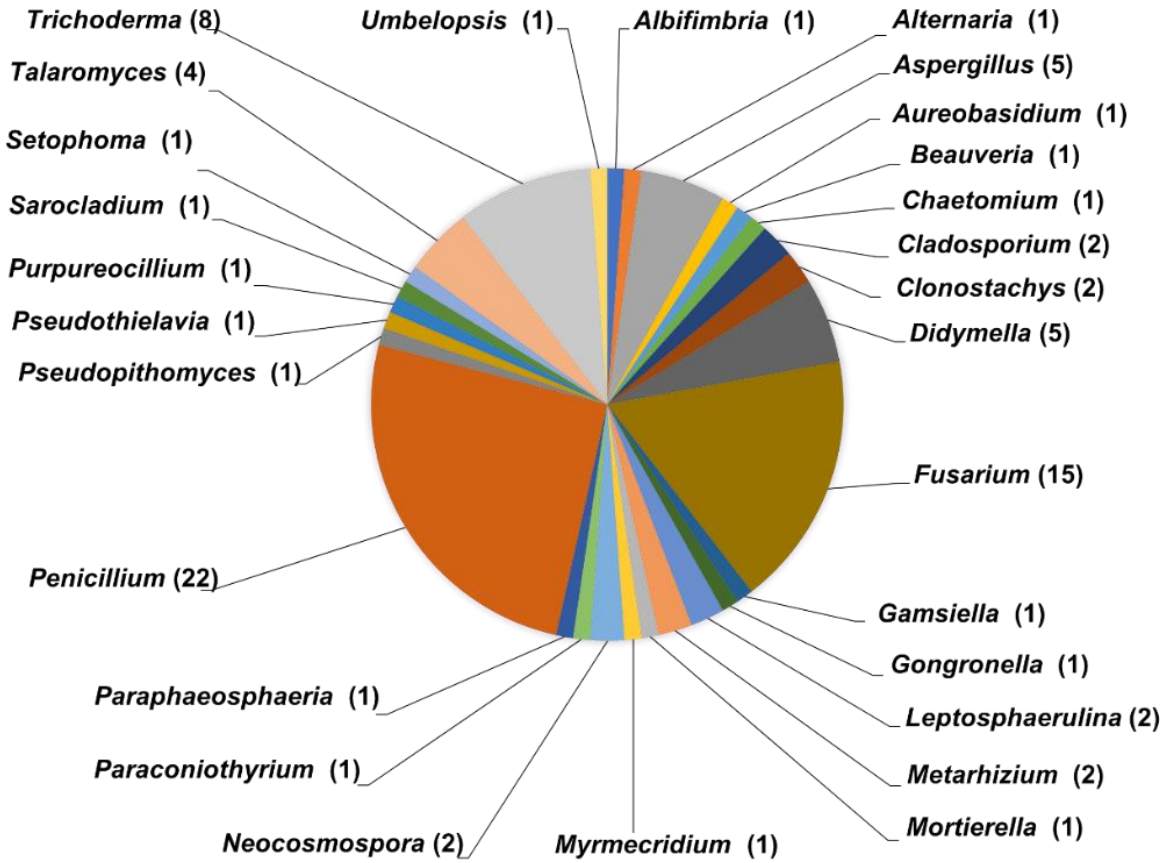

Figure 2: Genera that were found in maize agricultural soils. Numbers between brackets represent the number of species obtained for each genus.

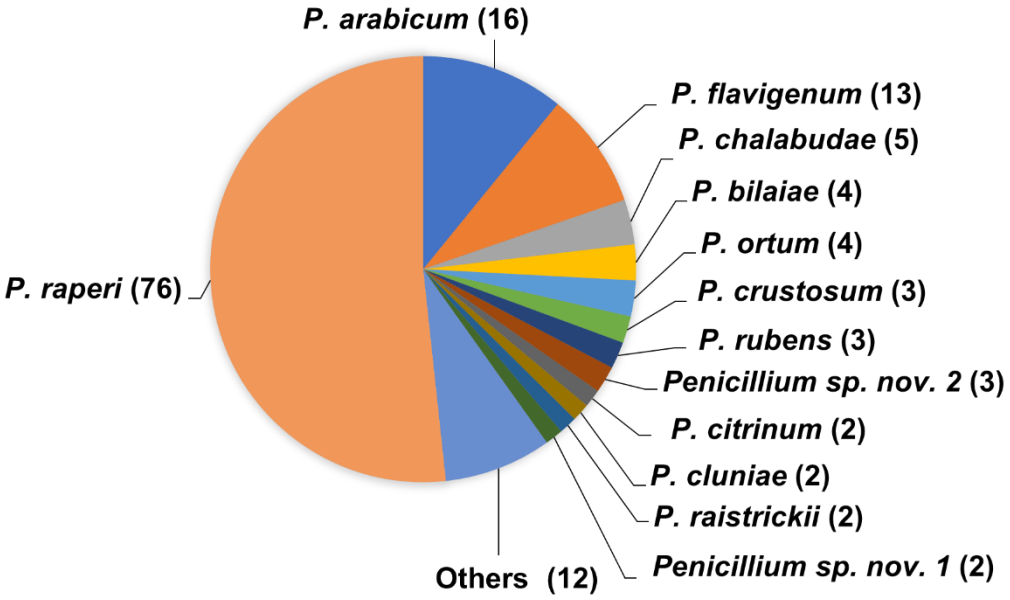

Figure 3: *Penicillium* species isolated from maize agricultural soils. Others = Species that had only one strain isolated (*Penicillium sp. nov.* 3, *P. atrovenetum*, *P. brevicompactum*, *P. desertorum*, *P. frequentans*, *P. griseofulvum*, *P. murcianum*, *P. olsonii*, *P. sacculum*, *P.*

sizovae, *P. striatisporum*, *P. yunnanense*). Numbers between brackets represent the number of strains obtained for each species.

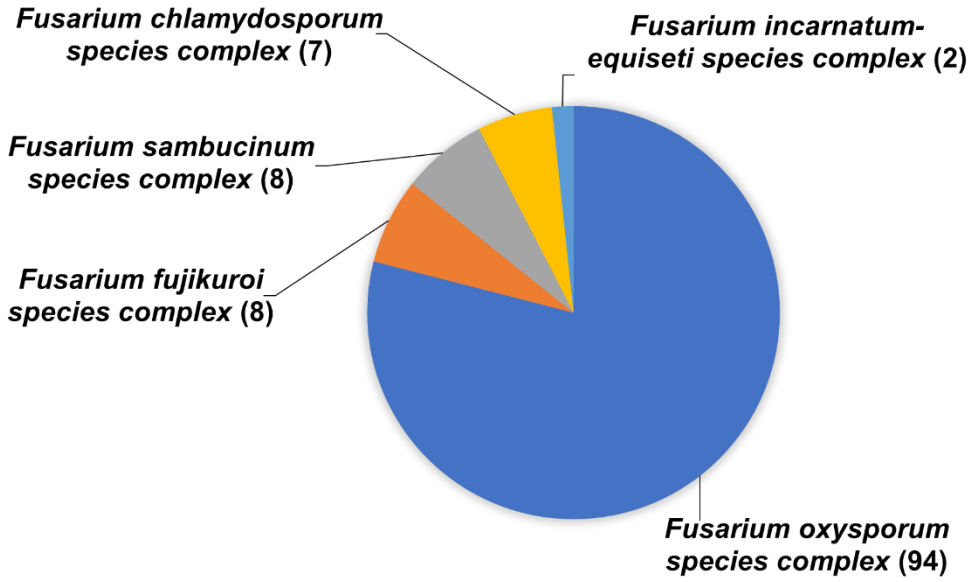

Figure 4: Species complexes isolated in maize agricultural soils. Numbers represent the number of strains obtained for each species complex.

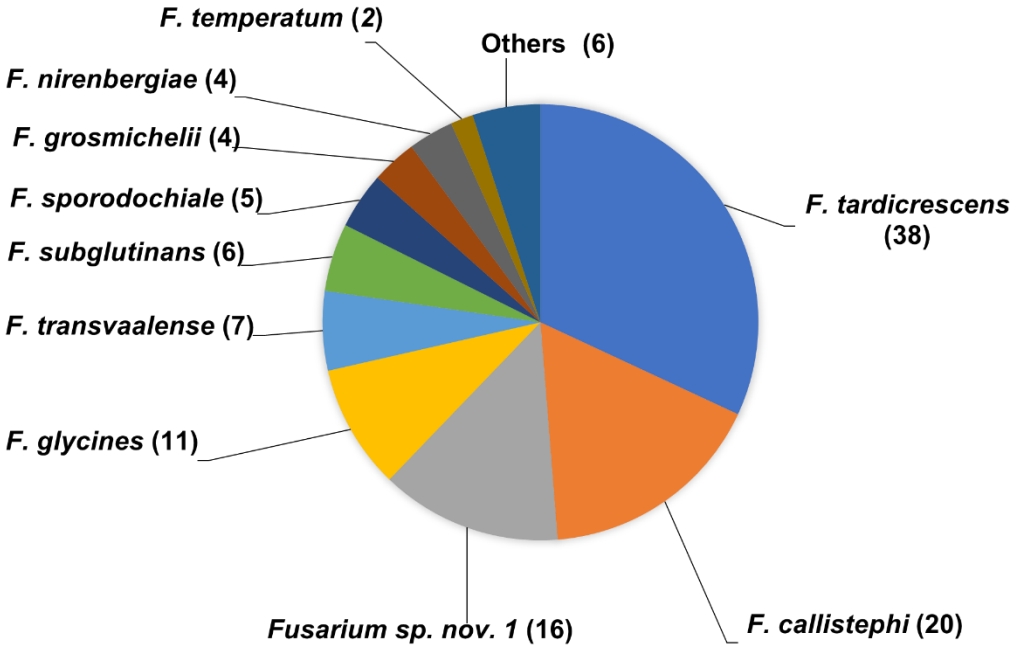

Figure 5: *Fusarium* species isolated in maize agricultural soils. "Other" include species that had only one strain isolated (*F. atrovinosum*, *F. boothii*, *F.chlamydosporum*, *F. clavus*, *F.*

equiseti, and *F. carminascens*). Numbers between brackets represent the number of strains obtained for each species.

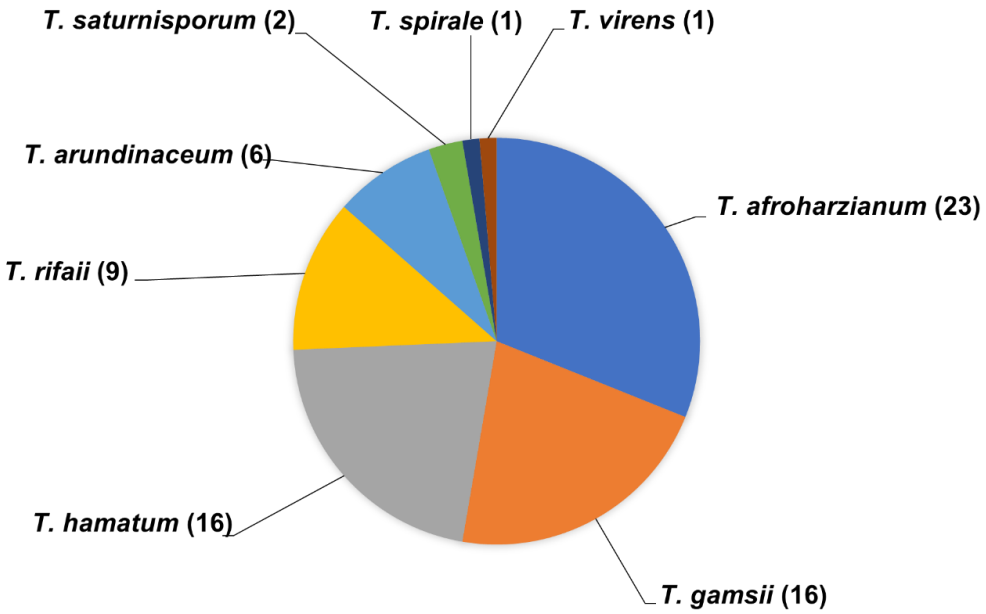

Figure 6: *Trichoderma* species isolated from maize agricultural soils. Numbers between brackets represent the number of strains obtained for each species.

Table 1: Primers used for PCR for different genera

Genus and marker (s)	Primer	Primer (5' to 3')	Reference
Aspergillus	CMD5	CCG AGT ACA AGG ARG CCT TC	(Hong *et al.*, 2006)
(*CaM*)	CMD6	CCG ATR GAG GTC ATR ACG TGG	(Hong *et al.*, 2006)
Cladosporium	EF1-728F	CATCGAGAAGTTCGAGAAGG	(Carbone & Kohn, 1999)
Trichoderma	EF2	GGARGTACCAGTSATCATGTT	(O'Donnell *et al.*, 1998)
(*tef1*)			
Fusarium	fusEF1	ATGGGTAAGGARGACAAGAC	(O'Donnell *et al.*, 1998)
(*tef1*)	fusEF2	GGARGTACCAGTSATCATGTT	(O'Donnell *et al.*, 1998)
Penicillium	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	(Glass & Donaldson, 1995)
(*tub2*/*BenA*)	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	(Glass & Donaldson, 1995)
Other genera	V9G	TTACGTCCCTGCCCTTTGTA	(De Hoog & Van den Ended, 1998)
(ITS)	LS266	GCATTCCCAAACAACTCGACTC	(Masclaux *et al.*, 1995)

Table 2: Thermocycler protocols followed for the amplification of identification markers

DNA region	Primer pairs	PCR thermocycle protocol
CaM	CMD5; CMD6	94 °C 5 min; 35 cycles of 95 °C 45 sec, 55 °C 45 sec, 72 °C 60 s; 72 °C 7 min
tub2/BenA	Bt2a; Bt2b	94 °C 5 min; 35 cycles of 95 °C 45 sec, 55 °C 45 sec, 72 °C 60 sec; 72 °C 7 min
tef1 (for *Fusarium*)	fusEF1; fusEF2	94 °C 5 min; 35 cycles of 94 °C 45 s, 52 °C 45 sec, 72 °C 90 sec; 72 °C 8 min
tef1 (for *Cladosporium* and *Trichoderma*)	EF1-728F; EF2	94 °C 5 min; 35 cycles of 94 °C 45 sec, 52 °C 45 sec, 72 °C 90 s; 72 °C 8 min
ITS	V9G; LS266	94 °C 5 min; 35 cycles of 95 °C 45 sec, 55 °C 45 sec, 72 °C 60 sec; 72 °C 7 min

Table 3: Genera and fungal species that were identified in 19 maize agricultural farms and CN collection numbers allocated to strains that were preserved.

Species name	Number of strains	Isolation frequency	CN number
Albifimbria	**1**		
Albifimbria verrucaria	1	1/19	CN030B3
Alternaria	**1**		
Alternaria alternata	1	1/19	CN042F7
Aspergillus	**9**		
Aspergillus calidoustus	1	1/19	CN029G4
Aspergillus dimorphicus	1	1/19	CN033A5
Aspergillus pseudodeflectus	4	4/19	CN051D8, CN069G5, CN079D9, CN094H1
Aspergillus terreus	2	1/19	CN079E1, CN069C1
Aspergillus tubingensis	1	1/19	CN069B8
Aureobasidium	**1**		
Aureobasidium melanogenum	1	1/19	CN029C9
Beauveria	**1**		
Beauveria bassiana	1	1/19	CN032F8
Chaetomium	**1**		

Chaetomium madrasense	1	1/19	CN042G2
Cladosporium	**21**		
Cladosporium cladosporioides	7	5/19	CN029D7, CN033A7, CN033A8, CN033A9, CN041H9, CN070B7, CN094B4
Cladosporium pseudocladosporioides	14	8/19	CN029D6, CN033A6, CN033B1, CN033B4, CN033B6, CN042F6, CN069D1, CN069E8, CN070B5, CN070B8, CN078G7, CN078G8, CN078H1, CN079D2
Clonostachys	**3**		
Clonostachys rhizophaga	1	1/19	CN082A8
Clonostachys rosea	2	1/19	CN070C3, CN070D3
Didymella	**7**		
Didymella dimorpha	1	1/19	CN042H3
Didymella glomerata	1	1/19	CN079C9
Didymella pedeiae	1	1/19	CN029I9
Didymella prosopidis	3	3/19	CN029D4, CN029G5, CN029G6
Didymella subherbarum	1	1/19	CN029C6
Fusarium	**119**		
Fusarium atrovinosum (FCSC)	1	1/19	CN038I2
Fusarium boothii (FSAMSC)	1	1/19	CN039E2
Fusarium chlamydosporum (FCSC)	1	1/19	CN038I4

Fusarium callispethi (FOSC)	20	8/19	CN038H8, CN038I3, CN039A3, CN039A4, CN039A9, CN039B6, CN039C3, CN039C7, CN039D8, CN051B5, CN051B6, CN051C2, CN051F7, CN081G1, CN081G6, CN081H9, CN081I1, CN081I7, CN082A2, CN082C4
Fusarium carminascens (FOSC)	1	1/19	CN081H8
Fusarium clavus (FIESC)	1	1/19	CN082B3
Fusarium equiseti (FIESC)	1	1/19	CN081G9
Fusarium glycines (FOSC)	11	5/19	CN039B1, CN081H7, CN038I8, CN038I9, CN039A1, CN039A2, CN039A5, CN039A6, CN039B4, CN051E8, CN081I3
Fusarium grosmichelii (FOSC)	4	3/19	CN038I6, CN039B5, CN081G3, CN081I6
Fusarium nirenbergiae (FOSC)	4	2/19	CN038H7, CN051F2, CN051F6, CN082B2
Fusarium sp. nov. 1 (FOSC)	16	6/19	CN038I7, CN039A7, CN039B3, CN039B9, CN039C4, CN039C6, CN039D4, CN039D6, CN039D9, CN039E1, CN051E5, CN081G4, CN081G5, CN081G8, CN081I8, CN082B7
Fusarium sporodochiale (FCSC)	5	2/19	CN039B7, CN039B8, CN051B9, CN051E4, CN051F3
Fusarium subglutinans (FFSC)	6	4/19	CN039E6, CN051C9, CN051D4, CN051D6, CN051F4, CN082B6,
Fusarium tardicrescens (FOSC)	38	11/19	CN038H6, CN038H9, CN038I1, CN039A8, CN039B2, CN039C1, CN039C2, CN039E3, CN039E4, CN051B7, CN051C1, CN051C3,

			CN051C4, CN051C5, CN051C6, CN051C7, CN051C8, CN051D1, CN051D3, CN051D5, CN051D7, CN051E2, CN051E6, CN051E7, CN051E9, CN081F9, CN081G2, CN081H3, CN081H5, CN081I2 CN081I5, CN082A1, CN082A3, CN082A4, CN082A6, CN082B5, CN082B8, CN082B9
Fusarium temperatum (FFSC)	2	1/19	CN081H4, CN081H6
Fusarium transvaalense (FSAMSC)	7	6/19	CN039C8, CN039C9, CN039D3, CN051B8, CN082A5, CN082B4, CN082C1
Gamsiella	**4**		
Gamsiella stylospora	4	2/19	CN033E8, CN033F8, CN094F3, CN094G7
Gongronella	**1**		
Gongronella butleri	1	1/19	CN094F8
Leptosphaerulina	**3**		
Leptosphaerulina briosiana	2	2/19	CN042H2, CN079A6
Leptosphaerulina chartarum	1	1/19	CN069C3
Metarhizium	**3**		
Metarhizium anisopliae	2	2/19	CN094G8, CN094H3
Metarhizium pinghaense	1	1/19	CN032G9
Mortierella	**1**		

Mortierella alpina	1	1/19	CN029I6
Myrmecridium	**1**		
Myrmecridium schulzeri	1	1/19	CN029G7
Neocosmospora	**26**		
Neocosmospora falciformis	2	2/19	CN038I5, CN081I4
Neocosmospora solani	24	15/19	CN038H5, CN039C5, CN039D1, CN039D2, CN039D5, CN039D7 CN039E5, CN039E7, CN051B2, CN051B3, CN051B4, CN051D2, CN051D9, CN051E1, CN051E3, CN051F5, CN081G7, CN081H1, CN081H2, CN082A7, CN082B1, CN082C2, CN082C3, CN082C5
Paraconiothyrium	**6**		
Paraconiothyrium thysanolaenae	6	3/19	CN069F1, CN069F8, CN070C6, CN070C7, CN070D2, CN078H3
Paraphaeosphaeria	**1**		
Paraphaeosphaeria sporulosa	1	1/19	CN078H5
Penicillium	**147**		
Penicillium arabicum	16	8/19	CN029C4, CN029F5, CN032G6, CN032H6, CN032H7, CN032H8, CN032I8, CN033A3, CN033A4, CN042B1, CN042E1, CN069G9, CN094C9, CN094E2, CN094E4, CN094E5
Penicillium atrovenetum	1	1/19	CN069H6
Penicillium bilaiae	4	3/19	CN029F7, CN032I3, CN042C6, CN094D9

Penicillium brevicompactum	1	1/19	CN033A2
Penicillium chalabudae	5	3/19	CN029C5, CN078F6, CN078G4, CN078I5, CN078I6
Penicillium citrinum	2	2/19	CN094D2, CN094E3
Penicillium cluniae	2	2/19	CN070C5, CN078H8
Penicillium crustosum	3	2/19	CN032F7, CN032H1, CN042B2
Penicillium desertorum	1	1/19	CN069B6
Penicillium flavigenum	13	6/19	CN029F6, CN029F8, CN029G2, CN029G3, CN042B3, CN042D1, CN042D4, CN042D8, CN042E6, CN042F2, CN069I5, CN070A5, CN078G3
Penicillium frequentans	1	1/19	CN079F3
Penicillium griseofulvum	1	1/19	CN069H7
Penicillium murcianum	1	1/19	CN069B4
Penicillium olsonii	1	1/19	CN029G1
Penicillium ortum	4	2/19	CN070A6, CN079E2, CN079E3, CN079E4
Penicillium raistrickii	2	1/19	CN042B4, CN042B7
Penicillium raperi	76	19/19	CN029C2, CN029C3, CN029C8, CN029H9, CN029I1, CN030A1, CN030A2, CN030A3, CN030A4, CN030A5, CN030A9, CN030B1, CN030B2, CN032F9, CN032G1, CN032G3, CN032G5, CN032G7, CN032G8, CN032H2, CN032H3, CN032H4, CN032H5, CN032H9, CN032I1, CN032I2, CN032I4, CN033A1, CN033E5, CN033F5,

			CN033G3, CN042C2, CN042F3, CN042G1, CN042G4, CN042G5, CN042G7, CN069A6, CN069B1, CN069F7, CN069F9, CN069G4, CN069H2, CN069H9, CN070D4, CN070D5, CN070D8, CN078G1, CN078I1, CN078I3, CN079A2, CN079A3, CN079A5, CN079B1, CN079B5, CN079B6, CN079B7, CN079F6, CN094C4, CN094C5, CN094C6, CN094C7, CN094C8, CN094D1, CN094D3, CN094D5, CN094D7, CN094D8, CN094E1, CN094E7, CN094E9, CN094F1, CN094F5, CN094F6, CN094H4
Penicillium rubens	3	3/19	CN032G2, CN042F4, CN070A2
Penicillium sacculum	1	1/19	CN070D9
Penicillium sizovae	1	1/19	CN029F9
Penicillium striatisporum	1	1/19	CN078H2
Penicillium yunnanense	1	1/19	CN079F2
Penicillium sp. nov. 1	2	1/19	CN069I8, CN078F7
Penicillium sp. nov. 2	3	1/19	CN070B2, CN079E8, CN079E9
Penicillium sp. nov. 3	1	1/19	CN069A9
Pseudopithomyces	**2**		
Pseudopithomyces karoo	2	2/19	CN069F2, CN079A8
Pseudothielavia	**1**		
Pseudothielavia arxii	1	1/19	CN042C9

Purpureocillium	**1**		
Purpureocillium lilacinum	1	1/19	CN070D7
Sarocladium	**1**		
Sarocladium zeae	1	1/19	CN030A7
Setophoma	**3**		
Setophoma terrestris	3	2/19	CN070E1, CN070E2, CN079A9
Talaromyces	**11**		
Talaromyces pinophilus	1	1/19	CN079B9
Talaromyces purpureogenus	6	5/19	CN032G4, CN032I5, CN032I6, CN079B2, CN094C1, CN094H2
Talaromyces stollii	3	2/19	CN032I7, CN032I9, CN042B5
Talaromyces veerkampii	1	1/19	CN094E6
Trichoderma	**73**		
Trichoderma afroharzianum	23	13/19	CN029B5, CN029B7, CN029E5, CN029E6, CN029F2, CN033C1, CN033C9, CN041H1, CN041I3, CN069D9, CN069E6, CN078I8, CN079C6, CN093H5, CN093H6, CN093H8, CN093I5, CN093I6, CN093I7, CN093I8, CN094A4, CN094A6, CN094B1
Trichoderma arundinaceum	6	4/19	CN078G5, CN079C2, CN093I9, CN094A1, CN094A2, CN094A3

Trichoderma gamsii	16	8/19	CN029B8, CN029C1, CN029E1, CN029E9, CN033C5, CN033C6, CN033D5, CN033D8, CN041G8, CN041I9, CN070B6, CN079C4, CN093G8, CN094A8, CN094A9, CN094B2
Trichoderma hamatum	16	6/19	CN029A9, CN029B2, CN029E4, CN041G1, CN041G2, CN041H3, CN041H5, CN093G5, CN093G6, CN093G7, CN093H1, CN093H2, CN093H3, CN093I2, CN093I3, CN093I4
Trichoderma rifaii	8	5/19	CN041G9, CN041I1, CN069C8, CN070B9, CN078G6, CN079C8 CN093H7, CN094A7
Trichoderma saturnisporum	2	1/19	CN093H9, CN093I1
Trichoderma spirale	1	1/19	CN029B3
Trichoderma virens	1	1/19	CN094A5
Umbelopsis	**11**		
Umbelopsis vinacea	11	5/19	CN029D2, CN029D3, CN029I4, CN029I5, CN033E6, CN033E7 CN033E9, CN033G4 CN042G6, CN070D6, CN079E7

*: Abbreviations: FCSC = *Fusarium chlamydosporum* species complex; FFSC = *Fusarium fujikuro*i species complex; FIESC = *Fusarium incarnatum-equiseti* species complex; FOSC = *Fusarium oxysporum* species complex; FSAMSC = *Fusarium sambucinum* species complex.

References

Acosta-Martinez V, Cotton J, Gardner T, Moore-Kucera J, Zak J, Wester D & Cox S (2014) Predominant bacterial and fungal assemblages in agricultural soils during a record drought/heat wave and linkages to enzyme activities of biogeochemical cycling. *Applied Soil Ecology* **84**: 69-82.

Adeyemi NO, Atayese MO & Olubode AA (2019) Identification and relative abundance of native arbuscular mycorrhizal fungi associated with oil-seed crops and maize (*Zea mays L*.) in derived savannah of Nigeria. *Acta fytotechn zootechn* **22**: 84-89.

Adnan M & Bilal HM (2020) Role of Boron Nutrition on Growth, Phenology and Yield of Maize (*Zea Mays* L.) Hybrids: A Review. *Open Access Journal of Biogeneric Science and Research* **4**: 1-8.

Angst G, Mueller KE, Nierop KG & Simpson MJ (2021) Plant-or microbial-derived? A review on the molecular composition of stabilized soil organic matter. *Soil Biology and Biochemistry* **156**: 108189. doi.org/10.1016/j.soilbio.2021.108189.

Aoki T, O'Donnell K & Geiser DM (2014) Systematics of key phytopathogenic *Fusarium* species: current status and future challenges. *Journal of General Plant Pathology* **80**: 189-201.

Bai J, Lin X, Yin R, Zhang H, Junhua W, Xueming C & Yongming L (2008) The influence of arbuscular mycorrhizal fungi on As and P uptake by maize (*Zea mays* L.) from As-contaminated soils. *Applied Soil Ecology* **38**: 137-145.

Baiyee B, Pornsuriya C, Ito S-i & Sunpapao A (2019) *Trichoderma spirale* T76-1 displays biocontrol activity against leaf spot on lettuce (Lactuca sativa L.) caused by *Corynespora cassiicola* or *Curvularia aeria*. *Biological control* **129**: 195-200.

Bell T, Gessner MO, Griffiths RI, McLaren JR, Morin PJ, Van Der Heijden M & van der Putten W (2009) Microbial biodiversity and ecosystem functioning under controlled conditions and in the wild. *Biodiversity, ecosystem functioning, and human wellbeing: an ecological and economic perspective* **30**: 121-133.

Beyers KM (2019) Quantifying twelve fungal isolates associated with maize root and crown rot complex in South Africa. Thesis, North-West University (South Africa).

Bissett J, Gams W, Jaklitsch W & Samuels GJ (2015) Accepted *Trichoderma* names in the year 2015. *IMA fungus* **6**: 263-295.

Carbone I & Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **91**: 553-556.

Cavaglieri L, Orlando J & Etcheverry M (2009) Rhizosphere microbial community structure at different maize plant growth stages and root locations. *Microbiological Research* **164**: 391-399.

Chávez-Díaz IF, Rios-Galicia B, Blanco-Camarillo M, Cruz-Cárdenas CI, Sandoval-Cancino G, Rojas-Anaya E, Gómez-Godínez LJ, Arteaga-Garibay RI & Zelaya-Molina LX (2021) Maize landrace rhizospheric fungi with biocontrol potential against four different *Fusarium* species complexes. *Biocontrol Science and Technology* **31**: 754-772.

Chehri K (2011) Occurrence of *Fusarium* species associated with economically important agricultural crops in Iran. *African Journal of Microbiology Research* **5**: 4043-4048.

Chen J-L, Sun S-Z, Miao C-P, Wu K, Chen Y-W, Xu L-H, Guan H-L & Zhao L-X (2016) Endophytic *Trichoderma gamsii* YIM PH30019: a promising biocontrol agent with hyperosmolar, mycoparasitism, and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of Panax notoginseng. *Journal of Ginseng Research* **40**: 315-324.

Cherry AJ, Banito A, Djegui D & Lomer C (2004) Suppression of the stem-borer Sesamia calamistis (Lepidoptera; Noctuidae) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. *International Journal of Pest Management* **50**: 67-73.

Chet I, Harman G & Baker R (1981) **Trichoderma hamatum**: Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. *Microbial Ecology* **7**: 29-38.

Classen AT, Sundqvist MK, Henning JA, Newman GS, Moore JA, Cregger MA, Moorhead LC & Patterson CM (2015) Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere* **6**: 1-21.

Cobo-Díaz JF, Baroncelli R, Le Floch G & Picot A (2019) A novel metabarcoding approach to investigate *Fusarium* species composition in soil and plant samples. *FEMS Microbiology Ecology* **95**: 1-13.

Cota LV, Maffia LA, Mizubuti ES, Macedo PE & Antunes RF (2008) Biological control of strawberry gray mold by *Clonostachys rosea* under field conditions. *Biological Control* **46**: 515-522.

Crous PW, Lombard L, Sandoval-Denis M, Seifert K, Schroers H-J, Chaverri P, Gené J, Guarro J, Hirooka Y & Bensch K (2021) *Fusarium*: more than a node or a foot-shaped basal cell. *Studies in Mycology* **98**: 100116.

Czembor E, Stępień Ł & Waśkiewicz A (2014) *Fusarium temperatum* as a new species causing ear rot on maize in Poland. *Plant Disease* **98**: 1001-1001.

De Hoog G & Van den Ended A (1998) Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *MYCOSES-BERLIN-* **41**: 183-190.

Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R & Ellis J (2012) The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology* **13**: 414-430.

Douriet-Angulo A, López-Orona C, López-Urquídez G, Vega-Gutiérrez T, Tirado-Ramírez M, Estrada-Acosta M, Ayala-Tafoya F & Yáñez-Juárez M (2019) Maize stalk rot caused by *Fusarium falciforme* (FSSC 3+ 4) in Mexico. *Plant Disease* **103**: 2951.

Eicker A (1970) Ecological observations on soil fungi. *South African Journal of Science* **66**: 327.

Evelin H, Kapoor R & Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Annals of botany* **104**: 1263-1280.

Fixen PE & West FB (2002) Nitrogen fertilizers: meeting contemporary challenges. *Ambio: A Journal of the Human Environment* **31**: 169-176.

Fu M, Li R, Guo C, Pang M, Liu Y & Dong J (2015) Natural incidence of *Fusarium* species and fumonisins B1 and B2 associated with maize kernels from nine provinces in China in 2012. *Food Additives and Contaminants: Part A* **32**: 503-511.

Gaddeyya G, Niharika PS, Bharathi P & Kumar PR (2012) Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. *Advances in Applied Science Research* **3**: 2020-2026.

Ge B, Wang B, Guo C, Sun S, Chen G, Wang X, Zhu Z & Duan C (2018) Composition and quantitative analysis of *Fusarium* species in maize rhizosphere soil. *Scientia Agricultura Sinica* **51**: 3683-3693.

Glass NL & Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and environmental microbiology* **61**: 1323-1330.

Gómez-Muñoz B, Jensen LS, De Neergaard A, Richardson A & Magid J (2018) Effects of Penicillium bilaii on maize growth are mediated by available phosphorus. *Plant and Soil* **431**: 159-173.

GrainSA (2020) GrainSA annual report [Online]. Available: https://www.grainsa.co.za/annualreport_2020/Grain%20SA%20Annual%20Report%202020/files/assets/common/downloads/publication.pdf [Accessed 19 November 2021]

GrainSA (2021) GrainSA annual report 2021 [Online]. Available: https://www.grainsa.co.za/annualreport_2021/Graan%20SA%20Jaarverslag%202021/ [Accessed 11 November 2020]

Hagn A, Pritsch K, Schloter M & Munch JC (2003) Fungal diversity in agricultural soil under different farming management systems, with special reference to biocontrol strains of *Trichoderma* spp. *Biology and fertility of soils* **38**: 236-244.

Harman GE, Howell CR, Viterbo A, Chet I & Lorito M (2004) *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nature reviews microbiology* **2**: 43-56.

Hong S-B, Cho H-S, Shin H-D, Frisvad JC & Samson RA (2006) Novel Neosartorya species isolated from soil in Korea. *International Journal of Systematic and Evolutionary Microbiology* **56**: 477-486.

Houbraken J, Kocsubé S, Visagie CM, Yilmaz N, Wang X-C, Meijer M, Kraak B, Hubka V, Bensch K & Samson R (2020) Classification of *Aspergillus*, *Penicillium*, *Talaromyces* and related genera (Eurotiales): An overview of families, genera, subgenera, sections, series and species. *Studies in Mycology* **96**: 141-153.

Howell C, Hanson L, Stipanovic R & Puckhaber L (2000) Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. *Phytopathology* **90**: 248-252.

Iqbal M, Dubey M, McEwan K, Menzel U, Franko MA, Viketoft M, Jensen DF & Karlsson M (2018) Evaluation of *Clonostachys rosea* for control of plant-parasitic nematodes in soil and in roots of carrot and wheat. *Phytopathology* **108**: 52-59.

Izquierdo I, Caravaca F, Alguacil M, Hernández G & Roldán A (2005) Use of microbiological indicators for evaluating success in soil restoration after revegetation of a mining area under subtropical conditions. *Applied Soil Ecology* **30**: 3-10.

Jeffries P, Gianinazzi S, Perotto S, Turnau K & Barea J-M (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils* **37**: 1-16.

Juan Z, Ting L, LIU W-c, ZHANG D-p, Dan D, WU H-l, ZHANG T-t & LIU D-w (2021) Transcriptomic insights into growth promotion effect of *Trichoderma afroharzianum* TM2-4 microbial agent on tomato plants. *Journal of Integrative Agriculture* **20**: 1266-1276.

Khan SA, Hamayun M, Yoon H, Kim H-Y, Suh S-J, Hwang S-K, Kim J-M, Lee I-J, Choo Y-S & Yoon U-H (2008) Plant growth promotion and *Penicillium citrinum*. *BMC Microbiology* **8**: 1-10.

Khonglah D, Devi HR & Dkhar M (2015) Diversity of Culture Dependent Mycoflora of the Rhizosphere and Non Rhizosphere Soil of Maize (*Zea Mays* L.). *International Journal of Advanced Agricultural Sciences and Technology* **4**: 86-95.

Koenning S, Frye J, Pataky J, Gibbs M & Cotton D (2007) First report of Phoma terrestris causing red root rot on sweet corn (Zea mays) in North Carolina. *Plant Disease* **91**: 1054-1054.

Küçük Ç & Kıvanç M (2011) In vitro interactions and fungal populations isolated from maize rhizosphere. *Journal of Biological Sciences* **11**: 492-495.

Laraba I, McCormick SP, Vaughan MM, Geiser DM & O'Donnell K (2021) Phylogenetic diversity, trichothecene potential, and pathogenicity within *Fusarium sambucinum* species complex. *Plos One* **16**: 1-5.

Leggett M, Newlands N, Greenshields D, West L, Inman S & Koivunen M (2015) Maize yield response to a phosphorus-solubilizing microbial inoculant in field trials. *The Journal of Agricultural Science* **153**: 1464-1478.

Lehmann A, Zheng W & Rillig MC (2017) Soil biota contributions to soil aggregation. *Nature Ecology & Evolution* **1**: 1828-1835.

Lombard L, Sandoval-Denis M, Lamprecht SC & Crous P (2019) Epitypification of *Fusarium oxysporum*–clearing the taxonomic chaos. *Persoonia-Molecular Phylogeny and Evolution of Fungi* **43**: 1-47.

Malmierca MG, Cardoza RE, Alexander NJ, McCormick SP, Collado IG, Hermosa R, Monte E & Gutiérrez S (2013) Relevance of trichothecenes in fungal physiology: disruption of tri5 in *Trichoderma arundinaceum*. *Fungal genetics and biology* **53**: 22-33.

Mao W, Carroll R & Whittington D (1998) Association of *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* in causing red root rot of corn. *Plant disease* **82**: 337-342.

Marasas W, Wehner F, Van Rensburg S & Van Schalkwyk D (1981) Mycoflora of corn produced in human esophageal cancer areas in Transkei, southern Africa. *Phytopathology* **71**: 792-796.

Maryani N, Lombard L, Poerba Y, Subandiyah S, Crous P & Kema G (2018) Phylogeny and genetic diversity of the banana Fusarium wilt pathogen F*usarium oxysporum f. sp. cubense* in the Indonesian centre of origin. *Studies in Mycology* **91**: 79-99.

Masclaux F, Guého E, De Hoog G & Christen R (1995) Phylogenetic relationships of human-pathogenic *Cladosporium* (*Xylohypha*) species inferred from partial LS rRNA sequences. *Journal of Medical and Veterinary Mycology* **33**: 327-338.

Michielse CB & Rep M (2009) Pathogen profile update: *Fusarium oxysporum. Molecular plant pathology* **10**: 311.

Mokhtari W, Chtaina N, Halmschlager E, Volgmayr H, Stauffer C & Jaklitsch W (2017) Potential antagonism of some *Trichoderma* strains isolated from Moroccan soil against three phytopathogenic fungi of great economic importance. *Revue Marocaine des Sciences Agronomiques et Vétérinaires* **5**: 248-254.

Morales-Rodríguez I, de Yañz-Morales MJ, Silva-Rojas HV, García-de-Los-Santos G & Guzman-de-Pena DA (2007) Biodiversity of *Fusarium* species in Mexico associated with ear rot in maize, and their identification using a phylogenetic approach. *Mycopathologia* **163**: 31-39.

Musokwa M, Mafongoya P & Lorentz S (2019) Evaluation of agroforestry systems for maize (*Zea mays*) productivity in South Africa. *South African Journal of Plant and Soil* **36**: 65-67.

Nesci A, Barros G, Castillo C & Etcheverry M (2006) Soil fungal population in preharvest maize ecosystem in different tillage practices in Argentina. *Soil and Tillage Research* **91**: 143-149.

Niehaus E-M, Münsterkötter M, Proctor RH, Brown DW, Sharon A, Idan Y, Oren-Young L, Sieber CM, Novák O & Pěnčík A (2016) Comparative "omics" of the *Fusarium fujikuroi* species complex highlights differences in genetic potential and metabolite synthesis. *Genome Biology and Evolution* **8**: 3574-3599.

Nyongesa BW, Okoth S & Ayugi V (2015) Identification key for *Aspergillus* species isolated from maize and soil of Nandi County, Kenya. *Advances in Microbiology* **5**: 205-229.

O'Donnell K, Kistler HC, Cigelnik E & Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences* **95**: 2044-2049.

Pfordt A, Schiwek S, Karlovsky P & von Tiedemann A (2020) *Trichoderma Afroharzianum* Ear Rot–A new disease on maize in Europe. *Frontiers in Agronomy* **2**: 1-7.

Raja HA, Miller AN, Pearce CJ & Oberlies NH (2017) Fungal identification using molecular tools: a primer for the natural products research community. *Journal of Natural Products* **80**: 756-770.

Ravindran K, Rajkuberan C, Prabukumar S & Sivaramakrishnan S (2015) Evaluation of pathogenicity of *Metarhizium anisopliae* TK-6 against developmental stages of Aedes aegypti and Culex quinquefasciatus. *Journal of Pharmaceutical and Biological Evaluations* **2**: 188-196.

Rheeder J & Marasas W (1998) *Fusarium* species from plant debris associated with soils from maize production areas in the Transkei region of South Africa. *Mycopathologia* **143**: 113-119.

Rodriguez MA, Cabrera G, Gozzo F, Eberlin M & Godeas A (2011) *Clonostachys rosea* BAFC3874 as a *Sclerotinia sclerotiorum* antagonist: mechanisms involved and potential as a biocontrol agent. *Journal of Applied Microbiology* **110**: 1177-1186.

Roux C & Van Warmelo K (1997) A survey of the mycobiota of a natural Karoo pasture. *Bothalia* **27**: 167-183.

Sampietro DA, Díaz CG, González V, Vattuone MA, Ploper LD, Catalán CAN & Ward TJ (2011) Species diversity and toxigenic potential of *Fusarium graminearum* complex isolates from maize fields in northwest Argentina. *International Journal of Food Microbiology* **145**: 359-364.

Samuels GJ (1996) *Trichoderma*: a review of biology and systematics of the genus. *Mycological Research* **100**: 923-935.

Sanna M, Pugliese M, Gullino ML & Mezzalama M (2022) First report of *Trichoderma afroharzianum* causing seed rot on maize in Italy. *Plant Disease* **106**: 1982.

Stummer B, Zhang Q, Zhang X, Warren R & Harvey P (2020) Quantification of *Trichoderma afroharzianum*, *Trichoderma harzianum* and *Trichoderma gamsii* inoculants in soil, the wheat rhizosphere and in planta suppression of the crown rot pathogen *Fusarium pseudograminearum*. *Journal of Applied Microbiology* **129**: 971-990.

Swamy SD, Mahadevakumar S, Hemareddy H, Amruthesh K, Mamatha S, Kunjeti SG, Swapnil R, Kumar TV & Lakshmidevi N (2020) First report of *Fusarium equiseti*–the incitant of post flowering stalkrot of maize (Zea mays L.) in India. *Crop Protection* **129**: 105035.

Swer H, Dkhar M & Kayang H (2014) Fungal population and diversity in organically amended agricultural soils of Meghalaya, India. *Journal of Organic Systems* **6**: 3-12.

Tripathi S, Srivastava P, Devi RS & Bhadouria R (2020) Influence of synthetic fertilizers and pesticides on soil health and soil microbiology. *Agrochemicals Detection, Treatment and Remediation* 25-54.

Wakelin SA, Warren RA, Harvey PR & Ryder MH (2004) Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. *Biology and Fertility of Soils* **40**: 36-43.

Widiati BR, Idrus MI & Imran AN (2015) Isolation and identification of vesicular arbuscular mycorrhizae (VAM) in the rhizosphere of maize (*Zea mays*) in the village of Lekopacing, Tanralili District of the Maros Regency. *International Journal of Science and Research (IJSR)* **4**: 760-765.

Yadav J, Verma J, Yadav S & Tiwari K (2011) Effect of salt concentration and pH on soil inhabiting fungus *Penicillium citrinum* Thom. for solubilization of tricalcium phosphate. *Microbiology journal* **1**: 25-32.

Yilmaz N, Sandoval-Denis M, Lombard L, Visagie CM, Wingfield BD & Crous PW (2021) Redefining species limits in the *Fusarium fujikuroi species complex*. *Persoonia-Molecular Phylogeny and Evolution of Fungi* **46**: 129-162.

Zhang L, Yang J, Niu Q, Zhao X, Ye F, Liang L & Zhang K-Q (2008) Investigation on the infection mechanism of the fungus *Clonostachys rosea* against nematodes using the green fluorescent protein. *Applied Microbiology and Biotechnology* **78**: 983-990.

Zhang X, Harvey PR, Stummer BE, Warren RA, Zhang G, Guo K, Li J & Yang H (2015) Antibiosis functions during interactions of *Trichoderma afroharzianum* and Trichoderma gamsii with plant pathogenic *Rhizoctonia* and *Pythium*. *Functional and Integrative Genomics* **15**: 599-610.

Zhao Y, Fu W, Hu C, Chen G, Xiao Z, Chen Y, Wang Z & Cheng H (2021) Variation of rhizosphere microbial community in continuous mono-maize seed production. *Scientific Reports* **11**: 1-13.

Chapter 3: Oomycete species in soils from the maize fields in the Free State and North West provinces of South Africa

Abstract

Maize is one of South Africa's most produced and economically important grain crops. The Free State and North West provinces are the major maize producing regions. Oomycetes can cause various diseases in maize, including seed rot, root rot, damping off, seedling blight and downy mildews. Many oomycetes are soilborne pathogens that may affect maize plants from pre-emergence to the seedling stage, However, knowledge on which species occurs in South African maize soils are lacking. The aim of this study was thus to survey oomycetes from soils collected across 19 maize farms in the Free State and North West provinces. Isolations were made using a baiting method, and subsequent pure cultures were preserved in a working CN collection. Isolates were identified based on sequences generated for the internal transcribed spacer rDNA (ITS) region of the ribosomal RNA operon. A total of 65 strains were isolated and identified as *Globisporangium irregulare*, *Globisporangium ultimum* and *Pythium torulosum*. *Globisporangium irregulare* was the most frequently isolated (n = 45; isolated from 19/19 farms), followed by *Gl. ultimum* (n = 15; isolated from 7/19 farms) and *Pythium torulosum* (n = 5; isolated from 4/19 farms). All three species have previously been reported to cause root rot or pre- and postemergence damping-off of maize in South Africa.

Keywords: Species identifications, DNA sequencing, Plant pathogens

Introduction

Oomycetes were originally classified as *Phycomycetes* within kingdom Fungi due to their filamentous growth patterns (Rossman & Palm, 2006). However, with modern molecular classifications, taxonomic revisions revealed that oomycetes are more closely related to the heterokont golden-brown algae classified in the *Stramenopiles* (Margulis & Schwartz, 1998, Baldauf *et al.*, 2000) and that they belong within kingdom *Chromista*. Numerous oomycetes are pathogens of a wide range of plants (Martin & Loper, 1999). Oomycetes that are often associated with maize plants include *Pythium* species, *Phytophthora* species, *Sclerophthora zeae* and *Peronosclerospora philippinensis*. Six *Phytophthora* species and *Pythium ultimum* (now known as *Globisporangium ultimum*) were considered to form part of the top ten oomycete plant pathogens (Kamoun *et al.*, 2015). However, *Pythium* species are mostly reported to be pathogenic to maize plants and there is limited information about *Phytophthora* diseases of maize.

Pythium contains many important soilborne pathogens that are a major cause for seed and seedling rot worldwide (Cacciola & Gullino, 2019) and can be found almost in all agricultural soils (Schroeder *et al.*, 2013). *Pythium* species overwinters as primary survival structures called oospores in plant and soil debris (Martin & Loper, 1999). When conditions are favourable, mycelium or sporangia is produced by the germinated oospores to infect seedling roots. *Pythium* species are active at elevated soil moisture levels, however, they are poor competitors compared to other soil-borne fungi under drier conditions (van Agtmaal *et al.*, 2015). Sporangia produce zoospores that can swim towards the host (Walker & van West, 2007). In maize, pathogenic *Pythium* species can cause seed rot, pre- and post-emergence damping off, stalk and root rot, and seedling blight (Chen, 1999, Chen *et al.*, 2005). Symptoms may also include necrosis on the root tips during early infection, or water-soaked lesions on the stalk and roots (Beckerman, 2010). Root rot caused by *Pythium* result in lowered maize yields, especially in poorly drained clay soils continuously planted to maize and/or that use conservation tillage systems (Broders *et al.*, 2009). Several studies have focused on the diversity of *Pythium* in maize seedlings and pathogenicity screening of these on maize plants (Rao *et al.*, 1978, Zhang & Yang, 2000, Soonthornpoct *et al.*, 2001, Schmidt *et al.*, 2020). This is also true for South Africa,

where surveys documenting *Pythium* and *Phytophthora* diversity have been conducted in various plants (Linde *et al.*, 1994, Gull *et al.*, 2004, Belbahri *et al.*, 2008, Binagwa *et al.*, 2016, Bose *et al.*, 2018, Hulbert *et al.*, 2019, Bose *et al.*, 2021). However, little is known about the diversity of these groups associated with maize rhizosphere soils.

Traditionally, oomycetes were classified and identified based on morphological characters such as the shape and type of sporangia (filamentous, inflated, globose), hyphal swelling and the way in which antheridia attach to oogonia (Schroeder *et al.*, 2013). However, overlapping of characters makes interpretation of such morphological data complicated, even for taxonomic experts (Levesque & De Cock, 2004).

Molecular identifications have become one of the most powerful tools for diversity studies. Since DNA sequencing became mainstream during the early 2000's, oomycete taxonomists have also adopted it in their species concepts. A comprehensive molecular phylogeny of the genus *Pythium* based on sequences of the internal transcribed spacer (ITS) region (ITS1_0.5S_ITS2) was first provided by Levesque and De Cock (2004). Uzuhashi et al. (2010) later revised the genus largely based on phylogenies of the large ribosomal DNA D1/D2 (LSU) and cytochrome oxidase II gene (Cox1), showing that *Pythium* strains resolved into five monophyletic clades and subsequently introducing the four genera: *Ovatisporangium*, *Globisporangium*, *Elongisporangium* and *Pilasporangium*.

The aim of this study was to complete a survey of oomycetes occurring in maize agricultural soils collected across the Free State and North West provinces by making use of molecular identifications. The objectives were to isolate, preserve and identify *Phytophthora* and *Pythium* species based on ITS DNA barcode sequences. The results of this study will broaden our foundational knowledge on oomycetes associated with maize agricultural soils in South African commercial farms.

Materials and methods

Soil collections

Surveys were conducted at 19 maize farms in the Free State (17 farms) and North West (two farms) provinces in March 2020. Farms were located in regions around

Bothaville, Henneman, Hertzogville, Reitz, Vrede, Wesselbron (in the Free State); and Ottosdal and Schwiezer-Reneke (in North West). At each farm, six soil samples were randomly collected. Soil was sampled around roots of maize plants, 10 cm below the surface using a hand shovel.

Oomycete Isolations

Oomycetes were isolated using the protocol of the Centre for the *Phytophthora* Science and Management (CPSM) as described by Burgess *et al.* (2021). For each farm, soil samples were homogenized in sterile distilled water (1:1 soil sample: distilled water) in plastic containers. These were left overnight for the soil to settle, after which a paper towel was used to removed particles and debris floating on top of the water. *Rosa alba* petals and *Hedera canariensis* leaves were used as baits and these were monitored regularly for lesions. Lesions were cut out with a sterile scalpel and plated onto *Phytophthora* selective medium (NARPH: cornmeal agar amended with nystatin, ampicillin, rifampicin, pentachloronitrobenzene and hymexazol) (Hüberli *et al.*, 2000) and a general growth media for oomycetes (NAR: cornmeal agar amended with nystatin, ampicillin, and rifampicin). The plates were incubated at ±21 °C and monitored daily for hyphal growth. Hyphal tips of the outgrowing mycelia were cut and sub-cultured onto carrot agar (Coffey & Coffey, 2015). Isolates from carrot agar were accessioned into internal working collection of Applied Mycology Group for Oomycetes (CN-Oom) collection and preserved in double sterilized distilled water in cryotubes and stored at room temperature (±21 °C).

DNA extractions

DNA extractions were made from 7–10 day old colonies grown on carrot agar, using the PrepMan^{TM} Ultra (Thermo Fisher Scientific, Warrington, UK). For this, mycelia of each strain were suspended into 70 µl PrepMan^{TM} Ultra in an eppendorf tube, vortexed for 30 sec and heated for 10 min at 100 °C. The eppendorf tubes were then centrifuged at 20800 g for 7 min. The supernatant containing DNA was transferred to a new eppendorf tube, and accessioned into a CN-DNA collection housed at the Forestry and Agricultural Biotechnology Institute (FABI) and stored at -20 °C.

PCR, DNA sequencing and identifications

For each isolate, ITS region was amplified in 25 µl reaction mixtures consisting of 17.5 µl MilliQ water, 5.0 µl BioLine 5X MyTaq Reaction Buffer (Bioline, England, UK), 0.50 µl of each primer (10 µM) and 0.50 µl BioLine MyTaq DNA polymerase and 1 µl of the extracted DNA. The primer pair ITS6 (5'-GAAGGTGAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990) were used for all amplifications. The thermocycle profile were as follows: Initial denaturation 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 60 sec, annealing at 55 °C for 60 sec; extension at 72 °C for 60 sec; final extension at 72 °C for 10 min. The band intensity and size of PCR products was checked using electrophoresis on 1% agarose gel stained with GelRed and 100 bp ladder (Thermo Fisher Scientific, Massachusetts, USA).

PCR products were purified using the ExoSAP-IT PCR Product Clean-up Reagent (Thermo Fisher Scientific, Massachusetts, USA). Briefly, a reaction of 2 µl ExoSAP-IT and 5 µl of PCR product was placed in a thermal cycle of: 37 °C for 15 min and 85 °C for 15 min. Purified PCR products were sequenced using the same primers used for PCR as follows: 7.4 µl sabax water, 2.1 µl sequencing buffer, 0.5 µl BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) and 1.0 µl primer. Thermocycle conditions consisted of initial denaturation at 94 °C for 5 min; 40 cycles of 96 °C for 30 sec; 50 °C for 10 sec; and 60 °C for 4 min. DNA was precipitated using Sodium Acetate (NaAc). Briefly, a 60 µl reaction of 8 µl sabax water, 2 µl NaAc and 50 µl absolute ethanol were added to each reaction. This was centrifuged at 3220 g for 30 min, and the supernatant was discarded. Subsequently, 150 µl of 70 % ethanol was added, then centrifuged at 3220 g for 10 min and the supernatant was discarded. This step was repeated twice. The plate was airdried in a laminar floor for 15 min, sealed and sent to the DNA Sanger Sequencing Facility at FABI for sequencing.

Contigs were assembled and base calls made in Geneious Prime v. 2019. To make identifications, sequences were compared to reference data previously deposited in RefSeq Target Loci and in GenBank databases of the National Center for Biotechnology Information (NCBI) using “Nucleotide BLAST” search.

Results

Oomycete isolations and identifications

A total of 65 oomycete strains were isolated, preserved, and identified using DNA sequence data. They represented *Globisporangium* and *Pythium*, as no *Phytophthora* were recovered from samples. The species identified were *Globisporangium irregulare* (n = 45), *Gl. ultimum* (n = 15) and *Pythium torulosum* (n = 5) (Figure 1; Table 1). *Globisporangium irregulare* was present at all farms, while *Gl. ultimum* was present at seven farms and *P. torulosum* at four farms (Table 2). Five strains of *Globisporangium ultimum* were isolated in farm JB Bothaville. *Pythium torulosum* was isolated in very low frequency, two strains were isolated at most in farm JF Bothaville.

Discussion

This study surveyed for oomycetes in maize rhizosphere soils from 19 farms in the Free State and North West provinces, using a culture dependent approach and identified strains based on ITS sequences. *Globisporangium* and *Pythium* were found and 65 strains that were obtained belonged to three species, *Globisporangium irregulare*, *Gl. ultimum*, and *Pythium torulosum*.

One of the most surprising findings was that *Phytophthora* was not detected in the soils tested. Rojas *et al.* (2019) previously reported *Phytophthora* species in maize soils and roots from Michigan using next-generation sequencing and culture dependent approach. For culture dependent approach, they cultured roots onto a semi-selective medium (CMA- PARPB: Cornmeal agar amended with pentachloronitrobenzene, ampicillin, rifampicin, pimaricin and benomyl). *Phytophthora* was not detected in the current survey even though the selective medium NARPH was used. Several *Globisporangium* and *Pythium* isolates were isolated in this medium, and if *Phytophthora* were present, it might have been missed due to its slower growth compared to *Pythium* (Ho, 2018). Future surveys may employ additional *Phytophthora* selective media like P10VP and P5ARPH for isolations or/and use culture independent approaches like metabarcoding.

The three species detected in this study partially agrees with those detected by Broders *et al.* (2009) who found *Pythium irregulare* (now known as *Globisporangium*

irregulare), *P. inflatum*, and *P. torulosum* as the dominant oomycetes (together with 21 other species) in maize and soybean rhizospheres in Ohio, USA. Using high throughput sequencing to study oomycete diversity in maize soils in Michigan, Rojas *et al.* (2019) identified all the three *Pythium* species and a few others. Jiang *et al.* (2012) recovered *Gl. irregulare* and *P. ultimum* (now known as *Globisporangium ultimum*) in maize-soybean rotation fields in six Illinois counties which we also recovered; however, they reported high abundance of *P. oopapillum* and *P. diclinum*. Zhang *et al.* (1998) who surveyed *Pythium* species in long-term maize and soybean cropping systems recovered three species (*Gl. ultimum*, *P. paroecandrum* and *P. torulosum*) in maize rhizosphere soils from three fields and *P. torulosum* was the most prevalent species. Slightly different results were obtained by Abdelzaher *et al.* (2004) who reported *P. delicense* and *P. oligandrum* as the most dominant species from maize rhizosphere in El-Minia, but recorded *P. irregulare* in very low numbers. Broders *et al.* (2009) speculated that *Pythium* diversity can be affected by the physical and chemical properties of soil such as soil type and pH. Blakney *et al.* (2022) also reported that soil chemistry and soil history play a role in structuring the oomycete communities in the rhizosphere.

All three species recovered during this survey are known plant pathogens. *Globisporangium irregulare* is one of the most widespread oomycete pathogens in temperate zones (Domsch *et al.*, 1980). In maize it commonly causes root rot (Harvey *et al.*, 2008). Mao *et al.* (1998) reported increased progression and severity of maize red root rot, basal stalk rot, and wilt caused by an interaction of *Gl. Irregulare* (as *P. irregulare*) and *Phoma terrestris* (a primary fungal pathogen for red root rot of maize), suggesting *Gl. irregulare* can significantly enhance crop damages as part of a disease complex. *Globisporangium ultimum* is mostly reported to cause damping off and root rot in different vegetables (Tojo *et al.*, 2001, Kubota *et al.*, 2006, Kida *et al.*, 2007, Alcala *et al.*, 2016). Mathre *et al.* (1999) reported pre-emergence damping off and stunted growth of sweet corn when they treated maize seeds with *Gl. ultimum* before planting. Robertson *et al.* (2013) who surveyed for *Pythium* that causes damping off of maize seedlings in Southeastern Iowa, reported nine species including *P. torulosum* and *Gl. irregulare* with the former reported as the most abundant and most aggressive pathogen towards maize seedlings. Tang *et al.* (2021) reported *P. tolurosum* as a causal agent of maize root rot in China.

Many South African maize farmers and especially small-scale farmers practice monoculture. Those who practice crop rotation usually crop rotate with soybean or sunflower in the same field. Lamprecht *et al.* (2020) mentioned crop rotating maize with sunflower and soybeans in the same field results to less or no effective management of *Pythium* as all the crops are susceptible. Several pathogenicity screenings of *Pythium* on both maize and soybeans have been conducted and different *Pythium* species exhibited a range of virulence potential on both crops (Broders *et al.*, 2007, Wei *et al.*, 2010, Zitnick-Anderson & Nelson Jr, 2015, Radmer *et al.*, 2017). A study conducted by Schmidt *et al.* (2020) revealed that maize plant can be infected by *Pythium* species but have less to no visible symptoms (e.g., reduced shoots). According to their findings, the level of virulence of *Pythium* species is influenced by the substrate it originated from. Based on literature, we can generally conclude that *Pythium* are more virulent to soybean than maize. For example, *Gl. irregulare* was highly virulent in soybean and moderately virulent in maize in a study conducted by Pitre (2004). Although maize plants are more resistant to *Pythium*, they might serve as a source of inoculum for soybean or other crop rotations.

Conclusion

The current study reported *Globisporangium* and *Pythium* species from agricultural soils. To obtain a better understanding of their distribution and role related to maize farms, future surveys should continue to focus on a wider geographic range and characterise communities by using both culture dependent and culture independent approaches. *Pythium* species that are found rhizosphere soils are not always pathogenic, therefore, future studies that will test the pathogenicity of the species obtained from this study need to be conducted.

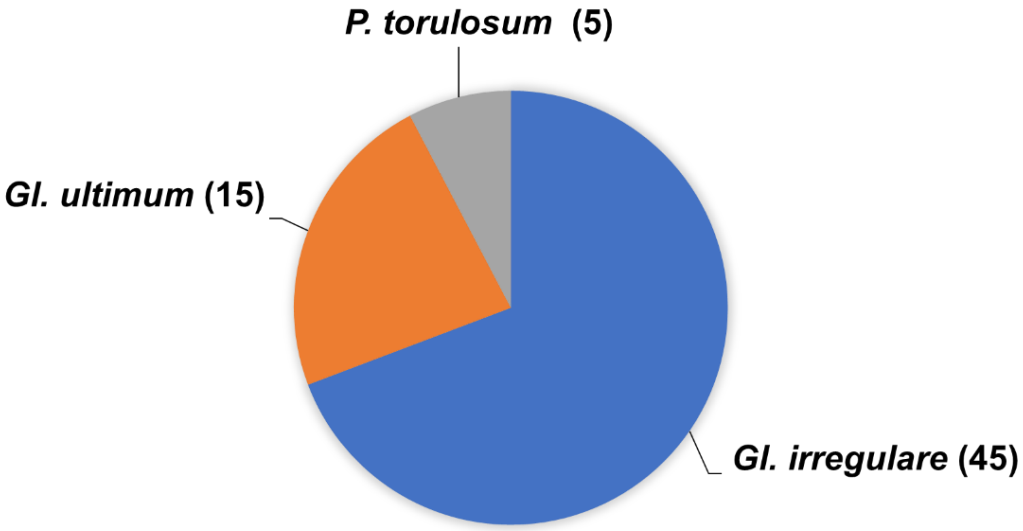

Figure 1: *Pythium* strains isolated from maize agricultural soils in Free State and North West. Numbers in brackets represent the number of strains isolated for each species.

Table 1: The NCBI GenBank 'BLAST Hit' of the oomycete species that were recovered from the maize commercial farms

CN number	Species Name	Farm region	Max score	Query coverage (%)	Percentage identity (%)	Accession length	Accession number
CN-Oom00A1	*Gl. Irregulare*	Bothaville	1753	99	99.28%	1034	MT377917.1
CN-Oom00A2	*Gl. Irregulare*	Bothaville	1724	99	99.07	979	HQ643583.1
CN-Oom001A3	*Gl. irregulare*	Bothaville	1725	99	99.47	996	MK794854.1
CN-Oom001A4	*Gl. irregulare*	Bothaville	1552	100	98.00	4212	AY598702.2
CN-Oom001A5	*Gl. irregulare*	Bothaville	1688	99	99.05	944	KU210485.1
CN-Oom001A6	*P. torulosum*	Bothaville	1553	100	99.65	874	MK794874.1
CN-Oom001A7	*Gl. ultimum*	Schwiezer-Reneke	1677	100	100	919	MK794642.1
CN-Oom001A8	*Gl. irregulare*	Bothaville	1772	99	99.09	1035	KU211360.1
CN-Oom001A9	*Gl. irregulare*	Bothaville	1711	100	99.16	1026	KU210458.1
CN-Oom001B1	*Gl. irregulare*	Bothaville	1712	99	99.16	1026	KU210458.1
CN-Oom001B2	*Gl. ultimum*	Schwiezer-Reneke	1677	100	100	915	MK794790.1
CN-Oom001B3	*Gl. irregulare*	Bothaville	1310	99	96.20	4212	AY598702.2
CN-Oom001B4	*Gl. irregulare*	Bothaville	1720	99	99.47	973	MK794815.1
CN-Oom001B5	*Gl. irregulare*	Bothaville	1718	97	99.37	1026	KU210458.1
CN-Oom001B6	*Gl. irregulare*	Bothaville	1760	99	99.38	1026	KU210458.1
CN-Oom001B7	*P. torulosum*	Bothaville	1463	100	99.05	874	MK794874.1
CN-Oom001B8	*Gl. irregulare*	Bothaville	1722	198	100	937	GQ410431.1
CN-Oom001B9	*Gl. irregulare*	Bothaville	1722	99	99.47	947	KU210458.1
CN-Oom00C1	*Gl. irregulare*	Bothaville	1757	100	99.79	4212	AY598702.2

CN-Oom00C2	*Gl. irregulare*	Bothaville	1727	97	99.07	982	MK794854.1
CN-Oom00C3	*Gl. ultimum*	Bothaville	1594	100	99.66	4212	AY598702.2
CN-Oom00C4	*Gl. irregulare*	Bothaville	1736	100	99.58	958	MW025225.1
CN-Oom00C5	*P. torulosum*	Bothaville	1556	100	99.48	874	MK794874.1
CN-Oom00C6	*Gl. irregulare*	Vrede	1653	100	98.52	984	KU210950.1
CN-Oom00C7	*Gl. irregulare*	Bothaville	2255	99	97.31	4212	AY598644.2
CN-Oom00C8	*Gl. irregulare*	Bothaville	2322	100	97.90	4201	HQ643515.2
CN-Oom00C9	*Gl. irregulare*	Reitz	2268	99	97.39	4212	AY598702.2
CN-Oom00D1	*Gl. irregulare*	Reitz	1491	100	95.84	4212	AY598702.2
CN-Oom00D2	*Gl. irregulare*	Bothaville	1670	100	98.83	983	HQ643622.1
CN-Oom00D3	*Gl. ultimum*	Schwiezer-Reneke	1594	100	99.66	915	MK794790.1
CN-Oom00D4	*Gl. ultimum*	Bothaville	2028	100	97.89	4113	AY598657.2
CN-Oom00D5	*Gl. irregulare*	Wesselbron	1430	99	96.28	1171	KX869911.1
CN-Oom00D6	*Gl. irregulare*	Wesselbron	1572	99	98.03	4212	AY598702.2
CN-Oom00D7	*Gl. irregulare*	Wesselbron	1613	99	97.57	1171	KX869911.1
CN-Oom00D8	*Gl. irregulare*	Hertzogville	1334	100	95.73	4212	AY598702.2
CN-Oom00D9	*Gl. irregulare*	Hertzogville	1027	100	96.51	4212	AY598702.2
CN-Oom00E1	*Gl. irregulare*	Hertzogville	1261	87	96.36	1031	KU211361.1
CN-Oom00E2	*Gl. ultimum*	Bothaville	1738	99	92.93	4113	AY598657.2
CN-Oom00E3	*Gl. ultimum*	Bothaville	1482	100	94.20	4113	AY598657.2
CN-Oom00E4	*Gl. irregulare*	Bothaville	2239	99	96.94	4212	AY598702.2
CN-Oom00E5	*Gl. irregulare*	Bothaville	656	100	98.51	4212	AY598702.2
CN-Oom00E6	*Gl. irregulare*	Bothaville	634	100	98.51	4212	AY598702.2

CN-Oom00E7	*Gl. ultimum*	Bothaville	1735	99	92.93	4113	AY598657.2
CN-Oom00E8	*Gl. irregulare*	Bothaville	651	99	98.64	4212	AY598702.2
CN-Oom00E9	*Gl. ultimum*	Bothaville	1728	99	92.93	4113	AY598657.2
CN-Oom00F1	*Gl. ultimum*	Bothaville	1441	100	100	840	MT177229.1
CN-Oom00F2	*Gl. irregulare*	Bothaville	1458	99	99.02	866	MT819402.1
CN-Oom00F3	*Gl. irregulare*	Bothaville	1074	100	97.03	4212	AY598702.2
CN-Oom00F4	*Gl. irregulare*	Schwiezer-Reneke	2194	100	97.33	4212	AY598702.2
CN-Oom00F5	*Gl. irregulare*	Bothaville	1724	99	99.37	1026	KU210458.1
CN-Oom00F6	*Gl. irregulare*	Henneman	660	92	96.95	1171	KX869911.1
CN-Oom00F7	*Gl. irregulare*	Henneman	1736	99	99.07	983	HQ643622.1
CN-Oom00F8	*Gl. irregulare*	Bothaville	1676	97	98.84	958	MW025225.1
CN-Oom00F9	*Gl. irregulare*	Schwiezer-Reneke	1483	99	99.04	938	KU209362.1
CN-Oom00G1	*Gl. ultimum*	Bothaville	1676	100	100	840	MT177229.1
CN-Oom00G2	*Gl. irregulare*	Bothaville	1703	99	99.06	1037	KU211361.1
CN-Oom00G3	*Gl. irregulare*	Ottosdal	1727	99	99.68	1035	KU211360.1
CN-Oom00G4	*Gl. irregulare*	Schwiezer-Reneke	1733	100	99.48	1035	KU211360.1
CN-Oom00G5	*Gl. irregulare*	Bothaville	1655	100	98.51	942	MK326412.1
CN-Oom00G6	*Gl. ultimum*	Bothaville	1348	100	100	840	MT177229.1
CN-Oom00G7	*Gl. ultimum*	Bothaville	1441	100	100	840	MT177229.1
CN-Oom00G8	*Gl. ultimum*	Bothaville	1222	100	100	840	MT177229.1
CN-Oom00G9	*Gl. ultimum*	Bothaville	1441	100	100	840	MT177229.1
CN-Oom00H1	*P. torulosum*	Bothaville	1456	99	99.25	1009	MN901168.1
CN-Oom00H2	*P. torulosum*	Henneman	1275	99	99.75	1009	MN901168.1

Table 2: Isolation frequencies of *Pythium* species from 19 agricultural farms in Free State and North West, South Africa

Province	**Farms regions**	**Sample number**	***Globisporangium irregulare*** 45 strains	***Pythium torulosum*** 5 strains	***Globisporangium ultimum*** 15 strains
	Bothaville	CS	3	-	2
Free State	Bothaville	CW	2	-	-
	Bothaville	DM	2	1	-
	Bothaville	DV	3	-	-
	Bothaville	JB	2	-	5
	Bothaville	JBL1	2	-	1
	Bothaville	JBL2	3	1	1
	Bothaville	JBL3	6	-	-
	Bothaville	JF	2	2	1
	Bothaville	JM	4	-	2
	Bothaville	LW	1	-	-
	Bothaville	PVZ	1	-	-
	Henneman	WM	2	1	-
	Hertzogville	MG	3	-	-
	Reitz	NC	2	-	-
	Vrede	PZ	1	-	-
	Wesselbron	IM	3	-	-
North West	Ottosdal	NR	1	-	-
	Schwiezer-Reneke	Jd P	2	-	3

References

Abdelzaher HM, Shoulkamy M & Yaser M (2004) Kinds, abundance and pathogenicity of *Pythium* species isolated from maize rhizosphere of various habitats in El-Minia Governorate, Egypt. *Mycobiology* **32**: 35-41.

Alcala AVC, Paulitz TC, Schroeder KL, Porter LD, Derie ML & du Toit LJ (2016) *Pythium* species associated with damping-off of pea in certified organic fields in the Columbia Basin of central Washington. *Plant Disease* **100**: 916-925.

Baldauf SL, Roger AJ, Wenk-Siefert I & Doolittle WF (2000) A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science* **290**: 972-977.

Beckerman JL (2010) Pythium root rot of herbaceous plants. *Grower talks* **74**: 74-76.

Belbahri L, McLeod A, Paul B, Calmin G, Moralejo E, Spies CF, Botha WJ, Clemente A, Descals E & Sánchez-Hernández E (2008) Intraspecific and within-isolate sequence variation in the ITS rRNA gene region of *Pythium mercuriale* sp. nov.(*Pythiaceae*). *FEMS microbiology letters* **284**: 17-27.

Binagwa PH, Bonsi CK, Msolla SN & Ritte II (2016) Morphological and molecular identification of *Pythium* spp. isolated from common beans (*Phaseolus vulgaris*) infected with root rot disease. *African Journal of Plant Science* **10**: 1-9.

Blakney AJ, Bainard LD, St-Arnaud M & Hijri M (2022) Soil chemistry and soil history significantly structure oomycete communities in Brassicaceae crop rotations. *bioRxiv*.

Bose T, Wingfield MJ, Roux J, Vivas M & Burgess TI (2018) Community composition and distribution of *Phytophthora* species across adjacent native and non-native forests of South Africa. *Fungal Ecology* **36**: 17-25.

Bose T, Hulbert JM, Burgess TI, Paap T, Roets F & Wingfield MJ (2021) Two novel *Phytophthora* species from the southern tip of Africa. *Mycological Progress* **20**: 755-767.

Broders K, Lipps P, Paul P & Dorrance A (2007) Characterization of *Pythium* spp. associated with corn and soybean seed and seedling disease in Ohio. *Plant disease* **91**: 727-735.

Broders K, Wallhead M, Austin G, Lipps P, Paul P, Mullen R & Dorrance A (2009) Association of soil chemical and physical properties with *Pythium* species diversity, community composition, and disease incidence. *Phytopathology* **99**: 957-967.

Burgess TI, López-Villamor A, Paap T, Williams B, Belhaj R, Crone M, Dunstan W, Howard K & Hardy GESJ (2021) Towards a best practice methodology for the detection of *Phytophthora* species in soils. *Plant Pathology* **70**: 604-614.

Cacciola SO & Gullino ML (2019) Emerging and re-emerging fungus and oomycete soil-borne plant diseases in Italy. *Phytopathologia Mediterranea* **58**: 451-472.

Chen J, Harman GE, COMIS A & CHENG GW (2005) Proteins related to the biocontrol of pythium damping-off in maize with *Trichoderma harzianum Rifai*. *Journal of Integrative Plant Biology* **47**: 988-997.

Chen W (1999) *Pythium* root rot and feeder root necrosis. *Compendium of Corn Diseases DG White, ed American Phytopathological Society, St Paul, MN*.

Coffey, M., & Coffey, M. (2015). PROTOCOL 07–23.1: Carrot agar (CA) or broth. *Laboratory Protocols for Phytophthora Species. The American Phytopathological Society*, 1.

Domsch KH, Gams W & Anderson T-H (1980) Compendium of soil fungi. London. *Academic Press*.

Gull C, Labuschagne N & Botha W (2004) *Pythium* species associated with wilt and root rot of hydroponically grown crops in South Africa. *African Plant Protection* **10**: 109-116.

Harvey P, Warren R & Wakelin S (2008) The Pythium–*Fusarium* root disease complex–an emerging constraint to irrigated maize in southern New South Wales. *Australian Journal of Experimental Agriculture* **48**: 367-374.

Ho HH (2018) The taxonomy and biology of *Phytophthora* and *Pythium*. *Journal Bacteriology Mycology* **6**: 40-45.

Hüberli D, Tommerup IC, Hardy GES (2000) False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi. Australasian Plant Pathology* **29**, 164–169.

Hulbert J, Paap T, Burgess TI, Roets F & Wingfield MJ (2019) Botanical gardens provide valuable baseline *Phytophthora* diversity data. *Urban Forestry & Urban Greening* **46**: 126461.

Jiang Y, Haudenshield J & Hartman G (2012) Characterization of *Pythium* spp. from soil samples in Illinois. *Canadian Journal of Plant Pathology* **34**: 448-454.

Kamoun S, Furzer O, Jones JD, Judelson HS, Ali GS, Dalio RJ, Roy SG, Schena L, Zambounis A & Panabières F (2015) The Top 10 oomycete pathogens in molecular plant pathology. *Molecular Plant Pathology* **16**: 413-434.

Kida K, Tojo M, Yano K & Kotani S (2007) First report of *Pythium ultimum var. ultimum* causing damping-off on okra in Japan. *Plant Pathology* **56**: 1042.

Kubota M, Nakasuji S, Shimizu M & Nishi K (2006) Damping-off of cabbage plug seedlings caused by *Pythium ultimum var. ultimum* in Japan. *Journal of General Plant Pathology* **72**: 123-125.

Lamprecht S, Phasoana T, Gokul A & Spies C (2020) Seedling diseases lead to poor establishment [Online]. Available: https://sagrainmag.co.za/2020/08/05/seedling-diseases-lead-to-poor-establishment/ [Accessed 19 August 2022]

Levesque CA & De Cock AW (2004) Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycological Research* **108**: 1363-1383.

Linde C, Kemp G & Wingfleld M (1994) *Pythium* and *Phytophthora* species associated with eucalypts and pines in South Africa. *European Journal of Forest Pathology* **24**: 345-356.

Mao W, Carroll R & Whittington D (1998) Association of *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* in causing red root rot of corn. *Plant Disease* **82**: 337-342.

Margulis L & Schwartz KV (1998) Five kingdoms: an illustrated guide to the phyla of life on earth, 3rd ed. *New York. W.H. Freeman* 1938-2011.

Martin FN & Loper JE (1999) Soilborne plant diseases caused by *Pythium* spp.: ecology, epidemiology, and prospects for biological control. *Critical Reviews in Plant Sciences* **18**: 111-181.

Mathre D, Cook R & Callan NW (1999) From discovery to use: traversing the world of commercializing biocontrol agents for plant disease control. *Plant Disease* **83**: 972-983.

Pitre JL (2004) *Characterization and pathogenicity of pythium isolates from continuous and rotational corn/soybean cropping systems in Mississippi*. Mississippi State University.

Radmer L, Anderson G, Malvick D, Kurle J, Rendahl A & Mallik A (2017) Pythium, *Phytophthora*, and *Phytopythium* spp. associated with soybean in Minnesota, their relative aggressiveness on soybean and corn, and their sensitivity to seed treatment fungicides. *Plant Disease* **101**: 62-72.

Rao B, Schmitthenner A, Caldwell R & Ellett C (1978) Prevalence and virulence of *Pythium* species associated with root rot of corn in poorly drained soil. *Phytopathology* **68**: 1557-1563.

Robertson AE, Matthiesen R & Ahmad A (2013) Nine species of *Pythium* associated with corn seeding blight in southeastern Iowa. *Nine* **4**: 15-2013.

Rojas J, Witte A, Noel Z, Jacobs J & Chilvers M (2019) Diversity and characterization of oomycetes associated with corn seedlings in Michigan. *Phytobiomes Journal* **3**: 224-234.

Rossman AY & Palm ME (2006) Why are *Phytophthora* and other Oomycota not true fungi? *Outlooks on Pest Management* **17**: 217.

Schmidt CS, Leclerque A, Pfeiffer T, Goessling JW, Orlik M, Jamshidi B, Saar K, Sellmann J, Siepe I & Koch E (2020) Pathogenicity of *Pythium* species to maize. *European Journal of Plant Pathology* **158**: 335-347.

Schroeder KL, Martin FN, de Cock AW, Lévesque CA, Spies CF, Okubara PA & Paulitz TC (2013) Molecular detection and quantification of Pythium species: evolving taxonomy, new tools, and challenges. *Plant Disease* **97**: 4-20.

Soonthornpoct P, Trevathan L, Gonzalez M & Tomaso-Peterson M (2001) Fungal occurrence, disease incidence and severity, and yield of maize symptomatic for seedling disease in Mississippi. *Mycopathologia* **150**: 39-46.

Tang X, Chen S, Yan X, Yuan H & Yang D (2021) First Report of *Pythium torulosum* Causing Corn Root Rot in Northeastern China. *Plant Disease* **105**: 712-712.

Tojo M, Hoshino T, Luz Herrero M, Sletner Klemsdal S & Tronsmo AM (2001) Occurrence of *Pythium ultimum var. ultimum* in a greenhouse on Spitsbergen Island, Svalbard. *European Journal of Plant Pathology* **107**: 761-765.

Uzuhashi S, Kakishima M & Tojo M (2010) Phylogeny of the genus *Pythium* and description of new genera. Mycoscience **51**: 337-365.

van Agtmaal M, van Os GJ, Hol WG, Hundscheid MP, Runia WT, Hordijk CA & De Boer W (2015) Legacy effects of anaerobic soil disinfestation on soil bacterial community composition and production of pathogen-suppressing volatiles. *Frontiers In Microbiology* **6**: 701.

Walker CA & van West P (2007) Zoospore development in the oomycetes. *Fungal Biology Reviews* **21**: 10-18.

Wei L, Xue A, Cober E, Babcock C, Zhang J, Zhang S, Li W, Wu J & Liu L (2010) Pathogenicity of *Pythium* species causing seed rot and damping-off in soybean under controlled conditions. *Phytoprotection* **91**: 3-10.

White TJ, Bruns T, Lee S & Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* **18**: 315-322.

Zhang B & Yang X (2000) Pathogenicity of *Pythium* populations from corn-soybean rotation fields. *Plant Disease* **84**: 94-99.

Zhang B, Chen W & Yang X (1998) Occurrence of *Pythium* species in long-term maize and soybean monoculture and maize/soybean rotation. *Mycological Research* **102**: 1450-1452.

Zitnick-Anderson KK & Nelson Jr BD (2015) Identification and pathogenicity of *Pythium* on soybean in North Dakota. *Plant Disease* **99**: 31-38.

Summary

Microbial communities present in agricultural soils play important roles in determining plant health and the total produce yield that a farmer will harvest. Soil that has a lower microbial diversity and which are dominated by pathogenic species, might lead to a loss of productivity due to the development of devastating diseases. With high diversity, the pathogen will have to compete for nutrients thus reducing the chances of infecting the plant. Therefore, it is important to understand the microbial communities that are present in agricultural ecosystems. This study used a culture-dependent approach to characterise fungal and oomycete communities and diversity present in maize rhizosphere soils in South Africa.

The first research chapter (chapter two) seeks to understand the fungal communities that are associated with maize rhizosphere and identify potential soilborne pathogens based on literature. There was a relatively diverse fungal community in rhizosphere soils. A total of 460 fungal strains were isolated that was found to belong to 28 genera and identified as 80 species. The most dominant fungi from collected soils were *Fusarium*, *Neocosmospora*, *Penicillium*, and *Trichoderma*. Species that dominated the rhizosphere soils included *Fusarium tardicrescens, Neocosmospora solani, Penicillium raperi*, and *Trichoderma afroharzianum*. Several species that were isolated (*Fusarium chlamydosporum, F. oxysporum, F. subglutinans, F. temperatum, Neocosmospora falciformis*, and *Setophoma terrestris*) have previously been reported to cause diseases of maize. Therefore, future studies are needed to explore the pathogenicity of obtained strains against maize. Several putative new *Penicillium* (three) and *Fusarium* (one) species were isolated and further studies of confirmation will be conducted.

The second research chapter (chapter three) identified oomycetes in maize rhizosphere soils that were collected from 19 farms in the Free State and North West provinces of South Africa. A total of 65 strains were identified as *Globisporangium irregulare*, *Gl. ultimum* and *Pythium torulosum*. *Globisporangium irregulare* which previously described as *Pythium irregulare* was the most abundant species detected across all farms. All three species have been reported as pathogens of maize. However, the presence of these species on the farm does not necessarily mean they are pathogenic to maize. Therefore, pathogenicity screening of obtained strains needs

to be conducted. This is the first survey to use modern taxonomic approaches using different identification markers to identify and characterise fungal and oomycete communities from maize soils in South Africa.