Population genetics of the maize foliar pathogen *Cercospora zeina* Crous & U. Braun in five countries of sub-Saharan Africa

by

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Declaration

I, David Livingstone Nsibo, hereby declare that the thesis, which I hereby submit for the award of the degree *Philosophiae Doctor* at the University of Pretoria, contains my own independent research and has not previously been submitted by me for a degree at this or any other tertiary Institution.

David Livingstone Nsibo

2019-09-17

Date

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Preface

Gray leaf spot (GLS) continues to thrive as a major foliar disease of maize that is responsible for more than 1 % global yield losses. Although two pathogens, *Cercospora zeina* and *C. zeae-maydis* are implicated as major causal pathogens of GLS worldwide, the former is the predominant species in Africa. Prior to our study, small-scale population genetic studies were previously conducted on *C. zeina*, originally known as *C. zeae-maydis* type II, in South Africa

and some parts of East Africa. These studies revealed that it is a highly diverse heterothallic pathogen, with cryptic sexual recombination as the major evolutionary force defining its population structure. There is a big knowledge gap, however, about the genetic diversity and evolutionary potential of this pathogen in the rest of Africa. The current study was, therefore, undertaken with the aim of determining the genetic diversity and population structure of *C. zeina* across maize-based cropping systems in five countries in sub-Saharan Africa, and to establish the evolutionary forces defining its population structure. We hypothesized that *C. zeina* was a highly diverse pathogen in Africa, with sexual recombination and migration being the major evolutionary factors defining its population structure.

The research findings presented in this thesis were based on field and molecular experiments conducted in the Molecular Plant-Pathogen Interactions (MPPI) research group at the Department of Plant and Soil Sciences, Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, South Africa. This thesis is presented in the form of five independent chapters with some degree of repetition because they were written in publication format.

Chapter 1 provides insights into the recent advances in the population biology and management of three major foliar pathogens of maize, *Cercospora zeina*, *Exserohilum turcicum* and *Bipolaris maydis*, in the African context. A comprehensive review is provided on the epidemiology, current pathogen molecular identification tools, pathogen population genetics and management of these three pathogens. In this chapter a combined life cycle for the three pathogens is presented and the chapter concludes by proposing more efficient management techniques for these pathogens.

Chapter 2 illustrates the role farming systems play in defining the population structure of *C. zeina* in South Africa's commercial and smallholder maize farming systems. It was accomplished by collecting isolates from KwaZulu-Natal, a GLS hotspot in South Africa. These were combined with isolates previously collected from commercial maize farms in the same region and subjected to species confirmation tests, mating type and microsatellite marker

genotyping and population genetics analysis. Results reveal that farming systems significantly influence the genetic diversity of the pathogen, with populations in the smallholder farms exhibiting a higher genetic diversity than those from commercial farms.

Chapter 3 examines the population genetics of *C. zeina* from five sub-Saharan countries, Kenya, South Africa, Uganda, Zambia, and Zimbabwe. This was accomplished by first confirming that *C. zeina* was the causal pathogen of GLS in Africa by analyzing more than 800 isolates collected from 25 different locations in these countries. This was followed by determining the genetic diversity and the role of sexual recombination in defining the population structure of the pathogen across these countries. Other evolutionary factors influencing the pathogen variability including gene flow, are also revealed. To determine *C. zeina*'s dispersal mechanisms around the continent, migration patterns of the pathogen are established.

In **Chapter 4**, isolates with different mating types were randomly selected and crossed in varying combinations to induce the sexual stage of *C. zeina* under different environmental conditions *in vitro* and *in planta*. The induction of the sexual stage was not successful. However, chlamydospores and other mycelial structures were observed for some of the crosses. Furthermore, the existence of population differentiation among mating types was tested to assess their evolutionary potential in nature and results showed no population differentiation among mating types.

The thesis concludes with **chapter 5,** which consists of the discussion, contributions, limitations and future research prospects of the different findings in the study. The prospect of *C. zeina* being the only GLS causal pathogen in Africa and the possible reasons for the pathogen having entered Africa through different entry points are discussed. Furthermore, based on previous studies, insights into the development of effective management strategies to counteract its high genetic diversity are explained. Future research, which may contribute to the current knowledge base, includes more comprehensive sampling and population genetic analyses of *C. zeina* from other countries in Africa where GLS is endemic. This could

especially be focused on entry points of maize into Africa and its comparison with *C. zeina* from other continents. This added knowledge could enable us to answer the major question of *C. zeina*'s origin.

The research outputs generated from this research up to date include:

Publications:

Nsibo D.L., Barnes, I., Kunene, N. T. & Berger, D. K**. Influence of farming practices on the population genetics of the maize pathogen *Cercospora zeina* in South Africa**. *Fungal Genetics and Biology* **125**, 36-44 10.1016/j.fgb.2019.01.005 (2019).

Presentations:

Nsibo D.L, Barnes I, and Berger DK **Population genetic structure and differentiation among *Cercospora zeina* geographic populations in Southern Africa.** 51st Congress of the South African Society for Plant Pathology; 2019 January 20-24; Cape Town, South Africa. (*Oral presentation*)

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Nsibo D.L, Barnes I, and Berger DK **Influence of farming practices on the population genetics of *Cercospora zeina* in South Africa.** Oral presentation at: Genomics Research Institute (GRI) Symposium. 2017 November 10; University of Pretoria, South Africa. (*Oral presentation*)

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Summary

Cercospora zeina, a causal pathogen of gray leaf spot (GLS) of maize, is responsible for up to 1 % of global yield losses. Previous small-scale population genetics studies have revealed that *C. zeina* is a highly diverse pathogen. Using microsatellite markers, we set out to determine the genetic diversity and population structure of 835 *C. zeina* isolates from five countries in Sub-Saharan Africa: Kenya, South Africa, Uganda, Zambia, and Zimbabwe, and to establish their evolutionary potential. Our findings revealed that populations from different farming systems exhibited partial, but significant population differentiation. Also, smallholder populations had a higher genetic diversity, number of private alleles and lower clonality than commercial populations. Country-level populations were highly diverse and structured, with migration occurring among populations. Interestingly, Zambia came out as a distinct population, with a lower genetic diversity, higher clonality and private alleles, indicative of a more recently introduced population. Our findings rejected the existing hypothesis of Durban harbor being the entry point of *C. zeina* into Africa, thus suggesting that the pathogen has other point(s) of entry. Additionally, signatures of cryptic sexual recombination were observed even though an attempt to induce the sexual stage in laboratory conditions was unsuccessful. We found no evidence for *C. zeae-maydis*, *C. zeina*'s sibling species in more than 1000 isolates collected from around Africa, thus confirming that *C. zeina* is the predominant species in Africa. Overall, this pathogen is a genetically well-established pathogen in Africa with its population structure being influenced by sexual recombination, migration and human activities. This study, therefore, provides a basis for effective monitoring of *C. zeina*'s dispersal and is a tool for designing more effective regional-specific management strategies to reduce the acquisition and movement of highly virulent strains that overcome host resistance or fungicide control.

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CHAPTER 1

Recent advances in the population biology and management of maize foliar fungal pathogens *Exserohilum turcicum*, *Cercospora zeina* and *Bipolaris maydis* in Africa.

1.1 INTRODUCTION

Globally, there is a progressive increase in CO_2 concentration and a rise in temperature. These have contributed to the increase in plant biomass and the rise in precipitation levels (Fuhrer, 2003; Girvetz *et al.*, 2019; Lee & Sheridan, 2018; Matros *et al.*, 2006; Pachauri *et al.*, 2014; Pangga *et al.*, 2004). Likewise, global changes in agricultural practices are on the rise, with a change in focus from crops with a high genotypic diversity on farms to genetically uniform crops (termed monoculture) (Zhan *et al.*, 2014). There is also increased adoption of conservation agricultural technologies such as minimum tillage (no-till), which concentrate plant residues on the surface of the field relatively undisturbed until the next planting season (Sumner *et al.*, 1981). All these abiotic and biotic changes combined create a favorable microclimate for crop pathogen (re)emergence leading to severe global outbreaks (Bateman *et al.*, 2007; Bebber, 2015; Bebber *et al.*, 2014; Dill-Macky & Jones, 2000; Fisher *et al.*, 2012) increasing the threat to global food production and security especially in Africa, Asia, and Latin America (Bongaarts, 2009).

Maize, rice, wheat, and sorghum are major contributors to food security, occupying 83 % of the world cereal area and 56 % of world arable land (Jaggard *et al.*, 2010). Their production is currently increasing at rates of 1.6, 1.0, 0.9 and 1.3 % respectively per year at a global scale (Ray *et al.*, 2013). These rates, however, are below the 2.4 % required per year, to meet the expected global production demand by 2050 (Ray *et al.*, 2013). According to FAOSTAT (2018), maize alone contributes 5.4 % of the global human calorie intake. This contribution is unfortunately threatened by 22.6 % global yield losses that occur annually (Oerke, 2006; Savary *et al.*, 2019). Twelve major maize pests and diseases recorded by Savary *et al.* (2019) are implicated in causing more than 1 % each of these losses (Table 1.1). Of these, northern corn leaf blight (NCLB), gray leaf spot (GLS), and southern corn leaf blight (SCLB) are the three major foliar fungal diseases with potential to cause more than 10 % yield losses if diagnosed inaccurately and managed ineffectively (Savary *et al.*, 2019). Here we review the

recent advances in the population biology and management of these three foliar diseases and their causal pathogens at a global scale, with Africa as the major focus.

Table 1.1: The 12 major pests and pathogens causing substantial maize yield losses globally and in sub-Saharan Africa as modified from Savary *et al.* (2019)

Pest or disease	Causal species	Global yield losses (%)	SSA yield losses (%)
Fusarium and *Gibberella* stalk rots	*Fusarium moniliforme*, and *Fusarium graminearum*	4.58	3.28
Fall armyworm	*Spodoptera frugiperda*	4.34	6.25
Northern corn leaf blight*	*Exserohilum turcicum*	2.68	1.02
Anthracnose stalk rots	*Colletotrichum graminicola*	1.65	Not reported
Fusarium and *Gibberella* ear rots	*F. moniliforme*, and *F. graminearum*	2.38	0.49
Southern rust	*Puccinia polysora*	1.15	1.40
Diabrotica (beetle and rootworms)	*Diabrotica balteata, Diabrotica longicornis, Diabrotica virgifera, Diabrotica speciosa*	0.98	Not reported
Gray leaf spot*	*Cercospora zeae-maydis,* and *Cercospora zeina*	0.94	Not reported
Common rust	*Puccinia sorghi*	0.75	2.5
Bacterial stalk rot	*Dickeya zeae*	0.64	Not reported
Southern corn leaf blight*	*Bipolaris maydis*	0.55	Not reported
Maize white spot	*Pantoea stewartii*	0.42	Not reported

*Foliar fungal diseases of maize

1.2 GLOBAL DISTRIBUTION AND CAUSAL AGENTS OF NCLB, GLS, AND SCLB

1.2.1 Northern corn leaf blight

Turcicum leaf blight (TLB) or northern corn leaf blight (NCLB), has persisted for more than 40 years as one of the major fungal foliar diseases of maize (Leonard & Suggs, 1974) and has recently been ranked the number one foliar disease of maize (Savary *et al.*, 2019). NCLB was discovered in Pharma Italy but was first well-documented in New Jersey in the United States of America (USA) in 1878 (Drechsler, 1923). In 1889, NCLB emerged as an outbreak in Connecticut (Drechsler, 1923) and has since appeared in many maize-growing countries in the Americas, Asia and Africa (Adipala *et al.*, 1995; Bashir *et al.*, 2018; Bergquist & Masias, 1974; Dingerdissen *et al.*, 1996; Kloppers & Tweer, 2009a; Muiru *et al.*, 2010a; Renfro & Ullstrup, 1976; Shi *et al.*, 2017)

In Africa, NCLB was first reported in Uganda in 1924 and has since been reported in all maize-producing countries in sub-Saharan Africa (Abebe & Singburaudom, 2006; Emechebe, 1975) (Figure 1.1). Its causal pathogen is an ascomycetes fungus *Exserohilum turcicum* (Pass.) Leonard & Suggs (teleomorph: *Setosphaeria turcica* (Luttrell) Leonard & Suggs) (Leonard & Suggs, 1974). There are twelve globally known physiological races of *E. turcicum* (Table 2), all of which have been reported to exist in Africa (Abadi *et al.*, 1989; Dong *et al.*, 2008; Ferguson & Carson, 2007; Jordan *et al.*, 1983; Moghaddam & Pataky, 1994; Muiru *et al.*, 2010a; Ramathani *et al.*, 2011; Sun *et al.*, 2005; Zhao *et al.*, 2008) (Table 1.2).

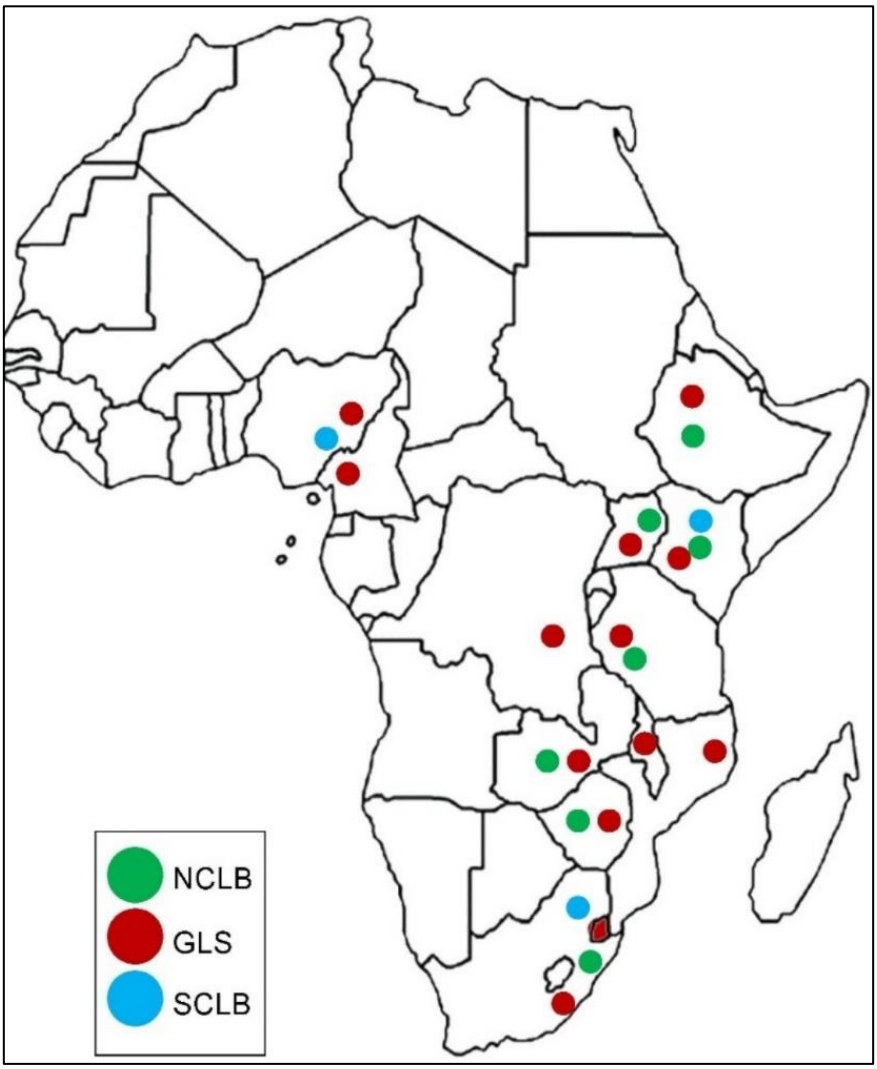

Figure 1.1: Distribution of NCLB, GLS and SCLB in Africa. Each of the diseases is color coded. Green: Northern corn leaf blight (NCLB) (Abebe & Singburaudom, 2006; Emechebe, 1975); Red: Gray leaf spot (GLS) (Meisel *et al.*, 2009; Okori *et al.*, 2003; Ward, Laing, *et al.*, 1997b); and Blue: Southern corn leaf blight (SCLB) (Mwangi, 1998; Rong & Baxter, 2006). GLS is the most widely distributed in Africa, followed by NCLB.

Table 1.2: The physiological races of *Exserohilum turcicum* and *Bipolaris maydis* and their global distribution

Disease	Races	Resistance gene in host that is overcome by the Race	Distribution	Authors
Northern corn leaf blight	0	Virulent to all known R genes	China, Kenya, Uganda, Germany, South Africa, United States, Zambia	(Craven & Fourie, 2011; Dong *et al.*, 2008; Muiru *et al.*, 2010b; Ramathani *et al.*, 2011; Sun *et al.*, 2005; Weems & Bradley, 2018; Zhao *et al.*, 2008)
	1	Ht1	Ohio, Central and Eastern United States, China, Kenya, Germany, Austria, Israel	(Abadi *et al.*, 1989; Dong *et al.*, 2008; Ferguson & Carson, 2007; Muiru *et al.*, 2010b; Ramathani *et al.*, 2011; Sun *et al.*, 2005; Weems & Bradley, 2018)
	2	Ht2	Ohio, Central and Eastern United States, Kenya, Uganda, China, Germany, Austria	(Jordan *et al.*, 1983; Muiru *et al.*, 2010b; Ramathani *et al.*, 2011; Sun *et al.*, 2005; Weems & Bradley, 2018; Zhao *et al.*, 2008)
	3	Ht3	Ohio, Central and Eastern United States, Kenya, China	(Dong *et al.*, 2008; Muiru *et al.*, 2010b; Ramathani *et al.*, 2011; Sun *et al.*, 2005; Zhao *et al.*, 2008)
	N	Htn1	Kenya, Uganda, China	(Dong *et al.*, 2008; Muiru *et al.*, 2010b; Sun *et al.*, 2005)
	12	Ht1 and Ht2	Kenya, China, Germany, the United States	(Dong *et al.*, 2008; Muiru *et al.*, 2010b; Sun *et al.*, 2005; Weems & Bradley, 2018; Zhao *et al.*, 2008)
	13	Ht1 and Ht3	Kenya, Germany, the United States	(Dong *et al.*, 2008; Muiru *et al.*, 2010b; Weems & Bradley, 2018; Zhao *et al.*, 2008)
	123	Ht1, Ht2 and Ht3	Kenya, China, United States	(Muiru *et al.*, 2010b; Sun *et al.*, 2005; Weems & Bradley, 2018; Zhao *et al.*, 2008)
	23	Ht2 and Ht3	Mexico, Kenya, Zambia, China, United States, Germany	(Dong *et al.*, 2008; Ferguson & Carson, 2007; Muiru *et al.*, 2010b; Sun *et al.*, 2005)
	23N	Ht2, Ht3, and Htn1	Mexico, Kenya, Uganda, Zambia, China, United States, Germany	(Dong *et al.*, 2008; Ferguson & Carson, 2007; Muiru *et al.*, 2010b; Sun *et al.*, 2005; Zhao *et al.*, 2008)
	13N	Ht1, Ht3 and Htn1	Kenya, China	(Muiru *et al.*, 2010b; Sun *et al.*, 2005)
	3N	Ht3 and Htn1	Kenya, China	(Muiru *et al.*, 2010b; Sun *et al.*, 2005)
Southern corn leaf blight	O	Virulent to all	All maize producing countries	(Mwangi, 1998; Smith *et al.*, 1970)
	T	cms-T	United States, South Africa	(Leonard, 1977a, 1977b; Rong & Baxter, 2006)
	C	cms-C	China	(Wei *et al.*, 1988)

1.2.2 Gray leaf spot

Gray leaf spot (GLS) is the second most economically important fungal foliar disease of maize after NCLB on a global scale and the most important foliar fungal disease in the USA and Canada (Mueller *et al.*, 2016). First reported in 1925 in Southern Illinois in the USA (Tehon & Daniels, 1925), GLS was of no economic importance until the late 1970s when its prevalence and severity became a significant threat to maize production in the USA (Latterell & Rossi, 1983). It has since been reported in the Americas (Juliatti *et al.*, 2009; Neves *et al.*, 2015; Zhu *et al.*, 2002), Africa (Kinyua *et al.*, 2010; Nega *et al.*, 2016; Nowell, 1998; Ward, Laing, *et al.*, 1997b; Ward & Nowell, 1994), and Asia (Liu & Xu, 2013; Manandhar *et al.*, 2011; Tiwari & Ferrara, 2007).

In Africa, GLS was first reported in 1988 in KwaZulu-Natal province, a GLS hotspot in South Africa (Ward, Laing, & Nowell, 1997) and has since been reported in other countries north of South Africa (Figure 1.1). Two pathogens cause GLS namely, *Cercospora zeae-maydis* Tehon & E.Y Daniels (Tehon & Daniels, 1925), and *Cercospora zeina* Crous & U. Braun (Crous & Braun, 2003). *Cercospora zeae-maydis* is predominant in North America and some parts of South America and Asia while *C. zeina* is predominant in Africa, some parts of Asia and the Eastern corn Belt of the USA (Crous & Braun, 2003; Crous *et al.*, 2006; Dunkle & Levy, 2000; Goodwin *et al.*, 2001; Meisel *et al.*, 2009; Okori *et al.*, 2003). Other pathogens associated with GLS include unidentified *Cercospora* sp. CPC 12062 reported in South Africa (Crous *et al.*, 2006) and *Cercospora sorghi* var. *maydis* reported in Kenya (Kinyua *et al.*, 2010) and Brazil (Neves *et al.*, 2015). The rest of the review will focus on *C. zeina*, which is the predominant pathogen in Africa.

1.2.3 Southern corn leaf blight

Southern corn leaf blight (SCLB), also known as southern leaf blight (SLB) and/or *Maydis* leaf blight (MLB), was first reported in the USA in 1923 (Drechsler, 1925). The disease only became a serious concern in the 1970s when its incidence and severity became a threat to

maize production (Smith *et al.*, 1970). Since then, SCLB incidence reports have emerged from Western Europe, Asia and Africa (Fisher *et al.*, 1976; Gregory *et al.*, 1979; Munjal & Kapoor, 1960; Singh & Srivastava, 2012; Ullstrup, 1972). *Bipolaris maydis* (Nisikado and Miyake) Shoemaker (teleomorph: *Cochliobolus heterostrophus* Drechs. ShoeMaker; synonym: *Helminthosporium maydis* Nisikado) is the causal pathogen of SCLB (Smith *et al.*, 1970). Previously called *C. heterostrophus, B. maydis* has been adopted as the most widely accepted species name by plant pathologists and taxonomists (Manamgoda *et al.*, 2014; Rossman *et al.*, 2013).

Globally, three physiological races (O, T and C) of *B. maydis* are known (Smith *et al.*, 1970; Wei *et al.*, 1988) (Table 1.2). Race O is the most common race in countries where SCLB is prevalent and infects the leaf blade. Race T is the most virulent, infecting the leaf sheaves, ear husks, grain, and leaf blades, especially of maize carrying the Texas male-sterile cytoplasm (T-cms), a trait which facilitates cross pollination and concomitant hybrid vigor (Levings 3rd, 1993). Race C was first reported in 1971 in China and has not been reported anywhere else in the world. It is virulent to C male-sterile cytoplasm (C-cms) maize hybrids and is postulated to be as virulent as race T in a majority of the C-cms hybrids grown in China (Bruns, 2017; Wei *et al.*, 1988).

Not much is known about SCLB in Africa. The first official report was an outbreak of SCLB in 1974 in Mpumalanga by the Mycology Unit and was confirmed at the Commonwealth Mycological Institute (now known as International Mycological Institute) (Rong & Baxter, 2006). This report resulted in the withdrawal of the T-cms maize germplasm from South Africa's breeding programs (Rong & Baxter, 2006). This maize germplasm secretes a mitochondrial protein (Urf13) which confers sensitivity to T toxin, a polyketide host-specific toxin produced by race T of *B. maydis* (Levings, 1990). No further incidences after the T-cms maize germplasm withdrawal have since been reported in the country. Later in a survey conducted by Mwangi (1998) during the 1995/96 season, SCLB was reported in the highlands west of the Rift Valley in Kenya. SCLB has also been reported in Nigeria, based on a seed-

testing assay conducted on 46,500 seeds that were taken from the informal seed systems. Results revealed the presence of *B. maydis* in 45 % of the tested seed (Biemond *et al.*, 2013). This is an indication that SCLB is present in Africa (Figure 1.1) but at levels where its severity and occurrence are still insignificant or where it has been misidentified as another disease in areas where it exists. More research is clearly needed to confirm the existence of the disease on the continent using the current morphological, molecular and phylogenetic tools available (see section 1.4).

Overall, all the first reports of NCLB, GLS, and SCLB emerged from the USA in the early twentieth century before any other country (Drechsler, 1923, 1925; Tehon & Daniels, 1925). The most plausible explanation for this could be that during this period when the first reports of these and many other diseases emerged, several Western countries such as the USA possessed the most sophisticated pathogen identification systems as a result of well-organized departments of agriculture and increased maize production in the early twentieth century (Duvick, 2001). This period steered genetic modernization and usage of improved cultural practices, leading to increased breeding and adoption of hybrid maize, thus, increasing the maize production area and area under monocultures, which if susceptible genotypes were planted presented a high disease risk (Dodd, 2000; Duvick, 2001). In addition, GLS is noted to be different from NCLB and SCLB with no physiological races existing amongst its causal pathogens. Designation of physiological races follows the “gene for gene” hypothesis. Races are designated based on the absence of virulence genes (effectors) such that the resistance proteins in the host are overcome resulting in symptom development on maize inbred lines harboring the resistance genes (Leonard *et al.*, 1989). Currently, virulence genes and their corresponding major resistance genes in the GLS-Maize pathosystem are unknown and as such there are no known physiological races in that pathosystem.

1.3 DISEASE EPIDEMIOLOGY AND ECONOMIC IMPACT OF NCLB, GLS, AND SCLB ON MAIZE

Disease epidemiology entails the understanding of the dynamics of disease development and increase in space and time (Milgroom & Peever, 2003). A plethora of biotic, abiotic and edaphic factors play a role in the weakening of host defenses as well as predispose the host to pathogen attack, thus causing disease (Milgroom & Peever, 2003). The knowledge of these predisposing factors and the epidemiological parameters such as infection efficiency, latent period, spore production among others, in disease development and the potential yield losses caused as a result of disease is therefore crucial in deciding on the nature of control strategies to adopt (de Vallavieille-Pope *et al.*, 2000; Milgroom & Peever, 2003). This section reviews the epidemiology of NCLB, GLS and SCLB and the key factors that favor their development as well as assesses their economic impact.

1.3.1 Northern corn leaf blight

Upon infection of maize, *E. turcicum* causes grayish lesions that start as chlorotic flecks and later mature into elliptical or cigar-shaped lesions of up to 2.5 to 17.5 cm in length (White, 1999) (Figure 1.2). Disease establishment occurs within 6 to 18 hours post infection, starting from the lower leaves moving up the plant throughout the growing season. Development of mature lesions occurs within 2 weeks of host-pathogen interaction under favorable environmental conditions. Conidia develop in these mature lesions and are spread to other plants during the growing season (Bentolila *et al.*, 1991; Levy & Cohen, 1983; White, 1999) (Table 1.3) (Figure 1.2). The pathogen is known to move into the vascular tissue, thus blocking water movement within the plant (Kotze *et al.*, 2019). This causes plant lodging and a reduction in photosynthetic leaf area, leading to 30 to 91 % yield losses, especially when severe infection occurs during the period of silking and grain fill (Jindal *et al.*, 2019; Kloppers & Tweer, 2009b; Nwanosike *et al.*, 2015; Tilahun *et al.*, 2012). NCLB is a splash- and wind-

borne disease, spreading conidia from old infected debris left in the fields and from secondary infections over long distances across fields (Schwartz & David, 2005).

1.3.2 Gray leaf spot

Under favorable conditions (Table 1.3), *C. zeina* invades the plant leaf tissue intracellularly within 4 to 7 days after the spore lands and attaches on the leaf surface and causes fully expanded lesions to develop within 1 to 2 weeks (Latterell & Rossi, 1983; Ward *et al.*, 1999) (Figure 1.2). Symptoms of infection first emerge as 1 to 3 mm irregularly shaped lesions surrounded by chlorotic borders and then mature into gray to tan linear rectangular lesions of 5 to 70 mm long with a width of 2 to 4 mm, that run parallel with leaf veins (Latterell & Rossi, 1983). Secondary conidia develop in these mature lesions which are then released, spreading to other plants or leaves during the growing season. This cycle of events is characteristic of a typical polycyclic disease. Extensive disease development results in coalescing of the lesions, blighting and necrosis of the leaf tissue. The disease generally progresses from the lower to the upper leaves, resulting in reduced photosynthetic area and plant lodging (Lennon *et al.*, 2016; Paul & Munkvold, 2005). Calculations made based on spore size (40 to 165 µm by 4 to 9 µm), wind speed (varies per location) and the height of vertical mixing of the atmosphere above the crop, estimate flight distances of GLS causal pathogens to range between 0.1 to 40 km as wind speed increases from 1 to 10 m/s (Ward *et al.*, 1999). In Nepal, the disease is reported to spread up to a distance of 80 to160 km annually, making it a fast spreading disease (Manandhar *et al.*, 2011). Reports from South Africa, Nepal and Brazil have shown that this disease accounts for up to 20 to 80 % of total yield losses (Manandhar *et al.*, 2011; Ward *et al.*, 1999), and is attributed to a reduction in photosynthetic leaf area and lodging (Latterell & Rossi, 1983).

1.3.3 Southern corn leaf blight

Bipolaris maydis infections on maize are race specific, with varying lesion structures. Upon interaction, the pathogen takes between 12 to 18 hours to infect its host under favorable

conditions (Singh & Srivastava, 2012) (Table 1.3). SCLB takes 2 to 3 days to form mature lesions that later release secondary inoculum to other plants (Singh & Srivastava, 2012) (Figure 1.2). Race O causes small diamond-shaped lesions that elongate into rectangular lesions delimited by veins to a length of 20 to 30 mm (Jeffers, 2004; Singh & Srivastava, 2012). These lesions later coalesce resulting in entire leaf blighting. Race T causes oval-shaped yellow to brown lesions that are larger than those of race O (Jeffers, 2004; Singh & Srivastava, 2012) and produces a T-cms-specific polyketide toxin (T toxin) that is specific to T-cms maize genotypes (Condon *et al.*, 2018). This race was implicated in causing the worst 1970 epidemic in the USA which destroyed more than 15 % of the maize crop (Hooker, 1974; Ullstrup, 1972) even though its origin and source of its unique toxin are still a mystery (Inderbitzin *et al.*, 2010; Turgeon & Baker, 2007; Turgeon & Lu, 2000). It is documented that SCLB thrives in hot and humid agroecosystems, preferring slightly higher temperatures (up to 30^{o}C) for initial infection than what is needed for NCLB (Warren, 1975) (Table 1.3). Yield losses of 10 to 40 % have been recorded due to SCLB infections, depending on race and environment, and there is a positive correlation between disease severity and maize yield (Bruns, 2017; Byrnes *et al.*, 1989; Fisher *et al.*, 1976; Singh & Srivastava, 2012).

For disease epidemiology therefore, growth and establishment of NCLB, GLS, and SCLB are favored by moderate temperatures between 20 to 30 ^{o}C and relative humidity above 90 %, leading to 10 to 80 % yield losses (Table 1.3). There is a need to establish the percentage co-occurrence of these three diseases at a plant, and field scale as well as at a larger spatial scale to model their combined potential yield losses. This will facilitate the development of management strategies that not only target a single but a combination of diseases.

1.4 DIAGNOSIS OF NCLB, GLS, SCLB AND IDENTIFICATION OF THEIR CAUSAL PATHOGENS

The need for diagnosis and identification of diseases and pathogens is becoming more critical due to the additive consequences of increased anthropogenic human activities such as global trade, and the fast-evolving pathogens due to climate change (Elad & Pertot, 2014; Hulme,

2009; Prakash *et al.*, 2014). Correct diagnosis of plant diseases and identification of causal pathogens at all stages in their life cycles are the crucial prelude to instituting proper management strategies. Likewise, failure to accurately diagnose diseases and correctly detect pathogens leads to inadequate employment of control measures, thus causing a reduction in crop yield and quality (Miller *et al.*, 2009). Like other plant diseases and pathogens, NCLB, GLS, and SCLB and their corresponding causal pathogens have been diagnosed and detected under field and greenhouse conditions based on symptoms (see sections 1.3), morphological characteristics of pathogens as well as molecular and phylogenetic differences.

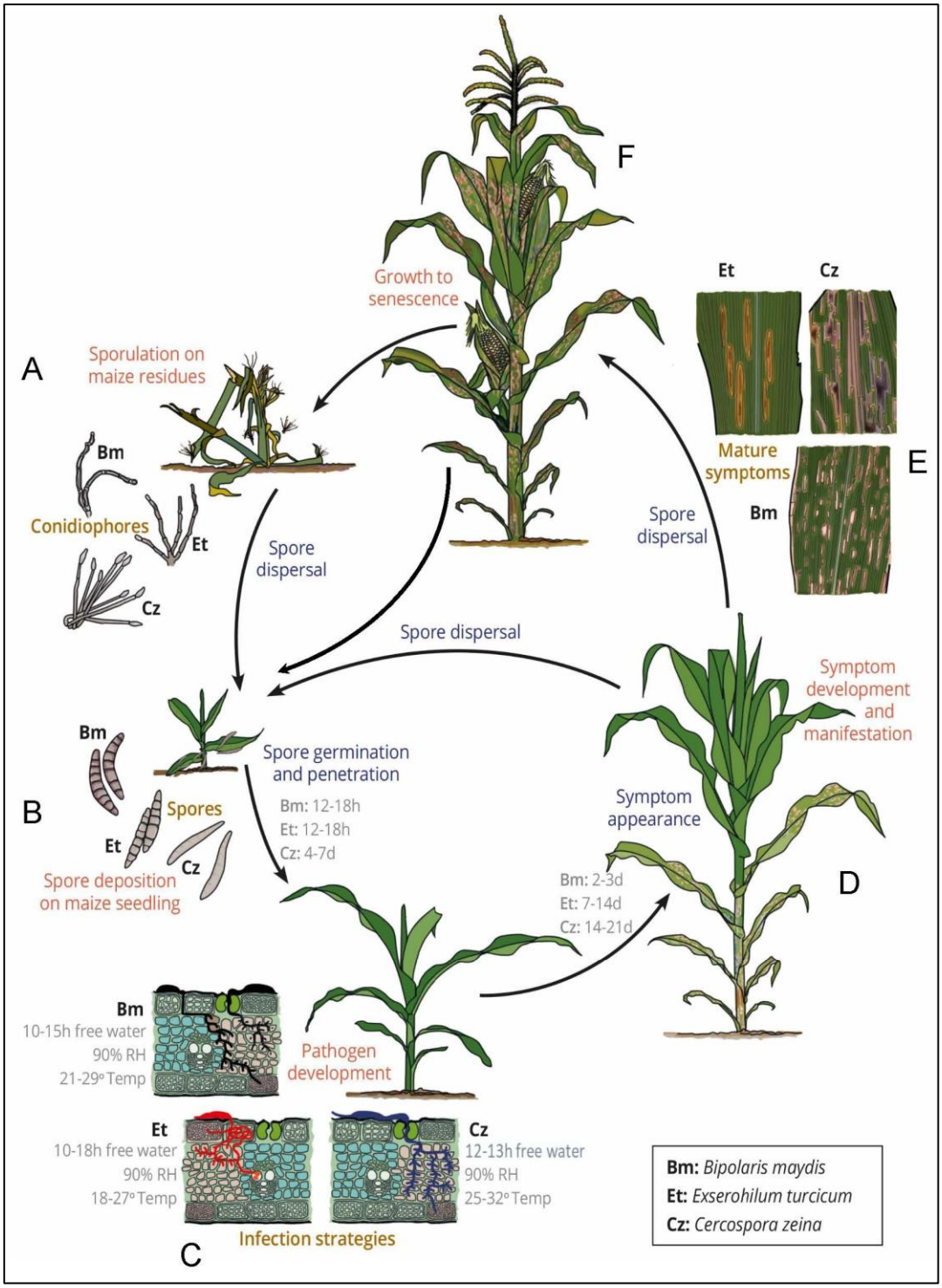

Figure 1.2: The asexual life cycles of *Exserohilum turcicum*, *Cercospora zeina* and *Bipolaris maydis*. A: Primary inoculum overwinters on the maize debris as conidiophores until the next growing season when they are dispersed in the form of conidia. B: Under favorable conditions, conidia are dispersed and land on the young maize plants. C: Conidia germinate and penetrate the plant cells and later develop into small chlorotic spots. D-E: Mature lesions later develop from the lower leaves to the younger leaves. These later give rise to conidia (secondary inoculum) which disperse to the younger plants and the cycle repeats. Et: *E. turcicum* (Bentolila *et al.*, 1991; Kotze *et al.*, 2019; Levy & Cohen, 1983; White, 1999); Cz: *C. zeina* (Latterell & Rossi, 1983; Ward *et al.*, 1999) and Bm: *B. maydis* (Jeffers, 2004; Singh & Srivastava, 2012).

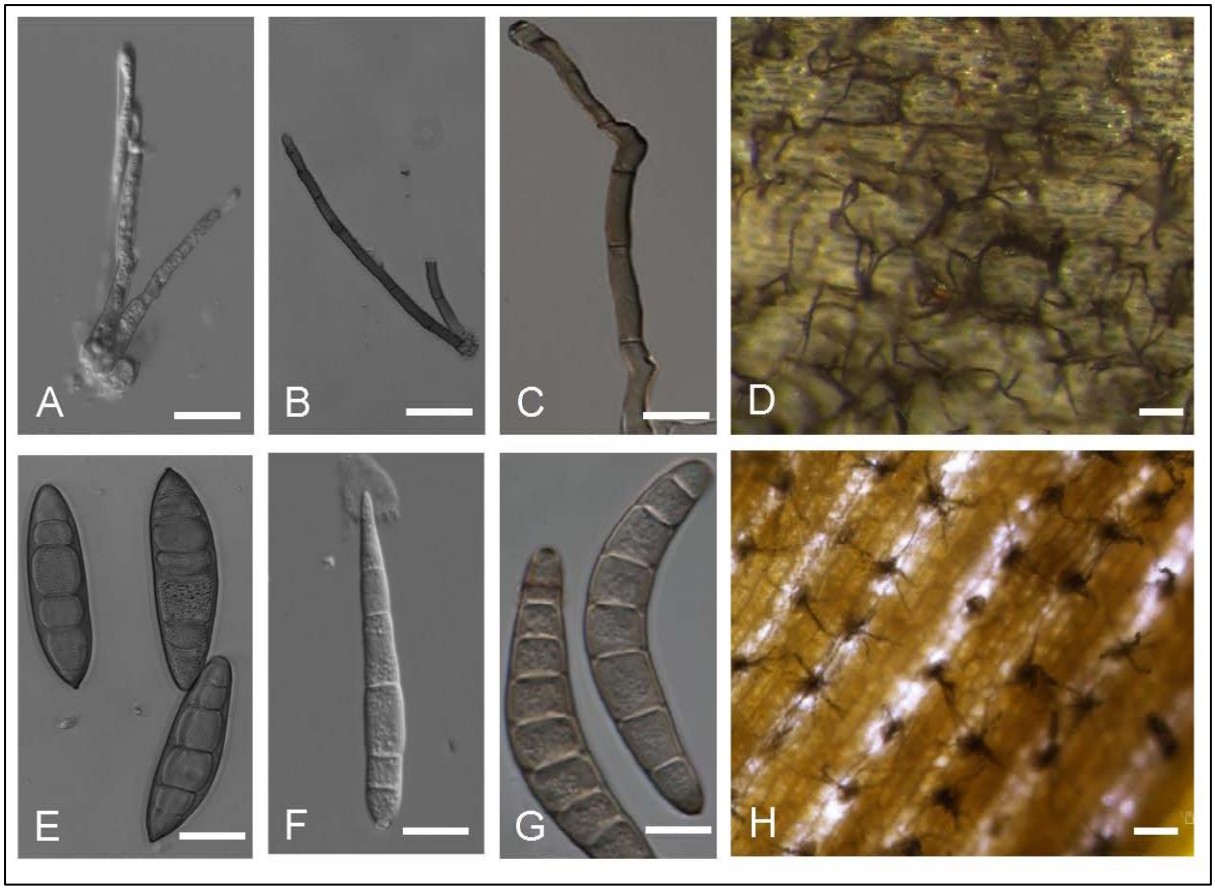

Figure 1.3: Asexual structures of *Exserohilum turcicum*, *Cercospora zeina*, and *Bipolaris maydis* on maize. A-C illustrates the conidiophores for A: *E. turcicum*; B: *C. zeina*; C: *B. maydis* as extracted from Manamgoda *et al.* (2012). D: *E. turcicum* conidia on leaf surface; E-G illustrates the conidia for E: *E. turcicum*; F: *C. zeina*. G: *B. maydis* as extracted from Manamgoda *et al.* (2012). H: *C. zeina* conidiophores coming out of the stomata of the leaf. Scale bars: A-C = 10 µm, D = 100 µm, E-G = 10 µm, H = 100 µm.

1.4.1 Field diagnosis of NCLB, GLS, and SCLB

Traditionally, plant disease diagnosis is done through conventional visual-field inspection of infected plant tissues (symptoms) by experienced technical human resources, following discrete disease rating scales to assess their severity (Bock *et al.*, 2010). Two standard scales, 1-5 and 1-9 where 1 = resistant and 5 or 9 = susceptible, are being used to rate the severity of NCLB (Abebe *et al.*, 2008; Asea *et al.*, 2009; Kumar *et al.*, 2011; Vivek *et al.*, 2010), GLS (Benson *et al.*, 2015; Berger *et al.*, 2014; Bubeck *et al.*, 1993; Chung *et al.*, 2011; Danson *et al.*, 2008; Munkvold *et al.*, 2001), and SCLB (Chung *et al.*, 2010; Chung *et al.*, 2011; Singh & Srivastava, 2012; Zwonitzer *et al.*, 2009) whose symptoms are unique (Table 1.3). Some plant pathologists have preferred using the “reversed” 1-9 scale with 1 = susceptible and 9 =

resistant (Bubeck *et al.*, 1993) while others have used it in the field and later converted it to the 1-5 scale using the formula: 0.5 * (disease score (1-9 scale) + 1) = disease score (1-5 scale) (Vivek *et al.*, 2010). These scales are used to assess disease severity by rating leaves from the ear leaf using the total percent leaf area covered with each disease symptoms by one or more disease raters. Disease ratings can further be manipulated to estimate area under the disease progress curve (AUDPC) or virulence index as has been illustrated in anthracnose of sorghum (Marley *et al.*, 2005; Mathur *et al.*, 1997; Rao *et al.*, 1998). While this traditional method has been refined over time, it is plagued by inherent unreliability of disease estimates observed between individual raters especially when it comes to disease symptoms that are small or unevenly distributed and is time consuming (Bock *et al.*, 2008; Bock *et al.*, 2010; Nutter Jr *et al.*, 1993; Poland & Nelson, 2011). Sometimes, morphological traits are misleading due to some similarities between disease symptoms. For instance, race O lesions of SCLB are sometimes mistaken for GLS while the initial symptoms of the three diseases (chlorotic spots) can potentially lead to misidentification (Figure 1.3).

Automated high-throughput digital imaging methods have been developed with the ability to produce quantitative phenotypic data across numerous time points and with greater accuracy, repeatability and precision than visual diagnosis (Mutka & Bart, 2015; Pauli *et al.*, 2016; Stewart & McDonald, 2014; Xie *et al.*, 2012). Additionally, machine-learning techniques are being used in diagnosing a wide range of plant diseases (Barbedo, 2013; Singh *et al.*, 2016) and are currently being used for NCLB, GLS and SCLB diagnosis. Digital image processing techniques, support vector machines (SVMs) and neural networks among other methods have been employed in diagnosing these maize diseases to an accuracy of up to 95.3 % (Chen & Wang, 2011; Kai *et al.*, 2007; QI *et al.*, 2016; Wang *et al.*, 2009; Xu *et al.*, 2015; Zhang, 2013). These have further been improved through deep learning (Guo *et al.*, 2016; LeCun *et al.*, 2015) to techniques such as convolutional neural networks (CNNs) that employ trainable filters and signals to achieve visual object, face and digit recognition with high accuracy (Serre *et al.*, 2007). Such approaches have been used to recognize NCLB lesions with 96.7 % accuracy

(DeChant *et al.*, 2017). These models can be mounted on to aerial or ground-based vehicles for plant phenotyping in addition to being employed in targeted management practices such as fungicide application (DeChant *et al.*, 2017). All these methods of disease diagnosis are a foundation to understanding pathogen ecology, epidemiology, and biology and their integration into management programs of several diseases will foster the prevention of epidemics.

Table 1.3: The epidemiological characteristics of NCLB, GLS and SCLB causal pathogens

	NCLB	GLS	SCLB
The first report (year and country)	1878, New Jersey, The United States (Drechsler, 1923)	1925, Illinois, The United States (Tehon & Daniels, 1925)	1923, The United States (Drechsler, 1925; Robert, 1953)
The first report in Africa	1924, Uganda (Emechebe, 1975)	1988, KwaZulu-Natal South Africa (Ward, Laing, & Nowell, 1997)	1974, Mpumalanga, South Africa (Rong & Baxter, 2006)
Causal pathogen (s) in Africa	*Exserohilum turcicum* (K.J. Leonard & Suggs)	*Cercospora zeina* (Crous & U. Braun)	*Bipolaris maydis* (Y. Nisikado and C. Miyake)
Pathogen lifestyle	Hemibiotroph (Ohm *et al.*, 2012)	Necrotroph (Benson *et al.*, 2015)	Necrotroph (Ohm *et al.*, 2012)
Lesion structure	Long oblong (cigar-shaped) tan or grayish (White, 1999)	Rectangular, tan to gray lesions delimited within veins (Latterell & Rossi, 1983)	Spindle-shaped (Race T) and rectangular parallel sided lesions (Race O) with chlorotic borders (Jeffers, 2004)
Lesion length	2.5 to 17.5 cm (White, 1999)	0.5 to 7 cm (Latterell & Rossi, 1983)	2 to 3 cm (Jeffers, 2004)
Asexual structures	Pale to olivaceous brown straight or slightly curved conidia with a hilum, 5-10 septa (Alcorn, 1988).	70-180 by 2-3 µm hyaline, 6-10 septa (Crous *et al.*, 2006)	Fusoid, straight or curved conidia with one germ tube from each end and protuberant conidial hilum, 2-8 septa (Alcorn, 1988)
Sexual structure	Pseudothecia reported in-vitro (Abadi *et al.*, 1993)	Unknown	Ascospores reported when intercrossed with other *Helminthosporium* spp (Nelson, 1960).
Teleomorph genera	*Setosphaeria* (Alcorn, 1988)	Unknown	*Cochliobolus* (Alcorn, 1988)
Optimal growth conditions	18-27^{o}C and high humidity	Temperature 22-30^{o}C, relative humidity is > 90 %	20-32^{o}C and high humidity
Toxin production in culture	Monocerin (Robeson & Strobel, 1982)	Cercosporin (*C. zeae-maydis*) (Wang *et al.*, 1998) Unknown toxin for *C. zeina*	Race T produces toxins I, II, III and IV (Karr *et al.*, 1974)

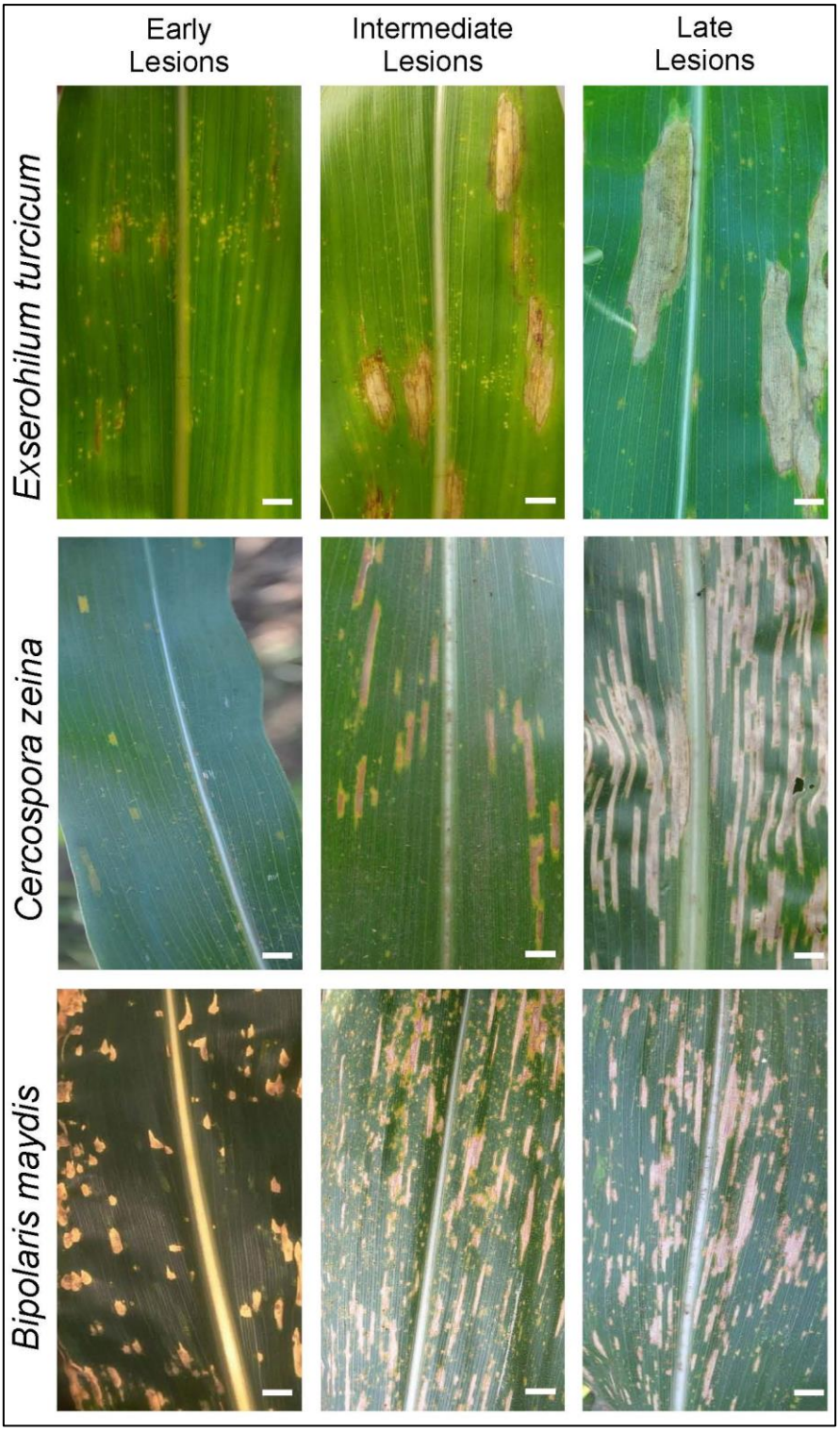

Figure 1.4: Symptomatic differences of NCLB, GLS and SCLB on maize. Each pathogen causes distinct disease symptoms at the intermediate and late stages of their infection cycle. Symptoms are prone to misidentification at the early stage. All three diseases exhibit chlorotic spots at their early stages, making them difficult to diagnose. At the intermediate to late stages, each disease assumes its distinct lesion shape (i.e. cigar-shaped lesions for NCLB; fine rectangular lesions for GLS and rectangular lesions with irregular margins for SCLB symptoms especially at the late stage. SCLB and GLS are not as clearly distinct as NCLB. Scale bars = 2 cm.

1.4.2 Morphological and physiological diagnosis and detection

The ability to identify causal pathogens of diseases is fundamental to understanding disease epidemiology, severity, their contribution to yield loss, and in many cases deciding on effective management strategies (McCartney *et al.*, 2003). For decades, pathogen identification has been based on conventional methods of isolation, culturing and laboratory identification. Morphological markers and taxonomic cues have resolved the difference between *E. turcicum* and *B. maydis,* pathogens that belong to *Helminthosporium*. Both pathogens are known to possess a hilum. *B. maydis* however, has an inconspicuous hilum (Alcorn, 1988) whereas *E. turcicum* has a truncate, strongly protruding hilum with an enveloping bubble (Leonard & Suggs, 1974) (Figure 1.4).

The Cercosporoid fungi are mainly distinguished based on the structure of the conidiogenous loci, hila and the pigmentation in their asexual structures (Crous & Braun, 2003). Based on morphology, Crous *et al.* (2006) summarize the features that distinguish between *C. zeae-maydis* and *C. zeina*, even though both pathogens cause undistinguishable symptoms on the plant. *C. zeina* conidia are characterized by their septate, hyaline, thin walls, smooth apex and thick darkened and refractive hila. These characteristics are like those of its sibling species *C. zeae-maydis*. However, they differ in conidia shape, with broad fusiform for *C. zeina* and broadly obclavate-subcyclindical for *C. zeae-maydis* (Crous *et al.*, 2006). They also differ in conidiophore length with *C. zeina* having shorter conidiophores (up to 100 µm) than *C. zeae-maydis* (up to 180 µm) and the time of growth, with *C. zeina* growing slower (colony reaches 10 to15 mm diameter after 3 weeks) than *C. zeae-maydis* (colony reaches 15 to 25 mm after 3 weeks) in culture (Crous *et al.*, 2006). Further, still, *C. zeae-maydis* produces a photoactive phytotoxin, cercosporin *in vitro* which plays a role in pathogenicity while *C. zeina* does not (Crous *et al.*, 2006; Goodwin *et al.*, 2001).

Studying disease diagnosis coupled with the morphological and physiological characteristics of NCLB, GLS, and SCLB causal pathogens has revealed that the diseases and their corresponding causal pathogens are variable in the symptoms caused and their infection

structures even though some symptoms present confusion in precise diagnosis. Physiologically, with an exception of *C. zeina* all the other pathogens produce toxins that are crucial in pathogenicity (Table 1.3). Swart *et al.* (2017) confirmed that the failure of *C. zeina* to produce cercosporin *in vitro* was due to a defective oxidoreductase gene *CTB-7*, a gene responsible for cercosporin synthesis in *C. zeae-maydis*. Since its spread and virulence on maize is significant, *C. zeina* is likely to be producing a different toxin that is yet to be characterized or deploys an unknown set of pathogenicity factors. Lastly, several of these conventional methods of diagnosis and identification rely on personal experience to interpret, making identification time consuming and impractical for cases where accurate species-level identification is needed and on time (McCartney *et al.*, 2003).

1.4.3 Molecular identification

More advanced methods to the conventional methods such as PCR-based amplification of nucleic acids and sequencing are increasingly being employed in *E. turcicum*, *C. zeina* and *B. maydis* identification. They are more sensitive, highly specific, faster and require no prior knowledge of the pathogen or expertise in the field of plant pathology (McCartney *et al.*, 2003; Ward *et al.*, 2004). The nuclear ribosomal DNAs (rDNAs), particularly the internal transcribed spacer (ITS), nested between conserved sequences of the 18S, 5.8S and 28S rRNA gene regions, has extensively been employed in the identification of *E. turcicum* (Goh *et al.*, 1998; Haasbroek *et al.*, 2014; Hernández-Restrepo *et al.*, 2018; Ramathani *et al.*, 2011; Weikert-Oliveira *et al.*, 2002), *C. zeina* (Bakhshi *et al.*, 2015; Crous *et al.*, 2006; Dunkle & Levy, 2000; Korsman *et al.*, 2012; Liu & Xu, 2013; Meisel *et al.*, 2009; Neves *et al.*, 2015) and *B. maydis* (Emami & Hack, 2002; Gogoi *et al.*, 2014; Goh *et al.*, 1998; Manamgoda *et al.*, 2012) and many other species. This is due to features such as high variability within the genus or among populations, small sizes (between 600 and 800 bp), faster evolution and the multiple copies available within a genome. These features thus allow for easy amplification even in small, dilute and degraded DNA (Gardes *et al.*, 1991; Lee & Taylor, 1992; Schoch *et al.*, 2012; White

et al., 1973). Through sequencing, the amplified regions have been compared to known sequences available on international databases such as EMBL and GenBank.

Other available gene regions for pathogen identification include the translation elongation factor 1-α, calmodulin, β-tubulin and mating types (Carbone & Kohn, 1999; James *et al.*, 2006; Walker *et al.*, 2012). Most of these gene regions are being employed in the identification of *E. turcicum* (Haasbroek *et al.*, 2014; Hernández-Restrepo *et al.*, 2018; Ramathani *et al.*, 2011), *C. zeina* (Bakhshi *et al.*, 2015; Meisel *et al.*, 2009; Muller *et al.*, 2016), and *B. maydis* (Leonard, 1974; Manamgoda *et al.*, 2012; Tan *et al.*, 2016; Turgeon *et al.*, 1995) in Africa and around the globe (Table 1.4).

Various species-specific PCR diagnostic tools, that do not require sequencing, have also been developed and are being used in the identification of these pathogens. For *E. turcicum*, the use of mating type genes is currently the only available species-specific diagnostic. Primers *MAT1-1*F, *MAT1-2*F and MAT_CommonR are used in a multiplex PCR assay in which more than one locus is simultaneously amplified in a single reaction (Henegariu *et al.*, 1997). The amplification of PCR products of 608 bp and 393 bp indicates the presence of *MAT1-1* and *MAT1-2*, respectively (Haasbroek *et al.*, 2014) (Table 1.5).

Species-specific PCR diagnostics have been developed and used to distinguish between the *Cercospora* species. The histone H3 gene region characterized by Crous *et al.* (2006) is able to distinguish three *Cercospora* species: *C. zeina*, *C. zeae-maydis* and *Cercospora* sp. Primers CzeaeHIST, CzeinaHIST and CmaizeHIST were employed in a multiplex assay to yield a 389-bp fragment that is present among all the three *Cercospora* species and a species-specific 284-bp fragment (Crous *et al.*, 2006). (Table 1.5). Furthermore, a cytochrome P450 reductase *(cpr1)* is also currently being used to differentiate *C. zeina* and *C. zeae-maydis* from other maize pathogens. Primers CPR1_2 forward and reverse of this gene region amplify *C. zeina* and *C. zeae-maydis* products of 164 bp, which is absent in other maize pathogens such as *E. turcicum*, *Phaeosphaeria maydis*, *Stenocarpella macrosporum* and many saprophytic organisms (Korsman *et al.*, 2012) (Table 5). Very recently the cercosporin toxin biosynthesis

(*CTB*)-*7* gene region primers, CTB-7 forward and reverse have been optimized and used to amplify 608-bp and 925-bp fragments, specific to *C. zeina* and *C. zeae-maydis*, respectively, and are not able to amplify (*CTB*)-*7* from other *Cercospora* species (Nsibo *et al.*, 2019; Swart *et al.*, 2017). *C. zeina* mating type gene regions have previously been characterized and primers CzMAT1 (forward and reverse) and Cz*MAT1-2*, (forward and reverse) amplify 631-bp and 409-bp fragments unique to *C. zeina MAT1-1* and *MAT1-2* respectively, with no amplification from any other tested species such as *C. zeae-maydis* (Muller et al., 2016) (Table 1.5).

For *B. maydis*, a multiplex mating type PCR assay was optimized for the determination of mating types of the pathogen. Primers MAT113 and MAT123 and MATcon5 were derived from *MAT1-1* and *MAT1-2* gene regions and these amplify 702-bp and 547-bp fragments unique to *B. maydis* (Gafur *et al.*, 1997) (Table 1.5).

1.5 MANAGEMENT OF NCLB, GLS, AND SCLB

Effective management of plant diseases requires a clear understanding of pathogen identification, ecology, epidemiology, and biology as reviewed in the previous sections. Any strategies developed should aim to interfere with the most vulnerable stages of the pathogen's life cycle to reduce the rate of disease development (Reddy *et al.*, 2013; Shah & Dillard, 2010; Ward & Nowell, 1998). Cultural practices, chemical usage and host genetic resistance are employed extensively in managing NCLB, GLS and SCLB.

1.5.1 Cultural control of NCLB, GLS, and SCLB

The three maize foliar diseases, NCLB, GLS and SCLB have similar management strategies including the use of tillage practices, rotation with non-host crops, and manipulation of environmental factors aimed at reducing the amount of initial inoculum of the pathogens in the fields (Ward & Nowell, 1998). Deep tillage is being used to offset the negative effects attributed to conservation tillage practices; practices that leave at least 30 % of the soil surface covered with crop residue at the end of the growing season, by ensuring burial of the pathogen

inoculum in the soil for a few months to kill them off (Huff *et al.*, 1988; Payne & Waldron, 1983). Rotations of at least two years with non-host crops are also being used to reduce these fungi to low levels, in addition to it improving the soil structure and nutrient levels thus increasing yields especially in seasons of low disease incidences (Ward & Nowell, 1998). Also, the removal of favorable environmental conditions (temperature, relative humidity, and leaf wetness) for pathogen development especially early in the growing season are crucial in hindering early-season disease development (Ward & Nowell, 1998). Although these cultural practices are useful in managing these diseases and may be effective in low-risk areas, they are not very effective in cases where the diseases are well established and there is abundance of external inoculum (Lipps *et al.*, 1998; Ward, Laing, *et al.*, 1997a; Ward & Nowell, 1998). Many of them involve the destruction of the soil structure which exposes the soil to erosion. They are also limiting due to lack of luxury to fallow the available farm land especially amongst smallholder farms, thus making them less effective (Karavina *et al.*, 2014).

1.5.2 Chemical control of NCLB, GLS and SCLB

Fungicide usage is one of the major strategies used in managing NCLB, GLS and SCLB. Broad-spectrum fungicides, particularly propiconazole, carbendazim, mancozeb, strobulurin, benomyl, flusilazole, and chlorothalonil, are being employed in managing NCLB, GLS and SCLB and are more effective in susceptible and moderately susceptible hybrids if one is to offset the costs associated with spraying (Reddy *et al.*, 2013; Shah & Dillard, 2010; Ward & Nowell, 1998). Fungicides such as fluazinam and strobulurin are used to effectively manage SCLB and NCLB, respectively, with fluazinam having a known efficacy reaching 98 % (Chen *et al.*, 2018; Shah & Dillard, 2010). Furthermore, Iturin A_2 from *Bacillus subtilis*, was discovered to have antifungal properties (Ye *et al.* (2012). This compound was concentrated and purified into an organic fungicide that registered 75 % efficacy against *B. maydis* (Ye *et al.*, 2012) and exhibits antifungal activity on other pathogens such as *Colletotrichum*, *Rhizoctonia*, *Alternata*, and *Verticillium* (Gong *et al.*, 2006; Kim *et al.*, 2010; Yu *et al.*, 2002). As such, it could be a

potential organic fungicide against other foliar maize pathogens such as *E. turcicum* and *C. zeina* and hence needs to be tested.

While fungicide usage is an effective management strategy, signatures of fungicide resistance have been registered owing to reduction in efficiency over time. Fungicide resistance has been recorded in a number of cereal pathogens including *Zymoseptoria tritici* (Garnault *et al.*, 2019), *Pyrenophora teres* f. *teres* (Ellwood *et al.*, 2019), and *Magnaporthe oryzae* (Bohnert *et al.*, 2018). Fungicidal resistance among the NCLB, GLS and SCLB pathogens is yet to be studied. Notably, chemical control is also unaffordable for the majority of smallholder farmers. There is a need to harness other more durable and affordable management strategies such as host resistance through breeding and using them in an integrated manner.

1.5.3 Breeding for resistance against NCLB, GLS and SCLB

Host plant resistance is by far the most cost-effective, environmentally friendly and easily adaptable approach to disease management in maize. For breeding, effective disease resistance depends on the effect and strength of the identified resistance genes in the host (Nelson *et al.*, 2018). These genes may act qualitatively or quantitatively to confer resistance against a given pathogen or group of pathogens. Qualitatively, resistance genes, known as major genes, confer complete or near-complete resistance while quantitative resistance involves multiple minor genes to confer small additive resistance effects (Jones & Dangl, 2006).

1.5.4 Qualitative breeding for resistance

Resistance in maize against NCLB is both qualitative and quantitative and can be used either separately or in combination. Qualitative genes can be dominant, following a gene-to-gene model (Ogliari *et al.*, 2005; Welz & Geiger, 2000) (Table 1.2). This form of resistance against *E. turcicum* is mediated by *Ht* resistance genes, a name derived from the previous species name *Helminthosporium turcicum* (Welz & Geiger, 2000). The four well-known *Ht* genes include *Ht1, Ht2, Ht3* and *Htn1*, of which the functions of *Ht2, Ht3* and *Htn1* are yet to be

characterized (Ogliari *et al.*, 2005; Van Staden *et al.*, 2001; Yin *et al.*, 2003). The *Ht1* gene encodes a wall-associated receptor-like kinase that confers resistance against race 12 (Hurni *et al.,* 2015). It is highly conserved in other *E. turcicum* alternative hosts, particularly sorghum, rice and foxtail millet (*Setaria italica*). *HtP* is another major *Ht* resistance gene that confers a wide range to resistance to *E. turcicum* races 123x and 23rx, which combine multiple virulence factors (Ogliari *et al.*, 2005). Recessive genes *ht4* and *rt* are also known to confer resistance to NCLB, and, unlike other genes that are race specific, these are postulated to confer resistance to a wide range of races (Ogliari *et al.*, 2005).

For GLS, a resistance locus, *GLS1* was characterized from breeding material derived from teosinte germplasm (Gevers, 1994). Tests for resistance through crosses between M162W (highly susceptible local inbred line) and TEOS1 (resistant multi-eared maize genotype) confirmed resistance. Although they proposed that *GLS1* was a major resistance gene against GLS, their data are not conclusive due to lack of further tests in numerous germplasm backgrounds. Furthermore, segments of a teosinte (*Zea mays* ssp. *parviglumis*) genome were introgressed into the background of maize line B73 to develop near-isogenic lines (NIL). Through quantitative trait loci (QTL) mapping of a segregating population from these NIL parents, QTL *Qgls8* situated on the same chromosome 8 was discovered and confirmed to also confer resistance to GLS (Zhang *et al.*, 2017).

Studying host resistance to *B. maydis* has revealed that some qualitative genes recessively confer resistance to its different races. The *rhm* gene identified by Smith and Hooker (1973) mainly confers resistance to race O strains, and, to a lesser extent race T of *B. maydis* (Chang & Peterson, 1995; Zaitlin *et al.*, 1993). In an experiment to functionally characterize this *rhm* gene, transposable elements were inserted into the dominant *Rhm* gene, a gene that confers susceptibility to maize lines. Mutation of *Rhm* into *rhm* resulted in maize lines gaining resistance against *B. maydis,* thus confirming its functionality in conferring resistance (Zaitlin *et al.*, 1993). More than two decades ago, Chang and Peterson (1995) proposed a two-gene model, and confirmed that homozygous mutations in two linked genes, *rhm1* and *rhm2,*

conferred resistance against *B. maydis* which was manifested by a reduction in lesion size. Later, *rhm1* was identified to associate with other known defense-related factors such as the pathogenesis-related gene, *PR1*, chitinase and peroxidase (Simmons *et al.*, 2001). While qualitative resistance plays a role against *B. maydis*, particularly on race O, the best remedy that has been applied previously against race T was to eliminate the use of T-cms maize germplasm from breeding programs (Leonard, 1977a, 1977b; Rong & Baxter, 2006). Resistance to race C is still unknown.

1.5.5 Quantitative breeding for resistance

Quantitative disease resistance is known to reduce disease severity and incidence rather than completely eliminating the disease (Poland *et al.*, 2009; Young, 1996). In recent years QTL mapping studies have characterized several traits in crops, including resistance to several plant pathogens (Bernardo, 2008; Xu & Crouch, 2008). This next section reviews studies that have been conducted on quantitative breeding against NCLB, GLS and SCLB.

Quantitative trait loci for resistance against NCLB spans the whole maize genome and is common in many inbred lines and cultivars (Chen *et al.*, 2016; Wang *et al.*, 2018; Welz & Geiger, 2000; Wende *et al.*, 2018; Wisser *et al.*, 2006). Through techniques such as genome-wide nested association mapping, QTLs with several potential candidate genes have been characterized and confirmed to confer resistance against NCLB (Poland *et al.*, 2011). Although many QTLs are known to confer resistance to a broad spectrum of *E. turcicum* races, some QTLs are known to confer race-specific resistance to NCLB (Chung *et al.*, 2010; Chung *et al.*, 2011).

Hot spots of QTLs conferring resistance to GLS span discrete regions of chromosomes 1, 2, 3, 4, 5, and 7 (Berger *et al.*, 2014; Lehmensiek *et al.*, 2001). Many of these QTLs come from bi-parental crosses between susceptible and resistant genotypes, tested under different disease pressures, germplasm backgrounds and environmental conditions (Balint-Kurti *et al.*, 2008; Berger *et al.*, 2014; Clements *et al.*, 2000; Danson *et al.*, 2008; Lehmensiek *et al.*, 2001).

Molecular markers linked to QTLs play a crucial role in their precise identification (Danson *et al.*, 2008; Lehmensiek *et al.*, 2001). For example, RFLPs and microsatellite markers were used to precisely locate four GLS resistance QTLs on chromosomes 1 (bin 1.05/06), 3 (bin 3.04), 5 (bins 5.03/04 and 5.05/06) from field trials on Zimbabwean inbred lines conducted in South Africa (Lehmensiek *et al.*, 2001). A majority of the QTLs are environment specific, however, many QTLs expressed in varying environments have been characterized (Berger *et al.*, 2014) and these can be introgressed into maize genotypes grown across different environments.

Major SCLB resistance QTLs characterized from different maize genotypes exist. Using mature recombinant inbred lines (RILs), Carson *et al.* (2004) found 11 QTLs spanning chromosomes 1, 2, 3, 4, 7 and 10, and are associated with SCLB resistance. More SCLB resistance QTLs are continuously being characterized from different maize genotypes at different maturity stages and spanning different chromosomes of maize (Balint-Kurti & Carson, 2006; Negeri *et al.*, 2011; Zwonitzer *et al.*, 2009).

Thus, qualitative and quantitative resistance is crucial in managing NCLB, GLS, and SCLB. Analysis of markers such as AFLPs, RFLPs and microsatellites that are linked to QTLs have allowed faster and more accurate identification of these QTLs. Also, identified QTLs confirm that resistance to NCLB, GLS and SCLB follows polygenic inheritance of resistance in maize. With an exception of GLS whose qualitative resistance is yet to be elucidated, maize breeders rely on both qualitative and quantitative resistance against NCLB and SCLB. Through molecular marker breeding, these and several other QTLs and resistance genes can be introgressed into high-yielding hybrids and inbred lines to enhance resistance, and thus increase yields.

Table 1.4: The universal molecular bar codes used in the identification of NCLB, GLS and SCLB causal pathogens

Locus	Definition	Primer Name	Oligonucleotide (5'→3'	Specificity	GenBank Numbers			Reference
					E. turcicum	***C. zeina***	***B. maydis***	
ITS	Internal transcribed spacer	ITS1	TCCGTAGGTGAACCTGCGG	Universal	NR_163537	DQ185081	NR_138224	(Berbee *et al.*, 1999; Goodwin *et al.*, 2001; Haasbroek *et al.*, 2014; Meisel *et al.*, 2009; Tang *et al.*, 2015; White, 1999)
		ITS4	TCCTCCGCTTATTGATATGC	Universal				
TEF1α	Translation elongation factor 1 alpha	EF1-728F	CATCGAGAAGTTCGAGAAGG	Universal	LT896674	DQ185093	KM093793	(Carbone & Kohn, 1999; Crous *et al.*, 2006; Meisel *et al.*, 2009; Neves *et al.*, 2015)
		EF1-986R	TACTTGAAGGAACCCTTACC	Universal				
CAL	Calmodulin	CAL-228F	GAGTTCAAGGAGGCCTTCTCCC	Universal	LT852468	DQ185117	HQ699077	(Carbone & Kohn, 1999; Crous *et al.*, 2006; Neves *et al.*, 2015)
		CAL-737R	CATCTTTCTGGCCATCATGG	Universal				
ACT	Actin	ACT-512F	ATGTGCAAGGCCGGTTTCGC	Universal	LT837686	DQ185105	AY748989	(Carbone & Kohn, 1999; Crous *et al.*, 2006)
		ACT-783R	TACGAGTCCTTCTGGCCCAT	Universal				
TUB	β-tubulin	Bt1a	TTCCCCCGTCTCCACTTCTTCATG	Universal	LT899336	-	KX835024	(Glass & Donaldson, 1995; Hernández-Restrepo *et al.*, 2018)
		Bt1b	GACGAGATCGTTCATGTTGAACTC	Universal				

These gene regions require PCR amplification, sequencing and phylogenetic analysis for species identification

Table 1.5: The species-specific molecular bar codes used in the identification of NCLB, GLS and SCLB causal pathogens

Locus	Definition	Primer Name	Oligonucleotide (5'→3')	Species	Amplicon size (bp)	Date Published
CPR*	Cytochrome P450 reductase	CPR1_1F	TCCACTCTCGCTCAATTCG	*C. zeina*	164	2012
		CPR1_1R	GCCTTCATCGCCATATGTTC			
		CPR1_1F	TCCACTCTCGCTCAATTCG	*C. zeae-maydis*	164	
		CPR1_1R	GCCTTCATCGCCATATGTTC			
CTB7	Cercosporin toxin biosynthesis 7	CTB7-F	AAGAGTGCTTGTGAATGG	*C. zeina*	618	2017
		CTB7-R	GATGCGGGTGAAGTAGAAA			
		CTB7-F	AAGAGTGCTTGTGAATGG	*C. zeae-maydis*	925	
		CTB7-R	GATGCGGGTGAAGTAGAAA			
HIST	Histone H3	CylH3F	AGG TCC ACT GGT GGC AAG	*Cercospora* species	389	2004 and 2006
		CylH3R	AGC TGG ATG TCC TTG GAC TG			
		CzeinaHIST	TCGAGTGGCCCTCACCGT	*C. zeina*	284	
		CzeaeHIST	TCGACTCGTCTTTCACTTG	*C. zeae-maydis*	284	
		CmaizeHIST	TCGAGTCACTTCGACTTCC	*Cercospora* sp.	284	
MAT	Mating types	Cz*MAT1-1*F	TCACCCTTTCACCGTACCCA	*C. zeina*	631	2016
		Cz*MAT1-1*R	CACCTGCCATCCCATCATCTC			
		Cz*MAT1-2*F	CGATGTCACGGAGGACCTGA		409	
		Cz*MAT1-2*R	GTGGAGGTCGAGACGGTAGA			
		*MAT1-1*F	CTCGTCCTTGGAGAAGAATATC	*E. turcicum*	608	2014
		*MAT1-2*F	GCTCCTGGACCAAATAATACA		393	
		MAT_CommonR	GTATTCCGTG TCCGCATT			
		MAT113	AGGTAGTTTGAGGTGAGGGCAGATGATG	*B. maydis*	702	1997
		MATcon5	TCTTTGTTTTCCTGTGACTGCCTGTTG			
		MAT123	CTGGGCTGATTGGGGGCTTGATAC		547	
		MATcon5	TCTTTGTTTTCCTGTGACTGCCTGTTG			

*Primers can distinguish between *C. zeina* and *C. zeae-maydis* based on differences in their quantitative PCR (qPCR) melting peaks (Korsman *et al.*, 2012)

1.6 RECENT ADVANCES IN POPULATION GENETICS OF NCLB, GLS AND SCLB CAUSAL PATHOGENS.

Pathogen survival is based on its ability to adapt to the constant environmental changes through evolution (McDonald, 1997). Therefore, management strategies to counteract their fast-changing lifestyles must be guided by understanding the genetics of populations rather than individual pathogens and how they will evolve in response to changing environments (McDonald, 1997). In this section, we provide insights into the population genetics of the maize foliar fungal pathogens, *E. turcicum*, *C. zeina,* and *B. maydis* and how this knowledge has helped shape management strategies.

1.6.1 Population genetics of *Exserohilum turcicum*

RAPD, AFLPs and microsatellite markers have clarified the population structure of *E. turcicum* globally. All reports from Asia, Europe, and Africa reveal that *E. turcicum* is a genetically and genotypically diverse pathogen, with populations from Asia and Africa exhibiting higher diversity than those in Europe (Borchardt *et al.*, 1998; Dong *et al.*, 2008; Haasbroek *et al.*, 2014; Human *et al.*, 2016; Muiru *et al.*, 2010a). European *E. turcicum* populations have low genetic diversity and partial population differentiation exists (Borchardt *et al.*, 1998). This is particularly noticeable between Eastern and Southern Europe owing to the presence of the Alps as major geographical barriers (Borchardt *et al.*, 1998). Therefore, geographic boundaries such as mountains and water bodies, many of which exist in many African countries, have the potential to influence *E. turcicum* population structure in Africa. Furthermore, sexual recombination is a major evolutionary factor driving the observed global population structure of *E. turcicum*, even in continents like Europe where rare sexual occurrences have been observed (Borchardt *et al.*, 1998; Fan *et al.*, 2007) (Table 1.3). To confirm occurrence of sexual recombination in *E. turcicum* populations, mating type genes were found to exist in equal distribution and frequency in many countries (Haasbroek *et al.*, 2014; Human *et al.*, 2016; Keller & Bergstrom, 1990; Weems & Bradley, 2018). Additionally,

the *E. turcicum* sexual stage was successfully induced on medium with mating stimulators such as Sach's medium with barley culm (Fan *et al.*, 2007).

Through population genetic analysis it is now possible to study the physiological race distribution and the potential for (re)-emergence of races in regions due to virulence variability (Fan *et al.*, 2007). Using RAPD markers, Dong *et al.* (2008) successfully illustrated that *E. turcicum* is highly diverse, and showed that physiological race groups within the same geographical locations clustered together. They confirmed that race O and race 1 of *E. turcicum* are the most abundant in Northern China. Surprisingly, they reported a rare emergence of race 123N, able to overcome four resistance genes in the available hybrids. In this study, Johnson grass (*Sorghum halepense*) and sorghum were confirmed as alternative hosts of *E. turcicum*. With the exception of Kenya (Borchardt *et al.*, 1998; Muiru *et al.*, 2010a) and South Africa (Human *et al.*, 2016; Nieuwoudt *et al.*, 2018), not much is known about the genetic diversity of the pathogen in Africa. There is therefore a need to decipher the population structure of *E. turcicum* in other African countries where *E. turcicum* is present on maize.

1.6.2 Population genetics of *Cercospora zeina*

Prior to distinguishing between the two GLS causal pathogens, several studies conducted on GLS referred to all infections as being caused by *C. zeae-maydis* (Latterell & Rossi, 1983; Lipps, 1998; Okori *et al.*, 2003; Ward *et al.*, 1999). As more studies based on taxonomy, molecular and phylogenetic tools emerged, it became evident that there were two sibling species, *C. zeina* (formerly known as *C. zeae-maydis* type II) and *C. zeae-maydis*. Using AFLPs, RFLPs and microsatellite markers, *C. zeina* was confirmed as the predominant GLS causal pathogen in Africa, with genetic relatedness to *C. zeina* in the Americas, and Asia (Dunkle & Levy, 2000; Liu & Xu, 2013; Muller *et al.*, 2016; Okori *et al.*, 2003; Wang *et al.*, 1998). *C. zeina* is a highly diverse pathogen in Africa, with weak to no defined population structure (Muller *et al.*, 2016; Okori *et al.*, 2003; Okori *et al.*, 2015). Given that maize is non-native to Africa, the dominance of *C. zeina* on the continent has been hypothesized to be as

a result of a host jump from another closely related host such as sorghum, or even an unidentified grass species (Crous *et al.*, 2006; Dunkle & Levy, 2000).

Sexual recombination accounts for a majority of the observed population structure (Muller *et al.*, 2016; Okori *et al.*, 2003). Although sexual structures have not been observed under field and laboratory conditions, cryptic sexual recombination has been suggested based on the presence of mating type genes in equal distribution and frequency in addition to the lack of linkage disequilibrium among haplotypes (Groenewald *et al.*, 2006; Muller *et al.*, 2016). Possible explanations for failure to induce or discover *C. zeina*'s sexual stage may include the absence of environmental parameters the pathogen encounters in nature to trigger sexual recombination. It could also be the failure to systematically monitor the development of an ascocarp in this presumably asexual pathogen (Dyer *et al.*, 1992) or fertility decline in the pathogen (Dyer & Paoletti, 2005). Gene flow is another evolutionary factor that has influenced the population structure of *C. zeina*. Several shared genotypes with a lack of population structure have been observed within and among geographical locations (Muller *et al.*, 2016; Okori *et al.*, 2003; Okori *et al.*, 2015), implying that there has been unrestricted movement of the pathogen genotypes across boundaries and over long distances.

There is still little information that is available concerning the evolutionary potential of *C. zeina* and therefore, a comprehensive study of the pathogen in all major maize producing countries in Africa is needed.

1.6.3 Population genetics of *Bipolaris maydis*

There is very limited information regarding the genetic diversity of *B. maydis*. RAPD markers have been used to understand the population genetic structure of *B. maydis,* especially in India from where the majority of the reports emerge. From India, *B. maydis* has been reported to be highly diverse with little to no population differentiation (Gogoi *et al.*, 2014; Gopi, 2008; Jahani *et al.*, 2011; Karimi, 2003), suggesting that gene flow plays a major evolutionary role in the pathogen's population structure. Furthermore, physiological race O is the most

predominant race in India (Gogoi *et al.*, 2014; Pal *et al.*, 2015), with high genetic variability among isolates of the same race (Gafur *et al.*, 2002; Pal *et al.*, 2015). Sexual recombination is another major evolutionary factor driving the observed genetic diversity (Gafur *et al.*, 2002; Gafur *et al.*, 1997).

SCLB is a potential threat to maize production in Africa even though it has only been reported in Kenya (Mwangi, 1998) and South Africa (Rong & Baxter, 2006) thus far. This is supported by the fact that *B. maydis* is both an air- and seed-borne pathogen (Aylor & Lukens, 1974; Biemond *et al.*, 2013; Manoj & Agarwal, 1998). Due to the increasing anthropogenic activities and global trade, unreported incidences of the pathogen in the rest of Africa are possible. Therefore, countries without SCLB need to be vigilant through establishment of phytosanitary regulations and bodies that test and ensure movement of healthy seed across geographical boundaries. Methods such as roguing, seed dressing and proper storage to minimize contamination are suggested as alternative ways of ensuring seed health (Biemond *et al.*, 2013).

The current population genetic studies conducted on *B. maydis* have depended upon dominant molecular markers, particularly RAPD to understand the genetics of the pathogen (Gogoi *et al.*, 2014; Jahani *et al.*, 2011). The availability of the *B. maydis* genome (Condon *et al.*, 2013), however, offers a unique opportunity to develop more robust molecular markers such as microsatellite and single-nucleotide polymorphism (SNP) markers that can be exploited to enable comprehensive studies of the pathogen from all the countries where the disease exists.

1.7 POPULATION GENETICS AND BREEDING FOR RESISTANCE AGAINST NCLB, GLS AND SCLB

Qualitative and quantitative disease resistance strategies either singly or in combination (McDonald & Linde, 2002), are important for the managing NCLB, GLS, and SCLB. These

strategies require screening of the developed advanced maize backgrounds across multiple environments as well as across several races and isolates with varying levels of virulence.

Qualitatively, major resistance genes have followed the gene-for-gene model and very few exhibit pleiotropic traits against more than one disease (Wisser *et al.*, 2011). Very recently, a maize glutathione S-transferase (*GST)* gene was implicated in disease resistance against NCLB, GLS, and SCLB (Wisser *et al.*, 2011). The *GST* gene was characterized as a multiple disease resistance gene belonging to a *GST* gene cluster (*GST1*, *GST2*, and *GST3*), that confers resistance to a number of diseases and that it possesses homologs that are involved in plant-pathogen interactions (Dean *et al.*, 2005; Wisser *et al.*, 2011). Previous studies associated the *GST1* gene with quantitative disease resistance due to its co-localization with the QTL for NCLB, GLS, and SCLB on maize chromosome 7 (Wisser *et al.*, 2006). A closer examination using SNP markers however, showed that the function of the *GST* gene was not attributable to the effect of the QTLs for NCLB, GLS and SCLB and, thus, may be a major resistance gene (Wisser *et al.*, 2011). A clear understanding and characterization of such multiple disease resistance genes as well as incorporating them into breeding programs will significantly reduce yield losses that are attributed to plant pathogens.

In cereals, many QTLs have been characterized from a diversity of crop lines and more remain unidentified. For example, in rice alone, Wisser *et al.* (2005) reported 94 QTLs from several studies, spanning half of the plant genome. Subsequently, maize hosts more than 437 QTLs associated with a number of diseases and these span 89 % of the maize genome (Wisser *et al.*, 2006; Yu *et al.*, 2008). This confirms that genes harboring small additive effects play a significant role in conferring resistance. Notably, clusters of genes in a QTL are also capable of conferring resistance to more than a single race within a species or even exhibit overlapping resistance to multiple species, even though some may have subtle effects (Hulbert, 1997; Nelson *et al.*, 2018).

In maize, several resistance-related unique QTLs linked to NCLB and SCLB have been discovered, some of which exhibit complementary resistance to both diseases (Li *et al.*, 2018).

Multiple other resistance QTLs associated with resistance to NCLB, GLS, and SCLB have been discovered (Lopez-Zuniga *et al.*, 2019). These function either individually or are associated with at least two diseases: NCLB and GLS; GLS and SCLB; as well as NCLB and SCLB; while others were associated with all three diseases at once (Lopez-Zuniga *et al.*, 2019). Combining several QTLs in a breeding program has been suggested for Ug99 races of wheat as the most durable strategy of resistance (Singh *et al.*, 2006) and would be the most durable strategy in maize against NCLB, GLS, and SCLB when integrated with other management strategies.

1.8 CONCLUSION

This review provides a summary of the recent advances in NCLB, GLS and SCLB ecology and epidemiology as well as the population biology and genetics of their causal pathogens in Africa. All three diseases are reported to exist on the continent and are threatening the continent's maize production and food security, even though up-to-date yield losses in Africa are debatable. NCLB, GLS and SCLB are polycyclic in nature and can infect maize under overlapping and favorable environmental conditions within a single growing season. With the increasing adoption of conservation agriculture and monocropping, there is a likelihood of these foliar disease to escalate to all maize-producing countries, owing to the accumulation of inoculum and shared dispersal mechanisms. Several management strategies at the commercial level, particularly cultural practices, fungicide usage and breeding for resistance are increasingly being adopted and used in Africa. However, since most of the farming in Africa is small scale, fungicide usage is not widespread due to its cost implications and the aftereffects on the soil and human health. As such, there is increasing adoption of breeding for resistance at the small-scale level, used in combination with cultural practices.

This review has further illustrated that there is limited knowledge available on the population biology and genetics of *E. turcicum*, *C. zeina* and *B. maydis* in Africa and thus the evolutionary potential of these pathogens to overcome resistance is not fully established. There is therefore

a need to conduct large scale sampling of isolates across the continent, to study their diversity as well as trace their migration patterns across the continent. Based on the review of the extant literature, this thesis is about studying the genetic diversity of *C. zeina* in Africa using microsatellite markers, with a view to understand its evolutionary potential and dispersal mechanisms. Small-scale population genetic studies have previously been conducted and their findings show that *C. zeina* is a highly diverse pathogen, with sexual recombination and gene flow playing a major role as its evolutionary drivers. Testing the hypothesis of genetic diversity, origin and evolutionary potential of *C. zeina* at a continental scale would, therefore, require population sampling that spans countries and different farming systems to gain more insight into the population subdivisions and genetic diversity. Such studies, if conducted on *C. zeina,* will contribute to the understanding of the pathogen's biology and provide some guidelines in the development of improved and more effective management strategies of GLS.

The major aim of this thesis, therefore, was to determine the genetic diversity and population structure of *C. zeina* across maize-based cropping systems in five countries in sub-Saharan Africa. Five hypotheses were tested: (i) *C. zeina* is a genetically diverse pathogen in Africa (ii) farming systems influence the genetic structure of *C. zeina* (iii) sexual recombination and gene flow define the evolutionary potential and population structure of the pathogen (iv) *C. zeina* migrated from South Africa, where it was first reported, into other African countries and (v) *C. zeina* is a heterothallic pathogen with the potential to successfully produce sexual spores *in vitro*. This research was accomplished through a collection of *C. zeina* isolates from individual smallholder farms spanning countries in Eastern Africa (Kenya and Uganda) and Southern Africa (South Africa, Zambia, and Zimbabwe).

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1.10 REFERENCES

Abadi, R., Levy, R., & Levy, Y. (1993). Mating types of *Exserohilum turcicum* in Israel. *Phytoparasitica, 21*(4), 315-320.

Abadi, R., Levy, Y., & Bar-Tsur, A. (1989). Physiological races of *Exserohilum turcicum* in Israel. *Phytoparasitica, 17*(1), 23-30.

Abebe, D., & Singburaudom, N. (2006). Morphological, cultural and pathogenicity variation of *Exserohilum turcicum* (pass) Leonard and Suggs isolates in maize (*Zea mays* l.). *Kasetsart Journal (Nat. Sci.), 40*, 341-352.

Abebe, D., Singburaudom, N., Sangchote, S., & Sarobol, E. (2008). Evaluation of maize varieties for resistance to northern leaf blight under field conditions in Ethiopia. *Kasetsart Journal of Nature and Science, 42*, 1-10.

Adipala, E., Takan, J., & Ogenga-Latigo, M. (1995). Effect of planting density of maize on the progress and spread of northern leaf blight from *Exserohilum turcicum* infested residue source. *European Journal of Plant Pathology, 101*(1), 25-33.

Alcorn, J. (1988). The taxonomy of "*Helminthosporium*" species. *Annual Review of Phytopathology, 26*(1), 37-56.

Asea, G., Vivek, B. S., Bigirwa, G., Lipps, P. E., & Pratt, R. C. (2009). Validation of consensus quantitative trait loci associated with resistance to multiple foliar pathogens of maize. *Phytopathology, 99*(5), 540-547.

Aylor, D. E., & Lukens, R. J. (1974). Liberation of *Helminthosporium maydis* spores by wind in the field. *Phytopathology, 64*(8), 1136-1138.

Bakhshi, M., Arzanlou, M., Babai-Ahari, A., Groenewald, J. Z., & Crous, P. W. (2015). Is morphology in *Cercospora* a reliable reflection of generic affinity. *Phytotaxa, 213*(1), 022-034.

Balint-Kurti, P., & Carson, M. (2006). Analysis of quantitative trait loci for resistance to southern leaf blight in juvenile maize. *Phytopathology, 96*(3), 221-225.

Balint-Kurti, P. J., Wisser, R., & Zwonitzer, J. C. (2008). Use of an advanced intercross line population for precise mapping of quantitative trait loci for gray leaf spot resistance in maize. *Crop Science, 48*(5), 1696-1704.

Barbedo, J. G. A. (2013). Digital image processing techniques for detecting, quantifying and classifying plant diseases. *SpringerPlus, 2*(1), 660.

Bashir, K., Kamaruzaman, S., & Khairulmazmi, A. (2018). First report of northern corn leaf blight disease caused by *Exserohilum turcicum* on *Zea mays* in Malaysia. *Journal of Molecular and Genetic Medicine, 12*(387), 1747-0862.1000387.

Bateman, G., Gutteridge, R., Gherbawy, Y., Thomsett, M., & Nicholson, P. (2007). Infection of stem bases and grains of winter wheat by *Fusarium culmorum* and *F. graminearum* and effects of tillage method and maize-stalk residues. *Plant Pathology, 56*(4), 604-615.

Bebber, D. P. (2015). Range-expanding pests and pathogens in a warming world. *Annual Review of Phytopathology, 53*, 335-356.

Bebber, D. P., Holmes, T., & Gurr, S. J. (2014). The global spread of crop pests and pathogens. *Global Ecology and Biogeography, 23*(12), 1398-1407.

Benson, J. M., Poland, J. A., Benson, B. M., Stromberg, E. L., & Nelson, R. J. (2015). Resistance to gray leaf spot of maize: genetic architecture and mechanisms elucidated through nested association mapping and near-isogenic line analysis. *PLoS Genetics, 11*(3), e1005045.

Bentolila, S., Guitton, C., Bouvet, N., Sailland, A., Nykaza, S., & Freyssinet, G. (1991). Identification of an RFLP marker tightly linked to the *Ht1* gene in maize. *Theoretical and Applied Genetics, 82*(4), 393-398.

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*, 964-977.

Berger, D. K., Carstens, M., Korsman, J. N., Middleton, F., Kloppers, F. J., Tongoona, P., & Myburg, A. A. (2014). Mapping QTL conferring resistance in maize to gray leaf spot disease caused by *Cercospora zeina*. *BMC Genetics, 15*, 60.

Bergquist, R. R., & Masias, O. (1974). Physiologic specialization in *Trichometasphaeria turcica f. sp. zeae* and *T. turcica f. sp. sorghi* in Hawaii. *Phytopathology, 64*(5), 645-649.

Bernardo, R. (2008). Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Science, 48*(5), 1649-1664.

Biemond, P., Oguntade, O., Stomph, T.-J., Kumar, P. L., Termorshuizen, A. J., & Struik, P. C. (2013). Health of farmer-saved maize seed in north-east Nigeria. *European Journal of Plant Pathology, 137*(3), 563-572.

Bock, C., Parker, P., Cook, A., & Gottwald, T. (2008). Visual rating and the use of image analysis for assessing different symptoms of citrus canker on grapefruit leaves. *Plant Disease, 92*(4), 530-541.

Bock, C., Poole, G., Parker, P., & Gottwald, T. (2010). Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. *Critical Reviews in Plant Sciences, 29*(2), 59-107.

Bohnert, S., Heck, L., Gruber, C., Neumann, H., Distler, U., Tenzer, S., Jacob, S. (2018). Fungicide resistance toward fludioxonil conferred by overexpression of the phosphatase gene MoPTP2 in *Magnaporthe oryzae*. *Molecular Microbiology*.

Bongaarts, J. (2009). Human population growth and the demographic transition. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*(1532), 2985.

Borchardt, D. S., Welz, H. G., & Geiger, H. H. (1998). Genetic structure of *Setosphaeria turcica* populations in tropical and temperate climates. *Phytopathology, 88*(4), 322-329.

Bronson, C. R. (1988). Ascospore abortion in crosses of *Cochliobolus heterostrophus* heterozygous for the virulence locus Tox1. *Genome, 30*(1), 12-18.

Bruns, H. A. (2017). Southern corn leaf blight: a story worth retelling. *Agronomy Journal, 109*(4), 1218-1224.

Bubeck, D., Goodman, M., Beavis, W., & Grant, D. (1993). Quantitative trait loci controlling resistance to gray leaf spot in maize. *Crop Science, 33*(4), 838-847.

Byrnes, K., Pataky, J., & White, D. (1989). Relationships between yield of three maize hybrids and severity of southern leaf blight caused by race O of *Bipolaris maydis*. *Plant Disease, 73*(10), 834-840.

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

Carson, M., Stuber, C., & Senior, M. (2004). Identification and mapping of quantitative trait loci conditioning resistance to southern leaf blight of maize caused by *Cochliobolus heterostrophus* race O. *Phytopathology, 94*(8), 862-867.

Chang, R.-Y., & Peterson, P. (1995). Genetic control of resistance to *Bipolaris maydis*: one gene or two genes? *Journal of Heredity, 86*(2), 94-97.

Chen, G., Wang, X., Long, S., Jaqueth, J., Li, B., Yan, J., & Ding, J. (2016). Mapping of QTL conferring resistance to northern corn leaf blight using high-density SNPs in maize. *Molecular Breeding, 36*(1), 4.

Chen, L., & Wang, L. (2011). Research on application of probability neural network in maize leaf disease identification. *Journal of Agricultural Mechanization Research, 33*(6), 145-148.

Chen, Y.-L., Mao, X.-W., Wang, J.-X., Wu, L.-Y., Zhou, M.-G., & Hou, Y.-P. (2018). Activity of the dinitroaniline fungicide fluazinam against *Bipolaris maydis*. *Pesticide Biochemistry and Physiology, 148*, 8-15.

Chung, C.-L., Jamann, T., Longfellow, J., & Nelson, R. (2010). Characterization and fine-mapping of a resistance locus for northern leaf blight in maize bin 8.06. *Theoretical and Applied Genetics, 121*(2), 205-227.

Chung, C.-L., Poland, J., Kump, K., Benson, J., Longfellow, J., Walsh, E., Nelson, R. (2011). Targeted discovery of quantitative trait loci for resistance to northern leaf blight and other diseases of maize. *Theoretical and Applied Genetics, 123*(2), 307-326.

Clements, M. J., Dudley, J., & White, D. (2000). Quantitative trait loci associated with resistance to gray leaf spot of corn. *Phytopathology, 90*(9), 1018-1025.

Condon, B. J., Elliott, C., González, J. B., Yun, S. H., Akagi, Y., Wiesner-Hanks, T., Turgeon, B. G. (2018). Clues to an evolutionary mystery: The genes for T-toxin, enabler of the devastating 1970 southern corn leaf blight epidemic, are present in ancestral species, suggesting an ancient origin. *Molecular Plant-Microbe Interactions, 31*(11), 1154-1165.

Condon, B. J., Leng, Y., Wu, D., Bushley, K. E., Ohm, R. A., Otillar, R., MohdZainudin, N. (2013). Comparative genome structure, secondary metabolite, and effector coding capacity across *Cochliobolus* pathogens. *PLoS Genetics, 9*(1), e1003233.

Craven, M., & Fourie, A. (2011). Field evaluation of maize inbred lines for resistance to *Exserohilum turcicum*. *South African Journal of Plant and Soil, 28*(1), 69-74.

Crous, P. W., & Braun, U. (2003). *Mycosphaerella* and its anamorphs: 1. Names published in *Cercospora and Passalora*: Centraalbureau voor Schimmelcultures (CBS).

Crous, P. W., Groenewald, J. Z., Groenewald, M., Caldwell, P., Braun, U., & Harrington, T. C. (2006). Species of *Cercospora* associated with grey leaf spot of maize. *Studies in Mycology, 55*, 189-197.

Danson, J., Lagat, M., Kimani, M., & Kuria, A. (2008). Quantitative trait loci (QTLs) for resistance to gray leaf spot and common rust diseases of maize. *African Journal of Biotechnology, 7*(18).

de Vallavieille-Pope, C., Giosue, S., Munk, L., Newton, A., Niks, R., Østergård, H., Sache, I. (2000). Assessment of epidemiological parameters and their use in epidemiological and forecasting models of cereal airborne diseases. *Agronomie*, *20 7*(2000), 715-727.

Dean, J., Goodwin, P., & Hsiang, T. (2005). Induction of glutathione S-transferase genes of *Nicotiana benthamiana* following infection by *Colletotrichum destructivum* and *C. orbiculare* and involvement of one in resistance. *Journal of Experimental Botany, 56*(416), 1525-1533.

DeChant, C., Wiesner-Hanks, T., Chen, S., Stewart, E. L., Yosinski, J., Gore, M. A., & Lipson, H. (2017). Automated identification of northern leaf blight-infected maize plants from field imagery using deep learning. *Phytopathology, 107*(11), 1426-1432.

Dill-Macky, R., & Jones, R. (2000). The effect of previous crop residues and tillage on Fusarium head blight of wheat. *Plant Disease, 84*(1), 71-76.

Dingerdissen, A., Geiger, H., Lee, M., Schechert, A., & Welz, H. (1996). Interval mapping of genes for quantitative resistance of maize to *Setosphaeria turcica*, cause of northern leaf blight, in a tropical environment. *Molecular Breeding, 2*(2), 143-156.

Dodd, J. (2000). *How to foresee corn disease outbreaks.* Paper presented at the 55th Annual Corn & Sorghum Industry Research Conference.

Dong, J., Fan, Y., Gui, X., An, X., Ma, J., & Dong, Z. (2008). Geographic distribution and genetic analysis of physiological races of *Setosphaeria turcica* in Northern China. *American Journal of Agricultural and Biological Science*.

Drechsler, C. (1923). Some graminicolons species of *Helminthosporium*. I. *Journal of Agricultural Research, 24*(8).

Drechsler, C. (1925). Leaf spot of maize caused by *Ophiobolus heterostrophus n.* sp., the ascigerons stage of a *Helminthosporium* exhibiting bipolar germination. *Journal of Agricultural Research, 31*(8).

Dunkle, L. D., & Levy, M. (2000). Genetic relatedness of African and United States populations of *Cercospora zeae-maydis*. *Phytopathology, 90*(5), 486-490.

Duvick, D. N. (2001). Biotechnology in the 1930s: the development of hybrid maize. *Nature Reviews Genetics, 2*(1), 69.

Dyer, P., & Paoletti, M. (2005). Reproduction in *Aspergillus fumigatus*: sexuality in a supposedly asexual species? *Medical Mycology, 43*(S1), 7-14.
Dyer, P. S., Ingram, D. S., & Johnstone, K. (1992). The control of sexual morphogenesis in the *Ascomycotina*. *Biological Reviews, 67*(4), 421-458.
Elad, Y., & Pertot, I. (2014). Climate change impacts on plant pathogens and plant diseases. *Journal of Crop Improvement, 28*(1), 99-139.
Ellwood, S. R., Piscetek, V., Mair, W. J., Lawrence, J. A., Lopez-Ruiz, F. J., & Rawlinson, C. (2019). Genetic variation of *Pyrenophora teres* f. *teres* isolates in Western Australia and emergence of a Cyp51A fungicide resistance mutation. *Plant Pathology, 68*(1), 135-142.
Emami, K., & Hack, E. (2002). Conservation of XYN11A and XYN11B xylanase genes in *Bipolaris sorghicola*, *Cochliobolus sativus*, *Cochliobolus heterostrophus*, and *Cochliobolus spicifer*. *Current Microbiology, 45*(4), 303-306.
Emechebe, A. M. (1975). Some aspects of crop diseases in Uganda. *Some aspects of crop diseases in Uganda.*
Fan, Y., Ma, J., Gui, X., An, X., Sun, S., & Dong, J. (2007). Distribution of mating types and genetic diversity induced by sexual recombination in *Setosphaeria turcica* in northern China. *Frontiers of Agriculture in China, 1*(4), 368-376.
FAOSTAT. (2018). Food and agricultural organization of the United Nations. http://www.fao.org/faostat/en (Accessed December 2018).
Ferguson, L., & Carson, M. (2007). Temporal variation in *Setosphaeria turcica* between 1974 and 1994 and origin of races 1, 23, and 23N in the United States. *Phytopathology, 97*(11), 1501-1511.
Fisher, D., Hooker, A., Lim, S., & Smith, D. (1976). Leaf infection and yield loss caused by four *Helminthosporium* leaf diseases of corn. *Phytopathology, 66*(8), 942-944.
Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L., & Gurr, S. J. (2012). Emerging fungal threats to animal, plant and ecosystem health. *Nature, 484*(7393), 186.
Fuhrer, J. (2003). Agroecosystem responses to combinations of elevated CO_2, ozone, and global climate change. *Agriculture, Ecosystems & Environment, 97*(1-3), 1-20.
Gafur, A., Mujim, S., & Aeny, T. N. (2002). Morphological and pathological variations in the Indonesian *Cochliobolus heterostrophus* (Pleosporaceae, Pleosporales, Euascomycetes). *Pakistan Journal of Biological Sciences, 5*(11), 1195-1198.
Gafur, A., Tanaka, C., Ouchi, S., & Tsuda, M. (1997). A PCR-based method for mating type determination in *Cochliobolus heterostrophus*. *Mycoscience, 38*(4), 455-458.
Gardes, M., White, T. J., Fortin, J. A., Bruns, T. D., & Taylor, J. W. (1991). Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. *Canadian Journal of Botany, 69*(1), 180-190.
Garnault, M., Duplaix, C., Leroux, P., Couleaud, G., Carpentier, F., David, O., & Walker, A. S. (2019). Spatiotemporal dynamics of fungicide resistance in the wheat pathogen *Zymoseptoria tritici* in France. *Pest Management Science,* https://doi.org/10.1002/ps.5360 .
Gevers, J. (1994). *GLS1*-A major gene for resistance to grey leaf spot in maize. *South African Journal of Science, 90*(7), 377-380.
Girvetz, E., Ramirez-Villegas, J., Claessens, L., Lamanna, C., Navarro-Racines, C., Nowak, A., & Rosenstock, T. S. (2019). Future climate projections in Africa: Where are we headed? *The Climate-Smart Agriculture Papers* (pp. 15-27): Springer.
Glass, N. L., & Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied Environmental Microbiology, 61*(4), 1323-1330.
Gogoi, R., Singh, S., Singh, P. K., Kulanthaivel, S., & Rai, S. (2014). Genetic variability in the isolates of *Bipolaris maydis* causing maydis leaf blight of maize. *African Journal of Agricultural Research, 9*(24), 1906-1913.

Goh, T., Hyde, K., & Lee, D. K. (1998). Generic distinction in the *Helminthosporium*-complex based on restriction analysis of the nuclear ribosomal RNA gene. *Fungal Diversity*.

Gong, M., Wang, J. D., Zhang, J., Yang, H., LU, X. F., Pei, Y., & Cheng, J. Q. (2006). Study of the antifungal ability of *Bacillus subtilis* strain PY-1 *in vitro* and identification of its antifungal substance (iturin A). *Acta Biochimica et Biophysica Sinica, 38*(4), 233-240.

Goodwin, S. B., Dunkle, L. D., & Zismann, V. L. (2001). Phylogenetic analysis of *Cercospora* and *Mycosphaerella* based on the internal transcribed spacer region of ribosomal DNA. *Phytopathology, 91*(7), 648-658.

Gopi, R. (2008). *Pathogenic and molecular variability in Bipolaris maydis incitant of Maydis leaf blight of maize* (Doctoral dissertation), Indian Agricultural Research Institute, New Delhi.

Gregory, L., Ayers, J., & Nelson, R. (1979). The influence of cultivar and location on yield loss in corn due to southern corn leaf blight caused by *Helminthosporium maydis* race T. *Plant Disease Reporter*.

Groenewald, M., Groenewald, J. Z., Harrington, T. C., Abeln, E. C., & Crous, P. W. (2006). Mating type gene analysis in apparently asexual *Cercospora* species is suggestive of cryptic sex. *Fungal Genetics and Biology, 43*(12), 813-825.

Guo, Y., Liu, Y., Oerlemans, A., Lao, S., Wu, S., & Lew, M. S. (2016). Deep learning for visual understanding: A review. *Neurocomputing, 187*, 27-48.

Haasbroek, M., Craven, M., Barnes, I., & Crampton, B. G. (2014). Microsatellite and mating type primers for the maize and sorghum pathogen, *Exserohilum turcicum*. *Australasian Plant Pathology, 43*(5), 577-581.

Henegariu, O., Heerema, N., Dlouhy, S., Vance, G., & Vogt, P. (1997). Multiplex PCR: critical parameters and step-by-step protocol. *BioTechniques, 23*(3), 504-511.

Hernández-Restrepo, M., Madrid, H., Tan, Y., Da Cunha, K., Gene, J., Guarro, J., & Crous, P. (2018). Multi-locus phylogeny and taxonomy of *Exserohilum*. *Persoonia: Molecular Phylogeny and Evolution of Fungi, 41*, 71.

Hooker, A. (1974). Cytoplasmic susceptibility in plant disease. *Annual Review of Phytopathology, 12*(1), 167-179.

Huff, C. A., Ayers, J., & Hill Jr, R. (1988). Inheritance of resistance in corn (*Zea mays*) to gray leaf spot. *Phytopathology, 78*(6), 790-794.

Hulbert, S. (1997). Structure and evolution of the rp1 complex conferring rust resistance in maize. *Annual Review of Phytopathology, 35*(1), 293-310.

Hulme, P. E. (2009). Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology, 46*(1), 10-18.

Human, M. P., Barnes, I., Craven, M., & Crampton, B. G. (2016). Lack of population structure and mixed reproduction modes in *Exserohilum turcicum* from South Africa. *Phytopathology, 106*(11), 1386-1392.

Hurni, S., Scheuermann, D., Krattinger, S. G., Kessel, B., Wicker, T., Herren, G., Ouzunova, M. (2015). The maize disease resistance gene *Htn1* against northern corn leaf blight encodes a wall-associated receptor-like kinase. *Proceedings of the National Academy of Sciences, 112*(28), 8780-8785.

Inderbitzin, P., Asvarak, T., & Turgeon, B. G. (2010). Six new genes required for production of T-toxin, a polyketide determinant of high virulence of *Cochliobolus heterostrophus* to maize. *Molecular Plant-Microbe Interactions, 23*(4), 458-472.

Jaggard, K. W., Qi, A., & Ober, E. S. (2010). Possible changes to arable crop yields by 2050. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 365*(1554), 2835-2851.

Jahani, M., Aggarwal, R., & Srivastava, K. (2011). Genetic differentiation of *Bipolaris* spp. based on random amplified polymorphic DNA markers. *Indian Phytopathology*, 61(4) 449-455.

James, T. Y., Kauff, F., Schoch, C. L., Matheny, P. B., Hofstetter, V., Cox, C. J., & Miadlikowska, J. (2006). Reconstructing the early evolution of fungi using a six-gene phylogeny. *Nature, 443*(7113), 818.

Jeffers, D. (2004). *Maize diseases: a guide for field identification*. 4th edition. Mexico, D.F.: CIMMYT.

Jindal, K. K., Tenuta, A. U., Woldemariam, T., Zhu, X., Hooker, D. C., & Reid, L. M. (2019). Occurrence and distribution of physiological races of *Exserohilum turcicum* in Ontario, Canada. *Plant Disease, 103*, 1450-1457.

Jones, J. D., & Dangl, J. L. (2006). The plant immune system. *Nature, 444*(7117), 323-329.

Jordan, E. G., Perkins, J. M., Schall, R., & Pedersen, W. (1983). Occurrence of race 2 of *Exserohilum turcicum* on corn in the central and eastern United States. *Plant Disease, 67*(10), 1163-1165.

Juliatti, F. C., Pedrosa, M. G., Silva, H. D., & da Silva, J. V. C. (2009). Genetic mapping for resistance to gray leaf spot in maize. *Euphytica, 169*(2), 227-238.

Kai, S., Xiaoyan, S., & Jianwei, J. (2007). Corn leaf disease recognition based on suport vector machine method. *Transactions of the Chinese Society of Agricultural Engineering, 2007*(1).

Karavina, C., Mandumbu, R., & Mukaro, R. (2014). Evaluation of three-way maize (*Zea mays* L) hybrids for yield and resistance to maize streak virus and turcicum leaf blight diseases. *Journal of Animal & Plant Sciences, 24*(1), 216-220.

Karimi, M. R. (2003). *Investigations on genetics of disease resistance on Zea mays-Drechslera maydis pathosystem and variability in D. maydis.* (Doctoral dissertation), Indian Agricultural Research Institute; New Delhi.

Karr, A. L., Karr, D. B., & Strobel, G. A. (1974). Isolation and partial characterization of four host-specific toxins of *Helminthosporium maydis* (race T). *Plant Physiology, 53*(2), 250-257.

Keller, N., & Bergstrom, G. (1990). Predominance in New York of isolates of *Exserohilum turcicum* virulent on maize with gene Htl. *Plant Disease, 74*(7).

Kim, P. I., Ryu, J., Kim, Y. H., & Chi, Y.-T. (2010). Production of biosurfactant lipopeptides Iturin A, fengycin and surfactin A from *Bacillus subtilis* CMB32 for control of *Colletotrichum gloeosporioides*. *Journal of Microbiology & Biotechnology, 20*(1), 138-145.

Kinyua, Z., Smith, J., Kibata, G., Simons, S., & Langat, B. (2010). Status of gray leaf spot disease in Kenyan maize production ecosystems. *African Crop Science Journal, 18*(4).

Kloppers, R., & Tweer, S. (2009). Northern corn leaf blight fact sheet. *PANNAR Seed (Pty) Ltd*, https://www.plantwise.org/FullTextPDF/2011/20117800335.pdf.

Korsman, J., Meisel, B., Kloppers, F. J., Crampton, B. G., & Berger, D. K. (2012). Quantitative phenotyping of gray leaf spot disease in maize using real-time PCR. *European Journal of Plant Pathology, 133*(2), 461-471.

Kotze, R., van der Merwe, C., Crampton, B., & Kritzinger, Q. (2019). A histological assessment of the infection strategy of *Exserohilum turcicum* in maize. *Plant Pathology, 68*(3), 504-512.

Kumar, S., Pardurange Gowda, K., Pant, S., Shekhar, M., Kumar, B., Kaur, B., & Parsanna, B. (2011). Sources of resistance to *Exserohilum turcicum* (Pass.) and *Puccinia polysora* (Underw.) incitant of turcicum leaf blight and polysora rust of maize. *Archives of Phytopathology and Plant Protection, 44*(6), 528-536.

Latterell, F. M., & Rossi, A. E. (1983). Gray leaf spot of corn: a disease on the move. *Plant Disease, 67*(8), 842-847.

LeCun, Y., Bengio, Y., & Hinton, G. (2015). Deep learning. *Nature, 521*(7553), 436.

Lee, C., & Sheridan, S. (2018). Trends in weather type frequencies across North America. *npj Climate and Atmospheric Science, 1*(1), 41.

Lee, S. B., & Taylor, J. W. (1992). Phylogeny of five fungus-like protoctistan *Phytophthora* species, inferred from the internal transcribed spacers of ribosomal DNA. *Molecular Biology and Evolution, 9*(4), 636-653.

Lehmensiek, A., Esterhuizen, A., Van Staden, D., Nelson, S., & Retief, A. (2001). Genetic mapping of gray leaf spot (GLS) resistance genes in maize. *Theoretical and Applied Genetics, 103*(5), 797-803.

Lennon, J. R., Krakowsky, M., Goodman, M., Flint-Garcia, S., & Balint-Kurti, P. J. (2016). Identification of alleles conferring resistance to gray leaf spot in maize derived from its wild progenitor species teosinte. *Crop Science, 56*(1), 209-218.
Leonard, K. (1974). *Bipolaris maydis* race and mating type frequencies in North Carolina. *Plant Disease Reporter, 58*(6), 529-531.
Leonard, K. (1977a). Races of *Bipolaris maydis* in the Southeastern US from 1974-1976. *Plant Disease Reporter, 61*(11), 914-915.
Leonard, K. (1977b). Virulence, temperature optima, and competitive abilities of isolines of races T and O of *Bipolaris maydis*. *Phytopathology, 67*(11), 1273-1279.
Leonard, K., Levy, Y., & Smith, D. (1989). Proposed nomenclature for pathogenic races of *Exserohilum turcicum* on corn. *Plant Disease, 73*(9), 776-777.
Leonard, K., & Suggs, E. G. (1974). *Setosphaeria prolata*, the ascigerous state of *Exserohilum prolatum*. *Mycologia, 66*(2), 281-297.
Levings 3rd, C. (1993). Thoughts on cytoplasmic male sterility in cms-T maize. *The Plant Cell, 5*(10), 1285.
Levings, C. S. (1990). The Texas cytoplasm of maize: cytoplasmic male sterility and disease susceptibility. *Science, 250*(4983), 942-947.
Levy, Y., & Cohen, Y. (1983). Biotic and environmental factors affecting infection of sweet corn with *Exserohilum turcicum*. *Phytopathology, 73*(5), 722-725.
Li, Y.-x., Chen, L., Li, C., Bradbury, P. J., Shi, Y.-s., Song, Y., & Li, Y. (2018). Increased experimental conditions and marker densities identified more genetic loci associated with southern and northern leaf blight resistance in maize. *Scientific Reports, 8*(1), 6848.
Lipps, P. (1998). Gray leaf spot: a global threat to corn production. *APSNet Feature*. doi: 10.1094/APSnetFeature-1998-0598
Lipps, P., White, D., Ayers, J., & Dunkle, L. (1998). Gray leaf spot of corn: update. A report from NCR-25 technical committee on corn and sorghum diseases. *The American Phytopathological Society. Published Online www. apsnet. org/online/feature/grayleaf/fullrprt. htm*.
Liu, K.-J., & Xu, X.-D. (2013). First report of gray leaf spot of maize caused by *Cercospora zeina* in China. *Plant Disease, 97*(12), 1656-1656.
Lopez-Zuniga, L. O., Wolters, P., Davis, S., Weldekidan, T., Kolkman, J. M., Nelson, R., & Wisser, R. (2019). Using maize chromosome segment substitution line populations for the identification of loci associated with multiple disease resistance. *G3: Genes, Genomes, Genetics, 9*(1), 189-201.
Manamgoda, D., Rossman, A. Y., Castlebury, L., Crous, P. W., Madrid, H., Chukeatirote, E., & Hyde, K. D. (2014). The genus *Bipolaris*. *Studies in Mycology, 79*, 221-288.
Manamgoda, D. S., Cai, L., McKenzie, E. H., Crous, P. W., Madrid, H., Chukeatirote, E., & Hyde, K. D. (2012). A phylogenetic and taxonomic re-evaluation of the *Bipolaris-Cochliobolus-Curvularia* complex. *Fungal Diversity, 56*(1), 131-144.
Manandhar, G., Ferrara, G., Tiwari, T., Baidya, S., Bajracharya, A., Khadge, B., & Narro, L. (2011). Response of maize genotypes to gray leaf spot disease (*Cercospora zeae-maydis*) in the hills of Nepal. *Agronomy Journal of Nepal, 2*, 93-101.
Manoj, K., & Agarwal, V. K. (1998). Location of seedborne fungi associated with discoloured maize seeds. *Indian Phytopathology, 51*(3), 247-250.
Marley, P. S., Diourte, M., Neya, A., & Rattunde, F. W. (2005). Sorghum anthracnose and sustainable management strategies in West and Central Africa. *Journal of Sustainable Agriculture, 25*(1), 43-56.
Mathur, K., Rao, V., & Thakur, R. (1997). Intra-population variability in *Colletotrichum sublineolum* infecting sorghum. *Journal of Mycology and Plant Pathology, 27*(3), 302-310.
Matros, A., Amme, S., Kettig, B., Buck-Sorlin, G. H., Sonnewald, U., & Mock, H. P. (2006). Growth at elevated CO_2 concentrations leads to modified profiles of secondary metabolites in tobacco cv. SamsunNN and to increased resistance against infection with potato virus Y. *Plant, Cell & Environment, 29*(1), 126-137.

McCartney, H. A., Foster, S. J., Fraaije, B. A., & Ward, E. (2003). Molecular diagnostics for fungal plant pathogens. *Pest Management Science:* formerly *Pesticide Science, 59*(2), 129-142.

McDonald, B. A. (1997). The population genetics of fungi: tools and techniques. *Phytopathology, 87*(4), 448-453.

McDonald, B. A., & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology, 40*(1), 349-379.

Meisel, B., Korsman, J., Kloppers, F. J., & Berger, D. K. (2009). *Cercospora zeina* is the causal agent of grey leaf spot disease of maize in Southern Africa. *European Journal of Plant Pathology, 124*(4), 577-583.

Milgroom, M. G., & Peever, T. L. (2003). Population biology of plant pathogens: the synthesis of plant disease epidemiology and population genetics. *Plant Disease, 87*(6), 608-617.

Miller, S. A., Beed, F. D., & Harmon, C. L. (2009). Plant disease diagnostic capabilities and networks. *Annual Review of Phytopathology, 47*, 15-38.

Moghaddam, P. F., & Pataky, J. (1994). Reactions of isolates from matings of races 1 and 23N of *Exserohilum turcicum*. *Plant Disease* 78, 767-771.

Mueller, D. S., Wise, K. A., Sisson, A. J., Allen, T. W., Bergstrom, G. C., Bosley, D. B., & Chilvers, M. I. (2016). Corn yield loss estimates due to diseases in the United States and Ontario, Canada from 2012 to 2015. *Plant Health Progress, 17*(3), 211-222.

Muiru, W., Koopmann, B., Tiedemann, A., Mutitu, E., & Kimenju, J. (2010a). Evaluation of genetic variability of Kenyan, German and Austrian isolates of *Exserohilum turcicum* using amplified fragment length polymorphism DNA marker. *Biotechnology, 9*(2), 204-211.

Muiru, W., Koopmann, B., Tiedemann, A., Mutitu, E., & Kimenju, J. (2010b). Race typing and evaluation of aggressiveness of *Exserohilum turcicum* isolates of Kenyan, German and Austrian origin. *World Journal of Agricultural Sciences, 6*(3), 277-284.

Muller, M. F., Barnes, I., Kunene, N. T., Crampton, B. G., Bluhm, B. H., Phillips, S. M., & Berger, D. K. (2016). *Cercospora zeina* from maize in South Africa exhibits high genetic diversity and lack of regional population differentiation. *Phytopathology, 106*(10), 1194-1205.

Munjal, E., & Kapoor, J. (1960). Some unrecorded diseases of sorghum and maize from India. *Current Science, 29*(11).

Munkvold, G., Martinson, C., Shriver, J., & Dixon, P. (2001). Probabilities for profitable fungicide use against gray leaf spot in hybrid maize. *Phytopathology, 91*(5), 477-484.

Mutka, A. M., & Bart, R. S. (2015). Image-based phenotyping of plant disease symptoms. *Frontiers in Plant Science, 5*, 734.

Mwangi, S. M. (1998). *Status of northen leaf blight, Phaeosphaeria maydis leaf spot, southern leaf blight, rust, maize streak virus and physiologic specialization of Exserohilum turcicum in Kenya.* (Doctoral dissertation) Virginia Tech.

Nega, A., Lemessa, F., & Berecha, G. (2016). Distribution and importance of maize gray leaf spot *Cercospora zeae-maydis* (Tehon and Daniels) in south and southwest Ethiopia. *Journal of Plant Pathology & Microbiology, 7*(362), 2.

Negeri, A. T., Coles, N. D., Holland, J. B., & Balint-Kurti, P. J. (2011). Mapping QTL controlling southern leaf blight resistance by joint analysis of three related recombinant inbred line populations. *Crop Science, 51*(4), 1571-1579.

Nelson, R. (1960). Evolution of sexuality and pathogenicity. 1. Interspecific crosses in the genus *Helminthosporium*. *Phytopathology, 50*, 375-377.

Nelson, R., Wiesner-Hanks, T., Wisser, R., & Balint-Kurti, P. (2018). Navigating complexity to breed disease-resistant crops. *Nature Reviews Genetics, 19*(1), 21.

Neves, D. L., Silva, C. N., Pereira, C. B., Campos, H. D., & Tessmann, D. J. (2015). *Cercospora zeina* is the main species causing gray leaf spot in southern and central Brazilian maize regions. *Tropical Plant Pathology, 40*(6), 368-374.

Nieuwoudt, A., Human, M., Craven, M., & Crampton, B. (2018). Genetic differentiation in populations of *Exserohilum turcicum* from maize and sorghum in South Africa. *Plant Pathology, 67*(7), 1483-1491.

Nowell, D. C. (1998). *Studies on ear rot and grey leaf spot of maize in South Africa.* (Doctoral dissertation), University of Natal, Pietermaritzburg.

Nsibo, D. L., Barnes, I., Kunene, N. T., & Berger, D. K. (2019). Influence of farming practices on the population genetics of the maize pathogen *Cercospora zeina* in South Africa. *Fungal Genetics and Biology, 125*, 36-44.

Nutter Jr, F., Gleason, M., Jenco, J., & Christians, N. (1993). Assessing the accuracy, intra-rater repeatability, and inter-rater reliability of disease assessment systems. *Phytopathology, 83*(8), 806-812.

Nwanosike, M., Mabagala, R., & Kusolwa, P. (2015). Effect of northern leaf blight (*Exserohilum turcicum*) severity on yield of maize (*Zea mays* L.) in Morogoro, Tanzania. *International Journal of Science and Research, 4*(9), 465-474.

Oerke, E.-C. (2006). Crop losses to pests. *The Journal of Agricultural Science, 144*(1), 31-43.

Ogliari, J. B., Guimarães, M. A., Geraldi, I. O., & Camargo, L. E. A. (2005). New resistance genes in the *Zea mays*: *Exserohilum turcicum* pathosystem. *Genetics and Molecular Biology, 28*(3), 435-439.

Ohm, R. A., Feau, N., Henrissat, B., Schoch, C. L., Horwitz, B. A., Barry, K. W., & Glaser, F. (2012). Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi. *PLoS Pathogens, 8*(12), e1003037.

Okori, P., Fahleson, J., Rubaihayo, P., Adipala, E., & Dixelius, C. (2003). Assessment of genetic variation among East African *Cercospora zeae-maydis*. *Journal of African Crop Science, 11*(2), 75-85.

Okori, P., Rubaihayo, P., Adipala, E., Fahieson, J., & Dixelius, C. (2015). Dynamics of *Cercospora zeina* populations in maize-based agro-ecologies of Uganda. *African Crop Science Journal, 23*(1), 45-57.

Pachauri, R. K., Allen, M. R., Barros, V. R., Broome, J., Cramer, W., Christ, R., & 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*: IPCC.

Pal, I., Singh, V., Gogoi, R., Hooda, K., & Bedi, N. (2015). Characterization of *Bipolaris maydis* isolates of different maize cropping zones of India. *Indian Phytopathology, 68*(1), 63-66.

Pangga, I., Chakraborty, S., & Yates, D. (2004). Canopy size and induced resistance in *Stylosanthes scabra* determine anthracnose severity at high CO2. *Phytopathology, 94*(3), 221-227.

Paul, P., & Munkvold, G. (2005). Influence of temperature and relative humidity on sporulation of *Cercospora zeae-maydis* and expansion of gray leaf spot lesions on maize leaves. *Plant Disease, 89*(6), 624-630.

Pauli, D., Chapman, S. C., Bart, R., Topp, C. N., Lawrence-Dill, C. J., Poland, J., & Gore, M. A. (2016). The quest for understanding phenotypic variation via integrated approaches in the field environment. *Plant Physiology, 172*(2), 622-634.

Payne, G., & Waldron, J. (1983). Overwintering and spore release of *Cercospora zeae-maydis* in corn debris in North Carolina. *Plant Disease, 67*(1), 87-89.

Poland, J. A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C., & Nelson, R. J. (2009). Shades of gray: the world of quantitative disease resistance. *Trends in Plant Science, 14*(1), 21-29.

Poland, J. A., Bradbury, P. J., Buckler, E. S., & Nelson, R. J. (2011). Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. *Proceedings of the National Academy of Sciences, 108*(17), 6893-6898.

Poland, J. A., & Nelson, R. J. (2011). In the eye of the beholder: the effect of rater variability and different rating scales on QTL mapping. *Phytopathology, 101*(2), 290-298.

Prakash, A., Rao, J., Mukherjee, A. K., Berliner, J., Pokhare, S. S., Adak, T., & Shashank, P. (2014). *Climate change: impact on crop pests*: Applied Zoologists Research Association (AZRA), Central Rice Research Institute.

Qi, Z., Jiang, Z., Yang, C., Liu, l., & Rao, Y. (2016). Identification of maize leaf diseases based on image technology. *Journal of Anhui Agricultural University, 43*(2), 325-330.

Ramathani, I., Biruma, M., Martin, T., Dixelius, C., & Okori, P. (2011). Disease severity, incidence and races of *Setosphaeria turcica* on sorghum in Uganda. *European Journal of Plant Pathology, 131*(3), 383-392.

Rao, V., Thakur, R., & Mathur, K. (1998). Morphological and pathogenic diversity among grain sorghum isolates of *Colletotrichum graminicola* in India. *Indian Phytopathology, 51*(2), 164-174.

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). Yield trends are insufficient to double global crop production by 2050. *PloS One, 8*(6), e66428.

Reddy, T. R., Reddy, P. N., Reddy, R. R., & Reddy, S. S. (2013). Management of Turcicum leaf blight of maize caused by *Exserohilum turcicum* in maize. *International Journal of Scientific and Research Publications, 3*(10), 1-4.

Renfro, B., & Ullstrup, A. (1976). A comparison of maize diseases in temperate and in tropical environments. *PANS, 22*(4), 491-498.

Robert, A. L. (1953). Some of the leaf blights of corn. *Year book of agriculture*, 380-385.

Robeson, D., & Strobel, G. (1982). Monocerin, a phytotoxin from *Exserohilum turcicum* (≡ *Drechslera turcica*). *Agricultural and Biological Chemistry, 46*(11), 2681-2683.

Rong, I. H., & Baxter, A. P. (2006). The South African national collection of fungi: celebrating a centenary 1905-2005. *Studies in Mycology, 55*, 1-12.

Rossman, A. Y., Manamgoda, D. S., & Hyde, K. D. (2013). (2233) Proposal to conserve the name *Bipolaris* against *Cochliobolus* (Ascomycota: Pleosporales: Pleosporaceae). *Taxon, 62*(6), 1331-1332.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology and Evolution*, 1.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., & Consortium, F. B. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences, 109*(16), 6241-6246.

Schwartz, H. F., & David, G. H. (2005). Sweet Corn XXXVI, *Helminthosporium* leaf blight. *High Plains IPM Guide,* a cooperative effort of the University of Wyoming, University of Nebraska, Colorado State University and Montana State University, https://www.cabdirect.org/cabdirect/FullTextPDF/2014/20143334606.pdf.

Serre, T., Wolf, L., Bileschi, S., Riesenhuber, M., & Poggio, T. (2007). Robust object recognition with cortex-like mechanisms. *IEEE Transactions on Pattern Analysis & Machine Intelligence*(3), 411-426.

Shah, D., & Dillard, H. (2010). Managing foliar diseases of processing sweet corn in New York with strobilurin fungicides. *Plant Disease, 94*(2), 213-220.

Shi, N., Du, Y., Ruan, H., Yang, X., Dai, Y., Gan, L., & Liu, X. (2017). First report of northern corn leaf blight caused by *Setosphaeria turcica* on Corn (*Zea mays*) in Fujian Province, China. *Plant Disease, 101*(5), 831.

Simmons, C. R., Grant, S., Altier, D. J., Dowd, P. F., Crasta, O., Folkerts, O., & Yalpani, N. (2001). Maize rhm1 resistance to *Bipolaris maydis* is associated with few differences in pathogenesis-related proteins and global mRNA profiles. *Molecular Plant-Microbe Interactions, 14*(8), 947-954.

Singh, A., Ganapathysubramanian, B., Singh, A. K., & Sarkar, S. (2016). Machine learning for high-throughput stress phenotyping in plants. *Trends in Plant Science, 21*(2), 110-124.

Singh, R., & Srivastava, R. (2012). Southern corn leaf blight-an important disease of maize: an extension fact sheet. *Indian Research Journal of Extension Education, 12*(2), 324-327.

Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M. G., Wanyera, R., & Ward, R. W. (2006). Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. *CAB reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 1*(54), 1-13.

Smith, D., & Hooker, A. (1973). Monogenic chlorotic-lesion resistance in corn to *Helminthosporium maydis* 1. *Crop Science, 13*(3), 330-331.
Smith, D., Hooker, A., & Lim, S. (1970). Physiologic races of *Helminthosporium maydis*. *Plant Disease Reporter, 54*, 819-822.
Stewart, E. L., & McDonald, B. A. (2014). Measuring quantitative virulence in the wheat pathogen *Zymoseptoria tritici* using high-throughput automated image analysis. *Phytopathology, 104*(9), 985-992.
Sumner, D. R., Doupnik Jr, B., & Boosalis, M. (1981). Effects of reduced tillage and multiple cropping on plant diseases. *Annual Review of Phytopathology, 19*(1), 167-187.
Sun, S.-q., Wen, L.-l., & Dong, J.-g. (2005). Identification of physiological races and mating type of *Exserohilum turcicum*. *Journal of Maize Sciences, 4*.
Swart, V., Crampton, B. G., Ridenour, J. B., Bluhm, B. H., Olivier, N. A., Meyer, J. M., & Berger, D. K. (2017). Complementation of *CTB7* in the maize pathogen *Cercospora zeina* overcomes the lack of *in vitro* cercosporin production. *Molecular Plant-Microbe Interactions, 30*(9), 710-724.
Taga, M., Bronson, C., & Yoder, O. (1985). Nonrandom abortion of ascospores containing alternate alleles at the Tox-1 locus of the fungal plant pathogen *Cochliobolus heterostrophus*. *Canadian Journal of Genetics and Cytology, 27*(4), 450-456.
Tan, Y. P., Crous, P. W., & Shivas, R. G. (2016). Eight novel *Bipolaris* species identified from John L. Alcorn's collections at the Queensland Plant Pathology Herbarium (BRIP). *Mycological Progress, 15*(10-11), 1203-1214.
Tang, L., Gao, Z., Yao, Y., & Liu, X. (2015). Identification and genetic diversity of formae speciales of *Setosphaeria turcica* in China. *Plant Disease, 99*(4), 482-487.
Tehon, L., & Daniels, E. (1925). Notes on the parasitic fungi of Illinois—II. *Mycologia, 17*(6), 240-249.
Tilahun, T., Wagary, D., Demissie, G., Negash, M., Admassu, S., & Jifar, H. (2012). *Maize pathology research in Ethiopia in the 2000s: A review.* Paper presented at the Meeting the challenges of global climate change and food security through innovative maize research.
Tiwari, T., & Ferrara, G. (2007). Maize GLS monitoring-a trip report of hill maize research program-CIMMYT: N.
Turgeon, B. G., & Baker, S. E. (2007). Genetic and genomic dissection of the *Cochliobolus heterostrophus* Tox1 locus controlling biosynthesis of the polyketide virulence factor T-toxin. *Advances in Genetics, 57*, 219-261.
Turgeon, B. G., & Lu, S.-W. (2000). Evolution of host specific virulence in *Cochliobolus heterostrophus*. In: Kronstad J.W. (eds) *Fungal Pathology*. *Springer, Dordrecht* (pp. 93-126).
Turgeon, B. G., Sharon, A., Wirsel, S., Yamaguchi, K., Christiansen, S. K., & Yoder, O. C. (1995). Structure and function of mating type genes in *Cochliobolus* sp. and asexual fungi. *Canadian Journal of Botany, 73*(S1), 778-783.
Ullstrup, A. (1972). The impacts of the southern corn leaf blight epidemics of 1970-1971. *Annual Review of Phytopathology, 10*(1), 37-50.
Van Staden, D., Lambert, C., & Lehmensiek, A. (2001). *SCAR markers for the Ht1, Ht2, Ht3 and HtN1 resistance genes in maize.* Paper presented at the Maize Genetics Conference Abstract.
Vivek, B. S., Odongo, O., Njuguna, J., Imanywoha, J., Bigirwa, G., & Pixley, K. (2010). Diallel analysis of grain yield and resistance to seven diseases of 12 African maize (*Zea mays* L.) inbred lines. *Euphytica, 172*(3), 329-340.
Walker, D. M., Castlebury, L. A., Rossman, A. Y., & White Jr, J. F. (2012). New molecular markers for fungal phylogenetics: two genes for species-level systematics in the Sordariomycetes (Ascomycota). *Molecular Phylogenetics and Evolution, 64*(3), 500-512.
Wang, J., Levy, M., & Dunkle, L. D. (1998). Sibling species of *Cercospora* associated with gray leaf spot of maize. *Phytopathology, 88*(12), 1269-1275.

Wang, J., Xu, Z., Yang, J., Lu, X., Zhou, Z., Zhang, C., & Yong, H. (2018). qNCLB7. 02, a novel QTL for resistance to northern corn leaf blight in maize. *Molecular Breeding, 38*(5), 54.
Wang, N., Wang, K., Xie, R., Lai, J., Ming, B., & Li, S. (2009). Maize leaf disease identification based on fisher discrimination analysis. *Scientia Agricultura Sinica, 42*(11), 3836-3842.
Ward, E., Foster, S. J., Fraaije, B. A., & McCartney, H. A. (2004). Plant pathogen diagnostics: immunological and nucleic acid-based approaches. *Annals of Applied Biology, 145*(1), 1-16.
Ward, J., Laing, M., & Cairns, A. (1997). Management practices to reduce gray leaf spot of maize. *Crop Science, 37*(4), 1257-1262.
Ward, J. M., Laing, M., & Nowell, D. (1997). Chemical control of maize gray leaf spot. *Crop Protection, 16*(3), 265-271.
Ward, J. M., & Nowell, D. (1994). *Epidemiology and management of grey leaf spot disease, a new disease of maize in South Africa.* Paper presented at the Proc. 11th South Africa Maize Breeding Symposium Cedara, South Africa March.
Ward, J. M., & Nowell, D. (1998). Integrated management practices for the control of maize grey leaf spot. *Integrated Pest Management Reviews, 3*(3), 177-188.
Ward, J. M., Stromberg, E. L., Nowell, D. C., & Nutter Jr, F. W. (1999). Gray leaf spot: a disease of global importance in maize production. *Plant Disease, 83*(10), 884-895.
Warren, H. (1975). Temperature effects on lesion development and sporulation after infection by races O and T of *Bipolaris maydis*. *Phytopathology, 65*(5), 623-626.
Weems, J. D., & Bradley, C. A. (2018). *Exserohilum turcicum* race population distribution in the north central United States. *Plant Disease, 102*(2), 292-299.
Wei, J.-K., Liu, K.-M., Chen, J.-P., Luo, P.-C., & Stadelmann, O. (1988). Pathological and physiological identification of race C of *Bipolaris maydis* in China. *Phytopathology, 78*(5), 550-554.
Weikert-Oliveira, R. C., Resende, M., Valério, H. M., Caligiorne, R. B., & Paiva, E. (2002). Genetic variation among pathogens causing" *Helminthosporium*" diseases of rice, maize and wheat. *Fitopatologia Brasileira, 27*(6), 639-643.
Welz, H., & Geiger, H. (2000). Genes for resistance to northern corn leaf blight in diverse maize populations. *Plant Breeding, 119*(1), 1-14.
Wende, A., Shimelis, H., & Gwata, E. T. (2018). Genetic variability for resistance to leaf blight and diversity among selected maize inbred lines: IntechOpen Limited.
White, D. G. (1999). *Compendium of corn diseases* (Vol. 78): APS press St. Paul, MN.
White, J., Calvert, O., & Brown, M. (1973). Ultrastructure of the conidia of *Helminthosporium maydis*. *Canadian Journal of Botany, 51*(10), 2006-2008.
Wisser, R. J., Balint-Kurti, P. J., & Nelson, R. J. (2006). The genetic architecture of disease resistance in maize: a synthesis of published studies. *Phytopathology, 96*(2), 120-129.
Wisser, R. J., Kolkman, J. M., Patzoldt, M. E., Holland, J. B., Yu, J., Krakowsky, M., & Balint-Kurti, P. J. (2011). Multivariate analysis of maize disease resistances suggests a pleiotropic genetic basis and implicates a *GST* gene. *Proceedings of the National Academy of Sciences, 108*(18), 7339-7344.
Wisser, R. J., Sun, Q., Hulbert, S. H., Kresovich, S., & Nelson, R. J. (2005). Identification and characterization of regions of the rice genome associated with broad-spectrum, quantitative disease resistance. *Genetics, 169*(4), 2277-2293.
Xie, W., Yu, K., Pauls, K. P., & Navabi, A. (2012). Application of image analysis in studies of quantitative disease resistance, exemplified using common bacterial blight–common bean pathosystem. *Phytopathology, 102*(4), 434-442.
Xu, L., Xu, X., Hu, M., Wang, R., Xie, C., & Chen, H. (2015). Corn leaf disease identification based on multiple classifiers fusion. *Transactions of the Chinese Society of Agricultural Engineering, 31*(14), 194-201.
Xu, Y., & Crouch, J. H. (2008). Marker-assisted selection in plant breeding: from publications to practice. *Crop Science, 48*(2), 391-407.

Ye, Y.-f., Li, Q.-q., Gang, F., Yuan, G.-q., Miao, J.-h., & Wei, L. (2012). Identification of antifungal substance (Iturin A2) produced by *Bacillus subtilis* B47 and its effect on southern corn leaf blight. *Journal of Integrative Agriculture, 11*(1), 90-99.

Yin, X., Wang, Q., Yang, J., Jin, D., Wang, F., Wang, B., & Zhang, J. (2003). Fine mapping of the *Ht2* (*Helminthosporium turcicum* resistance 2) gene in maize. *Chinese Science Bulletin, 48*(2), 165-169.

Young, N. (1996). QTL mapping and quantitative disease resistance in plants. *Annual Review of Phytopathology, 34*(1), 479-501.

Yu, G., Sinclair, J., Hartman, G., & Bertagnolli, B. (2002). Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. *Soil Biology and Biochemistry, 34*(7), 955-963.

Yu, J., Holland, J. B., McMullen, M. D., & Buckler, E. S. (2008). Genetic design and statistical power of nested association mapping in maize. *Genetics, 178*(1), 539-551.

Zaitlin, D., DeMars, S., & Ma, Y. (1993). Linkage of *rhm*, a recessive gene for resistance to southern corn leaf blight, to RFLP marker loci in maize (*Zea mays*) seedlings. *Genome, 36*(3), 555-564.

Zhan, J., Thrall, P. H., & Burdon, J. J. (2014). Achieving sustainable plant disease management through evolutionary principles. *Trends in Plant Science, 19*(9), 570-575.

Zhang, F. (2013). Recognition of corn leaf disease based on quantum neural network and combination characteristic parameter. *Journal of Southern Agriculture, 44*(8), 1286-1290.

Zhang, X., Yang, Q., Rucker, E., Thomason, W., & Balint-Kurti, P. (2017). Fine mapping of a quantitative resistance gene for gray leaf spot of maize (*Zea mays* L.) derived from teosinte (*Z. mays* ssp. *parviglumis*). *Theoretical and Applied Genetics, 130*(6), 1285-1295.

Zhao, H., Gao, Z.-g., Zhang, X.-f., Zhuang, J.-h., & Sui, H. (2008). Population of physiological races of *Setosphaeria turcica* and its dynamic analysis in China [J]. *Journal of Shenyang Agricultural University, 5*.

Zhu, X., Reid, L., Woldemariam, T., Tenuta, A., & Schaafsma, A. (2002). First report of gray leaf spot caused by *Cercospora zeae-maydis* on corn in Ontario, Canada. *Plant Disease, 86*(3), 327-327.

Zwonitzer, J. C., Bubeck, D. M., Bhattramakki, D., Goodman, M. M., Arellano, C., & Balint-Kurti, P. J. (2009). Use of selection with recurrent backcrossing and QTL mapping to identify loci contributing to southern leaf blight resistance in a highly resistant maize line. *Theoretical and Applied Genetics, 118*(5), 911-925.

CHAPTER 2

Influence of farming practices on the population genetics of the maize pathogen *Cercospora zeina* in South Africa

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2.1 ABSTRACT

Gray leaf spot (GLS) is an important foliar disease of maize. This disease, caused by *Cercospora zeina,* is prevalent in both smallholder and commercial maize farms in South Africa. Notably, smallholder practices are geared towards conservation agriculture, planting diverse maize genotypes within a field and avoiding chemical control. This study examined the population genetic structure of 129 *C. zeina* isolates from three smallholder farm sites in KwaZulu-Natal in South Africa using 13 microsatellite markers. These were analyzed, together with 239 isolates previously analyzed from four commercial farms in the same province, to determine whether farming systems influence the genetic diversity of *C. zeina.* In addition, we wanted to determine whether the smallholder farming system selected for distinct *C. zeina* haplotypes that had a greater genetic diversity than the commercial system. Overall, farming systems exhibited partial, but significant, population differentiation, contributing 10 % of the genetic variation observed. A 16 % genetic variation conferred between KwaNxamalala (smallholder) and Cedara (commercial) areas that are in proximity, confirmed this. Private alleles accounted for 29 % of the 52 alleles observed in smallholder farms. Smallholder farms harbored a higher gene and genotypic diversity, with a clonal fraction of only 13 % compared to 33 % in commercial farms. Mating type ratios indicative of sexual recombination and lower linkage disequilibrium in most smallholder populations were consistent with higher levels of diversity. This study suggests that commercial farming practices, such as fungicides and monoculture crop planting, may select for a narrower genetic diversity of the pathogen that is propagated by asexual reproduction. In contrast, management of GLS disease in smallholder farms should consider the greater diversity of pathogen genotypes, especially if future research shows that this equates to a greater diversity of pathogenicity alleles.

2.2 INTRODUCTION

Maize (*Zea mays* L.) is a key crop serving as a critical source of food, feed and bio-fuel globally (Ranum *et al.*, 2014). In Africa, maize is the number one cereal in terms of cultivated area and total grain production (FAOSTAT, 2016; McCann, 2005). South Africa is the leading producer of maize in Africa (FAOSTAT, 2016), with a yield of over 156 million tons being produced on 26 million hectares of land (GrainSA, 2018) commercially and on a smallholder scale.

Commercial maize farms in South Africa cultivate hybrid maize on farmlands greater than five hectares under extensive mechanization, irrigation and chemical usage for soil fertilization, disease and weed management (Mellor & Malik, 2017). This accounts for over 90 % of maize production in the country (DAFF, 2016). Conversely, smallholder farming is practiced by about 2.5 million farmers (Statistics, 2013) depending mainly on seed from previous seasons on up to five hectares of agricultural land per farmer (Gouse *et al.*, 2006). These are predominantly in the Eastern Cape and KwaZulu-Natal provinces, although the latter has both commercial and smallholder farms (DAFF, 2016; GrainSA, 2018). Smallholder farmers practice conservation agriculture with rainfall as their source of water (Thierfelder & Wall, 2012), conservation tillage, and minimal to no chemical usage (Gouse *et al.*, 2006; Walker & Schulze, 2006).

Both maize cropping systems in South Africa are subject to a range of foliar pathogens and pests, with grey leaf spot (GLS) disease caused by the fungus *Cercospora zeina* being one of the most important constraints (Berger *et al.*, 2014). This is a globally important foliar pathogen of maize (Crous *et al.*, 2006; Dunkle & Levy, 2000; Liu & Xu, 2013; Meisel *et al.*, 2009; Neves *et al.*, 2015). It is widely hypothesized that the adoption of reduced tillage in maize production has led to the observed increased incidence of GLS due to increased inoculum on maize stubble (Ward *et al.*, 1999). However, very little is known about the influence of cropping systems on the population genetics of maize fungal foliar pathogens such as *C. zeina*.

Cropping practices have been shown to have significant effects on foliar pests and pathogens in crops such as rice. One of the first large-scale field experiments that demonstrated the effect of host genotype was in the rice-Blast pathosystem in China (Zhu *et al.*, 2000). Susceptible cultivars planted

in mixtures with resistant hybrids yielded 84 % more grain and exhibited 96 % less disease than in monocultures. Furthermore, genetic diversity of the Blast fungal pathogen was greater in mixtures than in fields with single cultivars (Zhu *et al.*, 2000).

Most studies that compare conventional cropping with organic methods have catalogued the diversity of types of pathogens, microbiomes or pests, rather than the diversity within a species (Adams *et al.*, 2017). For example, changes in the foliar fungal microbiomes of wild poplar trees that were relocated to a warmer environment showed that not only climatic differences, but also host genotype, had a significant effect on the diversity of fungal microbiomes in poplar leaves (Bálint *et al.*, 2015). Soil microbiomes, rather than foliar microbiomes are also more commonly studied, such as the evaluation of maize grown in organic compared to conventional soil fertility schemes (Lazcano *et al.*, 2013). In another study, five maize/soybean cropping systems were assessed for arthropod but not fungal diversity (Adams *et al.*, 2017). Foliar arthropod diversity was significantly greater in the “organic reduced till” soybean plots, and this was attributed to increased weed cover which provided a favorable microclimate.

Several aspects of potato cropping systems have been shown to have an effect on the population dynamics of the foliar oomycete pathogen *Phytophthora infestans.* In Estonia, organic potato production was dominated by the A2 mating type of the pathogen in contrast to the predominant A1 mating type in conventional production, which was also characterized by a fourfold greater number of metalaxyl fungicide resistant isolates (Runno-Paurson *et al.*, 2010). The phytosanitary quality of potatoes in Estonia was shown to affect race diversity of the *P. infestans* pathogen, where it was higher in plots planted with lower quality seed potatoes (Runno-Paurson *et al.*, 2013). Similar results were observed in Poland, where microsatellite diversity analysis was conducted on *P. infestans* collected from four production regions that varied in cultivation practices. Isolates from the Mlochow region, which is characterized by intensive potato farming, formed a distinct cluster, were more clonal, and had greater frequency of metalaxyl resistance (Brylińska *et al.*, 2016).

The development of fungicide resistance also appears to be dependent upon the population structure of *P. infestans* in a particular region. For example, in Ireland, where the pathogen is predominantly clonal, no evidence for selection of metalaxyl fungicide resistance was found and this was attributed

to the relatively low genetic diversity in the population (Stellingwerf *et al.*, 2018). In contrast to Ireland, selection for metalaxyl resistance was observed in “center of origin” Mexican populations of *P. infestans*, where there is high diversity and sexual recombination is common (Grünwald *et al.*, 2006).

The effect of potato host genotype was also shown to depend on the *P. infestans* population structure in different locations. In Ireland, where the pathogen is predominantly clonal, it was observed that host genotypes carrying specific resistance genes led to selection of the predominant clonal lineage of the pathogen (EU_13A2) (Stellingwerf *et al.*, 2018). In another study, on the island of Jersey where a single potato cultivar Jersey Royal has been grown for a century, researchers expected host genotype to have a strong influence on the population structure of *P. infestans* (Glais *et al.*, 2014). However, long distance gene flow, facilitated by prevailing winds from nearby France, was shown to be the predominant factor in determining the population diversity of the pathogen on the island.

Host adaptation by the wheat foliar pathogen *Zymoseptoria tritici* (previously *Mycosphaerella graminicola*) has also been cited as a driver of pathogen population sub-division (Zhan *et al.*, 2002). This was most evident in populations of this pathogen from Iran where genetically distinct populations were identified from different cropping regions in the country (Abrinbana *et al.*, 2010). Apart from the role of geographic barriers, host adaptation was noted as an important driver of genetic diversity. In contrast, a study in Ireland that compared *Z. tritici* isolates collected from four wheat cultivars ranging in host resistance genes showed no effect on neutral marker diversity in the pathogen (Welch *et al.*, 2018). However, the authors did observe variation in accessory chromosome structure, pointing to potential selection of pathogenicity functions by host genotype.

Taken together, these reports indicate that cropping practices can have a significant influence on foliar pathogen populations and highlight the importance of regional studies on specific crop-pathogen interactions. Studies have focused on a few main crops and diseases in the Northern hemisphere, however, to our knowledge, there have been no other studies on foliar pathogens of maize in Africa. We aimed, therefore, to test whether cropping practices have an influence on GLS populations by focusing on a maize growing region in South Africa (KwaZulu-Natal Province) that is a hotspot for GLS. We hypothesize that farming systems would influence the genetic diversity and population structure of *C. zeina* in commercial and small-holder farms. Microsatellite and mating-

type gene analysis of more than 128 isolates from each of the two farming systems were used to test this hypothesis.

2.3 MATERIALS AND METHODS

2.3.1 Fungal isolates and DNA extraction

Maize leaves exhibiting symptoms of gray leaf spot were collected from smallholder maize fields in the Hlanganani (30° 4' 12.5616" S; 29° 42' 2.3472"E), Ntabamhlophe (near Estcourt) (29° 5' 53.1312" S; 29° 42' 23.6412"E) and KwaNxamalala (29° 36' 29.16" S; 30° 13' 27.9228" E) districts of KwaZulu-Natal (KZN) province, South Africa, over two seasons in 2015 and 2016 (Figure 2.1) (see Appendix A for data). The sampling method of collecting from 50 plants at each site was as described in Muller et al (2016), although the average field size from small-holder sites was 2 hectares (ha), whereas it was double that size for commercial farm sites. In this study, a population was defined as the set of isolates obtained from one of the seven geographically separated field collection sites (Figure 2.1). Monoconidial spores typical of *Cercospora* species were isolated from individual GLS lesions under a dissecting microscope at 90x magnification using a sterile hypodermic needle, and fungal cultures were cultured and genomic DNA extracted as previously described (Muller *et al.*, 2016). In some cases, more than one isolate was obtained from the same GLS lesion, and the proportions thereof have been reported in results.

2.3.2 PCR amplification and microsatellite genotyping

PCR of a cercosporin toxin biosynthesis *ctb7* gene region was used as a diagnostic test as described by Swart *et al.* (2017) to evaluate whether all fungal isolates from GLS lesions collected from smallholder maize farms in KZN province, South Africa were *C. zeina*. Microsatellite genotyping was performed on all the isolates generated in this study using 13 previously developed and characterized microsatellite primer pairs (Muller *et al.*, 2016) (Table 2.1). In addition, genotyping data for the same 13 microsatellite markers for *C. zeina* isolates from commercial farms in KZN province, South Africa (Figure 1) was obtained from Muller *et al.* (2016).

Table 2.1: Microsatellite and polymorphic loci for *Cercospora zeina* isolates from smallholder and commercial farms in KwaZulu-Natal province, South Africa.

Locus [a]	N_{SF}[b]	N_{CF}[c]	Common alleles between farming systems	H_e[d]	E_5[e]	PIC[f]
CzSSR01	5 (1)	4	152, 155, 158, 161	0.51	0.84	0.41
CzSSR02	1	2 (1)	345	0.11	0.32	0.02
CzSSR04	6 (2)	4	459, 462, 465,468	0.55	0.81	0.48
CzSSR05	4 (1)	3	242, 245, 248	0.51	0.89	0.41
CzSSR06	3 (1)	2	223, 236	0.49	0.94	0.37
CzSSR07	9 (4)	7 (2)	209, 212, 226, 230, 233	0.53	0.61	0.47
CzSSR08	2	2	199, 202	0.45	0.82	0.37
CzSSR10	4 (1)	3	175, 191, 194	0.44	0.74	0.35
CzSSR12	7 (3)	4	236, 240, 244, 248	0.23	0.43	0.31
CzSSR13	1	1	329	-	-	0.00
CzSSR15	6[g] (2)	2	245, 251	0.50	0.78	0.43
CzSSR17	2	2	474, 480	0.47	0.94	0.36
CzSSR18	2	2	134, 137	0.46	0.92	0.36
Total	**52 (15)**	**38 (3)**		**0.40**	**0.75**	**0.33**

[a] CzSSR: *C. zeina* simple sequence repeat (SSR). Primer sequences in Muller *et al.* (2016).
[b] N_{SF}: Number of alleles per locus from 129 isolates from smallholder farms as reported in this study. Number of private alleles (only observed in one farming system and only one site of that farming system) are shown in brackets.
[c] N_{CF}: Number of alleles per locus from 239 isolates from commercial farms as reported by Muller *et al* (2016). Number of private alleles (as defined above) are shown in brackets.
[d] H_e: Nei's unbiased measure of gene diversity (Nei, 1978) per locus for smallholder and commercial farm isolates.
[e] E_5: population evenness estimating uniform genotype distribution for small-holder and commercial farm isolates, $E_5 = 1$ means genotypes occur at equal frequency, regardless of richness (Grünwald *et al.*, 2003).
[f] PIC: Polymorphic information content for smallholder and commercial farm isolates.
[g] Two alleles were observed in two smallholder sites and therefore not counted as private.

2.3.3 Measures of genetic diversity

Cercospora zeina isolates were grouped in populations according to their district of collection and farming system (Figure 1, Table 2). Data were divided into non-clone corrected (containing all isolates including clones) and clone-corrected isolates (containing only one unique multilocus genotype (MLG) per location). Non-clone-corrected data were used to calculate number of private alleles (P_a), average number of alleles/locus (N_a), effective number of alleles (N_e), number of multilocus genotypes (MLG), expected MLGs at the smallest sample size (eMLG), genetic evenness (E_5) and genotypic diversity (I, G) using the R package poppr (Kamvar *et al.*, 2014). Private allelic richness (P_{rar}) and allelic richness (Ra) were estimated with rarefaction to the smallest sample size

of 20, using the ADZE v1.0 software (Szpiech *et al.*, 2008). Alleles were classified as private if they were only present at one site of either the smallholder or commercial farming system and were not present in the other farming system. Clonal fraction (CF) was calculated by CF = 1 – (number of unique genotypes/total number of isolates) (Zhan *et al.*, 2003).

Genotypic diversity, which is the measure of genotypic richness and abundance, was evaluated based on two indices; Shannon-Wiener Index of MLG diversity, I (Shannon, 2001), and Stoddart and Taylor's index of MLG diversity, G (Stoddart & Taylor, 1988). Genetic evenness (E_5), which is a measure of distribution of genotypic abundances without depending on the number of genotypes in a population, was also calculated using the poppr package in R (Kamvar *et al.*, 2014). E_5 values range from 0 to 1, representing uneven and equal distributions of genotypes, respectively (Grünwald *et al.*, 2003; Ludwig & Reynolds, 1988). Nei's unbiased gene diversity (H_e), was calculated from the clone-corrected dataset using the equation, $H_e = 1-\sum x_\alpha^2$, where x_α is the frequency of the α^{th} allele (Nei, 1978). T-tests for significant difference of genetic diversity metrics between smallholder and commercial farms were calculated in Microsoft Excel.

2.3.4 Linkage disequilibrium and mating type frequencies

Multilocus v1.3b (Agapow & Burt, 2001) software with 1000 randomizations was used to estimate the index of association indices, I_A and rBarD. A previously optimized multiplex PCR assay (Muller *et al.*, 2016) was used to allocate mating types to all studied *C. zeina* isolates. Chi-square goodness of fit tests of MAT idiomorph frequencies per site were performed to test for any deviations from the expected 1:1 ratio of *MAT* genes under random mating.

2.3.5 Population genetic differentiation, gene and genotypic flow

To estimate gene migration amongst populations over time, gene flow (N_m) was computed using GenAlEx v6.5 (Peakall & Smouse, 2012). This index estimates the average number of alleles that migrate over time across loci based on Phi values between each pair of populations. Higher Phi values indicate more divergence amongst populations. Population differentiation was evaluated using analysis of molecular variance (AMOVA), with 1000 permutations to test the null hypothesis of free gene flow between and within populations. To visualize variation amongst *C. zeina* populations,

principal coordinate analysis (PCoA) implemented in GENALEX v6.5 (Peakall & Smouse, 2012) was performed using Nei's unbiased genetic distances (Nei, 1978).

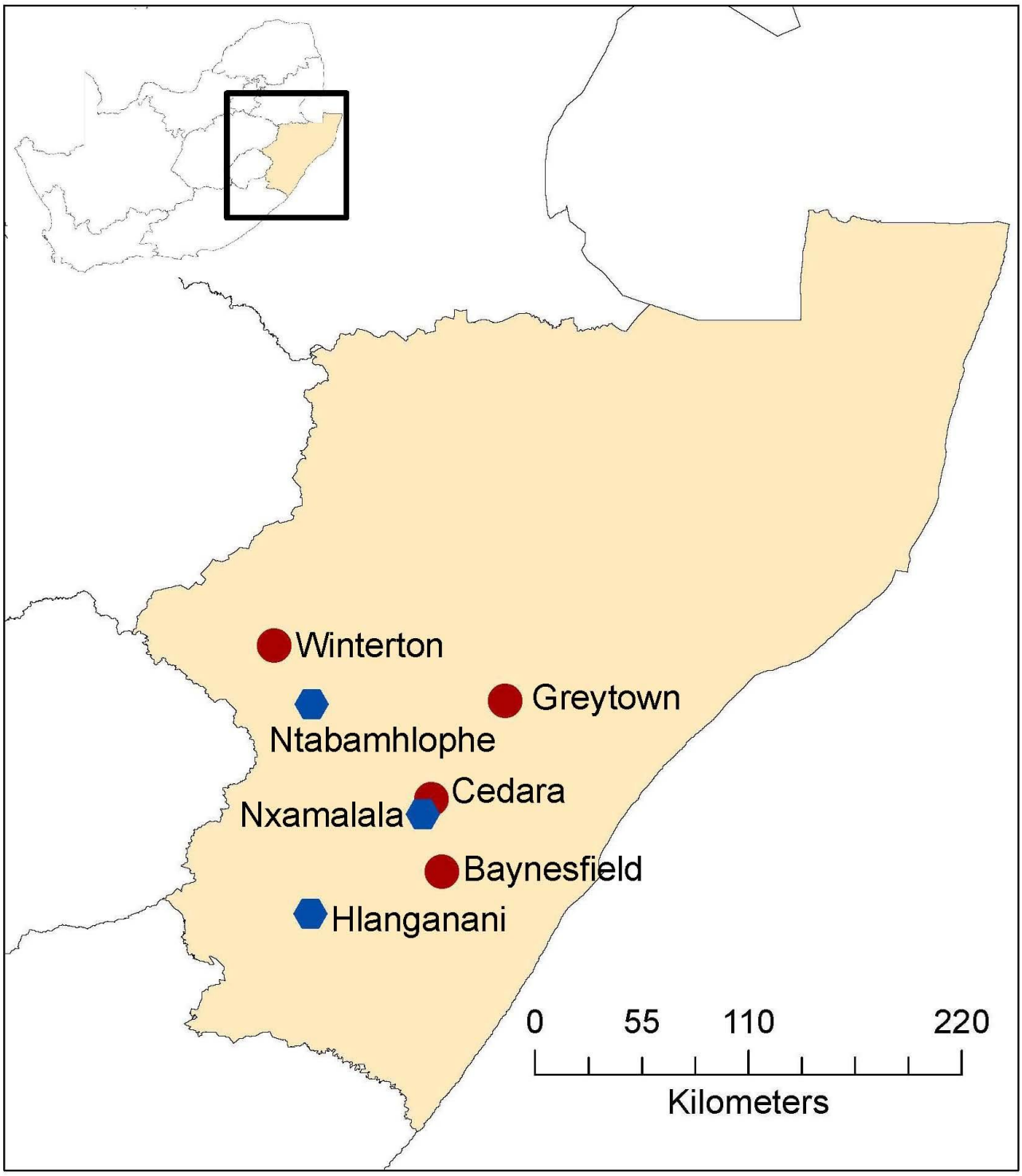

Figure 2.1: Collection sites of *Cercospora zeina* isolates from smallholder (blue symbols; this study) and commercial (red symbols); (Muller *et al.*, 2016) maize growing sites in KwaZulu-Natal province of South Africa. The inset shows the position of the province in South Africa.

2.3.6 Population genetic structure

Bayesian-model clustering method implemented in STRUCTURE v 2.3.4 (Falush *et al.*, 2007; Pritchard *et al.*, 2002) was used to estimate individual clustering of *C. zeina* isolates and their level of relatedness. All isolates in each population (with clones removed) were analyzed using an Admixture model with correlated allele frequencies without prior information on sample location. Simulations were run using clustering models with 20 independent runs, K = 1 to 20, with 1 000 000 Monte Carlo Markov chain (MCMC) iterations and a burn-in of 100 000. The best K value was determined by examining the log probability [ln Pr (X|K)] and rate of change, ΔK, for a particular cluster over multiple runs (Evanno *et al.*, 2005). Clusters were estimated using membership coefficient matrices for each value of K using CLUMPAK and graphically represented using DISTRUCT (Kopelman *et al.*, 2015).

To further determine whether farming systems shape the population structure of *C. zeina*, a minimum spanning network was built using non-clone-corrected data for all isolates from smallholder and commercial farms. This network was constructed in R based on Bruvo's distance (Bruvo *et al.*, 2004), which assumes a stepwise mutation model in the calculation of genetic distances among isolates (Ruibal *et al.*, 2017). Network visualization was performed using the imsn function in R and displayed in 2D using the package magrittr.

2.4 RESULTS

2.4.1 *Cercospora zeina* identification and microsatellite genotyping

In total, 129 single-spore isolates were obtained from GLS infected maize leaves collected in three smallholder farm sites in KwaZulu-Natal (Hlanganani, KwaNxamalala and Ntabamhlophe) (Figure 2.1). All isolates were confirmed to be *C. zeina*, since they produced a diagnostic *ctb7* gene product of 618 bp (Supplementary Figure 2.1).

Eleven out of thirteen microsatellite loci were polymorphic between isolates of *C. zeina* from smallholder farms, having levels of polymorphism ranging from two alleles for CzSSR08, CzSSR17 and CzSSR18 to nine alleles for CzSSR07 (Table 2.1) (Supplementary Table 2.1). A lower number

of alleles were observed for the same loci for *C. zeina* isolates from the four commercial maize populations in KwaZulu-Natal (Table 2.1; (Muller *et al.*, 2016). Gene diversity of the combined dataset from smallholder and commercial isolates ranged from 0.11 (CzSSR02) to 0.55 (CzSSR04) (Table 2.1). Nine of the thirteen markers had genetic evenness across the combined dataset of 0.74 or more, indicating that they had a relatively even distribution of alleles for each of these loci (Table 1). Ten of the markers had a polymorphic information content (PIC) greater than or equal to 0.35 (Table 2.1).

Table 2.2: Indices of genetic variability for *Cercospora zeina* populations from seven sites of commercial and smallholder maize farms in KwaZulu-Natal, South Africa.

Population	N[d]	P_a[e]	P_{rar}[f]	N_a[g]	N_e[h]	R_a[i]	I[j]	G[k]	E_5[l]	CF (%)[m]	MLG[n]	eMLG[o]	H_e[p]
Hlanganani	72	12	0.16 ± 0.05	3.69	1.97	2.89 ± 0.39	0.74	57.6	0.94	11	64	19.4	0.45
Ntabamhlophe[a]	20	2	0.15 ± 0.08	2.62	2.12	2.21 ± 0.21	0.70	12.5	0.90	25	15	15.0	0.49
KwaNxamalala[a]	37	1	0.05 ± 0.02	2.54	1.79	2.22 ± 0.21	0.62	30.4	0.95	11	33	18.9	0.41
Sub-total (smallholder)[b]	**129**	**15**	**0.12 ± 0.05**	**2.95**	**1.96**	**2.44 ± 0.27**	**0.69**	**33.5**	**0.93**	**13**	**112**	**17.8**	**0.45**
Baynesfield[c]	36	2	0.06 ± 0.03	2.54	1.74	2.18 ± 0.18	0.56	16.2	0.72	25	27	16.1	0.40
Cedara[c]	55	0	0.03 ± 0.01	2.39	1.63	2.18 ± 0.19	0.55	36.4	0.88	18	45	18.4	0.36
Graytown[c]	128	1	0.02 ± 0.01	2.39	1.75	2.02 ± 0.19	0.50	16.5	0.38	44	72	15.5	0.38
Winterton[c]	20	0	0.01 ± 0.01	1.85	1.64	1.71 ± 0.13	0.49	18.2	0.97	8	19	19.0	0.35
Sub-total (commercial)[b]	**239**	**3**	**0.03 ± 0.02**	**2.29**	**1.67**	**2.17 ± 0.18**	**0.52**	**19.9**	**0.74**	**33**	**163**	**17.3**	**0.37**

[a] Smallholder farm population.

[b] Sub-totals shown for columns N, Pa, MLG; Averages shown for all other columns.

[c] Commercial farm population. Data from Muller *et al.* (2016).

[d] N: Number of isolates per area of collection.

[e] P_a: Number of private alleles per locus.

[f] P_{rar}: Number of private alleles after rarefaction to the smallest sample size of 20.

[g] N_a: Average number of alleles per locus.

[h] N_e: Number of effective alleles per locus.

[i] R_a: Allelic richness after rarefaction to the smallest sample size of 20.

[j] I: Shannon-Wiener index of MLG diversity (Shannon, 2001).

[k] G: Stoddart and Taylor's index of MLG diversity (Stoddart & Taylor, 1988).

[l] E_5: Population evenness estimating uniform genotype distribution, $E_5 = 1$ means genotypes occur at equal frequency, regardless of richness (Grünwald *et al.*, 2003).

[m] CF: Clonal fraction as calculated by CF = 1 - (number of unique genotypes/total number of isolates), expressed as a percentage.

[n] MLG: Number of multilocus genotypes observed.

[o] eMLG: Number of expected MLGs at the smallest sample size based on rarefaction.

[p] H_e: Nei's unbiased measure of gene diversity (Nei, 1978) calculated using clone-corrected data set.

2.4.2 Population genetic diversity

From 129 smallholder isolates genotyped, 52 alleles were obtained across the 13 microsatellite loci (Table 2.1). Fifteen of these (29%) were private alleles, in contrast to the 239 commercial farm isolates (Muller *et al.*, 2016), which had only three private alleles (Table 2.2). The number of private alleles calculated after rarefaction to the smallest sample size (20) was significantly greater for smallholder farm isolates (p=0.04, t-test). The average number of effective alleles (N_e) was significantly greater for smallholder farms (N_e = 1.96) than commercial farms (N_e = 1.67) (p = 0.03, t-test). Allelic richness (R_a) adjusted to the smallest sample size was higher on average in populations from smallholder farms (2.44) than commercial farms (2.17) although the difference was not significant. The Shannon-Weiner index of genotypic diversity was significantly greater in smallholder than commercial farms (I = 0.69 vs 0.52, respectively; p = 0.01, t-test) (Table 2.2). The Stoddart-Taylor index of diversity was greater in smallholder compared to commercial farms, although the difference was not significant (G = 33.5 vs 19.9, respectively; Table 2.2).

The clonal fraction was 19 % lower in smallholder farms (13 %) compared to commercial farms (32 %) (Table 2.2). It was expected that isolates from the same lesion would be more likely to be clones, however this could not be the reason for greater clonality in commercial farms since they had a lower proportion of isolates from the same lesion (7 %) compared to smallholder farms (22 %) (Table 2.2). After removing clones, 275 multilocus genotypes (MLGs) (112 from smallholder and 163 from commercial farms) were identified (Table 2.2). Nei's measure of gene diversity (H_e) for the clone-corrected dataset was higher at all smallholder farm sites (Table 2.2), and significantly different from commercial sites (p = 0.02, t-test).

2.4.3 Mating type frequencies and linkage disequilibrium

A total of 368 *C. zeina* isolates was screened with a multiplex PCR assay using mating type primer pairs (Muller *et al.*, 2016). Each screened isolate showed either a 631-bp or 409-bp product for *MAT1-1* (160 isolates) and *MAT1-2* (208), respectively (Table 2.3). Isolates exhibited a 0.95 (X^2 = 0.07) and 0.68 (X^2 = 8.47) mating type ratio for smallholder and commercial farms, respectively. The overall distribution for KwaZulu-Natal province (smallholder and commercial farms) did not deviate

significantly from a 1:1 ratio (X^2 = 6.26) (Table 2.4). Looking at the individual populations, two (Hlanganani, Ntabamhlophe) of the three smallholder farms, and two (Cedara, Winterton) of the four commercial farms exhibited a 1:1 mating type ratio (Table 2.3). *MAT1-2* was predominant in the commercial farms.

The test for linkage disequilibrium (LD) using the microsatellite data of the clone-corrected set of 275 isolates by I_A and rBarD at $p < 0.001$ showed that the combined commercial populations exhibited significant LD (I_A = 0.13, $p < 0.001$), whereas the combined smallholder populations did not (I_A = 0.06, $p = 0.02$). However, there was not a significant difference between the I_A or rBarD values for the smallholder compared to commercial farm isolates (t-test, $p > 0.05$, data not shown). The only individual population that showed significant LD was Ntabamhlophe, however the sample size was low (15 isolates after clone correction) (Table 2.3).

Table 2.3: Mating type frequencies and tests for linkage disequilibrium at microsatellite loci from *Cercospora zeina* isolates from commercial and smallholder maize farms in South Africa using the non-clone-corrected data set.

Population	**N[a]**	***MAT1-1***	***MAT1-2***	**Ratio**	**χ^2 (p-value)[b]**	**I_A (p-value)[c]**	**rBarD (p-value)[d]**
Hlanganani	72	30	42	0.71	2.00 (0.06)	0.09 (0.010)	0.01 (0.010)
Ntabamhlophe	20	9	11	0.82	0.20 (0.55)	1.13 (<0.001)	0.11 (<0.001)
KwaNxamalala	37	24	13	1.85	3.27 (0.002)	0.10 (0.10)	0.01 (0.096)
Sub-total (smallholder)	**129**	**63**	**66**	**0.95**	**0.07 (0.711)**	**0.44 (0.035)**	**0.13 (0.035)**
Baynesfield	36	11	25	0.44	5.44 (0.01)	0.53 (<0.001)	0.05 (<0.001)
Cedara	55	23	32	0.72	1.47 (0.11)	0.24 (<0.001)	0.02 (<0.001)
Graytown	128	52	76	0.68	4.5 (0.01)	0.84 (<0.001)	0.09 (<0.001)
Winterton	20	11	9	1.22	0.20 (0.50)	0.28 (0.040)	0.03 (0.040)
Sub-total (commercial)	**239**	**97**	**142**	**0.68**	**8.47 (<0.001)**	**0.515 (0.010)**	**0.052 (<0.001)**
Total (smallholder + commercial)	368	160	208	0.77	6.26 (0.36)	0.48 (0.022)	0.05 (0.022)

[a] N: Number of isolates per site (not clone-corrected).

[b] X^2: Chi-square value based on the 1:1 mating type ratio and one degree of freedom for $p < 0.01$.

[c] I_A: index of association for $p < 0.001$. [d] rBarD: standardized index of association for $p < 0.001$.

Table 2.4: Analysis of molecular variance (AMOVA) of microsatellite data for *Cercospora zeina* isolates grouped by farming systems (commercial and smallholder) in KwaZulu-Natal, South Africa.

Source	df[a]	SS[b]	MS[c]	Estimated Variance	percentage of total Variance	Phi-Statistic[d]	Value (p-value)	N_m[e]
Non-clone-corrected data set between farming systems								
Between farming systems	1	51	51	0.27	10	PhiRT	0.12 (0.001)	3.62
Between populations	5	25	5	0.06	2	PhiPT	0.08 (0.001)	5.46
Within populations	353	850	2	2.41	88			
Clone-corrected data set between farming systems								
Between farming systems	1	38	38	0.25	9	PhiRT	0.09 (0.001)	4.32
Between populations	5	21	4	0.05	2	PhiPT	0.10 (0.001)	6.50
Within populations	259	679	3	2.62	89			
Non-clone-corrected data set of KwaNxamalala (smallholder) and Cedara (commercial)								
Between Cedara and KwaNxamalala	1	22	22	0.45	16	PhiPT	0.16 (0.001)	2.70
Within populations	90	216	2	2.40	84			
Clone-corrected data set of KwaNxamalala (smallholder) and Cedara (commercial)								
Between Cedara and KwaNxamalala	1	19	19	0.44	15	PhiPT	0.15 (0.001)	2.81
Within populations	76	188	3	2.47	85			

[a] df: Degrees of freedom.

[b] SS: Sum of squared observations.

[c] MS: Mean of squared observations.

[d] PhiRT: proportion of total genetic variance between farming systems; PhiPT: Proportion of the total genetic variance between populations.

[e] N_m: Absolute number of migrants per generation between different farming systems or populations.

2.4.4 Population genetic differentiation and gene flow

Analysis of molecular variance (AMOVA) of the microsatellite data for all the isolates from both farming systems showed that genetic variation was contributed at all levels (systems, sites (populations) and isolates) (Table 2.4). However, the over-riding source of variation was due to differences within populations, i.e. between individual isolates, accounting for 88 % and 89 % of variation for non-clone-corrected and clone-corrected datasets, respectively (Table 2.4). Farming systems made a significant contribution to variation, with 10 % (non-clone-corrected) and 9 % (clone- corrected) variability attributed to farming systems with Phi = 0.12 (non-clone-corrected) and 0.09 (clone-corrected) for $p < 0.001$ (Table 2.4). Variation between the seven populations contributed only 2 % to the variation for both non-clone-corrected and clone corrected datasets. Consistent with this, there was a higher estimation of gene flow between populations (N_m = 5.46 (non-clone corrected) and 6.50 (clone-corrected), than between farming systems (N_m = 3.62 (non-clone corrected) and 4.32 (clone-corrected)). Furthermore, AMOVA analysis of two populations in close proximity (Cedara (commercial) and KwaNxamalala (smallholder) indicated that farming systems contributed 15 % of the variability (Phi = 0.15 for $p < 0.001$), and these two sites had lower gene flow (N_m = 2.70 for non-clone corrected and 2.81 for clone corrected) (Table 2.4).

2.4.5 Population genetic structure

Both principal coordinates analysis (PCoA) and STRUCTURE were used to visualize the patterns of variation between farming systems using the clone-corrected dataset. The PCoA results revealed that isolates from the two farming systems mostly grouped together, consistent with the AMOVA analysis that more than 80 % of variation was between isolates (Figure 2.2A). However, the first principal component (x axis) shows outlier isolates from commercial and smallholder sites on the right- and left-hand sides, respectively. A finer-scale analysis between isolates from Cedara (commercial) and KwaNxamalala (smallholder), revealed partial population differentiation on the PCoA plot (Figure 2.2B).

From the structure analysis, the highest likelihood of the ΔK index, and posterior probability, was K = 2 (ΔK = 766.09) using the admixture model best suited to the data (Figure 2.3A). The STRUCTURE plot shows haplotype differences and partial structure existing between commercial and smallholder isolates (Figure 2.3B).

The global spanning network with the non-cloned-corrected microsatellite dataset showed that although there are some haplotypes that are shared between systems and between populations, there was some level of separation between isolates from commercial and smallholder farming systems (Figure 2.4). The network diagram also illustrated that the commercial populations are more clonal than the smallholder populations.

2.5 DISCUSSION

The main findings from this study were that (i) *C. zeina* isolates from smallholder farms in KwaZulu-Natal province of South Africa exhibited a high genetic diversity, and this was greater than that seen from *C. zeina* isolates from commercial farms (Muller *et al.*, 2016); and (ii) partial population differentiation was seen between isolates from smallholder and commercial farms, indicating that differences in farming practices influence the population biology of the pathogen.

2.5.1 High genetic diversity in smallholder farms

Cercospora zeina populations from smallholder farms showed a higher genetic diversity than has been reported in commercial farms (Muller *et al.*, 2016). This indicates that the pathogen is relatively well established within the country ever since the first report in 1988 in KwaZulu-Natal (Ward *et al.*, 1999) and is now widespread throughout the country. Notably, populations with higher genetic diversity are postulated as probable centers of origin. This has been shown on a continental level for pathogens such as *P. infestans* originating from Mexico (Fry *et al.*, 1992) and *Z. tritici* from Europe and the Middle East (Banke *et al.*, 2004). *C. zeina* has in the past been postulated to have originated in Africa and not the United States based on its higher genetic diversity in Africa (Dunkle & Levy, 2000). However, despite the diversity that we

observe in smallholder farms in KwaZulu-Natal province, it is not conclusive that this is the center of introduction of *C. zeina* haplotypes observed in South Africa.

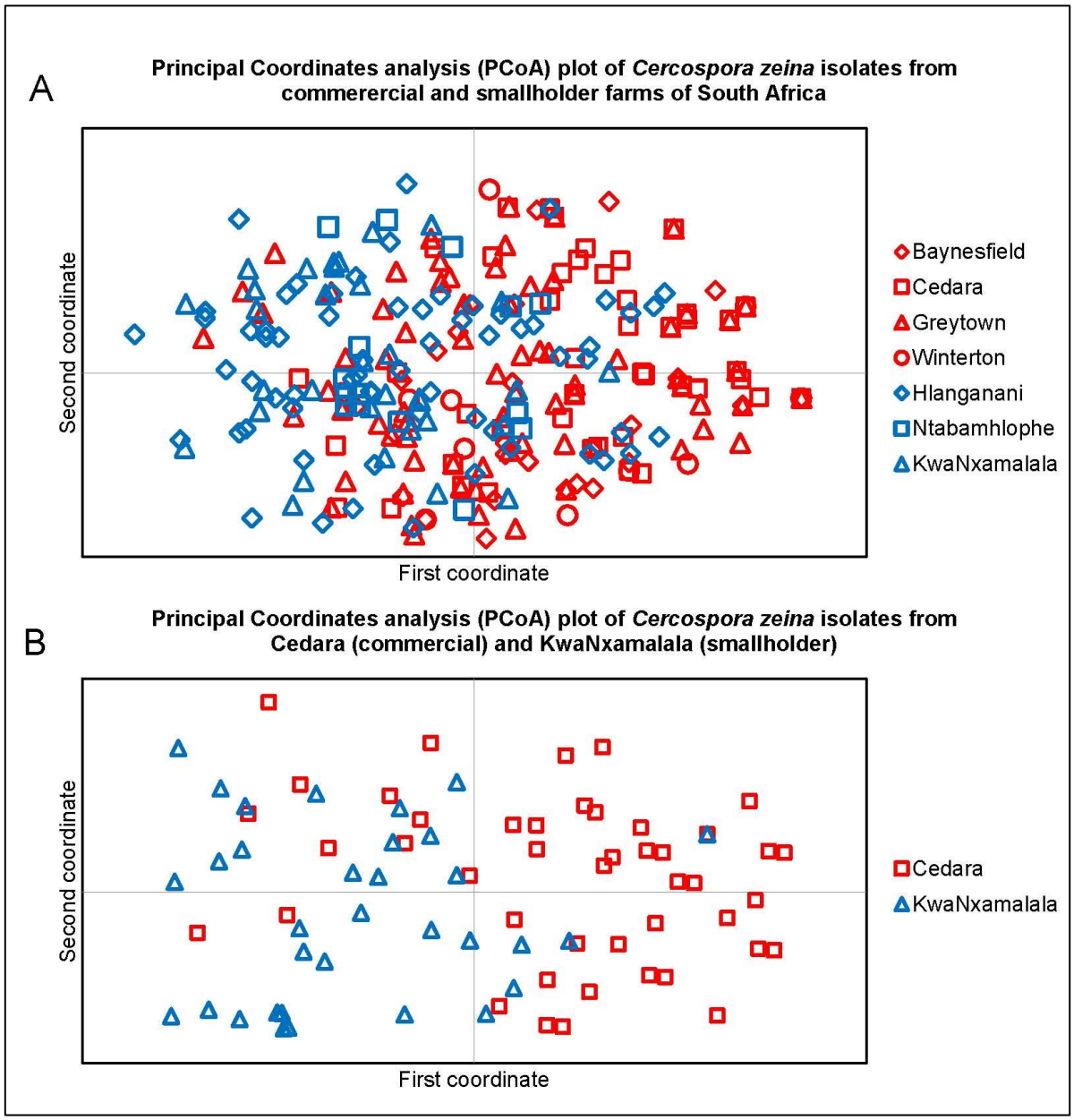

Figure 2.2: Principal coordinate analysis (PCoA) of *Cercospora zeina* isolates from KwaZulu-Natal, South Africa. A: PCoA of 275 *C. zeina* isolates from three smallholder (blue symbols; Hlanganani, KwaNxamalala, Ntabamhlophe) and four commercial (red symbols; Baynesfield, Cedara, Graytown, Winterton) farm sites. The first and second principle components explain 13 % and 24 % of the variation, respectively, based on Nei's genetic distance (Nei, 1987) using GenAlEx software v6.5 (Peakall & Smouse, 2012). B: PCoA of 78 *C. zeina* isolates from adjacent (9 km apart) smallholder (blue symbol; KwaNxamalala) and commercial (red symbol; Cedara) farm sites. The first and second principle components explain 17 % and 29 % of the variation, respectively. Clones were not included in the PCoA analysis.

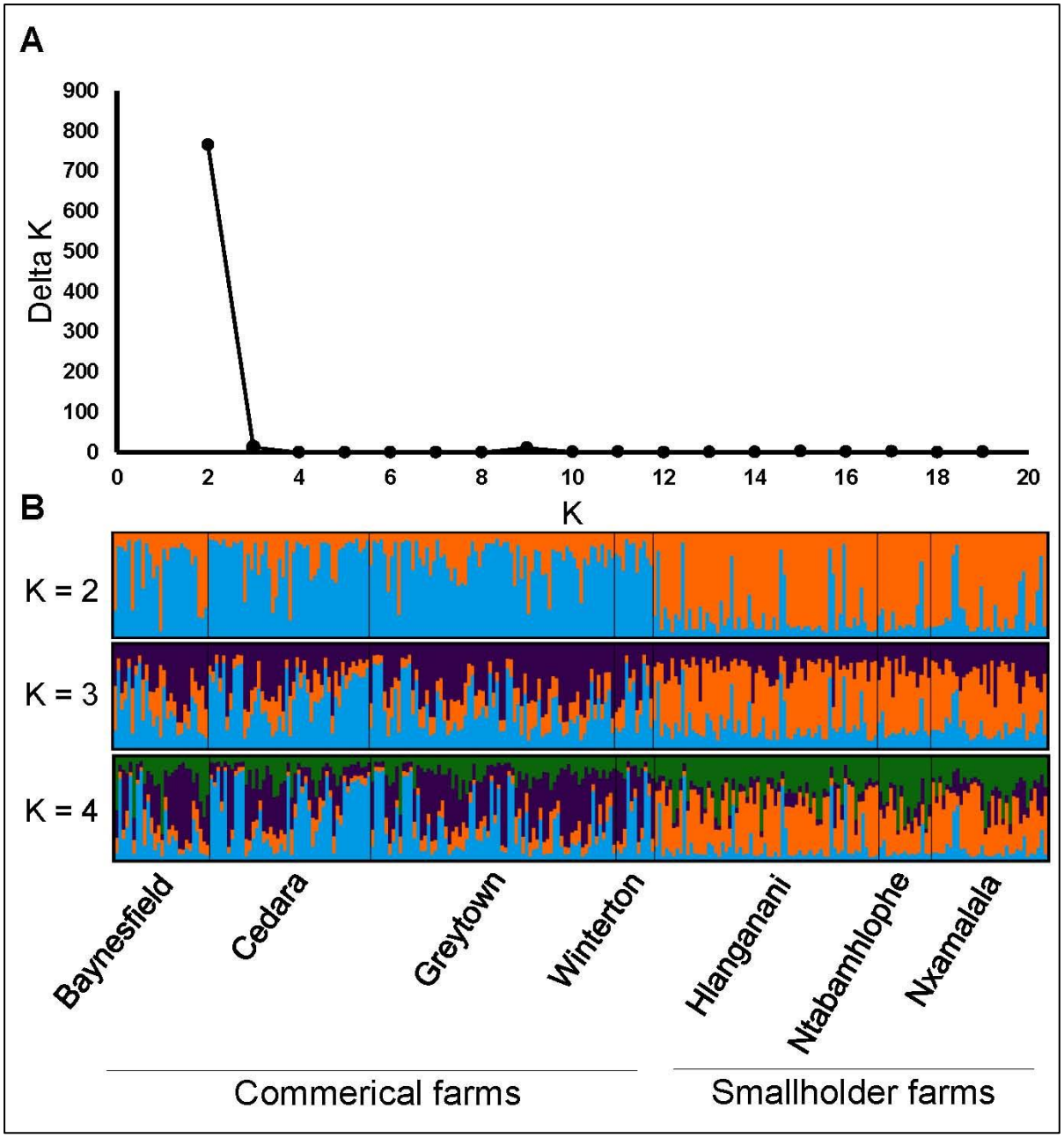

Figure 2.3: Population structure of *Cercospora zeina* isolates from KwaZulu-Natal inferred using STRUCTURE. The dataset included 275 multilocus haplotypes (clone-corrected) determined using 13 microsatellite loci, analyzed using an admixture model in STRUCTURE v2.3. A: Graph to estimate the optimal K value based on the formula Delta K = mean (|L"(K)|) / stdev [L (K)]. B: Graphical representation of isolates sorted by population for commercial (Baynesfield, Cedara, Graytown, Winterton) and smallholder (Hlanganani, KwaNxamalala, Ntabamhlophe) populations for K=2 after analysis with STRUCTURE v2.3.4 and visualized using DISTRUCT. Each isolate is represented by a vertical line fragmented into K colored sections with length proportional to each of the K inferred clusters.

2.5.2 Partial population structure between smallholder and commercial farms

Our data presents evidence of partial population differentiation between *C. zeina* populations across farming systems in South Africa. This is shown by a 10 % total genetic divergence and a relatively low absolute number of migrants, N_m = 3.62 between farming systems. These results were supported by the PCoA and STRUTURE results. The analysis of populations from neighboring sites Cedara and KwaNxamalala further corroborated our findings. However, the 88 % of genetic variance observed between isolates within the province revealed that gene flow plays a crucial role in the diversity of the pathogen. Past research has shown that gene flow influences the admixture of *Cercospora beticola* populations into a single mega-population, and the same was shown for *Cercospora sojina* (Groenewald *et al.*, 2008; Kim *et al.*, 2013). *Cercospora zeina* is a known foliar pathogen with no seed transmission recorded (Dunkle & Levy, 2000; Ward *et al.*, 1999) and its movement between fields is mainly by the dispersal of airborne conidia (Ward *et al.*, 1999). *Cercospora* spp. conidia are estimated to travel up to 40 km by wind, resulting in disease progression across a landscape at a rate of 80 to 160 km per year (Ward *et al.*, 1999).

2.5.3 Reasons for partial population structure between smallholder and commercial farms

Our findings indicated that differences in farming practices influence the population biology of *C. zeina*, which is the only causal pathogen of GLS in South Africa (Meisel *et al.*, 2009; Muller *et al.*, 2016). In a study by Neves *et al.* (2015) however, climate and farming systems had no substantial effect on the diversity of species associated with GLS, where *C. zeae-maydis*, *C. zeina* and *Cercospora sorghi* var. *maydis* were isolated from diseased maize. Smallholder farmers in Africa plant a diversity of maize varieties (land-races and hybrids) and often use farmer saved seed for the next season (Sibiya *et al.*, 2013). These may harbor a diversity of host resistance genes, which may explain, to some extent, the wider diversity of the fungus that we observed. Commercial farms on the other hand depend on monoculture production

using maize hybrids with a limited number of host resistance genes against GLS (Berger *et al.*, 2014). Pathogen haplotypes that can overcome the resistance are able to survive and these may exhibit a lower genetic diversity compared to smallholder farms as shown in this study. The influence of maize genotype on pathogen diversity could not be assessed in this study, since the identity of the maize varieties and their GLS resistance rating was not recorded or available at collection time.

Smallholder farms depend on manual weed control (Sibiya *et al.*, 2013). This may create a more humid environment due to delayed weeding thus providing a favorable microclimate for pathogen establishment on its host. In contrast, commercial farms use better weed management systems that create a less humid environment for fungal development. In South Africa, there is increased use of roundup-ready maize (Mathews *et al.*, 2018) and also mechanized and pre-emergence herbicide treatment. These allow for better management of the micro-environment between plants and may result in reduced pathogen populations. In addition, canopy cover and thus humidity may differ between farming systems due to other agronomic factors, such as planting density, maize architecture and fertilization regimes (Tivoli *et al.*, 2013).

Overall, the practice of conservation agriculture by smallholder farms has been implicated in the increased prevalence of a number of plant fungal diseases such as rice blast (Raveloson *et al.*, 2018). The increased adoption of reduced tillage in maize production has long been considered a reason for the increased incidence of GLS disease of maize in South Africa, due to the over-wintering of the pathogen on maize stubble (Ward *et al.*, 1999). On the other hand, the continued use of fungicides by commercial farms (Mellor & Malik, 2017) and the planting of resistant monocropped genotypes represent strong selection pressures that could narrow the genetic diversity of *C. zeina* in the cropping system (Brown, 2015).

2.5.4 Higher clonality in commercial farms

In this study, higher clonality was detected in the commercial farms than in smallholder farms, and this was not a result of sampling bias. The same sampling strategy was followed in both farming systems in which samples were collected at intervals in transects across each field site. The only difference was that commercial farm sites were, on average, twice as large as small-holder sites. This could lead to greater diversity in commercial sites; however, our results showed the opposite. Another factor is whether isolates were obtained from the same lesions, however our sampling had a greater number of same-lesion isolates from smallholder sites than commercial sites.

Greater clonality in commercial farms might be partly due to host resistance since commercial farms have access to hybrids with quantitative resistance. Evidence of selection pressure due to host resistance has been reported in many pathogens including *P. infestans* (Stellingwerf *et al.*, 2018) and *Z. tritici* (Welch *et al.*, 2018). Secondly, fungicide resistance in the pathogen may also explain clonality, since a resistant mutation would be maintained in a population with higher rates of asexual reproduction, as was seen for the *C. zeina* commercial farm isolates. Resistance to strobilurin-based fungicides has been reported in other *Cercospora* species, namely *C. sojina* and *C. beticola* (Bolton *et al.*, 2013; Shrestha *et al.*, 2017). Fungicides based on the same chemistry are deployed in commercial maize fields in South Africa, although resistance in the maize gray leaf spot pathogen has not been reported to our knowledge. Overall, greater clonality of *C. zeina* isolates within commercial farms may be as a result of selection by fungicides and larger areas of genetically homogenous maize.

2.5.5 Evidence for sexual recombination in *Cercospora zeina* populations

Recombination is a potential mechanism driving the observed diversity of *C. zeina* in South Africa's maize cropping systems. This study reports that most pairs of loci within smallholder farm isolates exhibited low LD since the indices of I_A and rBarD for the combined smallholder populations did not differ significantly from zero. In contrast, there was significant LD in the

combined commercial farm populations which is consistent with the higher clonality. In addition, commercial farms appeared to have a larger number of *MAT1-2* than *MAT1-1* isolates. This may be due to the practices in this farming system that favor clonality thus resulting in bias towards one mating type. More studies are needed to establish the level of virulence of isolates with different mating types from both farming systems. *Cercospora zeina* has been reported to have a high genotypic diversity (Muller *et al.*, 2016). Although no sexual stage has been reported in *C. zeina*, this suggests a cryptic sexual reproductive stage as has been proposed for other *Cercospora* species (Grünwald *et al.*, 2006; Shrestha *et al.*, 2017). Sexual recombination has been reported in many other heterothallic fungal pathogens (Dale *et al.*, 2011; Hayden & Howlett, 2005; Laraba *et al.*, 2017; Young *et al.*, 2018).

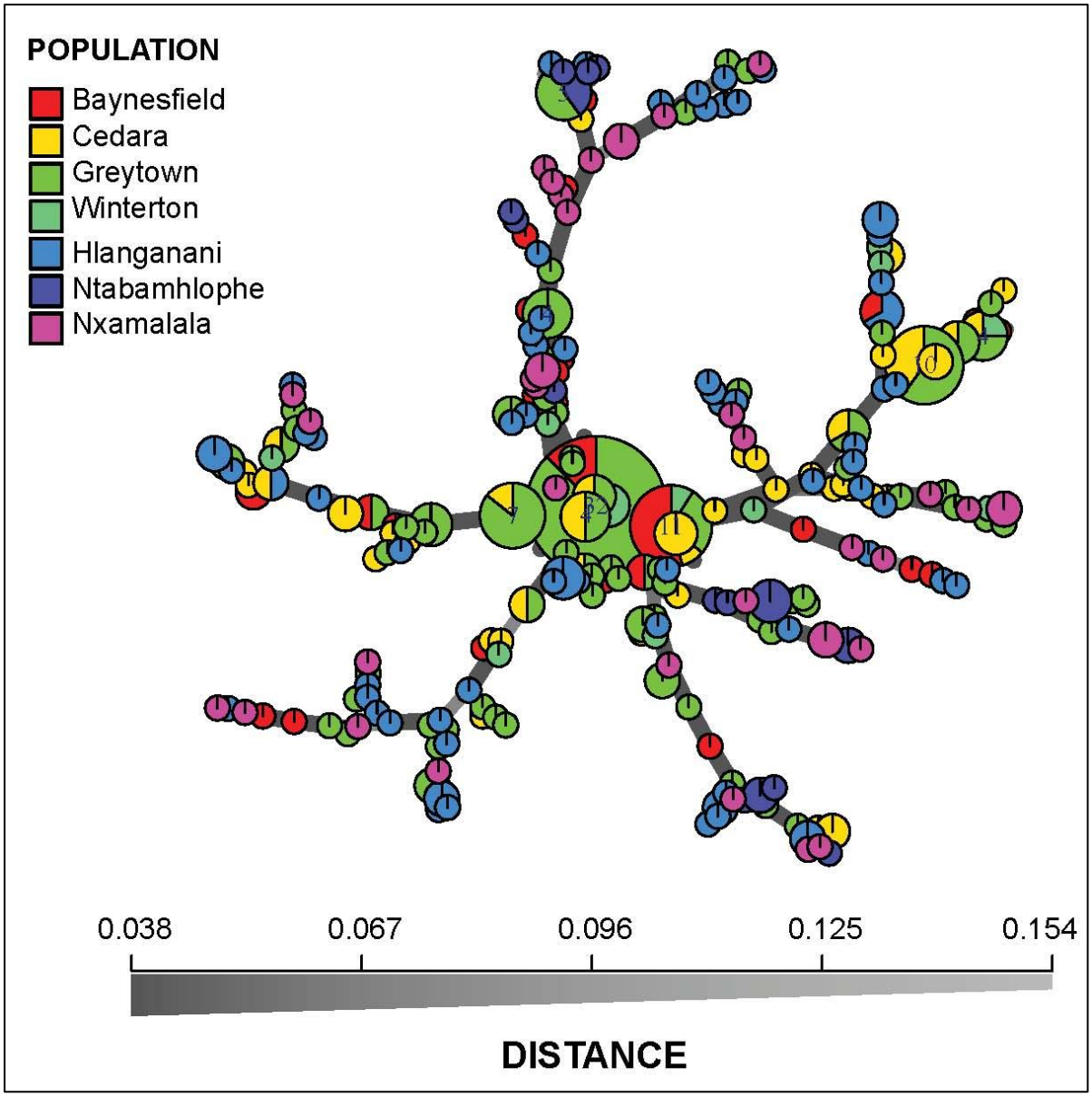

Figure 2.4: Minimum spanning network showing the relationship between multilocus genotypes of *Cercospora zeina*. Data is shown for 368 isolates (non-cloned corrected) from smallholder and commercial farms, color-coded by population. The network was constructed in R based on Bruvo's distance (Bruvo *et al.*, 2004) with the scale shown below the figure. Node sizes correspond to the number of isolates with a particular haplotype.

2.6 CONCLUSIONS

This study has provided support for the hypothesis that farming systems influence the population genetics and structure of *C. zeina* in South Africa. We therefore propose the adoption of extensive integrated disease management strategies in both smallholder and commercial farming systems. For commercial systems that can afford fungicides, there is a

need to use a variety of fungicides, since a clonal population of resistant individuals would propagate more rapidly under selection with a single fungicide. Although conservation agriculture is crucial especially in smallholder farming systems, there is a need for tillage or fallow seasons to reduce the accumulation of the primary infection inoculum and the rate of sexual recombination occurring in the pathogen.

The above management strategies must augment other strategies such as field sanitation and cultural practices that exploit host resistance more effectively. Four strategies for combining host resistance genes with the aim to delay resistance-breaking pathogens were recently modelled using the cereal-rust pathosystem, namely pyramiding in a single genotype, mosaics, mixtures or rotation (Rimbaud *et al.*, 2018). The outcome was that pyramiding was the most durable, but the pathogen's evolutionary potential was a critical factor in determining the most effective strategy. Taking this to the maize-GLS pathosystem, this highlights the importance of understanding the role of cropping systems on the population genetics of *C. zeina* in a particular region, and the need for a better understanding of maize resistance mechanisms against this pathogen (Christie *et al.*, 2017; Meyer *et al.*, 2017).

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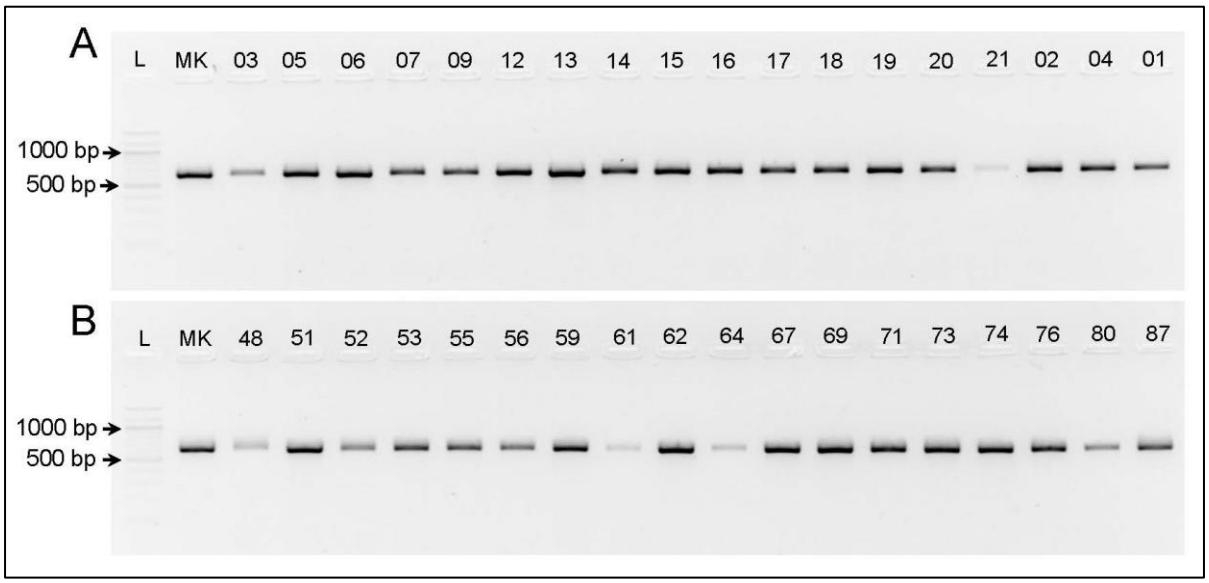
2.8 SUPPLEMENTARY DATA



Supplementary Figure 2.1: Identification of *Cercospora zeina* based on the amplification of *ctb7* gene region. PCR products visualized on agarose gels of representative isolates from Ntabamhlophe (panel A) and KwaNxamalala (panel B). Lane L: Quick-Load® 100 bp DNA Ladder (New England BioLabs Inc.); Lane MK: *C. zeina* CMW 25467 (positive control); Lanes with numbers: codes for the different fungal isolates from the different sites of collection. The expected *C. zeina ctb7* amplicon is 618 bp.

2.9 REFERENCES

Abrinbana, M., Mozafari, J., Shams-Bakhsh, M., & Mehrabi, R. (2010). Genetic structure of *Mycosphaerella graminicola* populations in Iran. *Plant Pathology, 59*(5), 829-838.

Adams, P. R. I., Orr, D. B., Arellano, C., & Cardoza, Y. J. (2017). Soil and foliar arthropod abundance and diversity in five cropping systems in the coastal plains of North Carolina. *Environmental Entomology, 46*(4), 771-783.

Agapow, P. M., & Burt, A. (2001). Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes, 1*(1-2), 101-102.

Bálint, M., Bartha, L., O'Hara, R. B., Olson, M. S., Otte, J., Pfenninger, M., & Schmitt, I. (2015). Relocation, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. *Molecular Ecology, 24*(1), 235-248.

Banke, S., Peschon, A., & McDonald, B. A. (2004). Phylogenetic analysis of globally distributed *Mycosphaerella graminicola* populations based on three DNA sequence loci. *Fungal Genetics and Biology, 41*(2), 226-238.

Berger, D. K., Carstens, M., Korsman, J. N., Middleton, F., Kloppers, F. J., Tongoona, P., & Myburg, A. A. (2014). Mapping QTL conferring resistance in maize to gray leaf spot disease caused by *Cercospora zeina*. *BMC Genetics, 15*, 60.

Bolton, M. D., Rivera, V., & Secor, G. (2013). Identification of the G143A mutation associated with QoI resistance in *Cercospora beticola* field isolates from Michigan, United States. *Pest Management Science, 69*(1), 35-39.

Brown, J. K. M. (2015). Durable Resistance of Crops to Disease: A Darwinian perspective. *Annual Review of Phytopathology, 53*(1), 513-539.

Brylińska, M., Sobkowiak, S., Stefańczyk, E., & Śliwka, J. (2016). Potato cultivation system affects population structure of *Phytophthora infestans*. *Fungal Ecology, 20*, 132-143.

Christie, N., Myburg, A. A., Joubert, F., Murray, S. L., Carstens, M., Lin, Y. C., & Ntuli, J. F. (2017). Systems genetics reveals a transcriptional network associated with susceptibility in the maize–grey leaf spot pathosystem. *The Plant Journal, 89*(4), 746-763.

Crous, P. W., Groenewald, J. Z., Groenewald, M., Caldwell, P., Braun, U., & Harrington, T. C. (2006). Species of *Cercospora* associated with grey leaf spot of maize. *Studies in Mycology, 55*, 189-197.

DAFF. (2016). Crop estimates *Republic of South Africa Department of Agriculture, Forestry and Fisheries*, Available from: http://www.nda.agric.za/docs/Cropsestimates/Media%20Aug%202016.pdf (Accessed December 2018).

Dale, A., Lewis, K., & Murray, B. (2011). Sexual reproduction and gene flow in the pine pathogen *Dothistroma septosporum* in British Columbia. *Phytopathology, 101*(1), 68-76.

Dunkle, L. D., & Levy, M. (2000). Genetic relatedness of African and United States populations of *Cercospora zeae-maydis*. *Phytopathology, 90*(5), 486-490.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology, 14*(8), 2611-2620.

Falush, D., Stephens, M., & Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes, 7*(4), 574-578.

FAOSTAT. (2016). Statistical databases and data-sets of the Food and Agricultural Organization of the United Nations. *Available from:* http://www.fao.org/faostat/en/#data/QC (Accessed December 2018).

Fry, W., Goodwin, S., Matuszak, J., Spielman, L., Milgroom, M., & Drenth, A. (1992). Population genetics and intercontinental migrations of *Phytophthora infestans*. *Annual Review of Phytopathology, 30*(1), 107-130.

Glais, I., Montarry, J., Corbiere, R., Pasco, C., Marquer, B., Magalon, H., & Andrivon, D. (2014). Long-distance gene flow outweighs a century of local selection and prevents local adaptation in the Irish famine pathogen *Phytophthora infestans*. *Evolutionary Applications, 7*(4), 442-452.

Gouse, M., Pray, C., Schimmelpfennig, D., & Kirsten, J. (2006). Three seasons of subsistence insect-resistant maize in South Africa: have smallholders benefited? *Available from:* http://www.agbioforum.org/v9n1/v9n1a02-gouse.htm (Accessed May 2018).

GrainSA. (2018). Production information of white and yellow maize per province. doi:http://www.grainsa.co.za/pages/industry-reports/production-reports

Groenewald, M., Linde, C., Groenewald, J. Z., & Crous, P. W. (2008). Indirect evidence for sexual reproduction in *Cercospora beticola* populations from sugar beet. *Plant Pathology, 57*(1), 25-32.

Grünwald, N. J., Goodwin, S. B., Milgroom, M. G., & Fry, W. E. (2003). Analysis of genotypic diversity data for populations of microorganisms. *Phytopathology, 93*(6), 738-746.

Grünwald, N. J., Sturbaum, A. K., Montes, G. R., Serrano, E. G., Lozoya-Saldaña, H., & Fry, W. E. (2006). Selection for fungicide resistance within a growing season in field populations of *Phytophthora infestans* at the center of origin. *Phytopathology, 96*(12), 1397-1403.

Hayden, H. L., & Howlett, B. J. (2005). Genetic structure of a population of the fungus *Leptosphaeria maculans* in a disease nursery of *Brassica napus* in Australia. *Current Genetics, 48*(2), 142-149.

Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ, 2*, e281.

Kim, H., Newell, A. D., Cota-Sieckmeyer, R. G., Rupe, J. C., Fakhoury, A. M., & Bluhm, B. H. (2013). Mating-type distribution and genetic diversity of *Cercospora sojina* populations

on soybean from Arkansas: evidence for potential sexual reproduction. *Phytopathology, 103*(10), 1045-1051.
Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources, 15*(5), 1179-1191.
Laraba, I., Boureghda, H., Abdallah, N., Bouaicha, O., Obanor, F., Moretti, A., O'Donnell, K. (2017). Population genetic structure and mycotoxin potential of the wheat crown rot and head blight pathogen *Fusarium culmorum* in Algeria. *Fungal Genetics and Biology, 103*, 34-41.
Lazcano, C., Gómez-Brandón, M., Revilla, P., & Domínguez, J. (2013). Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. A field study with sweet corn. *Biology and Fertility of Soils, 49*(6), 723-733.
Liu, K. J., & Xu, X. D. (2013). First report of gray leaf spot of maize caused by *Cercospora zeina* in China. *Plant Disease, 97*(12), 1656-1656.
Ludwig, J. A., & Reynolds, J. F. (1988). *Statistical ecology: a primer in methods and computing* (Vol. 1): John Wiley & Sons.
Mathews, J. A., Kruger, L., & Wentink, G. J. (2018). Climate-smart agriculture for sustainable agricultural sectors: The case of Mooifontein. *Jàmbá: Journal of Disaster Risk Studies, 10*(1), 1-10.
McCann, J. C. (2005). *Maize and grace: Africa's encounter with a new world crop, 1500-2000*: Harvard University Press.
Meisel, B., Korsman, J., Kloppers, F. J., & Berger, D. K. (2009). *Cercospora zeina* is the causal agent of grey leaf spot disease of maize in southern Africa. *European Journal of Plant Pathology, 124*(4), 577-583.
Mellor, J. W., & Malik, S. J. (2017). The impact of growth in small commercial farm productivity on rural poverty reduction. *World Development, 91*, 1-10.
Meyer, J., Berger, D. K., Christensen, S. A., & Murray, S. L. (2017). RNA-Seq analysis of resistant and susceptible sub-tropical maize lines reveals a role for kauralexins in resistance to grey leaf spot disease, caused by *Cercospora zeina*. *BMC Plant Biology, 17*(1), 197.
Muller, M. F., Barnes, I., Kunene, N. T., Crampton, B. G., Bluhm, B. H., Phillips, S. M., & Berger, D. K. (2016). *Cercospora zeina* from maize in South Africa exhibits high genetic diversity and lack of regional population differentiation. *Phytopathology, 106*(10), 1194-1205.
Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics, 89*(3), 583-590.
Nei, M. (1987). *Molecular evolutionary genetics*: Columbia university press.
Neves, D. L., Silva, C. N., Pereira, C. B., Campos, H. D., & Tessmann, D. J. (2015). *Cercospora zeina* is the main species causing gray leaf spot in southern and central Brazilian maize regions. *Tropical Plant Pathology, 40*(6), 368-374.
Peakall, R., & Smouse, P. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and researchd-an update. *Bioinformatics* 28, 2537e2539.
Pritchard, J. K., Wen, W., & Falush, D. (2002). Documentation for STRUCTURE software: Version 2. *University of Chicago, Chicago,* Available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.323.9675&rep=rep1&type=pdf (Accessed May 2018).
Ranum, P., Peña-Rosas, J. P., & Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences, 1312*(1), 105-112.
Raveloson, H., Ratsimiala Ramonta, I., Tharreau, D., & Sester, M. (2018). Long-term survival of blast pathogen in infected rice residues as major source of primary inoculum in high altitude upland ecology. *Plant Pathology, 67*(3), 610-618.
Rimbaud, L., Papaïx, J., Barrett, L. G., Burdon, J. J., & Thrall, P. H. (2018). Mosaics, mixtures, rotations or pyramiding: What is the optimal strategy to deploy major gene resistance? *Evolutionary Applications, 11*(10), 1791-1810.

Runno-Paurson, E., Hannukkala, A. O., Kotkas, K., Koppel, M., Williams, I. H., & Mänd, M. (2013). Impact of phytosanitary quality of seed potato and temporal epidemic progress on the phenotypic diversity of *Phytophthora infestans* populations. *American Journal of Potato Research, 90*(3), 245-254.

Runno-Paurson, E., Remmel, T., Ojarand, A., Aav, A., & Mänd, M. (2010). The structure of *Phytophthora infestans* populations from organic and conventional crops. *European Journal of Plant Pathology, 128*(3), 373-383.

Shannon, C. E. (2001). A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing and Communications Review, 5*(1), 3-55.

Shrestha, S. K., Cochran, A., Mengistu, A., Lamour, K., Castro-Rocha, A., & Young-Kelly, H. (2017). Genetic diversity, QoI fungicide resistance, and mating type distribution of *Cercospora sojina*—Implications for the disease dynamics of frogeye leaf spot on soybean. *PLoS One, 12*(5), e0177220.

Sibiya, J., Tongoona, P., Derera, J., & Makanda, I. (2013). Smallholder farmers' perceptions of maize diseases, pests, and other production constraints, their implications for maize breeding and evaluation of local maize cultivars in KwaZulu-Natal, South Africa. *African Journal of Agricultural Research, 8*(17), 1790-1798.

Statistics, S. A. (2013). Census 2011: Agricultural households. Available from: http://www.statssa.gov.za/census/census_2011/census_products/Agricultural_Households.pdf (Accessed May 2018).

Stellingwerf, J., Phelan, S., Doohan, F., Ortiz, V., Griffin, D., Bourke, A., & Mullins, E. (2018). Evidence for selection pressure from resistant potato genotypes but not from fungicide application within a clonal *Phytophthora infestans* population. *Plant Pathology*. doi:doi.org/10.1111/ppa.12852

Stoddart, J. A., & Taylor, J. F. (1988). Genotypic diversity: estimation and prediction in samples. *Genetics, 118*(4), 705-711.

Swart, V., Crampton, B. G., Ridenour, J. B., Bluhm, B. H., Olivier, N. A., Meyer, J. M., & Berger, D. K. (2017). Complementation of *CTB7* in the maize pathogen *Cercospora zeina* overcomes the lack of *in vitro* cercosporin production. *Molecular Plant-Microbe Interactions, 30*(9), 710-724.

Szpiech, Z. A., Jakobsson, M., & Rosenberg, N. A. (2008). ADZE: a rarefaction approach for counting alleles private to combinations of populations. *Bioinformatics, 24*(21), 2498-2504.

Thierfelder, C., & Wall, P. (2012). Effects of conservation agriculture on soil quality and productivity in contrasting agro-ecological environments of Zimbabwe. *Soil Use and Management, 28*(2), 209-220.

Tivoli, B., Calonnec, A., Richard, B., Ney, B., & Andrivon, D. (2013). Current knowledge on plant/canopy architectural traits that reduce the expression and development of epidemics. *European Journal of Plant Pathology, 135*(3), 471-478.

Walker, N., & Schulze, R. (2006). An assessment of sustainable maize production under different management and climate scenarios for smallholder agro-ecosystems in KwaZulu-Natal, South Africa. *Physics and Chemistry of the Earth, Parts A/B/C, 31*(15), 995-1002.

Ward, J. M., Stromberg, E. L., Nowell, D. C., & Nutter Jr, F. W. (1999). Gray leaf spot: a disease of global importance in maize production. *Plant Disease, 83*(10), 884-895.

Welch, T., Feechan, A., & Kildea, S. (2018). Effect of host resistance on genetic structure of core and accessory chromosomes in Irish *Zymoseptoria tritici* populations. *European Journal of Plant Pathology, 150*(1), 139-148.

Young, C., Bock, C., Charlton, N., Mattupalli, C., Krom, N. D., Bowen, J. K., & Wood, B. W. (2018). Evidence for sexual reproduction: identification, frequency and spatial distribution of *Venturia effusa* (pecan scab) mating type idiomorphs. *Phytopathology, 108*(7), 837-846.

Zhan, J., Mundt, C. C., Hoffer, M. E., & McDonald, B. A. (2002). Local adaptation and effect of host genotype on the rate of pathogen evolution: an experimental test in a plant pathosystem. *Journal of Evolutionary Biology, 15*(4), 634-647.

Zhan, J., Pettway, R. E., & McDonald, B. A. (2003). The global genetic structure of the wheat pathogen *Mycosphaerella graminicola* is characterized by high nuclear diversity, low mitochondrial diversity, regular recombination, and gene flow. *Fungal Genetics and Biology, 38*(3), 286-297.

Zhu, Y., Chen, H., Fan, J., Wang, Y., Li, Y., Chen, J., Mundt, C. C. (2000). Genetic diversity and disease control in rice. *Nature, 406*(6797), 718-722.

CHAPTER 3

Population genetic structure and migration patterns of a maize pathogenic fungus, *Cercospora zeina,* in Africa

3.1 ABSTRACT

Cercospora zeina is a causal pathogen of gray leaf spot (GLS) disease of maize in Africa. Recent studies have reported a high genetic diversity of *C. zeina* in South Africa, attributed to sexual recombination and gene flow. However, no comprehensive population genetic studies have been conducted on the pathogen in the rest of Africa where GLS exists. This study aimed to employ population genetics tools on *C. zeina* populations from Kenya, South Africa, Uganda, Zambia, and Zimbabwe, to determine its population genetic structure, evolutionary potential and migration routes. Here we hypothesized that *C. zeina* is highly variable, and that sexual recombination and migration play key roles in defining its population structure. To test these hypotheses, 835 isolates were genotyped using 11 microsatellite markers. Our results provide evidence that *C. zeina* is a genetically variable pathogen and is demographically structured, with signatures of cryptic sexual recombination and migration among populations. Our results further established the direction and levels of gene flow of the pathogen, with South Africa being the greatest donor of migrants and Zambia having a distinct population with minimum migrants. This study provides a basis for effective monitoring of *C. zeina*'s dispersal and is a tool for designing more effective management strategies that limit the acquisition and movement of host and fungicide resistance traits and highly virulent strains.

3.2 INTRODUCTION

Persistence of diseases in new or well-established crop ecosystems occurs through natural processes, particularly splash- or wind-blown spores, insect vectors, soil and seed, as well as human-mediated activities such as global travel and trade, monoculture and conservation agriculture (Aylor, 2003; Brown & Hovmøller, 2002; Ingwell *et al.*, 2012; Ward & Nowell, 1998). Consequently, these diseases are a component of global crop yield losses of over 20 to 40 % and cause a notable reduction in yield quality and marketability (Savary *et al.*, 2012). Thus, it is imperative to have a solid understanding of plant diseases and the evolution, biology,

ecology, and epidemiology of their causal pathogens to counteract crop yield losses, through effective management strategies.

Gray leaf spot (GLS) disease is an economically important foliar disease of maize (*Zea mays*). First reported in KwaZulu-Natal, South Africa in 1988, GLS has since been reported in Cameroon (1995), DR Congo (1996), Kenya (1995), Uganda (1994), Zambia (1995) and Zimbabwe (1995) (Dunkle & Levy, 2000; Ward *et al.*, 1999). Other countries where GLS is present include Ethiopia, Malawi, Mozambique, Nigeria, Swaziland, and Tanzania (Ward *et al.*, 1999). It is identified as a major threat to food security, causing from 20 to 80 % country-specific yield losses (Manandhar *et al.*, 2011; Ward *et al.*, 1999). Its emergence on the African continent is still speculative since its host, maize is non-native to Africa. There are two existing hypotheses for the continental occurrence of GLS in Africa. Ward *et al.* (1999) proposed that it might have emerged with maize importation into Africa through the Durban harbor in South Africa, one of the major routes of trade into the continent. Alternatively, the pathogen might have undergone a host shift from a native host such as sorghum (*Sorghum bicolor*) or an unknown grass onto maize (Dunkle & Levy, 2000).

In sub-Saharan Africa, GLS is caused by an ascomycete fungus, *Cercospora zeina* Crous & U. Braun. It is a host-specific hemibiotroph with no reports of any other hosts except maize (Crous *et al.*, 2006; Meisel *et al.*, 2009; Muller *et al.*, 2016). Like many other *Cercospora* species (Groenewald *et al.*, 2006), *C. zeina* is generally known to be an asexual species. It is a wind- and splash-borne pathogen, dispersing its conidia that overwinter on maize debris left in fields to distances of up to 40 to 160 km (Manandhar *et al.*, 2011; Ward *et al.*, 1999). Other pathogens associated with GLS include *Cercospora zeae-maydis*, *Cercospora sorghi* var. *maydis* and *Cercospora* sp. CPC 12062 (Crous *et al.*, 2006; Dunkle & Levy, 2000; Kinyua *et al.*, 2010). *C. zeae-maydis* has not been reported on the African continent, while the rest have scarcely been isolated from maize (Crous *et al.*, 2006).

Population genetics studies using neutral molecular markers have contributed to the understanding of the biology of plant pathogens, their evolution, and epidemiology, and thus

have significantly informed the development of effective disease management strategies (Grünwald & Goss, 2011; McDonald, 2015; McDonald & Mundt, 2016; Milgroom & Peever, 2003). A few studies have described the genetic diversity of *C. zeina* in Africa, of which a substantial genetic diversity is reported among isolates from Uganda, Kenya, Rwanda, Zimbabwe and South Africa, with the latter exhibiting the highest diversity (Dunkle & Levy, 2000; Muller *et al.*, 2016; Okori *et al.*, 2003; Okori *et al.*, 2015). Further studies in South Africa showed the influence that farming systems can have on the population structure of *C. zeina*. Partial population differentiation between the two systems was observed, with populations from smallholder farms revealing a higher genetic diversity and lower clonality than populations from commercial farms (Nsibo *et al.*, 2019).

Gene flow in combination with migration are evolutionary factors that substantially define the population structure of several *Cercospora* species. Its impact on the population structure is estimated based on the migration rates (N_m) (i.e. number of isolates that would be exchanged between populations per generation to account for the observed population differentiation) of individuals (Giraud *et al.*, 2008). Gene flow has influenced the population structure of *Cercospora beticola* in Greece, with N_m values ranging from 4.21 to 14.77 (Moretti *et al.*, 2006) and *Cercospora sorghi* in Uganda, with average N_m = 20 (Okori *et al.*, 2004). It has further influenced the population structure of *C. zeina* in South Africa (Muller *et al.*, 2016), Kenya, Rwanda and Uganda (Okori *et al.*, 2003). In the latter study based on AFLP and RFLP analysis, fungal isolates from the three African countries did not form genetically distinct populations but were differentiated from a distinct set of isolates from the USA. This could possibly be due to the African isolates being *C. zeina*, and the distinct USA clade being *C. zeae-maydis*.

Presence of mating genes is another fundamental evolutionary force implicated in driving the population structure of many *Cercospora* species including *C. zeae-maydis*, *C. beticola* (Groenewald *et al.*, 2006), and *C. sojina* (Kim *et al.*, 2013). Sexual recombination is responsible for the generation of new genotypes and random association of alleles at different

loci, resulting in high genotypic diversity and low linkage disequilibrium among populations (Liu *et al.*, 1996). In South Africa, *C. zeina* mating type genes have been characterized and found to support the hypothesis of random mating in most of the populations from commercial and smallholder maize farms. This result in addition to the lack of linkage disequilibrium and high genotypic diversity alludes to the existence of cryptic sexual recombination even though no sexual stage has been discovered under field or laboratory environments (Goodwin *et al.*, 2001; Muller *et al.*, 2016; Nsibo *et al.*, 2019). Overall, sexual recombination is believed to define ancestral populations (Schurko & Logsdon Jr, 2008). Its existence among *C. zeina* populations in South Africa, in comparison to other African countries, coupled with a higher genetic diversity may provide some indication of the ancestral origin of *C. zeina* in Africa.

To comprehensively understand the population genetics of *C. zeina* and answer questions of evolution, biology and dispersal, mega population sampling and genetic analysis among different countries in Africa is necessary. Following from Muller *et al.* (2016) and Nsibo *et al.* (2019) studies, this study aimed to employ population genetics tools on *C. zeina* populations from Kenya, South Africa, Uganda, Zambia, and Zimbabwe, to determine its population genetic structure and migration routes. The specific objectives included: (i) to confirm that *C. zeina* is the causal pathogen of GLS in Sub-Saharan Africa; (ii) to determine the genetic diversity of *C. zeina* in smallholder farms in the five populations; (iii) to understand the role of sexual recombination in the evolution of the pathogen; (iv) to determine whether population structure exists in *C. zeina* populations in comparison to previous studies; and, (v) to determine the migration patterns of *C. zeina* in Sub-Saharan Africa.

3.3 MATERIALS AND METHODS

3.3.1 Sample location and isolation of *Cercospora zeina*

A total of 835 isolates was collected from five sub-Saharan countries from Eastern and Southern Africa (Table 3.1). All the samples collected from a country constituted a population and as such a population was defined as a collection of isolates from several geographically

separated fields within a country. Within a country, isolates were collected from fields that were more than 50 km apart. The sampling strategy used in this study followed Muller *et al.* (2016) and isolations were made as described by Nsibo *et al.* (2019). Cultures were grown and sub-cultured on V8 growth medium (3 g of Calcium Carbonate, 20 g of bacteriological agar and 200 mL of V8 vegetable juice [Campbell Soup Co., Camden, New Jersey] and 1000 mL of double distilled water). This growth medium was supplemented with 50 µg/ml of cefotaxime antibiotic (Aspen Pharmacare, Durban, South Africa). Cultures were incubated at 28 ^{o}C for 12 to 14 weeks.

3.3.2 Species identification and molecular genotyping

Genomic DNA was extracted from 12 to 14 week-old mycelia through a modified CTAB protocol (Lee *et al.*, 1988). The DNA quality and quantity were assessed using a NanoDrop Spectrophotometer (NanoDrop Technologies). The cercosporin toxin biosynthesis (*CTB*)-*7* gene region (Forward: 5'-AAGAGTGCTTGTGAATGG-3', Reverse: 5'-GATGCGGGTGAAGTAGAAA-3'), was used as a diagnostic marker to confirm the identity of the isolates as *C. zeina*, following conditions described by Swart *et al.* (2017). Furthermore, morphological characteristics, typical of *Cercospora* species, were also assessed using microscopy.

Microsatellite loci amplification was performed using a set of 11 of the 14 microsatellite loci characterized by Muller *et al.* (2016) (Supplementary Table 3.1) in five multiplex reactions. Combinations of primer pairs that allowed for multiplexing was based on differences in amplicon size and a fluorescent label attached to the forward primer. Using the 2720 Applied Biosystems Thermal Cycler (ThermoFisher Scientific Inc, USA), PCR amplification was performed, with an initial denaturation period of 95 ^{o}C for 10 min, followed by 10 cycles at 95 ^{o}C for 30 s, 60 ^{o}C for 45 s, 72 ^{o}C for 60 s, 25 cycles at 95 ^{o}C for 30 s, 62 ^{o}C for 45 s, and 60 s at 72 ^{o}C. This was followed by a final extension step at 60 ^{o}C for 30 min. Amplicons were separated on 2 % agarose gel (Roche Diagnostics) stained with Ethidium bromide and run with a DNAMARK™ 500-bp size standard (G-Biosciences). After amplification, PCR reactions

were pooled into two panels as described by Muller *et al.* (2016). Subsequent fragment analysis was performed against the LIZ500 size standard (Applied BioSystems) using the ABI 3500xl Genetic analyzer (Applied BioSystems) as follows: 1 µl of pooled PCR products were added to a mixture of 0.14 µl of LIZ500 size standard and 9.86 µl of Hi-Di formamide (Applied BioSystems). Allele detection was performed using GENESCAN software (Applied BioSystems) and these were manually scored using GENEMAPPER software v 4.1 (Applied BioSystems).

3.3.3 Genetic diversity among populations of *Cercospora zeina*

To study the population genetic structure of *C. zeina* between populations, two datasets were generated: a non-clone-corrected (one which included all data) and a clone-corrected (one with only a single representative of each genotype) data sets. Using the non-clone-corrected data set, the total number of alleles, and private alleles (alleles only present in a single population) were computed for each population across all 11 markers using GenAlEx v 6.501 (Peakall & Smouse, 2012). Additionally, private allele richness (average number of private alleles per locus) and allelic richness (average number of alleles per locus) were computed using ADZE software (Szpiech *et al.*, 2008). Individuals that were scored with the same alleles at all 11 microsatellite loci were termed as clones. The clone-corrected dataset thus consisted of isolates with unique multilocus genotypes (MLG), including a representative of each clone. The clonal faction (CF) (proportion of genotypes originating from asexual reproduction) per population was calculated as follows: CF = 1 - [number of unique MLG / total number of isolates] (Zhan *et al.*, 2003). Measures of genotypic diversity were quantified based on three indices: Shannon-Weiner Index of MLG diversity (I) (Shannon, 2001); Stoddart and Taylor's index (G) (Stoddart & Taylor, 1988); and Simpson's Index of MLG diversity (λ) (Simpson, 1949); using the R package poppr (Kamvar *et al.*, 2014). With the clone-corrected dataset, Nei's gene diversity (frequency of alleles per locus in a population) was determined using GenAlEx v 6.501 (Peakall & Smouse, 2012).

3.3.4 Population structure and distribution of genetic variation

All analyses below were conducted using the clone-corrected data set. Principal coordinate analysis (PCoA) was performed to visualize any patterns of variation between populations based on the standardized approach of pairwise Nei's genetic distances, using GenAlEx v 6.501 (Peakall & Smouse, 2012). To determine genetic relatedness among individuals, a model-based Bayesian clustering algorithm, implemented in STRUCTURE v 2.3.4 (Pritchard *et al.*, 2002) was used to determine the optimal number of K clusters. While clustering, this method assumes no prior knowledge of geographic locations that may artificially cluster genotypes (Dutech *et al.*, 2010). Posterior probabilities were estimated using 20 independent runs with inferred partitions (K = 1 to 20), 1000000 Monte Carlo Markov chain (MCMC) iterations and a burn-in of 100000, assuming an admixture model. Log probability and rate of change, ΔK, for a particular cluster over multiple runs were used to determine the best K value (Evanno *et al.*, 2005). The graphical visualization of the STRUCTURE result was performed using CLUMPAK software (Kopelman *et al.*, 2015), with each vertical line representing an inferred ancestry of each isolate.

3.3.5 Population differentiation

The existence of levels of population differentiation was evaluated using a hierarchical analysis of molecular variance (AMOVA) performed in GenAlEx v 6.501 (Peakall & Smouse, 2012). Total genetic variation was partitioned at three levels: among regions (i.e. Eastern and Southern Africa); among populations (Kenya, South Africa, Uganda, Zambia, and Zimbabwe); and among genotypes within populations. The significance of covariance components at each level was then tested at 1000 permutations. The total genetic variation among regions was considered in this study because of the differences in growing seasons that exist between Eastern and Southern Africa (Supplementary Figure 3.1). We thus examined the influence of maize growing seasons in Eastern and Southern Africa on the population structure of *C. zeina*.

3.3.6 Mating type gene analysis and tests for recombination

Mating type genes, *MAT1-1* and *MAT1-2*, were amplified using primers (*MAT1-1*F 5'-TCACCCTTTCACCGTACCCA-3', *MAT1-1*R 5'-CACCTGCCATCCCATCATCTC-3' *MAT1-2*F 5'-CGATGTCACGGAGGACCTGA-3' and *MAT1-2*R 5'- GTGGAGGTCGAGACGGTAGA-3') developed by Muller *et al.* (2016). These were multiplexed in a single reaction. Presence of the *MAT1-1* and *MAT1-2* idiomorphs was confirmed by the amplification of fragments of 631 bp (for *MAT1-1*) and 409 bp (for *MAT1-2*). Random mating was tested using the chi-square test (p = 0.05). Deviation from a 1:1 ratio would mean a rejection of the null hypothesis of random mating (Milgroom, 1996). To further test random mating, the association between two loci (Index of Association, I_A) was tested after 1000 permutations using Multilocus software v 1.3 (Agapow & Burt, 2001). The index of association ranges between zero and one, where zero is no association between two loci. Unbiased rBarD, another index that measures linkage disequilibrium independent of the number of loci was also calculated (Ahmadpour *et al.*, 2018). Finally, the proportion of compatible (PrCP) loci was determined using the software Multilocus v 1.3 (Agapow & Burt, 2001). This test assumes that two loci are compatible when mutation as an evolutionary force, effectively accounts for all the observed genotypes without presuming reproduction or homoplasy. For phylogenetically compatible loci, PrCP approaches one, indicative of a lack of recombination. On the other hand, the existence of phylogenetic incompatibility (i.e. PrCP < 1) suggests recombination within two loci (Chowdhary *et al.*, 2011).

Table 3.1: Geographic origin, elevation, collector, year of collection, and number of isolates of *Cercospora zeina* studied.

Country	District	Latitude (º)	Longitude (º)	Altitude (m)	Collector (s)	Growing season	Number of isolates
South Africa	Hlanganani	29.70055556	-30.07015611	1066	D.L. Nsibo & D.K. Berger	Mar-2015	72
South Africa	Ntabamhlophe	29.70666667	-29.09809194	1494	D.L. Nsibo & D.K. Berger	Mar-2015	20
South Africa	KwaNxamalala	30.22444444	-29.60810000	1145	D.L. Nsibo & D.K. Berger	Mar-2015	37
South Africa	Mbizana	29.97277778	-30.91210694	822	D.L. Nsibo & D.K. Berger	Mar-2015	50
South Africa	Mbizana	29.97138889	-30.91894806	672	D.L. Nsibo & D.K. Berger	Mar-2015	30
South Africa	Ntabankulu	29.52694444	-30.89276389	941	D.L. Nsibo & D.K. Berger	Mar-2015	45
South Africa	Mthatha	29.12750000	-31.56646111	767	D.L. Nsibo & D.K. Berger	Mar-2016	31
Zambia	Chilanga	28.00166667	-14.08560694	1146	B. Banda, ZARI Lusaka	Apr-2015	29
Zambia	Chisamba	28.01805556	-15.05081833	1106	B. Banda, ZARI Lusaka	Apr-2015	64
Zimbabwe	KweKwe	31.21833333	-17.67353000	1341	N. Chiuraise, Seed Co.	Apr-2017	21
Zimbabwe	Mutare	32.34666667	-18.54953000	1070	N. Chiuraise, Seed Co.	Apr-2017	27
Zimbabwe	Chinhoyi	30.14361111	-17.21249000	1162	N. Chiuraise, Seed Co.	Apr-2017	8
Zimbabwe	Harare	31.06138889	-17.70880000	1528	N. Chiuraise, Seed Co.	Apr-2017	40
Zimbabwe	Harare	30.03777778	-17.42514000	1466	N. Chiuraise, Seed Co.	Apr-2017	44
Kenya	Bungoma	34.51666667	-0.48426167	1396	D.L. Nsibo	Dec-2016	12
Kenya	Kakamega	34.62944444	0.21725000	1463	D.L. Nsibo	Dec-2016	22
Kenya	Kisumu	34.65111111	0.04584333	1336	D.L. Nsibo	Dec-2016	8
Kenya	Siaya	34.46750000	0.05024500	1332	D.L. Nsibo	Dec-2016	6
Kenya	Vihiga	34.77277778	0.11681667	1621	D.L. Nsibo	Dec-2017	7
Kenya	Kericho	35.13750000	-0.42646833	1842	D.L. Nsibo	Dec-2017	18
Kenya	Kericho	35.40583333	-0.30053833	2270	D.L. Nsibo	Dec-2017	23
Kenya	Kitale	35.10888889	0.89254167	1680	D.L. Nsibo & D. Omondi	Dec-2017	24
Kenya	Transzoia	34.99972222	1.03288167	1857	D.L. Nsibo & D. Omondi	Dec-2017	16
Kenya	Transzoia	34.99000000	0.98686500	1932	D.L. Nsibo & D. Omondi	Dec-2017	10
Uganda	Fortportal	30.33416667	0.66020500	1453	D.L. Nsibo	Nov-2016	28
Uganda	Fortportal	30.37944444	0.74348000	1563	D.L. Nsibo	Nov-2016	21
Uganda	Kapchorwa	34.51611111	1.37480500	2377	D.L. Nsibo	Nov-2016	17
Uganda	Lira	32.92888889	2.29781833	1087	D.L. Nsibo	Nov-2016	18
Uganda	Masaka	31.66416667	-0.30331000	1247	D.L. Nsibo	Jun-2017	46
Uganda	Wakiso	32.62694444	0.52725500	1131	D.L. Nsibo	Nov-2016	22
Uganda	Wakiso	32.60777778	0.50286333	965	D.L. Nsibo	Jun-2017	19

3.3.7 Migration events of *Cercospora zeina* populations

To determine the existence of any significant correlation between Nei's genetic distances and geographic distance, a Mantel test was performed in GenAlEx v 6.501 (Peakall & Smouse, 2012) with 1000 permutations using the clone-corrected dataset.

An effective number of migrants (N_m) between populations was assessed, to determine the level of gene flow between countries. This was computed in GenAlEx v 6.501 (Peakall & Smouse, 2012), and expressed in units of genetically effective genotypes following the equation: N_m = [(1 / PhiPT) - 1] / 2. To estimate contemporary migration among the five populations, Migrate-N v 3.4.4 (Beerli, 2006) was used on the clone-corrected dataset. This software performs a maximum likelihood estimation of population size and inter-population migration using the coalescence theory (Atallah *et al.*, 2010). This theory assumes neutral evolution, lack of recombination and no change in population size and migration rates over time. Migration, M, was estimated based on migrants per generation between populations as M = N_em where m denotes the proportion of migrants per population (Beerli, 2006). Four scenarios: 1) full migration; 2) Southern African countries pooled into one population, migrating to individual Eastern African countries; 3) Eastern African countries pooled into one population, migrating to individual Southern African countries; and 4) panmixia (all populations assumed to be one mega population), were examined using the Brownian motion approximation to the stepwise mutation model. In all scenarios, five parallel static chains were run, with temperatures 1.0, 1.5, 3.0 and 1000000. Runs included a burn-in of 50000 steps with 10000 steps recorded at intervals of 50 steps. Finally, a 95 % confidence interval for the maximum likelihood profiles produced was estimated (Banke & McDonald, 2005).

3.4 RESULTS

3.4.1 Species identification

All isolates derived from single spores on GLS lesions (Table 3.1), grew into broadly fusiform, hyaline, thin-walled conidia with five to ten septa, typical of *C. zeina* as described by Crous *et*

al. (2006) (Figure 3.1). Additionally, the amplification of the *CTB-7* gene region produced a band size of 618 bp (Figure 3.2) for all isolates, consistent with *C. zeina*.

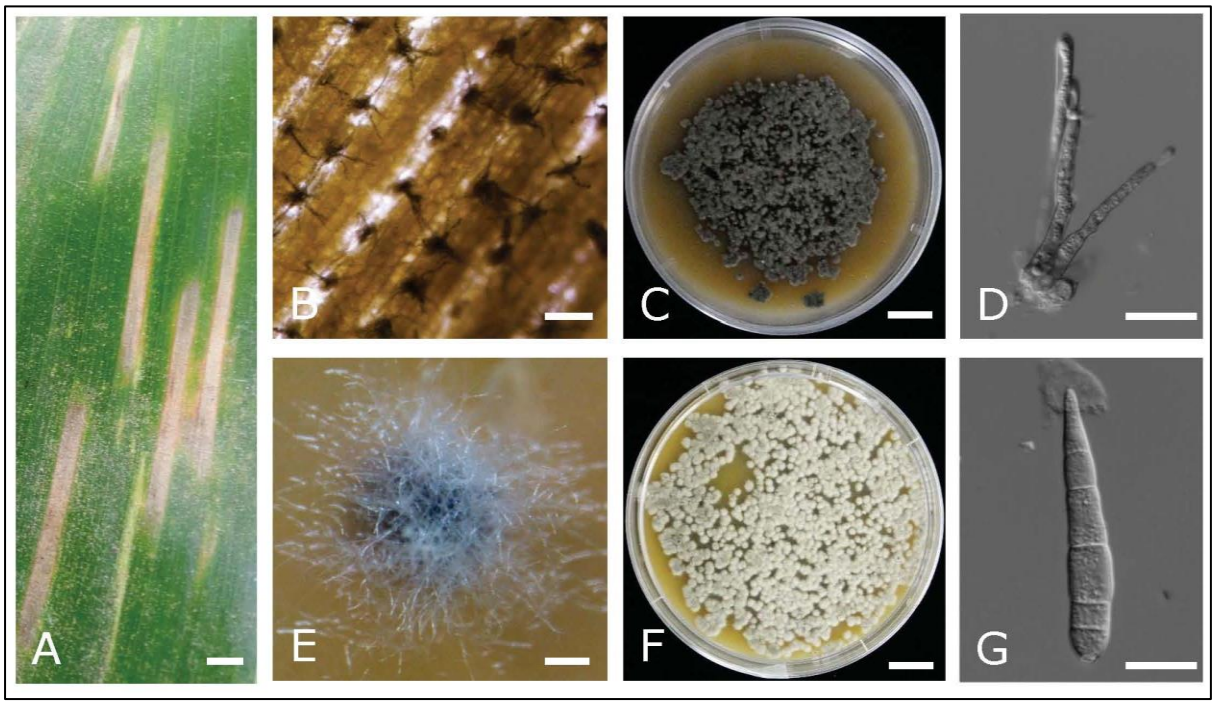


Figure 3.1: Morphological characteristics of *Cercospora zeina*: A: Mature rectangular lesions of gray leaf spot disease of maize. B: Conidiophores emerging from the leaf stomata. C: Sporulating culture on V8 medium. D: Conidiophores. E: A single colony growing on V8 medium. F: Vegetative culture on V8 medium. G: A single conidium. Scale bars: A = 2 cm, B = 20 µm, C and F = 0.5 cm, D and G = 10 µm, E = 0.3 cm

3.4.2 Genetic diversity among populations of *Cercospora zeina*

A total of 124 microsatellite alleles were inferred from 835 isolates, and these ranged from three to 21 per locus (Supplementary Table 3.1). Except for one marker, the levels of genetic diversity among loci were relatively high (0.03 to 0.76) and genetic evenness ranged from 0.30 to 0.89 (Supplementary Table 3.1). From 835 isolates, 768 multilocus genotypes (MLGs) were identified, highly dependent on the population sample size. All populations had a relatively high frequency of private alleles (6.5 to 12.1) and an allelic richness that ranged between 2.37 to 3.15 (Table 3.2). The percentage of polymorphic loci was high in all populations and ranged from 91 % for Uganda and Zambia to 100 % for the rest of the populations. Except for South Africa (0.10) and Zambia (0.20), the clonal fraction was consistently low for the remaining populations (0.02 to 0.06). Furthermore, all indices of genotypic diversity indicated high

diversity: Shannon-Weiner's index (I) ranged from 0.78 (Zambia) to 1.00 (Uganda); Stoddart and Taylor's index (G) ranged from 62 (Zambia) to 216 (South Africa) and Simpson's index (λ) ranged from 0.98 (Zambia) to 1.00 (South Africa) with no significant differences. Nei's gene diversity was high for all populations and ranged from 0.42 (Kenya) to 0.52 (Uganda) (Table 3.2). There were no significant differences in genetic diversity amongst all populations except for Uganda which had a significantly higher gene diversity (0.52) than Kenya (0.42) ($p = 0.006$, t test). At a regional scale, Southern Africa had a higher percentage of private alleles (25.8 %) and allelic richness (6.79) than Eastern Africa (23.4 % and 6.56 respectively). In all cases, Southern Africa had higher genetic diversity than Eastern Africa, however, with no statistical significance (Table 3.2).

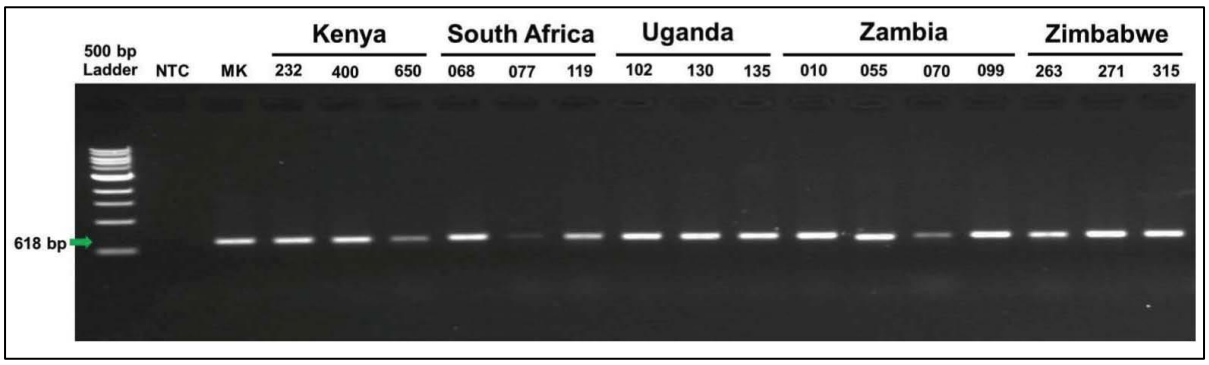


Figure 3.2: Agarose gel electrograph of the *CTB7* amplicon from *Cercospora zeina*. Amplicons produced with CTB7 primers. DNAMARK™ 500-bp size standard (G-Biosciences) was used as ladder. NTC: Non-template control; MK: *C. zeina* CMW25467 from Zambia (Swart *et al.*, 2017) as a positive control. Numbers represent different Isolates tested from the different countries.

3.4.3 Population structure and distribution of genetic variation

The PCoA plots based on Nei's unbiased genetic distance indicated varying levels of population structure in accordance to principal coordinates one and two. Analysis of all five populations together showed distinct clustering between Eastern Africa (Kenya and Uganda) and Southern Africa (South Africa, Zambia and Zimbabwe) with limited gene flow among regions (Figure 3.3A). Except for South Africa and Zimbabwe which exhibited a substantial overlap, Zambia was particularly distinct from the rest of Southern Africa (Figure 3.3B). Additionally, there was limited gene flow between Kenya and Uganda (Figure 3.3C). Overall,

there was distinct differentiation between populations, except for the overlap between the South Africa and Zimbabwe populations.

Applying individual-based Bayesian clustering showed that populations were separated into three genetic groups (K = 3, ΔK = 528.72) (Figure 3.4A). At this K value, isolates from South Africa and Zimbabwe comprised one genetic group and Zambia was a distinct population from the Southern Africa populations. Similarly, isolates from Kenya and Uganda grouped as one population. In all genetic groups, limited genotypic flow occurred. At K = 4, Southern Africa populations grouped into geographically defined clusters while Kenya and Uganda remained in one genetic group. At K = 5, results were consistent with the five distinct populations with limited admixture. Overall, there is support for population structure with the existence of limited admixture between populations (Figure 3.4B).

3.4.4 Population differentiation

Existence of population differentiation between the five populations was supported by AMOVA (Table 3.3). Although most of the genetic variability occurred among isolates within populations (64 to 93 %), substantial variability existed among regions (also representing the growing seasons) (5 %), and populations (5 to 35 %) (Table 3.3). To further support the existence of population differentiation, the highest Φ_{PT} values existed between Kenya and Zambia (Φ_{PT} = 0.36, p ≤ 0.001), supported by the lowest number of migrants (N_m = 0.90), followed by Zambia and Uganda (Φ_{PT} = 0.34, p < 0.001), supported by a low number of migrants (N_m = 0.97). In contrast, the lowest population differentiation was between South Africa and Zimbabwe (Φ_{PT} = 0.07, p < 0.001), supported by the highest number of migrants (N_m = 6.92) (Table 3.4). Comparisons within regions revealed that Eastern Africa which is characterized by two maize growing seasons (Supplementary Figure 3.1) exhibited a higher within-region gene flow (N_m = 3.10) compared to Southern Africa which only has a single maize growing season (N_m = 1.93) (results not shown). This confirms that growing seasons have an influence on the population genetic structure of *C. zeina* in sub-Saharan Africa.

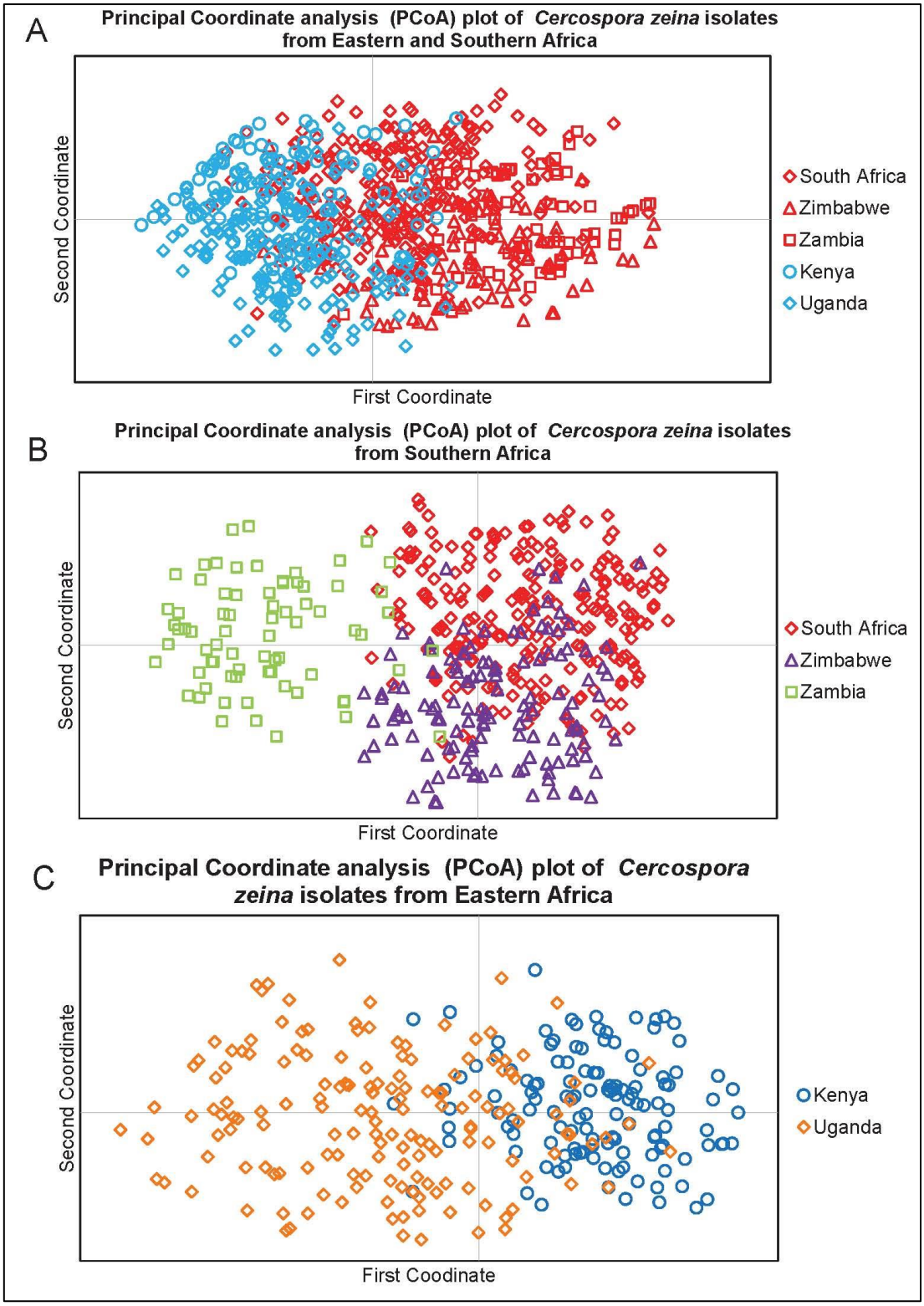


Figure 3.3: Principal coordinate analysis (PCoA) of *Cercospora zeina* isolates from five populations (countries): A: PCoA of 768 isolates from three Southern African (Red) and two Eastern African (Light blue) countries. B: PCoA of 463 isolates from South Africa (Red), Zambia (Green) and Zimbabwe (Purple). C: PCoA of 305 isolates from Kenya (Light Blue) and Uganda (Orange). The first and second principal coordinates explained 13 and 21 % (A); 10 and 20 % (B); and 13 and 22 % (C), of the variance, respectively, based on Nei's genetic distance (Nei, 1987) calculated with GenAlEx software (Peakall & Smouse, 2012). Different shapes represent individual populations (Kenya, South Africa, Uganda, Zambia; and Zimbabwe). A clone-corrected dataset was used to construct the plots.

Table 3.2: Indices of genetic diversity for *Cercospora zeina* populations from five African countries collected in 2015, 2016 and 2017.

Country	N[a]	MLG[b]	P_a[c]	%P_a[d]	R_a[e]	%P[f]	CF[g]	I[h]	G[i]	Λ[j]	H_e[k]
South Africa	285	257	8	6.5	2.75	100	0.10	0.84	216	1.00	0.49
Zambia	93	74	11	8.9	2.72	91	0.20	0.78	62	0.98	0.47
Zimbabwe	140	132	8	6.5	2.63	100	0.06	0.85	102	0.99	0.49
Kenya	146	139	15	12.1	2.37	91	0.05	0.82	104	0.99	0.42
Uganda	169	166	9	7.3	3.15	100	0.02	1.00	147	0.99	0.52
Southern Africa*	**518**	**463**	**32**	**25.8**	**6.79**	**100**	**0.11**	**1.04**	**373**	**1.00**	**0.55**
Eastern Africa¥	**315**	**305**	**29**	**23.4**	**6.56**	**100**	**0.03**	**1.02**	**244**	**1.00**	**0.51**

[a]N: Number of isolates per collection area.
[b]MLG: Number of multilocus genotypes observed.
[c]P_a: Number of private alleles per locus
[d]%Pa: percentage of private alleles per locus
[e]R_a: Allelic richness after rarefaction to the smallest sample size of 93
[f]%P: percentage polymorphism
[g]CF: Clonal fraction as calculated by CF = 1 - (number of unique genotypes/total number of isolates), expressed as a percentage (Zhan *et al.*, 2003).
[h]I: Shannon-Wiener index of MLG diversity (Shannon, 2001).
[i]G: Stoddart and Taylor's index of MLG diversity (Stoddart & Taylor, 1988).
[j]λ: Simpson's index of MLG diversity (Simpson, 1949).
[k]H_e: Nei's unbiased measure of gene diversity (Nei, 1978), the only metric in Table 3 calculated using clone-corrected data set.
*Southern Africa: South Africa, Zambia and Zimbabwe populations combined
¥Eastern Africa: Uganda and Kenya populations combined

Table 3.3: Analysis of molecular variance (AMOVA) of microsatellite data for 768 *Cercospora zeina* isolates separated based on regions, countries, and isolates.

Source	df[a]	SS[b]	MS[c]	Estimated variance	% of the total variance
Southern and Eastern Africa					
Between regions	1	144	144	0.15	5%
Among countries	3	219	73	0.50	15%
Within isolates	763	2028	3	2.66	80%
Southern Africa					
Between countries	2	153	76	0.53	16%
Among populations*	9	39	4	0.05	1%
Within isolates	451	1194	3	2.65	82%
Eastern Africa					
Between countries	1	66	66	0.41	13%
Among populations	11	45	4	0.07	2%
Within isolates	292	751	3	2.57	84%
South Africa and Zambia					
Between countries	1	116	116	0.96	26%
Among populations	6	29	5	0.06	2%
Within isolates	323	853	3	2.64	72%
South Africa and Zimbabwe					
Between countries	1	31	31	0.15	5%
Among populations	8	34	4	0.05	2%
Within isolates	379	1010	3	2.66	93%
South Africa and Kenya					
Between countries	1	64	64	0.33	11%
Among populations	12	45	4	0.04	2%
Within isolates	382	974	3	2.55	87%
South Africa and Uganda					
Between countries	1	105	105	0.49	15%
Among populations	9	49	5	0.07	2%
Within isolates	412	1114	3	2.70	83%
Zambia and Zimbabwe					
Between countries	1	99	99	1.00	27%
Among populations	4	14	4	0.04	1%
Within isolates	200	525	3	2.63	72%
Zambia and Kenya					
Between countries	1	130	130	1.31	35%
Among populations	8	25	3	0.04	1%
Within isolates	203	490	2	2.41	64%
Zambia and Uganda					
Between countries	1	140	140	1.30	32%
Among populations	5	29	6	0.10	2%
Within isolates	233	629	3	2.70	66%
Zimbabwe and Kenya					
Between countries	1	93	93	0.66	21%
Among populations	10	30	3	0.03	1%
Within isolates	259	647	2	2.50	78%
Zimbabwe and Uganda					
Between countries	1	107	107	0.68	20%
Among populations	7	34	5	0.07	2%
Within isolates	289	786	3	2.72	78%

[a]df: Degrees of freedom.
[b]SS: Sum of squared observations.
[c]MS: Mean of squared observations.
* The population is defined as a collection of isolates from several geographically separated fields within a country

3.4.5 Mating types and linkage disequilibrium

All isolates produced unique bands corresponding to either *MAT1-1* (631 bp) or *MAT1-2* (409 bp) respectively (Appendix A). Furthermore, both mating types segregated in equal proportions in four of the five populations and hence the hypothesis of random mating could not be rejected in Kenya, South Africa, Uganda and Zimbabwe (Table 3.5). Similarly, at the region level, the hypothesis of random mating could not be rejected when comparing Eastern to Southern Africa. To test for linkage disequilibrium, all populations except Kenya and Uganda were in linkage disequilibrium at p = 0.05 (Table 3.5). At the regional level, both Eastern and Southern Africa were not in linkage equilibrium (p < 0.001). The PrCP values ranged from 0 (p = 1.000) to 0.22 (p = 0.153) (Table 3.5) on a scale of zero to one, suggesting that sexual recombination occurs.

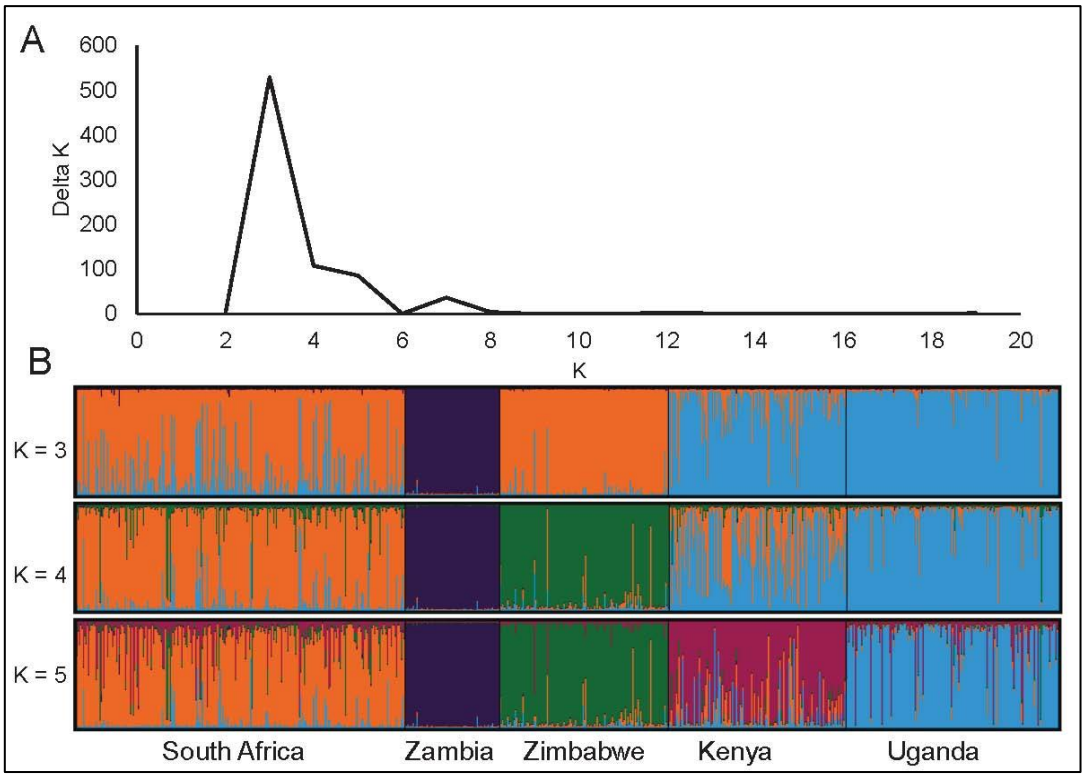


Figure 3.4: Population genetic structure of 768 clone-corrected *Cercospora zeina* isolates using eleven microsatellite markers. A: Graph showing the optimal ΔK value indicating the most likely number of *C. zeina* populations (K = 3, 4 and 5). B: Graphical representation of isolates based on ΔK = 3, 4 and 5, respectively. These clusters are defined by five color patterns, Orange, Purple, Green, Red and Sky Blue. Analysis was performed using STRUCTURE v 2.3.4 (Pritchard et al., 2002) and visualized using DISTRUCT in CLUMPAK software (Kopelman et al., 2015). Each vertical bar represents one of the 768 isolates partitioned into K inferred clusters.

3.4.6 Migration patterns and the effect of geographic distance

The Mantel test with correlation coefficient showed a positive correlation between geographic and genetic distances among all populations (r = 0.15, p = 0.001), suggesting that populations that are more distant from each other tended to be less closely related.

Migration events amongst genotypes were first assessed using the full migration scenario, with 20 pairwise comparisons tested (5 x 5 matrix for Kenya, South Africa, Uganda, Zambia, and Zimbabwe) (Table 3.6), and this is depicted on a map in Figure 3.5. Kenya and Uganda had higher theta values (theta = 1.78 and 1.30 respectively) than South Africa (theta = 0.20), Zambia (theta = 0.02) and Zimbabwe (theta = 0.26), suggesting that Eastern Africa possessed a higher effective population size than Southern Africa. Estimates of recent migration rates ranged from 0.14 (South Africa into Zambia and Zambia into Zimbabwe) to 18.54 (South Africa into Zimbabwe) (Table 3.6). The largest donor of migrants was South Africa followed by Uganda. Populations from Zimbabwe and Zambia emerged as the largest sink populations receiving migrants from South Africa and Zimbabwe respectively. Consequently, Zambia was the highest sink population, with the most migrants coming from Zimbabwe (Table 3.6) and the lowest donor with no migrants into South Africa.

The second scenario assessed was migration from the Eastern African countries (pooled) to individual Southern African countries, in which 12 comparisons were tested (4 x 4 matrix for South Africa, Zambia, Zimbabwe, and Eastern Africa). The theta value for this scenario was 4.14. Estimates of recent migration rates ranged from 0.00 (Zambia to South Africa) to 17.54 (Eastern Africa to South Africa) (Supplementary Table 3.2). This revealed that Eastern Africa donated the most immigrants, with South Africa (M = 17.54) being the highest recipient followed by Zimbabwe (M = 13.62). Zambia on the other hand was the lowest recipient of immigrants from this region (M = 0.06).

The third scenario assessed was migration from the Southern African countries (pooled) to individual Eastern African countries, in which six comparisons were tested (3 x 3 matrix for

Southern Africa, and Kenya and Uganda). This scenario gave a theta value of 5.10. Southern Africa donated the highest immigrants to Uganda (M = 14.30) (Supplementary Table 3.3). The posterior probabilities were above 0.90 for all scenarios tested except for panmixia (all populations assumed to be one mega population), and thus the latter analysis is not reported. In general, the three scenarios tested indicated considerable movement of *C. zeina* genotypes between the Southern and Eastern Africa regions.

Table 3.4: Measure of pairwise estimates of population differentiation (ϕ_{PT}, above diagonal) and the effective migrants (N_m, below diagonal; bold) from the five populations of *Cercospora zeina*.

Population	South Africa	Zambia	Zimbabwe	Kenya	Uganda
South Africa	-	0.28	0.07	0.13	0.15
Zambia	**1.29**	-	0.28	0.36	0.34
Zimbabwe	**6.92**	**1.26**	-	0.22	0.20
Kenya	**3.38**	**0.90**	**1.82**	-	0.14
Uganda	**2.40**	**0.97**	**1.96**	**3.12**	-

3.5 DISCUSSION

This is the first comprehensive study to infer the genetic structure of *C. zeina* from smallholder maize farms in five sub-Saharan African countries: Kenya, South Africa, Uganda, Zambia, and Zimbabwe. In this study, the major findings were: (i) *C. zeina* was confirmed as the causal pathogen of GLS in Africa with no *C. zeae-maydis* and other GLS-associated causal pathogens recovered from Africa; (ii) the pathogen exhibited substantially higher levels of genetic diversity than previously reported; (iii) genetic structure existed among *C. zeina* populations, with Zambia being distinct from the rest of the populations; (iv) cryptic sexual recombination can be inferred even though no sexual stage has been identified; (v) long-distance migration of *C. zeina* genotypes has occurred in Sub-Saharan Africa, with South Africa being the major donor of genotypes to all populations except Zambia. Finally, the hypotheses that *C. zeina* would have originated from South Africa and the pathogen having an alternative indigenous host could not be confirmed in this study.

Table 3.5: Estimates of mating type frequencies and tests of linkage disequilibrium (index of association I_A and the modified rBarD statistics) of *Cercospora zeina* isolates within five countries in Africa.

Country	N[a]	*MAT1-1*	*MAT1-2*	*MAT1-1 / MAT1-2*[b]	χ^2[c]	I_A[d]	rBarD[e]	PrCP[f]
South Africa	285	138	147	0.94	0.28 (0.594)	0.02 (0.071)	0.002 (0.071)	0.16 (0.007)
Zambia	93	36	57	0.63	4.74 (0.029)	0.02 (0.322)	0.002 (0.322)	0.18 (1.000)
Zimbabwe	140	66	74	0.89	0.46 (0.499)	0.04 (0.092)	0.004 (0.092)	0.07 (0.790)
Kenya	146	79	67	1.18	0.99 (0.321)	0.11 (0.036)	0.01 (0.036)	0.22 (0.153)
Uganda	169	71	98	0.72	3.98 (0.045)	0.15 (0.001)	0.02 (0.001)	0.00 (1.000)
Southern Africa*	**518**	**240**	**278**	**0.86**	**2.89 (0.094)**	**-0.53 (<0.001)**	**-0.06 (<0.001)**	**0.07 (0.083)**
Eastern Africa¥	**315**	**150**	**165**	**0.91**	**0.62 (0.429)**	**0.21 (<0.001)**	**0.02 (<0.001)**	**0.00 (1.000)**

[a]N: Number of isolates per collection area.

[b]Ratio of observed *MAT1-1* and *MAT1-2* genotypes by collection area and corresponding [c]χ^2 values to test for the null hypothesis of 1:1 distribution ratio with one degree of freedom (p-values in parentheses). The non-clone-corrected dataset was used for this estimation

[d]I_A: index of association (p-values in parentheses) and [e]rBarD (p-values in parentheses), statistic values, calculated by 999 permutations using Multilocus software (Agapow & Burt, 2001). The clone-corrected dataset was used for these calculations.

[f]PrCP: Proportion of incompatibility loci as determined by Multilocus software v 1.3 (Agapow & Burt, 2001). PrCP approaches one when there is a lack of recombination. PrCP close to zero suggests sexual recombination within two loci (Chowdhary *et al.*, 2011) (p-values in parentheses).

*Southern Africa: South Africa, Zambia and Zimbabwe populations combined

¥Eastern Africa: Uganda and Kenya populations combined

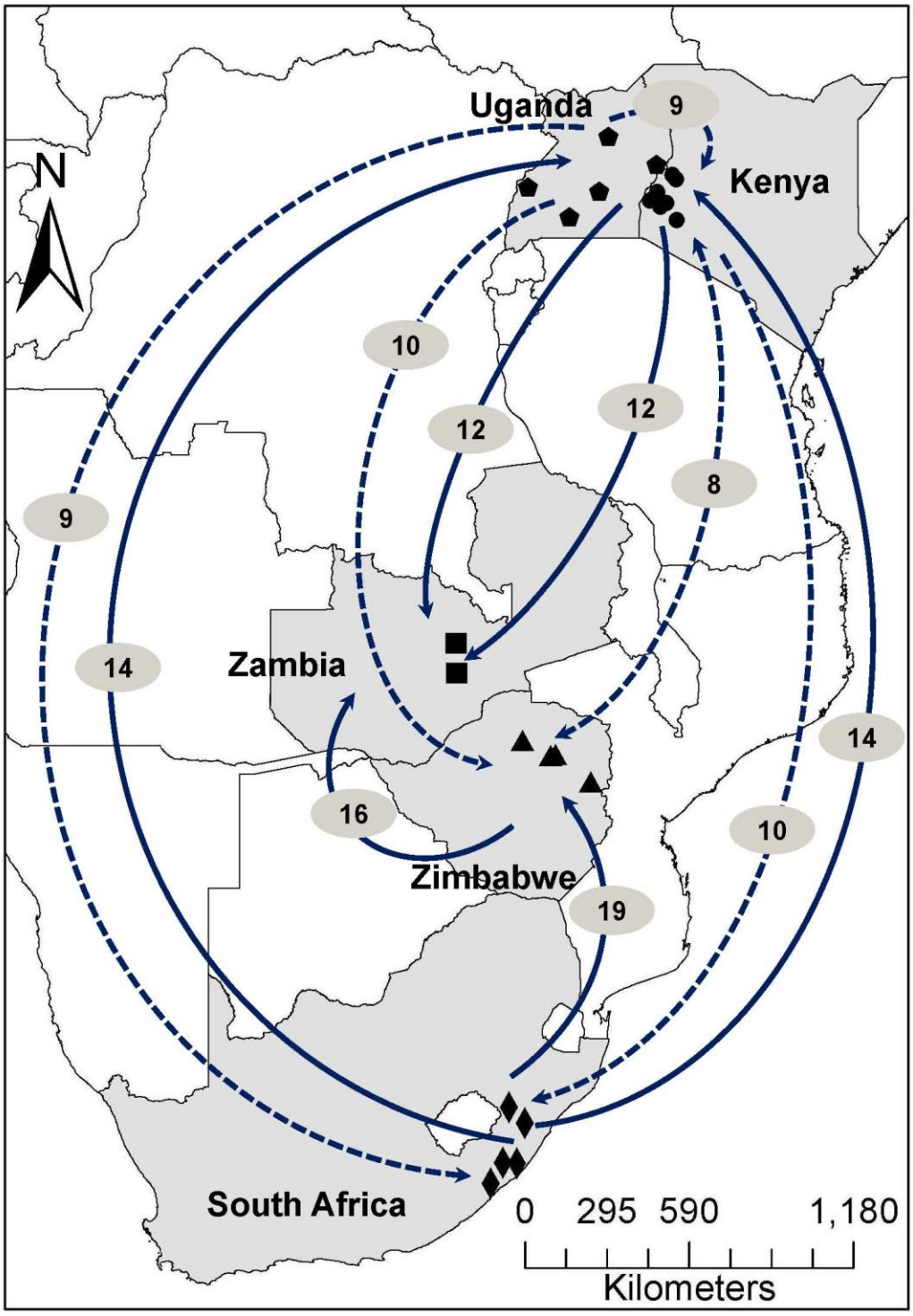


Figure 3.5: Migration patterns of *Cercospora zeina* populations in five countries in Sub-Saharan Africa. Mutation-scaled migration rates (M = m/μ) between pairs of five *C. zeina* populations, Kenya, South Africa, Uganda, Zambia, and Zimbabwe, based on full migration scenario, as determined by Migrate-N v 3.4.4 (Beerli, 2006), are indicated by arrows (based on the full migration scenario). Arrows are only shown for those directions with migration rate (M) values greater or equal to the median value (8). The corresponding M values are shown in gray ellipses on each arrow. Dotted arrows: Migration routes with M ≥ 8. Solid arrows: Migration routes with M ≥ 10. South Africa was the highest donor of migrants into Zimbabwe, Kenya and Uganda, whereas Zimbabwe was the highest donor into Zambia (see Table 3.6).

Table 3.6: Population estimates of theta (θ) and mutation-scaled migration rates (M = m/μ) between pairs of five *Cercospora zeina* populations based on full migration scenario.

Source/Sink[a] Populations	Theta	South Africa	Zambia	Zimbabwe	Kenya	Uganda
South Africa	0.20	-	0.14 (0-0.48)	18.54 (17.92-19.96)	14.30 (12.92-18.32)	13.54 (11.00-15.20)
Zambia	0.02	0.00 (0.00-0.00)[b]	-	0.14 (0.00-0.52)	4.78 (4.36-5.20)	2.22 (1.68-2.80)
Zimbabwe	0.26	3.90 (3.12-5.48)	16.06 (12.08-19.04)	-	7.66 (5.6-8.84)	7.06 (5.08-9.12)
Kenya	1.78	9.82 (8.68-11.28)	12.42 (8.12-16.60)	7.42 (5.84-8.60)	-	5.90 (5.44-8.88)
Uganda	1.30	8.50 (6.76-9.28)	11.70 (8.92-16.60)	10.14 (9.08-11.44)	9.18 (8.00-10.32)	-

[a]Donor (Source) populations are on the left column while the sink (receiving) populations are across.

[b]M values are represented as the mode value while the 95 % confidence intervals are in parentheses.

3.5.1 *Cercospora zeina*, the causal pathogen of gray leaf spot in Africa

In this study, we first established the causal pathogen of GLS in Africa using both morphological and molecular tools. Morphologically, fungal cultures, conidiophores, and conidia were typical of *C. zeina*, thus, corroborating previous studies (Crous *et al.*, 2006; Dunkle & Levy, 2000; Meisel *et al.*, 2009). Using the *CTB-7* gene region characterized by Swart *et al.* (2017) as a diagnostic tool, *C. zeina* was further confirmed at a molecular level, as the causal pathogen of GLS in Africa. Even though several other pathogens are associated with GLS, particularly, *C. zeae-maydis*, *C. sorghi* var. *maydis* and *Cercospora* sp. CPC 12062 (Crous *et al.*, 2006; Kinyua *et al.*, 2010; Neves *et al.*, 2015), none of these were identified in the five populations studied. Contrary to these findings, Nega *et al.* (2016) reported *C. zeae- maydis* as the causal pathogen of GLS in a survey conducted in the corn belt of Ethiopia. However, no morphological, physiological, cultural or molecular evidence was reported in that study.

3.5.2 High levels of genetic diversity among *Cercospora zeina* populations.

All populations studied in Africa exhibited a very high gene and genotypic diversity, thus, revealing that *C. zeina* is evolutionarily stable and well established on the continent (Nsibo *et al.*, 2019). Indeed, these results are consistent with previous studies which found high genetic diversity of *C. zeina* in Africa and the United States (Dunkle & Levy, 2000; Okori *et al.*, 2003; Okori *et al.*, 2015). Similarly a high genetic diversity of *C. zeina* was reported in South Africa (Muller *et al.*, 2016; Nsibo *et al.*, 2019), thus, there is congruence between previous studies and this study. On the contrary, the pathogen is reported to exhibit a substantially low level of genetic diversity in Brazil (Brunelli *et al.*, 2008; Neves *et al.*, 2015).

High genetic diversity has been used to infer centers of origin in other cereal pathogens such as *Z. tritici*, whose genetic diversity was highest in the Middle East, its presumed center of origin, and lowest in continents of introduction such as America and Australia (Banke *et al.*,

2004). In this current study, however, the center of origin could not be resolved based on genetic diversity. This was due to lack of significant differences in the population- or region-specific genetic diversities except for Uganda and Kenya, where the former had a significantly higher gene diversity. More studies from individual countries and continents are therefore needed to completely resolve *C. zeina*'s center of origin.

We propose several factors that may be contributing to the observed high genetic diversity in Africa. Firstly, as with crops such as apples (Ebrahimi *et al.*, 2016), different smallholder maize farms depend on a diversity of host genotypes that are in most cases country-specific due to ecological diversity, and rainfall patterns. This is supported by the presence of different maize breeding companies that produce seed based on their countries' agro-ecologies, growing seasons and farmer preferences among several other factors (Access to Seeds Foundation, 2017). As such, we hypothesize that the observed high genetic diversity could be due to the adaptability of the pathogen to the variable maize genotypes.

Other factors may include both asexual and sexual reproduction (Groenewald *et al.*, 2008; Milgroom, 1996; Whitlock, 2000). Asexual reproduction contributes to the genetic diversity of pathogens through events such as normal mutations occurring within gene combinations as well as multiple origins of genotypes into a population through gene flows, thus leading to high allele frequencies (Bengtsson, 2003; Groenewald *et al.*, 2008). Sexual recombination on the other hand naturally gives rise to new allele combinations in each generation, thus, resulting into highly variable populations that often act to select against groups of deleterious alleles (McDonald & Linde, 2002; Whitlock, 2000). Sexual recombination is also implicated in increasing intragenic recombination, thus creating new alleles (Zhan *et al.*, 2003). Based on these findings, we propose that these two forms of reproduction influence the genetic diversity observed among *C. zeina* populations.

3.5.3 Limited gene and genotype flow contribute to *Cercospora zeina* population structure

Analysis of molecular variance has revealed the existence of significant population differentiation among all populations ($\Phi_{PT} > 0.07$), supported by a low number of migrants (N_m) between populations. This is consistent with a previous study where partial population differentiation between *C. zeina* populations from two different farming systems in South Africa was detected (Nsibo *et al.*, 2019). Similar results were also observed in *C. zeina* populations from Kenya, Rwanda and Uganda (Okori *et al.*, 2003). In other *Cercospora* species, population differentiation has been reported to exist in *C. beticola* populations from among fields and between cropping systems of *Beta vulgaris* (Vaghefi *et al.*, 2017). Also, *C. kikuchii* from different plant tissue (leaves or seed) exhibits low but significant population differentiation (Cai & Schneider, 2008). Although, the effect of farming systems and different plant genotypes and tissue were not tested in the current study, there is enough evidence that *C. zeina*, like other *Cercospora* species, is genetically stable and may adapt to different environments, farming systems, plant tissues and genotypes.

The observed high genetic differentiation in this current study may also be partly due to long physical, and geographical distances and barriers existing between populations. Geographical barriers such as water bodies and mountains have been shown to restrict pathogen movement between countries thus limiting population admixture (Ebrahimi *et al.*, 2016). Additionally, local adaptation to different climates, crop rotation patterns, and cultural practices have been implicated in driving population differentiation of fungal pathogens (Abrinbana *et al.*, 2010; El Chartouni *et al.*, 2011; Siah *et al.*, 2018). Thus, we propose that these factors are playing a similar role in shaping the population differentiation of *C. zeina*. These results are consistent with a previous study on *Z. tritici*, which revealed a high genetic differentiation between States and no genetic structure observed within individual populations (Kabbage *et al.*, 2009). This further confirms the lack of population structure within individual populations of *C. zeina* that was observed in this study.

Our population structure results were visualized and supported by PCoA results and the existence of clusters identified by individual-based Bayesian clustering. Three clusters were inferred based on ΔK = 3 with limited admixture. Additionally, our results have confirmed that *C. zeina* populations in Africa are not part of a single panmictic population. The clustering of South Africa and Zimbabwe into one population together with the clustering of the Eastern Africa populations observed at ΔK = 3 may be due to pathogen adaptation to the growing seasons of maize in the two regions. Unlike Southern Africa which has one maize-growing season, Eastern Africa has two maize-growing seasons dictated by long and short rains (FAO/GIEWS, 2018) (Supplementary Figure 3.1). These may influence pathogen persistence across seasons, owing to a combination of frequent signatures of recombination events and gene flow. The long-distance dispersal of genetically fit genotypes over a wide area (McDonald & Linde, 2002) will thus force South Africa and Zimbabwe into one mega population as well as the Eastern Africa populations into one mega population. In general, the observed population structure and high genetic diversity in this study resemble those of other well-characterized cereal pathogens including *Z. tritici* (Abrinbana *et al.*, 2010; Boukef *et al.*, 2012; Grandaubert *et al.*, 2017; Siah *et al.*, 2018), and *R. commune* (Linde *et al.*, 2009; Salamati *et al.*, 2000), that are characterized by high genetic diversities and existence of genetic structure.

3.5.4 Evidence of cryptic sexual recombination among *Cercospora zeina* isolates

The results of high genotypic diversity, low linkage disequilibrium, and the existence of equal proportions of mating types suggest that sexual recombination plays a significant role in shaping the population structure of *C. zeina* in Africa. Even though no sexual stage has been reported in *C. zeina*, these results are consistent with previous findings that *Cercospora* species undergo cryptic sexual recombination (Groenewald *et al.*, 2006; Groenewald *et al.*, 2008; Kim *et al.*, 2013). These findings have significant implications for the management of GLS. Firstly, the occurrence of sexual recombination may give rise to novel genotypes with advantageous allele combinations, that are potentially more adaptable and able to overcome

host resistance (El Chartouni *et al.*, 2011; Milgroom, 1996). Secondly, sexual recombination can also increase fungicide resistance, capable of spreading across populations for generations. For example, it has been hypothesized that quinone outside inhibitor (QoI) fungicide resistance among *C. sojina* populations is partly due to a sexual recombination event that occurred in the past (Kim *et al.*, 2013; Shrestha *et al.*, 2017). Thus, testing *C. zeina*'s potential to develop host and fungicide resistance owing to sexual recombination is important.

The clonal fraction of Zambia was higher than the rest of the populations studied. High clonality may suggest that Zambia lacked the conducive environmental conditions to permit sexual recombination or that this population has lost some alleles over time. The latter may explain the presence of a bias towards *MAT1-2*. This evolutionary behavior is very common in recently introduced populations from their areas of origin owing to mainly anthropogenic activities (Taylor *et al.*, 1999). In previous studies, several ascomycetes such as *Fusarium circinatum* (pitch canker fungus) (Wikler & Gordon, 2000), *Cryphonectria parasitica* (chestnut blight fungus) (Milgroom *et al.*, 2008), *Dothistroma septosporum* (*Dothistroma* needle blight fungus) (Barnes *et al.*, 2014) have exhibited high clonality and a shift towards a single mating type when compared to their centers of origin. We therefore propose that *C. zeina* in Zambia was recently introduced from an unknown source and so more studies are needed to establish its source populations.

3.5.5 Evidence for migration patterns of *Cercospora zeina* within African countries.

The direction and levels of gene flow of *C. zeina* genotypes among populations provided evidence for directional migration. For all the scenarios tested, excluding panmixia, the posterior probabilities were above 0.90, suggesting that there has been migration of many genotypes amongst the five populations. From the first report of GLS in Africa in 1988, in South Africa (Ward, Laing, & Rijkenberg, 1997), the disease has since been reported in Uganda (1994), Zimbabwe (1995), Kenya (1995), Zambia and many other African countries (Nowell, 1997; Wang *et al.*, 1998; Ward, Laing, & Cairns, 1997). Of the five populations, South Africa

is the highest donor of immigrants, fitting the description of the oldest population, similar to previous reports on *Z. tritici* where its older populations donated more immigrants into the recently established populations than did the younger populations (Banke & McDonald, 2005). For a population to fit the description of an ancestral population however, it must also exhibit the highest genetic diversity, and effective population size. Since none of the populations in this study meets those criteria, our findings cannot resolve yet *C. zeina*'s center of origin in Africa. Thus, the reports of identification of GLS in Africa do not reflect the time of introduction of the pathogen. A more comprehensive study involving isolates from all other African countries where GLS is endemic is, therefore, needed to effectively monitor its trends of dispersal across the continent. Migration was most observed from South Africa into Zimbabwe (19) and less from Zimbabwe into South Africa (4). The most plausible explanation is that South Africa experiences earlier build-up of inoculum since its planting season starts earlier than that of Zimbabwe (Supplementary Figure 3.1). Presence of prevailing winds from South Africa to Zimbabwe disperse this inoculum into the young maize fields in Zimbabwe. It is therefore important to test and understand the movement patterns of the winds and other environmental factors in the region. Additionally, migration estimates revealed Zambia as the main sink population, receiving immigrants from especially Zimbabwe, Uganda, and Kenya. This may suggest that Zambia is a younger and more recent population when compared to other geographically distant populations (Banke & McDonald, 2005).

Cercospora zeina is a known splash-dispersed pathogen within fields and has been reported to spread across regions by wind up to 160 km (Manandhar *et al.*, 2011). Notably, however, this short distance alone may not explain the observed migration patterns. We hypothesize therefore, that in addition to the splash and wind dispersal of the pathogen, anthropogenic movement of the pathogen over long geographic distances occurs. This is through infected corn residues including sheaths and husks that accompany imported or exported maize as reported by Ward *et al.* (1999). Such movement contributed to the distribution of *D. septosporum* into non-native pine populations in the Southern Hemisphere (Barnes *et al.*,

2014). It also contributed to the founder populations of the seed-borne pathogen *R. secalis* of barley into South Africa, California and New Zealand (Linde *et al.*, 2009). Additional isolates from other Sub-Saharan African countries are needed to completely resolve the migration patterns and the ancestral origin of *C. zeina* in Africa.

3.6 CONCLUSION

This study provides the most comprehensive analysis of the genetic diversity and population genetic structure of *C. zeina*, coupled with migration pattern estimations in sub-Saharan Africa, using microsatellite markers. Our results provide evidence that *C. zeina* is a genetically divergent pathogen and is a demographically structured pathogen of maize, with signatures of cryptic sexual recombination. The discovered migration patterns provide information on the direction and levels of gene flow of the pathogen, which is critical in reconstructing the original introduction of *C. zeina* into pathogen-free corn-growing countries in Africa. This study provides a basis for effective monitoring of the pathogen's dispersal and is a tool for modelling emergence events into other countries where it has not been reported. Our results could not confirm South Africa as the pathogen's center of spread into Africa even though GLS was first reported there. Furthermore, there is a need to investigate the population genetic structure of *C. zeina* from all other sub-Saharan African countries where the pathogen has been previously reported to fully reconstruct its evolutionary history. Ultimately, this will facilitate the designing of more effective management strategies of *C. zeina*, thus, limiting the acquisition and movement of host and fungicide resistance traits and highly virulent strains across the continent and globally. Tracing how gene flow and migration define the pathogen's populations in Africa was one of the aims of this study as a basis for tracking its movement and spread across the continent.

3.7 ACKNOWLEDGEMENTS

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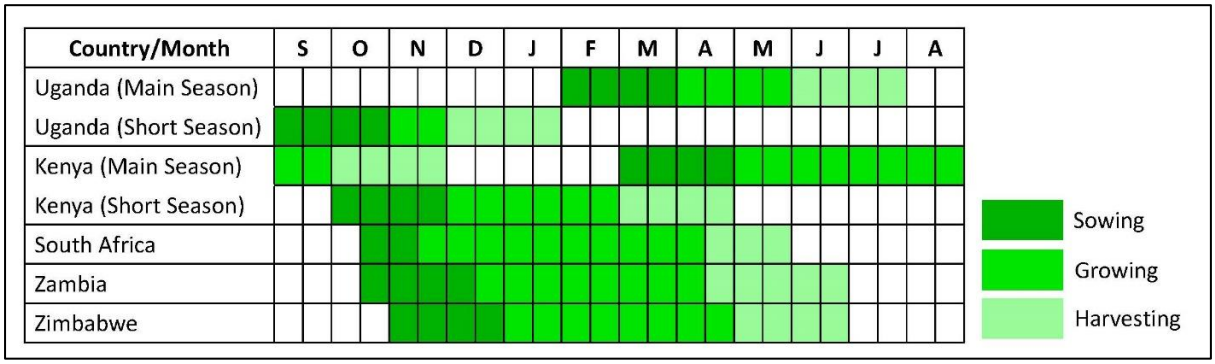
3.8 SUPPLEMENTARY DATA



Supplementary Figure 3.1: Growing seasons of maize in Kenya, South Africa, Uganda, Zambia and Zimbabwe: Kenya and Uganda have two growing seasons, with the first season being longer than the second season. South Africa, Zambia and Zimbabwe are characterized by single growing seasons. Green = Sowing period. Light green = Growing period. Pale green = Harvesting period. S to A: Months of the year starting from September and ending in August

Supplementary Table 3.1: Genetic characteristics of 11 microsatellite markers of 833 isolates of *Cercospora zeina*

Primer name	Accession number[a]	Forward primer	Reverse primer	Size	Number of alleles	E_5	H_e
CzSSR01	KP015832	AATTAATCGTAAGCACGACGA	CTCCCTCCACAACCACAACT	150-160	9	0.74	0.55
CzSSR04	KP015834	GGTTAGCGTGTAGCCGAGTT	CGACCAAGTGCTTGTCAAC	450-460	10	0.73	0.56
CzSSR05	KP015835	CTTCGACTACGTTGCGTTGA	AGCCCTTGACAGCACTGACT	230-240	8	0.70	0.65
CzSSR06	KP015836	CAGAAAGAAGGCACCAAAGC	GAGCAGGTTTAGTCGGAGGA	220-250	14	0.66	0.66
CzSSR07	KP015837	CAAGAATGCCAATGATGCTG	GTCTCCTTTCTGGCGAAGTG	200-240	21	0.60	0.76
CzSSR08	KP015838	GTAACTCCGCGAGATTCCTG	AGCAGCAGCAGCAGTAACAA	190-200	13	0.69	0.69
CzSSR10	KP015839	GCGTTACTTCGAAGGTGCTT	GTTGGTCGTTTGTTTTGTCCT	170-190	16	0.60	0.66
CzSSR12	KP015841	GAAGGCTTTTCTCTCGCAAA	TTGTCCCTCGGTCGCTTAT	230-240	14	0.62	0.58
CzSSR13	KP015842	GAGAGATAGTTGCGGCGT	GATGATGATTTGAGGAGTGTTG	320-330	5	0.30	0.03
CzSSR15	KP015844	CATTCTTTGTCCGCGTTC	CACTCACTTCCCACATAC	240-250	11	0.59	0.56
CzSSR18	KP015847	ATGCGTCAAAATCACACTTTC	AAAGCGTCTCCTCATCGATAC	130-140	3	0.89	0.53
Total					**124**	**0.65**	**0.57**

CzSSR: *C. zeina* simple sequence repeat (SSR). Primer sequences in Muller *et al.* (2016).

H_e: Nei's unbiased measure of gene diversity (Nei, 1978) per locus for all isolates.

E_5: Population evenness estimating uniform genotype distribution for 835 isolates, E5 = 1 means genotypes occur at equal frequency, regardless of richness (Grünwald *et al.*, 2003).

Supplementary Table 3.2: Population estimates of Theta (θ) and mutation-scaled migration rates (M = m/μ) between pairs of five *Cercospora zeina* populations based on Eastern African countries pooled together and migrating into individual Southern African countries scenario

Source/Sink[a] Populations	Theta	Eastern Africa	South Africa	Zambia	Zimbabwe
Eastern Africa	4.14	-	17.54 (15.44-19.04)	0.06 (0.00-0.44)	13.62 (11.32-16.64)
South Africa	0.82	13.5 (12.56-15.24)	-	0.10 (0.00-0.44)	15.62 (14.36-18.04)
Zambia	0.22	2.14 (1.56-3.60)	0.00 (0.00-0.00)	-	1.10 (0.64-1.48)
Zimbabwe	0.58	5.86 (5.36-6.44)	5.34 (4.52-6.6)	2.10 (1.40-2.76)	-

[a]Donor (Source) populations are on the left column while the sink (receiving) populations are across.
[b]M values are represented as the mode value while the 95 % confidence intervals are in parentheses.

Supplementary Table 3.3: Population estimates of Theta (θ) and mutation-scaled migration rates (M = m/μ) between pairs of five *Cercospora zeina* populations based on Southern African countries pooled together and migrating into individual Eastern African countries scenario.

Source/Sink[a] Populations	Theta	Southern Africa	Kenya	Uganda
Southern Africa	5.10	-	11.94 (8.48-13.24)	14.30 (12.52-16.72)
Kenya	0.54	0.00 (0.00)	-	9.34 (8.36-11.20)
Uganda	0.86	9.46 (9.00-9.92)	6.30 (5.08-8.08)	-

[a]Donor (Source) populations are on the left column while the sink (receiving) populations are across.
[b]M values are represented as the mode value while the 95 % confidence intervals are in parentheses.

3.9 REFERENCES

Abrinbana, M., Mozafari, J., Shams-Bakhsh, M., & Mehrabi, R. (2010). Genetic structure of *Mycosphaerella graminicola* populations in Iran. *Plant Pathology, 59*(5), 829-838.

Access to Seeds Foundation. (2017). Identifying leading seed companies in Eastern and Southern Africa. https://www.accesstoseeds.org/app/uploads/2018/2003/Leading-seed-companies-in-Eastern-and-Southern-Africa_DEF.pdf.

Agapow, P. M., & Burt, A. (2001). Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes, 1*(1-2), 101-102.

Ahmadpour, A., Castell-Miller, C., Javan-Nikkhah, M., Naghavi, M., Dehkaei, F., Leng, Y., & Zhong, S. (2018). Population structure, genetic diversity, and sexual state of the rice brown spot pathogen *Bipolaris oryzae* from three Asian countries. *Plant Pathology, 67*(1), 181-192.

Atallah, Z. K., Maruthachalam, K., Du Toit, L., Koike, S. T., Davis, R. M., Klosterman, S. J., & Subbarao, K. V. (2010). Population analyses of the vascular plant pathogen *Verticillium dahliae* detect recombination and transcontinental gene flow. *Fungal Genetics and Biology, 47*(5), 416-422.

Aylor, D. E. (2003). Spread of plant disease on a continental scale: role of aerial dispersal of pathogens. *Ecology, 84*(8), 1989-1997.

Banke, S., & McDonald, B. (2005). Migration patterns among global populations of the pathogenic fungus *Mycosphaerella graminicola*. *Molecular Ecology, 14*(7), 1881-1896.

Banke, S., Peschon, A., & McDonald, B. A. (2004). Phylogenetic analysis of globally distributed *Mycosphaerella graminicola* populations based on three DNA sequence loci. *Fungal Genetics and Biology, 41*(2), 226-238.

Barnes, I., Wingfield, M. J., Carbone, I., Kirisits, T., & Wingfield, B. D. (2014). Population structure and diversity of an invasive pine needle pathogen reflects anthropogenic activity. *Ecology and Evolution, 4*(18), 3642-3661.

Beerli, P. (2006). Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics, 22*(3), 341-345.

Bengtsson, B. O. (2003). Genetic variation in organisms with sexual and asexual reproduction. *Journal of Evolutionary Biology, 16*(2), 189-199.

Boukef, S., McDonald, B. A., Yahyaoui, A., Rezgui, S., & Brunner, P. C. (2012). Frequency of mutations associated with fungicide resistance and population structure of *Mycosphaerella graminicola* in Tunisia. *European Journal of Plant Pathology, 132*(1), 111-122.

Brown, J. K., & Hovmøller, M. S. (2002). Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science, 297*(5581), 537-541.

Brunelli, K. R., Dunkle, L. D., Sobrinho, C. A., Fazza, A. C., & Camargo, L. E. (2008). Molecular variability in the maize grey leaf spot pathogens in Brazil. *Genetics and Molecular Biology, 31*(4), 938-942.

Cai, G., & Schneider, R. (2008). Population structure of *Cercospora kikuchii*, the causal agent of *Cercospora* leaf blight and purple seed stain in soybean. *Phytopathology, 98*(7), 823-829.

Chowdhary, A., Hiremath, S. S., Sun, S., Kowshik, T., Randhawa, H. S., & Xu, J. (2011). Genetic differentiation, recombination and clonal expansion in environmental populations of *Cryptococcus gattii* in India. *Environmental Microbiology, 13*(7), 1875-1888.

Crous, P. W., Groenewald, J. Z., Groenewald, M., Caldwell, P., Braun, U., & Harrington, T. C. (2006). Species of *Cercospora* associated with gray leaf spot of maize. *Studies in Mycology, 55*, 189-197.

Dunkle, L. D., & Levy, M. (2000). Genetic relatedness of African and United States populations of *Cercospora zeae-maydis*. *Phytopathology, 90*(5), 486-490.

Dutech, C., Fabreguettes, O., Capdevielle, X., & Robin, C. (2010). Multiple introductions of divergent genetic lineages in an invasive fungal pathogen, *Cryphonectria parasitica*, in France. *Heredity, 105*(2), 220.

Ebrahimi, L., Fotuhifar, K.-B., Javan Nikkhah, M., Naghavi, M.-R., & Baisakh, N. (2016). Population genetic structure of apple scab (*Venturia inaequalis* (Cooke) G. Winter) in Iran. *PLoS One, 11*(9), e0160737.

El Chartouni, L., Tisserant, B., Siah, A., Duyme, F., Leducq, J.-B., Deweer, C., & Halama, P. (2011). Genetic diversity and population structure in French populations of *Mycosphaerella graminicola*. *Mycologia, 103*(4), 764-774.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology, 14*(8), 2611-2620.

FAO/GIEWS. (2018). Global information and early warning system: Country briefs. http://www.fao.org/giews/countrybrief/ (Last accessed May, 2019).

Giraud, T., Enjalbert, J., Fournier, E., Delmotte, F., & Dutech, C. (2008). Population genetics of fungal diseases of plants. *Parasite, 15*, 449-454.

Goodwin, S. B., Dunkle, L. D., & Zismann, V. L. (2001). Phylogenetic analysis of *Cercospora* and *Mycosphaerella* based on the internal transcribed spacer region of ribosomal DNA. *Phytopathology, 91*(7), 648-658.

Grandaubert, J., Dutheil, J., & Stukenbrock, E. (2017). The genomic rate of adaptation in the fungal wheat pathogen *Zymoseptoria tritici*. *bioRxiv*. doi: https://doi.org/10.1101/176727

Groenewald, M., Groenewald, J. Z., Harrington, T. C., Abeln, E. C., & Crous, P. W. (2006). Mating type gene analysis in apparently asexual *Cercospora* species is suggestive of cryptic sex. *Fungal Genetics and Biology, 43*(12), 813-825.

Groenewald, M., Linde, C., Groenewald, J. Z., & Crous, P. W. (2008). Indirect evidence for sexual reproduction in *Cercospora beticola* populations from sugar beet. *Plant Pathology, 57*(1), 25-32.

Grünwald, N. J., Goodwin, S. B., Milgroom, M. G., & Fry, W. E. (2003). Analysis of genotypic diversity data for populations of microorganisms. *Phytopathology, 93*(6), 738-746.

Grünwald, N. J., & Goss, E. M. (2011). Evolution and population genetics of exotic and re-emerging pathogens: novel tools and approaches. *Annual Review of Phytopathology, 49*.

Ingwell, L. L., Eigenbrode, S. D., & Bosque-Pérez, N. A. (2012). Plant viruses alter insect behavior to enhance their spread. *Scientific Reports, 2*, 578.

Kabbage, M., Leslie, J. F., Hulbert, S. H., & Bockus, W. W. (2009). Comparison of natural populations of *Mycosphaerella graminicola* from single fields in Kansas and California. *Physiological and Molecular Plant Pathology, 74*(1), 55-59.

Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ, 2*, e281.

Kim, H., Newell, A. D., Cota-Sieckmeyer, R. G., Rupe, J. C., Fakhoury, A. M., & Bluhm, B. H. (2013). Mating-type distribution and genetic diversity of *Cercospora sojina* populations on soybean from Arkansas: evidence for potential sexual reproduction. *Phytopathology, 103*(10), 1045-1051.

Kinyua, Z., Smith, J., Kibata, G., Simons, S., & Langat, B. (2010). Status of grey leaf spot disease in Kenyan maize production ecosystems. *African Crop Science Journal, 18*(4), 183 - 194.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources, 15*(5), 1179-1191.

Lamesch, P., Berardini, T. Z., Li, D., Swarbreck, D., Wilks, C., Sasidharan, R., Huala, E. (2011). The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. *Nucleic Acids Research*, *40*(D1), D1202-D1210

Lee, S., Milgroom, M., & Taylor, J. (1988). A rapid, high yield mini-prep method for isolation of total genomic DNA from fungi. *Fungal Genetics Reports, 35*(1), 23.
Linde, C. C., Zala, M., & McDonald, B. A. (2009). Molecular evidence for recent founder populations and human-mediated migration in the barley scald pathogen *Rhynchosporium secalis*. *Molecular Phylogenetics and Evolution, 51*(3), 454-464.
Liu, Y.-C., Cortesi, P., Double, M. L., MacDonald, W. L., & Milgroom, M. G. (1996). Diversity and multilocus genetic structure in populations of *Cryphonectria parasitica*. *Phytopathology, 86*(12), 1344-1351.
Manandhar, G., Ferrara, G., Tiwari, T., Baidya, S., Bajracharya, A., Khadge, B., & Narro, L. (2011). Response of maize genotypes to gray leaf spot disease (*Cercospora zeae-maydis*) in the hills of Nepal. *Agronomy Journal of Nepal, 2*, 93-101.
McDonald, B., & Linde, C. (2002). The population genetics of plant pathogens and breeding strategies for durable resistance. *Euphytica, 124*(2), 163-180.
McDonald, B. A. (2015). How can research on pathogen population biology suggest disease management strategies? The example of barley scald (*Rhynchosporium commune*). *Plant Pathology, 64*(5), 1005-1013.
McDonald, B. A., & Mundt, C. C. (2016). How knowledge of pathogen population biology informs management of *Septoria tritici* blotch. *Phytopathology, 106*(9), 948-955.
Meisel, B., Korsman, J., Kloppers, F. J., & Berger, D. K. (2009). *Cercospora zeina* is the causal agent of grey leaf spot disease of maize in Southern Africa. *European Journal of Plant Pathology, 124*(4), 577-583.
Milgroom, M. G. (1996). Recombination and the multilocus structure of fungal populations. *Annual Review of Phytopathology, 34*(1), 457-477.
Milgroom, M. G., & Peever, T. L. (2003). Population biology of plant pathogens: the synthesis of plant disease epidemiology and population genetics. *Plant Disease, 87*(6), 608-617.
Milgroom, M. G., Sotirovski, K., Spica, D., Davis, J. E., Brewer, M. T., Milev, M., & Cortesi, P. (2008). Clonal population structure of the chestnut blight fungus in expanding ranges in southeastern Europe. *Molecular Ecology, 17*(20), 4446-4458.
Moretti, M., Karaoglanidis, G., Saracchi, M., Fontana, A., & Farina, G. (2006). Analysis of genotypic diversity in *Cercospora beticola* Sacc. field isolates. *Annals of Microbiology, 56*(3), 215-221.
Muller, M. F., Barnes, I., Kunene, N. T., Crampton, B. G., Bluhm, B. H., Phillips, S. M., & Berger, D. K. (2016). *Cercospora zeina* from maize in South Africa exhibits high genetic diversity and lack of regional population differentiation. *Phytopathology, 106*(10), 1194-1205.
Nega, A., Lemessa, F., & Berecha, G. (2016). Distribution and importance of maize grey leaf spot *Cercospora zeae-maydis* (Tehon and Daniels) in south and southwest Ethiopia. *Journal of Plant Pathology and Microbiology, 7*(362), 2.
Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics, 89*(3), 583-590.
Nei, M. (1987). *Molecular evolutionary genetics*: Columbia university press.
Neves, D. L., Silva, C. N., Pereira, C. B., Campos, H. D., & Tessmann, D. J. (2015). *Cercospora zeina* is the main species causing gray leaf spot in southern and central Brazilian maize regions. *Tropical Plant Pathology, 40*(6), 368-374.
Nowell, D. C. (1997). *Studies on ear rot and gray leaf spot of maize in South Africa.* (Doctoral dissertation), University of Natal, Pietermaritzburg.
Nsibo, D. L., Barnes, I., Kunene, N. T., & Berger, D. K. (2019). Influence of farming practices on the population genetics of the maize pathogen *Cercospora zeina* in South Africa. *Fungal Genetics and Biology, 125*, 36-44.
Okori, P., Fahleson, J., Rubaihayo, P., Adipala, E., & Dixelius, C. (2003). Assessment of genetic variation among East African *Cercospora zeae-maydis*. *African Crop Science Journal, 11*(2), 75-85.
Okori, P., Rubaihayo, P., Adipala, E., Fahieson, J., & Dixelius, C. (2015). Dynamics of *Cercospora zeina* populations in maize-based agro-ecologies of Uganda. *African Crop Science Journal, 23*(1), 45-57.

Okori, P., Rubaihayo, P., Ekwamu, A., Fahleson, J., & Dixelius, C. (2004). Genetic characterization of *Cercospora sorghi* from cultivated and wild sorghum and its relationship to other *Cercospora* fungi. *Phytopathology, 94*(7), 743-750.

Peakall, R., & Smouse, P. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and researchd-an update. *Bioinformatics, 28*, 2537-2539.

Pritchard, J. K., Wen, W., & Falush, D. (2002). Documentation for STRUCTURE software: Version 2. *University of Chicago, Chicago.*

Salamati, S., Zhan, J., Burdon, J. J., & McDonald, B. A. (2000). The genetic structure of field populations of *Rhynchosporium secalis* from three continents suggests moderate gene flow and regular recombination. *Phytopathology, 90*(8), 901-908.

Savary, S., Ficke, A., Aubertot, J.-N., & Hollier, C. (2012). Crop losses due to diseases and their implications for global food production losses and food security. *Food Security*, *4*(4), 519-537.

Schurko, A. M., & Logsdon Jr, J. M. (2008). Using a meiosis detection toolkit to investigate ancient asexual "scandals" and the evolution of sex. *Bioessays, 30*(6), 579-589.

Shannon, C. E. (2001). A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing and Communications Review, 5*(1), 3-55.

Shrestha, S. K., Cochran, A., Mengistu, A., Lamour, K., Castro-Rocha, A., & Young-Kelly, H. (2017). Genetic diversity, QoI fungicide resistance, and mating type distribution of *Cercospora sojina*—Implications for the disease dynamics of frogeye leaf spot on soybean. *PLoS ONE, 12*(5), e0177220.

Siah, A., Bomble, M., Tisserant, B., Cadalen, T., Holvoet, M., Hilbert, J.-L., & Reignault, P. (2018). Genetic structure of *Zymoseptoria tritici* in Northern France at region, field, plant, and leaf layer scales. *Phytopathology, 108*(9), 1114-1123.

Simpson, E. H. (1949). Measurement of diversity. *Nature*, *163*(4148), 688.

Stoddart, J. A., & Taylor, J. F. (1988). Genotypic diversity: estimation and prediction in samples. *Genetics, 118*(4), 705-711.

Swart, V., Crampton, B. G., Ridenour, J. B., Bluhm, B. H., Olivier, N. A., Meyer, J. M., & Berger, D. K. (2017). Complementation of *CTB7* in the maize pathogen *Cercospora zeina* overcomes the lack of *in vitro* cercosporin production. *Molecular Plant-Microbe Interactions, 30*(9), 710-724.

Szpiech, Z. A., Jakobsson, M., & Rosenberg, N. A. (2008). ADZE: a rarefaction approach for counting alleles private to combinations of populations. *Bioinformatics, 24*(21), 2498-2504.

Taylor, J., Jacobson, D., & Fisher, M. (1999). The evolution of asexual fungi: reproduction, speciation and classification. *Annual Review of Phytopathology, 37*(1), 197-246.

Vaghefi, N., Nelson, S. C., Kikkert, J. R., & Pethybridge, S. J. (2017). Genetic structure of *Cercospora beticola* populations on *Beta vulgaris* in New York and Hawaii. *Scientific Reports, 7*(1), 1726.

Wang, J., Levy, M., & Dunkle, L. D. (1998). Sibling species of *Cercospora* associated with gray leaf spot of maize. *Phytopathology, 88*(12), 1269-1275.

Ward, J., & Nowell, D. (1998). Integrated management practices for the control of maize grey leaf spot. *Integrated Pest Management Reviews, 3*(3), 177-188.

Ward, J. M., Laing, M., & Cairns, A. (1997). Management practices to reduce gray leaf spot of maize. *Crop Science, 37*(4), 1257-1262.

Ward, J. M., Laing, M., & Rijkenberg, F. (1997). Frequency and timing of fungicide applications for the control of gray leaf spot in maize. *Plant Disease, 81*(1), 41-48.

Ward, J. M., Stromberg, E. L., Nowell, D. C., & Nutter Jr, F. W. (1999). Gray leaf spot: a disease of global importance in maize production. *Plant Disease, 83*(10), 884-895.

Whitlock, M. C. (2000). Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. *Evolution, 54*(6), 1855-1861.

Wikler, K., & Gordon, T. R. (2000). An initial assessment of genetic relationships among populations of *Fusarium circinatum* in different parts of the world. *Canadian Journal of Botany, 78*(6), 709-717.

Zhan, J., Pettway, R. E., & McDonald, B. A. (2003). The global genetic structure of the wheat pathogen *Mycosphaerella graminicola* is characterized by high nuclear diversity, low mitochondrial diversity, regular recombination, and gene flow. *Fungal Genetics and Biology, 38*(3), 286-297.

CHAPTER 4

Population genetic analysis of mating type genes at field scale indicates cryptic sex in phytopathogenic fungus *Cercospora zeina*.

4.1 ABSTRACT

Cercospora zeina is a heterothallic ascomycete, having both *MAT1-1* and *MAT1-2* idiomorphs. *Cercospora zeina*'s mating strategy and the role of sexual recombination has not been extensively studied in many African countries and, like other *Cercospora* species, its sexual stage is still unknown. The aim of this study was to investigate the role of sexual recombination in defining the genetic structure of 25 *C. zeina* populations from five countries in sub-Saharan Africa using 11 microsatellite markers. An attempt was made to also induce the sexual stage under laboratory conditions. Our results showed that populations have a low linkage disequilibrium and exhibit a high genotypic diversity. Both mating types (MAT1 and MAT2) existed in equal frequencies and there was also a lack of genetic differentiation and phylogenetic association between mating types. Although we found genetic evidence for sexual recombination, no sexual stage was induced under the laboratory or greenhouse conditions tested. Results of this study imply that sexual recombination plays a central role in the biology of *C. zeina*. With sexual recombination, the pathogen can acquire novel combinations of genetic variation available from the inter-mating population. This can give rise to transgressive segregation with potential for more virulent strains which overcome host resistance genes or have resistance to fungicides. Consistent monitoring of *C. zeina* and a systematic search for its sexual stage is therefore needed to further shed light on the biology of the pathogen.

4.2 INTRODUCTION

Plant pathogenic fungi undergo either asexual or sexual reproduction to transmit their genetic material to the next generation (Chen & McDonald, 1996; Taylor *et al.*, 1999; Taylor *et al.*, 2017). Approximately 20 % of known fungal species may rely solely on asexual reproduction (Dyer & O'Gorman, 2011). Many of their populations are largely characterized by a high degree of clonality, low number of genotypes at relatively high frequencies, and high linkage disequilibrium between alleles at different loci (Giraud *et al.*, 2008). Asexuality is suggested to

be gained through the loss or reduction in the pathogen's sexual potential (Möller & Stukenbrock, 2017). Such lifestyle changes have been recorded in clonal lineages of pathogens, including the wheat yellow rust fungus *Puccinia striiformis* formae speciales (f. sp) *tritici* (Ali *et al.*, 2014; Jin *et al.*, 2010) and the rice blast pathogen *Magnaporthe oryzae* (Couch *et al.*, 2005), but whose ancestral populations are characterized by sexual recombination (Ali *et al.*, 2010; Saleh *et al.*, 2012).

Sexual recombination exists in all four major fungal phyla (Lee *et al.*, 2010) and is characterized by the existence of a higher genetic diversity based on a plethora of recombinant genotypes that arise from intra-gene recombination and gene rearrangements (Ellegren & Parsch, 2007; Lin *et al.*, 2005; Milgroom, 1996; Sommerhalder *et al.*, 2010). Existence of high genotypic variability in sexually reproducing fungi facilitates rapid pathogen evolution especially in response to different environmental stresses, and management strategies such as host resistance and fungicide usage (McDonald & Linde, 2002; Meng *et al.*, 2015). Sexual reproduction can purge deleterious and undesired alleles. It also gives rise to stress-tolerant sexual structures with a higher dispersal potential when compared to asexual reproduction (Meng *et al.*, 2015; Whitlock, 2000).

The discovery of the sexual state of many pathogenic fungi both in nature and under laboratory conditions is still rare (Meng *et al.*, 2015; Turgeon, 1998). However, genome sequencing combined with the characterization of mating type genes and population genetic analysis has permitted the inference of cryptic sexual reproduction among fungal pathogens whose sexual stage is unknown (Bihon *et al.*, 2012; Bihon *et al.*, 2014; Kim *et al.*, 2013; Kück & Pöggeler, 2009). Sexually reproducing fungi can exist as either homothallic (self-fertile or self-compatible) or heterothallic (self-incompatible or outcrossing) (Coppin *et al.*, 1997; Kronstad & Staben, 1997; Lin & Heitman, 2007; Wilson *et al.*, 2015). In both sexual lifestyles, mating type (*MAT*) genes, *MAT1-1* and *MAT1-2*, are involved in addition to many other sex-related genes (Bihon *et al.*, 2014; Coppin *et al.*, 1997; Martin *et al.*, 2011; Turgeon & Yoder, 2000; Wilken *et al.*, 2014). These are highly dissimilar DNA sequences located on the *MAT1* locus,

and termed idiomorphs instead of alleles (alternate forms of the same genes), to emphasize their dissimilarity (Metzenberg & Glass, 1990). The *MAT1-1* gene encodes a protein with an alpha box, whilst the *MAT1-2* gene encodes a regulatory protein with a DNA binding domain of the high mobility group (HMG) family (Turgeon & Yoder, 2000). Homothallic organisms harbor both alpha box and HMG encoding genes in the same individual, either tightly linked at a single *MAT* locus or present at two *MAT* loci within a genome while for heterothallic species, each self-sterile individual possesses genes from a different *MAT* idiomorph which when combined results into successful mating (Wilson *et al.*, 2015).

The discovery of *MAT* genes has facilitated the direct crossing of isolates of known opposite mating types to induce fully functional sexual reproductive stages in fungi otherwise thought to be only asexual (Houbraken & Dyer, 2015). A model example is the human pathogen *Aspergillus fumigatus* where isolates of opposite mating type were crossed on a range of growth media in the dark at different temperatures. Cleistothecia containing recombinant ascospores were successfully induced on oatmeal agar incubated at 30 ^{o}C for 6-12 months (O'Gorman *et al.*, 2009). The induction of the sexual state has since then been reduced to 4 weeks by choosing mating type strains that exhibit highest growth rate (Sugui *et al.*, 2011). Discoveries of sexual stages have also been made in other supposedly asexual cereal pathogens including *Septoria passerinii* (Ware *et al.*, 2007) and *Magnaporthe oryzae* (Saleh *et al.*, 2012).

The genus *Cercospora* has a total of 659 species, many of which are pathogenic to a wide range of plant genera and families (Crous & Braun, 2003). Most of these species have no known sexual stages from nature and efforts to induce the sexual state *in vitro* have so far been unsuccessful (Bolton *et al.*, 2012; Groenewald *et al.*, 2006; Groenewald *et al.*, 2008; Kim *et al.*, 2013). The *Cercospora* genus is, however, known to form a monophyletic group within the *Mycosphaerella* (Crous *et al.*, 2001; Goodwin *et al.*, 2001) and as such it has been suggested that if a sexual stage of any *Cercospora* species does exist, it would likely be a member of *Mycosphaerella* (Bolton *et al.*, 2012; Crous & Braun, 2003; Groenewald *et al.*,

2008) or produce a *Mycosphaerella*-like sexual stage. *Cercospora* species for which a sexual stage has been identified in *Mycosphaerella* include *M. fijiensis* (*Cercospora fijiensis* = *Paracercospora fijiensis*), *M. musicola* (*Cercospora musae* = *Psuedocercospora musae*), and *M. arachidis* (*Cercospora arachidicola*) (Goodwin *et al.*, 2001). The morphological description of these sexual stages conforms to the description of the sexual stage of other *Mycosphaerella* species such as *Mycosphaerella graminicola* (Halama, 1996). It comprises pseudothecia with bitunicate asci containing eight irregularly arranged two-celled, hyaline, elliptical ascospores (Halama, 1996).

Cercospora zeina is a heterothallic ascomycete, having both *MAT1-1* and *MAT1-2* idiomorphs existing in nature in South Africa (Muller *et al.*, 2016; Nsibo *et al.*, 2019). However, its mating strategy has not been extensively studied in other African countries and, like other *Cercospora* species, its sexual stage is still unknown. The aim of this study was to investigate the role of sexual recombination in defining the genetic structure of 25 *C. zeina* populations from around five countries in Sub-Saharan Africa and to attempt to induce its sexual stage under laboratory conditions.

4.3 MATERIALS AND MATHODS

4.3.1 Collection and culturing of *Cercospora zeina* isolates

Gray leaf spot symptomatic maize leaves were collected from 25 locations across five Sub-Saharan countries between 2015 and 2017 (see Appendix B). Isolates from a single field were considered as a single population. A single conidium was isolated directly from one or two lesions per leaf per plant, using a sterilized hypodermic needle under a 90X magnification stereomicroscope as described by Nsibo *et al.* (2019). Each conidium was then cultured on V8 growth media (3 g of Calcium Carbonate, 20 g of Bacteriological Agar and 200 mL of V8 vegetable juice [Campbell Soup Co., Camden, New Jersey] and 1000 mL of double distilled water), supplemented with a final concentration of 50 ug/mL of cefotaxime antibiotic (Aspen

Pharmacare, Durban, South Africa), for 12 to 13 weeks until enough mycelia was produced for further analysis.

4.3.2 DNA extraction

Genomic DNA was extracted from 12 to 14 week-old mycelia through a modified CTAB protocol (Lee *et al.*, 1988) and DNA quality and quantity were assessed using a NanoDrop Spectrophotometer (NanoDrop Technologies).

4.3.3 Mating type characterization

Mating types were confirmed by PCR amplification following conditions optimized by Muller *et al.* (2016). Primer pair CzMAT1 (forward and reverse) amplifies part of the α-domain, generating a single amplicon of 631 bp in isolates possessing the *MAT1-1* idiomorph. Primer pair C*zMAT1-2* (forward and reverse) amplifies a segment of the HMG box, generating an amplicon of 409 bp from isolates harboring the *MAT1-2* idiomorph. PCR conditions used were as described by Nsibo *et al.* (2019). PCR products were separated by electrophoresis on a 2 % agarose gel at 80 V for 60 minutes and visualized by GelDoc system (Bio-Rad Laboratories Ltd., South Africa). Mating type for each isolate was determined based on size of the PCR amplicon.

4.3.4 Data and statistical analysis

In total, 11 microsatellite markers were used for allele amplification from all studied isolates and alleles were scored as described by Nsibo *et al.* (2019) (see chapters 2 and 3). Multilocus genotypes (MLG) were characterized by pooling together all alleles across 11 loci for each studied isolate. The total numbers of alleles were obtained using GenAlEx v 6.501 (Peakall & Smouse, 2012). Estimates of genotypic diversity based on the Shannon-Weiner index of MLG diversity (Shannon, 2001) were performed using GenAlEx v 6.501 (Peakall & Smouse, 2012) to test the effect of independent assortment and recombination on population structure, and to determine the pathogen's evolutionary potential.

Genetic differentiation between the two mating types at each population was estimated using G_{ST} (Nei, 1978) executed using poppr (Kamvar *et al.*, 2014). This was to test for existence of any association between isolates from the same mating type within a population. To further test population genetic structure, a phylogenetic tree using 46 randomly selected genotypes from the two mating types was constructed using UPGMA. A neighbor joining phylogenetic tree of all studied isolates representing each mating type was further constructed based on Nei's unbiased genetic distance using poppr (Kamvar *et al.*, 2014). This was to test for the existence of genetic convergence within the mating types. Visualization of both phylogenetic trees was performed using Fig Tree software v 1.4 (Rambaut, 2009).

The null hypothesis of a 1:1 ratio of the two mating types, as is characteristic of randomly recombining populations (Milgroom, 1996), was tested by estimating the mating type frequencies and significance was determined using the Chi-square (χ^2) test (Everitt, 1992) at $p = 0.05$. To further test the hypothesis, gametic disequilibrium was evaluated using the index of association (I_A), based on 1000 permutations using Multilocus v 1.3 (Agapow & Burt, 2001). Clonal fraction, which is defined as the proportion of isolates originating from asexual reproduction within a population, was calculated using the formula, 1 - [(number of MLGs) / (total number of isolates)].

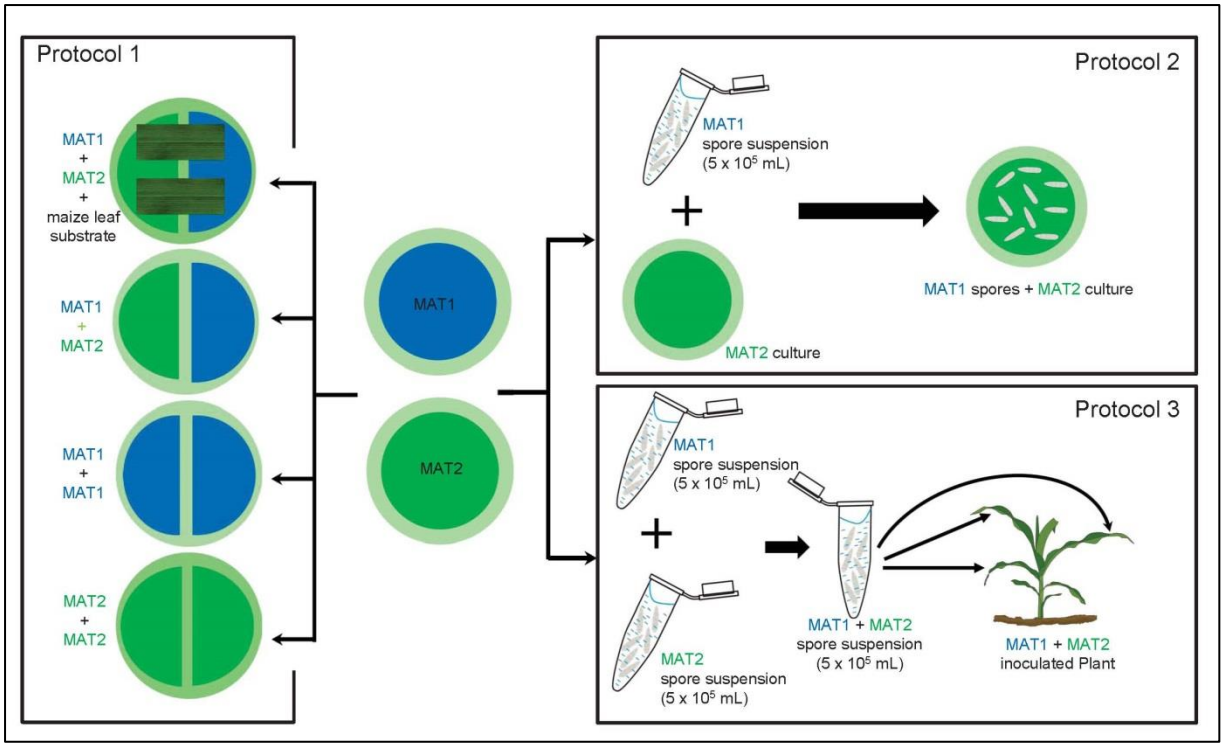


Figure 4.1: Schematic for the mating type crosses using three protocols: Protocol 1: MAT1 and MAT2 crossed in different combinations: MAT1 + MAT2 + maize leaf substrate; MAT1 + MAT2; MAT1 + MAT1; MAT2 + MAT2 divided into two replicates. Replicate 1 incubated in the dark at room temperature for six months. Replicate 2 first incubated in the dark at room temperature for three months and later transferred to 4 ^{o}C in the dark for another three months. Protocol 2: MAT1 prepared as a spore suspension to a concentration of 5 x 10^5 per mL which are spread on MAT2 cultures and incubated at 22 and 28 ^{o}C in alternating 12 h dark / 12 h fluorescent white light for 6 months. Protocol 3: Spore suspensions of both MAT1 and MAT2 prepared and mixed together to a concentration of 5 x 10^5 per mL and inoculated on three leaves on different B73 maize plants and incubated throughout a growing season and left until the wintering period. At the end of each protocol, sexual structures were searched for under a stereomicroscope. Blue: MAT1. Light green: Growth media. Dark green: MAT2.

4.3.5 Sexual crosses of *Cercospora zeina*

To induce the sexual stage of *C. zeina*, several crosses using randomly selected isolates and different protocols were made (Figure 4.1). Four MAT1 isolates (2015.ZA.NTA.016, Mkushi.V1A, 2017.UG.034, 2017.KE.335) and four MAT2 isolates (2015.ZA.NTA.067, 2016.UG.121, 2015.ZM.070 and 2017.UG.NML.111) were used for the mating type crossing assay. Two other *Cercospora* species of unknown mating type; *C. zeae-maydis* isolate 2018.US.Czm.028 and *Cercospora sorghi* 2018.KE.Cs.001 were used to test for interspecies hybridization. Four growth media were used including MEA (30 g of malt extract, 5 g of bacteriological agar, and 1000 mL of double-distilled water, balanced at pH 5.4) (Merck (Pty)

Ltd, Modderfontein, South Africa), czapek dox agar (49.01 g of czapek and 1000 mL of double-distilled water) (Sigma-Aldrich, St. Loius, USA), V8 growth medium and oatmeal agar (OMA) (72.5 g of oat meal and 1000 mL of double-distilled water) (Sigma-Aldrich, St. Loius, USA).

Following the Gross *et al.* (2012) protocol (Figure 4.1 protocol 1), maize leaves sterilized by autoclaving were used as a substrate to imitate natural conditions. Inoculum with either the same mating type or with opposite mating types were placed 5 cm apart on a petri dish containing growth media and left to grow for 14 days. The maize leaves were then placed on top of the sporulating cultures. Plates were divided into two replicates: replicate one was incubated in the dark at room temperature for six months. Replicate two was incubated under similar conditions as above for three months and thereafter subjected to stress by transferring it to 4 ^{o}C for another three months. After six months, the material was then observed under a stereomicroscope to look for the sexual structures

The protocol of Zhou *et al.* (2018) was also tested for the induction of the sexual stage. Using the same growth media as stated above, both mating types were grown separately at 22 and 28 ^{o}C, under alternating 12 h dark / 12 h fluorescent white light. After 14 days, conidia of MAT1 were resuspended in 5 mL of 0.02 % Tween 20 (Merck (Pty) Ltd, Modderfontein, South Africa). About 5 x 10^{5} mL^{-1} spore suspension was uniformly spread over MAT2. Crosses were incubated at 22 and 28 ^{o}C under the lighting conditions stated above for six months. Material was then observed under a stereomicroscope to look for the sexual structures (Figure 4.1 protocol 2).

Induction of a sexual stage *in planta* was tested (Figure 4.1 protocol 3). Conidia from 14-day-old cultures grown on V8 medium was resuspended in 0.02 % Tween 20 (Merck (Pty) Ltd, Modderfontein, South Africa) to a concentration of 5 x 10^{5} conidia mL^{-1}. Mating type crossing combinations were set up as follows: Four MAT1 x MAT2; two MAT1 x MAT1; two MAT2 x MAT2 and one negative control with only distilled water. Each MAT combination was applied on three leaves (four-leaf stage) per potted B73 maize plant. Two plants were inoculated with distilled water as negative controls. All plants were kept growing in a phytotron for four months

at 28 ^{o}C and above 90 % relative humidity. The senesced plants were then transferred into the open screen house to allow the pathogen to overwinter under natural conditions. Leaf samples were then searched for presence of ascospores under a stereomicroscope.

4.3.6 Mapping of the microsatellite and mating type markers to the genome

The nucleotide sequences of the microsatellite and mating type markers were located on the recently Pacific BioSciences (PacBio) re-sequenced *C. zeina* (CMW25467) genome (Berger et al., unpublished) using a local BLASTn search. The alignment was viewed on the Integrative Genomics Viewer (Thorvaldsdóttir *et al.*, 2013). This was to confirm the presence of these markers on the current genome and to visualize their location within the contigs.

4.4 RESULTS

4.4.1 Mating type frequency and gametic disequilibrium

A total of 835 isolates of *C. zeina* collected from 25 populations were assayed for mating type distribution and frequencies. Upon PCR amplification, all isolates produced a unique amplicon of either 631 or 409 bp, corresponding to MAT1 or MAT2, respectively (Figure 4.2). Both mating types were detected in all 25 populations sampled (Figure 4.3). The frequency of MAT1 ranged from 0.14 in Vihiga to 0.75 in Bungoma with an average of 0.45. MAT2 ranged from 0.25 in Chinhoyi and Bungoma to 0.86 in Vihiga with an average of 0.48 (Table 4.1). A 1:1 ratio of mating types, as expected under random mating, was attained with all populations at $p < 0.05$ except for those at KwaNxamalala, and Masaka (Table 4.1). Additionally, based on the significantly low index of association, the hypothesis of random mating was retained in 15 of 25 populations at $p < 0.05$ (Table 4.2).

Table 4.1: *Cercospora zeina* mating type frequencies and their corresponding Chi-square test (χ^2) from 25 populations collected between 2015 and 2017.

Country	Populations	Number of isolates	Year of collection	Mating type frequencies		χ^2 test (p-value) within fields
				MAT1	MAT2	
Kenya	Bungoma	12	2016	0.75	0.25	3.00 (0.08)
	Kakamega	22	2016	0.50	0.50	0.00 (1.00)
	Kericho	41	2017	0.59	0.41	1.20 (0.27)
	Kisumu	8	2016	0.63	0.38	0.50 (0.48)
	Siaya	6	2016	0.50	0.50	0.00 (1.00)
	Vihiga	7	2016	0.14	0.86	3.57 (0.06)
	Kitale	24	2017	0.63	0.38	1.50 (0.22)
	Transzoia	26	2017	0.42	0.58	0.62 (0.43)
South Africa	Hlanganani	72	2015	0.42	0.58	2.00 (0.16)
	KwaNxamalala*	37	2015	0.62	0.30	4.24 (0.04)
	Mbizana	80	2015	0.49	0.51	0.05 (0.82)
	Mthatha	31	2016	0.52	0.48	0.03 (0.86)
	Ntabamhlophe	20	2015	0.45	0.55	0.20 (0.66)
	Ntabankulu	45	2015	0.47	0.53	0.20 (0.66)
Uganda	Fortportal	49	2016	0.49	0.51	0.02 (0.89)
	Kapchorwa	17	2016	0.47	0.53	0.06 (0.81)
	Lira	18	2016	0.44	0.56	0.22 (0.64)
	Masaka*	46	2017	0.32	0.73	7.04 (0.01)
	Wakiso	41	2017	0.41	0.59	1.20 (0.27)
Zambia	Chilanga	29	2015	0.41	0.59	0.86 (0.35)
	Chisamba	64	2015	0.38	0.63	4.00 (0.05)
Zimbabwe	Chinhoyi	8	2017	0.75	0.25	2.00 (0.16)
	Harare	84	2017	0.45	0.55	0.76 (0.38)
	KweKwe	21	2017	0.38	0.62	1.19 (0.28)
	Mutare	27	2017	0.52	0.48	0.04 (0.85)

* Populations that deviate from the 1:1 ratio of random mating at $p < 0.05$, based on mating type distribution and frequencies.

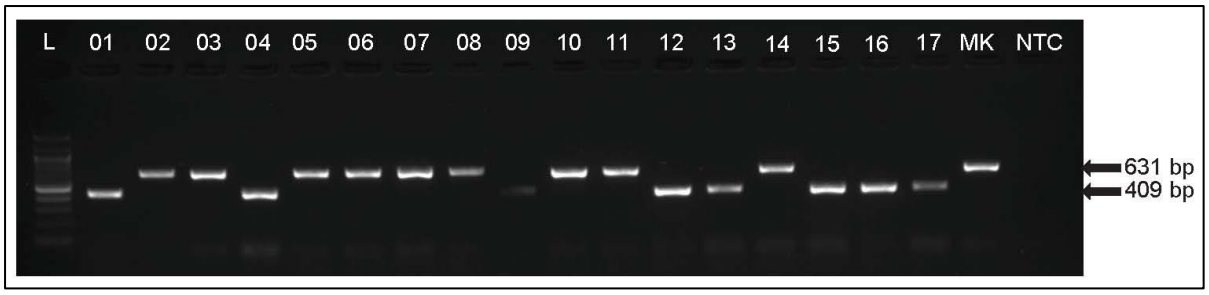


Figure 4.2: Agarose gel electrograph of mating type amplicons of *Cercospora zeina*: Amplicon sizes of 631 bp represent MAT1 individuals and those of 409 bp represent MAT2 individuals. A DNAMARK™ 100 bp size standard (G-Biosciences) was used as ladder in the first lane (L). 01-17 are individual *C. zeina* isolates screened for mating types. MK: *C. zeina* CMW25467 isolate (MAT1) used as the positive control. NTC: Non-template control.

4.4.2 Genetic diversity in *Cercospora zeina*

There were no clones observed in nine of the 25 populations while the clonal fractions for the remaining 14 populations ranged from 0.03 in Mthatha to 0.25 in Ntabamhlophe, with an average of 0.08 for the combined populations (Table 4.2). The genotypic diversity measured

based on standardized Shannon-Weiner index of MLG diversity ranged from 0.32 in Kisumu to 0.98 in Fortportal, with an average Shannon-Weiner index of MLG diversity of 1.00 when all isolates were pooled together (Table 4.2). Both results are consistent with the occurrence of sexual recombination.

Among the 835 isolates analyzed, 768 MLGs (92 %) were retrieved, with the most common genotypes being detected five (1 genotype) and seven (1 genotype) times (Figure 4.4A). Among the 768 MLGs, 735 were observed in at least one of the 25 locations sampled whereas 33 MLGs were observed in more than one location (Figure 4.4B). In total, 750 (97.7 %) genotypes were observed in one of the three sampling years, 2015, 2016 and 2017. A total of 17 genotypes were observed in two of the three sampling years and 10 of the 17 were observed in both 2016 and 2017 (data not shown). One (0.1 %) genotype was recovered in every sampling year (Figure 4.4C). This suggests that the frequency of shared genotypes was reduced over the sampling years.

4.4.3 Population differentiation and phylogenetic relatedness between MAT1 and MAT2 isolates

Population differentiation (G_{ST}) between MAT1 and MAT2 ranged from 0.01 in Kericho to 0.50 in Vihiga (Table 4.2). The highest G_{ST} values were observed in Chinhoyi, Kisumu, Siaya, and Vihiga where populations had a sample size of less than ten genotypes. The overall G_{ST} value after combining all populations was 0.01 (Table 4.2). A majority of the mating types were randomly distributed in phylogenetic clades (Figure 4.5 A and B) which is indicative of a lack of association between isolates that share a mating type.

Table 4.2: Tests of gametic disequilibrium, genotypic diversity and population differentiation of 25 *Cercospora zeina* populations collected between 2015 and 2017.

Country	Populations	Sample size	Clonal fraction	Shannon index	Index of association I_A (p-value)	G_{ST} between mating types
Kenya	Bungoma	12	0.00	0.47	0.01 (0.655)	0.12
	Kakamega	22	0.09	0.67	0.08 (0.054)	0.10
	Kericho*	41	0.07	0.70	0.22 (0.014)	0.01
	Kisumu*	8	0.13	0.32	0.47 (0.009)	0.45
	Kitale	24	0.04	0.71	0.11 (0.613)	0.06
	Siaya	6	0.00	0.45	0.28 (0.215)	0.26
	Transzoia*	26	0.00	0.70	0.05 (0.006)	0.03
	Vihiga	7	0.00	0.34	0.13 (0.648)	0.50
South Africa	Hlanganani*	72	0.13	0.77	0.02 (0.022)	0.09
	KwaNxamalala	37	0.14	0.63	0.04 (0.613)	0.09
	Mbizana*	80	0.08	0.79	0.07 (0.025)	0.03
	Mthatha*	31	0.03	0.74	0.01 (0.445)	0.04
	Ntabamhlophe*	20	0.25	0.73	0.71 (0.001)	0.12
	Ntabankulu	45	0.04	0.73	0.01 (0.294)	0.02
Uganda	Fortportal	49	0.00	0.98	0.14 (0.051)	0.03
	Kapchorwa	17	0.00	0.77	0.25 (0.050)	0.09
	Lira	18	0.06	0.68	0.10 (0.058)	0.16
	Masaka	46	0.00	0.78	0.02 (0.043)	0.07
	Wakiso*	41	0.05	0.85	0.25 (0.001)	0.07
Zambia	Chilanga*	29	0.17	0.72	0.20 (0.001)	0.03
	Chisamba	64	0.22	0.72	0.04 (0.309)	0.05
Zimbabwe	Chinhoyi	8	0.00	0.56	0.31 (0.166)	0.27
	Harare*	84	0.05	0.88	0.16 (0.001)	0.04
	KweKwe*	21	0.19	0.75	0.84 (0.001)	0.18
	Mutare	27	0.00	0.73	0.01 (0.447)	0.05

*Populations that deviated from random mating at $p < 0.05$, based on the Index of association (I_A).

4.4.4 Sexual crosses of *Cercospora zeina* isolates

The MAT1 and MAT2 pairings under the conditions tested did not result in the production of sexual structures (pseudothecia with bitunicate asci containing two-celled ascospores). In many cases, a zone of compatibility barrier was observed based on the failure of mycelia from the opposite mating types to make contact (Figure 4.6A and 4.6B). Only conidiophores and conidia were observed from the *in-planta* experiment (Figure 4.6E to 4.6H). Chlamydospores and other mycelial-based structures were observed in some of the crosses between different MAT types, suggesting that the sexual stage was not successfully induced (Figure 4.6I to 4.6L).

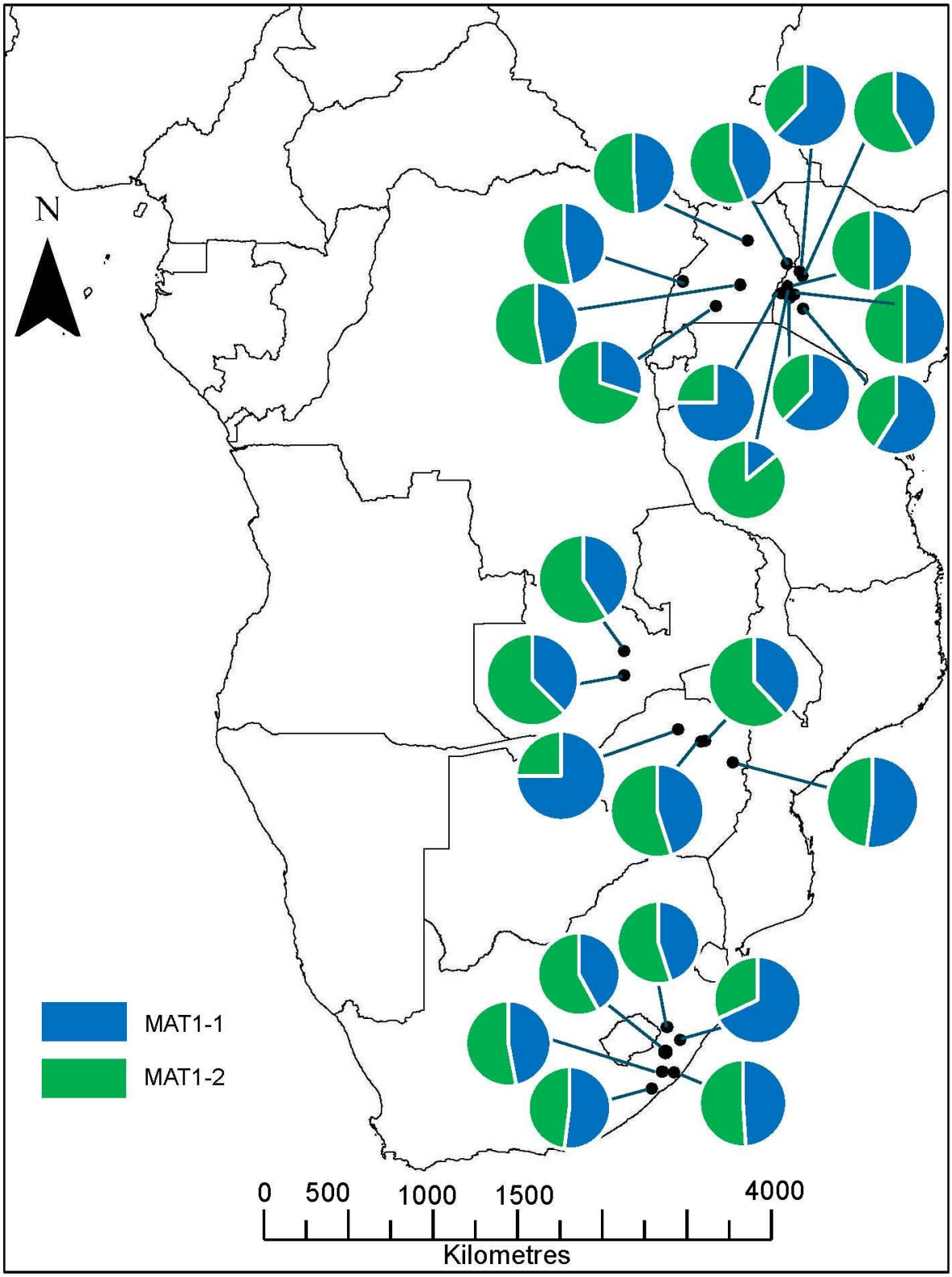


Figure 4.3: Map showing the spatial scale distribution of mating type idiomorphs in 25 locations in five sub-Saharan African countries. Pie charts represent the distribution of mating types in Kenya, South Africa, Uganda, Zambia, and Zimbabwe. Blue: MAT1 and Green: MAT2.

4.4.5 Mapping of the microsatellite markers and *MAT1-1* gene to the *Cercospora zeina* genome

All the 11 microsatellite markers and *MAT1-1* gene were successfully mapped to the *C. zeina* CMW25467 PacBio re-sequenced genome and were present on different contigs and positions (Figure 4.7). Three markers are located on contig Czeina_00002F, which is speculated to be one of the fully assembled chromosomes (Kabwe & Berger, personal communication). Interestingly, the mating type gene *MAT1-1* was found in proximity to microsatellite marker CzSSR18 on contig Czeina_00001F.

4.5 DISCUSSION

In total, 11 microsatellite and two mating type idiomorphs were employed in this study to indirectly assess the relative importance of both asexual and sexual reproduction on the genetic structure of *C. zeina* populations. From this study, the major findings include: i) sexual recombination plays a central role in the disease epidemic and evolution of *C. zeina,* ii) there is a lack of genetic differentiation and phylogenetic association between mating type genes *MAT1-1* and *MAT1-2* of the pathogen and; iii) no sexual stage was induced under laboratory or greenhouse conditions.

4.5.1 Equal mating type frequencies and low linkage disequilibrium

Isolates with equal frequencies of mating types exist in 23 of the 25 populations tested, thus retaining the null hypothesis of a 1:1 ratio of random mating. This therefore maximizes the probability of sexually compatible *C. zeina* individuals to recombine in nature. Equal mating type frequencies have also been reported previously in *C. zeina* populations from South Africa and in other *Cercospora* species (Groenewald *et al.*, 2006; Kim *et al.*, 2013; Muller *et al.*, 2016; Nsibo *et al.*, 2019). Equal mating type frequencies among *C. zeina* populations may be attributed to frequency-dependent selection (May *et al.*, 1999). This is a balancing type of selection that favors rare alleles by increasing the probability of individuals harboring them to

find compatible partners for recombination, which homogenizes the mating type frequencies (Richman, 2000; Zhan *et al.*, 2002).

Mating types occurred at slightly different frequencies in KwaNxamalala and Masaka. These populations were either skewed towards MAT1 or MAT2. This suggests that one mating type expresses no advantage over the other and argues against any selection bias in favor of one mating type. This is consistent with the lack of selection bias among mating types that has been previously reported in *Rhynchosporium secalis*, the scald pathogen of barley (Linde *et al.*, 2003). In a sexually recombining population as is suggested for *C. zeina*, the frequency of the dominant mating type can reduce its chances of recombining while increasing that of the rare mating type until the probability of finding a compatible partner is maximized for the rare mating type (Sommerhalder *et al.*, 2006).

Results have further revealed low levels of linkage disequilibrium among populations which suggests that a majority of the alleles were randomly associated across populations. The hypothesis of random mating was therefore, supported and we can, in addition to the observed equal mating type frequencies, conclude that genetic recombination likely occurs often and substantially drives the observed genetic diversity of the pathogen at a local and continental scale. This is consistent with the previous reports on *C. zeina* and other *Cercospora* species which have shown signatures of sexual recombination based on high genetic diversity, equal mating type frequencies and low levels of linkage disequilibrium (Groenewald *et al.*, 2006; Kim *et al.*, 2013; Muller *et al.*, 2016; Nsibo *et al.*, 2019). There is also consistency with previous findings on other cereal pathogens whose sexual stages are known including *Zymoseptoria tritici* (Zhan *et al.*, 2003) and *Fusarium graminearum* (Zeller *et al.*, 2004).

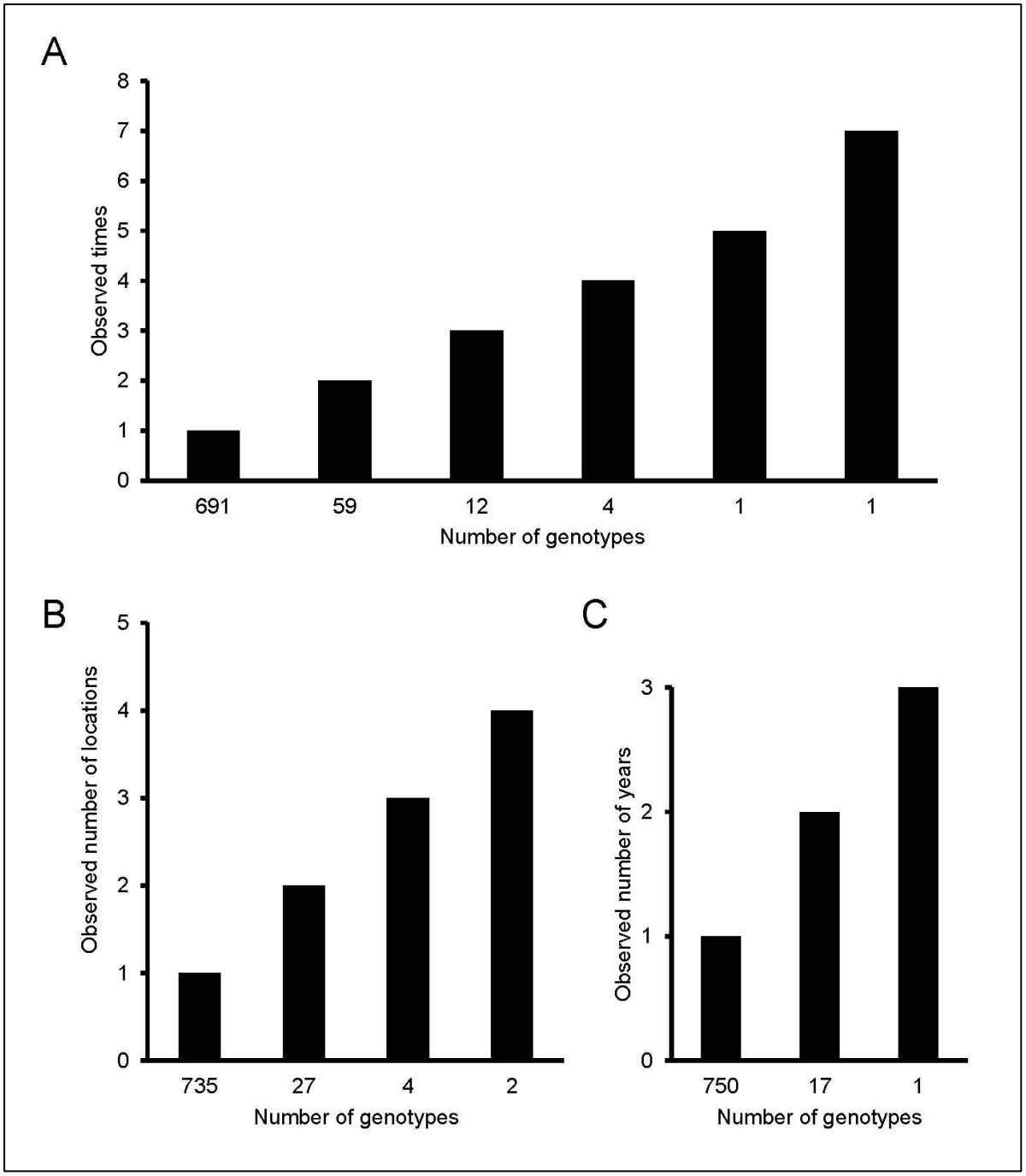


Figure 4.4: Spatial distribution of 768 multilocus genotypes recovered from 835 isolates collected from 25 *Cercospora zeina* populations: These were collected between 2015 and 2017. A: Number of times different genotypes were observed over the sampling years. The highest number of genotypes was observed only once during the sampling years while two genotypes were each observed five and seven times respectively. B: Locations in which the different genotypes were observed during the sampling years. Most genotypes were observed in a single location while the most widespread genotypes were observed in four locations. C: Number of years in which identical genotypes were observed.

4.5.2 Varying levels of clonality among *Cercospora zeina* populations

Clonality analysis revealed that 8 % (67/835) identical genotypes were recovered from different populations with clonality between 13 and 25 %. These results confirm that asexual reproduction, although weakly represented, may influence the population structure of *C. zeina* through the sharing of genotypes within and among fields over short distances of 50 km within the same countries. Our findings are consistent with other studies where a high level of clonality was reported to exist among *C. zeina* populations from commercial farms in South Africa, owing to the use of resistant maize genotypes and fungicides as well as producing abundant conidia as its mechanism of dispersal (Muller *et al.*, 2016; Nsibo *et al.*, 2019). Prevalence of clones in any given population can promote divergence between alleles within loci as a result of mutational events that can occur over time within the genomes of pathogens (Birky, 1996; Goyeau *et al.*, 2007). Our results showing low levels of clonality could, therefore, be explained by mutation as another evolutionary force that may shape the population structure of *C. zeina*.

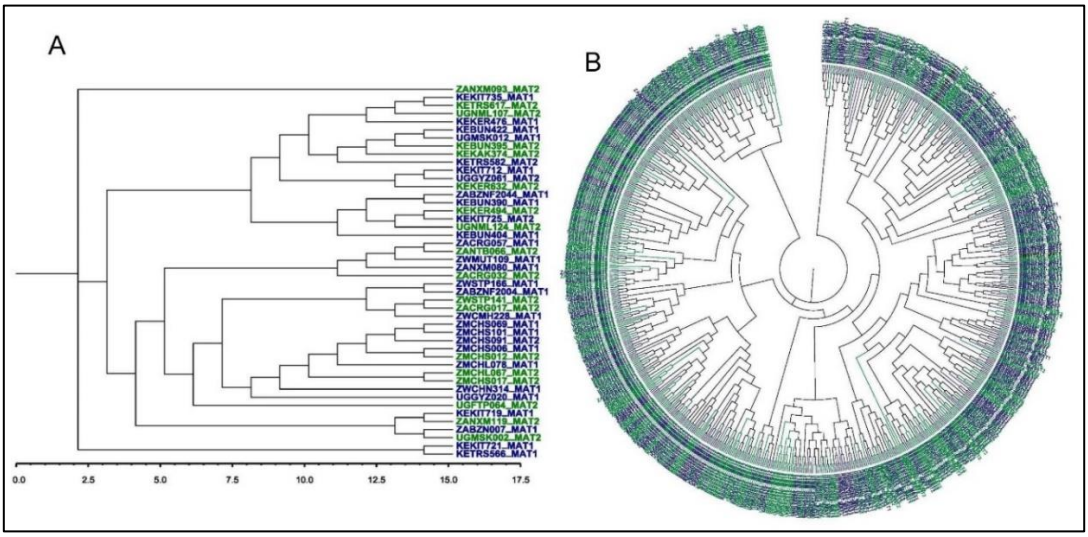


Figure 4.5: Phylogenetic relatedness of mating type genes of *Cercospora zeina*. A: Phylogenetic tree of 46 randomly selected *C. zeina* isolates carrying either *MAT1-1* or *MAT1-2* idiomorphs. Tree was constructed using microsatellite data by UPGMA and visualized using Fig Tree software v1.4 (Rambaut, 2009). B: Neighbor joining tree of all the 835 *C. zeina* isolates assigned with definite mating types. Tree was constructed using Nei's genetic distance using GenAlEx software (Peakall & Smouse, 2012) and a phylogenetic tree reconstructed using poppr. In both trees, mating types were randomly distributed, suggesting that there is no genetic association between isolates from the same mating types. Blue = MAT1, and Green = MAT2.

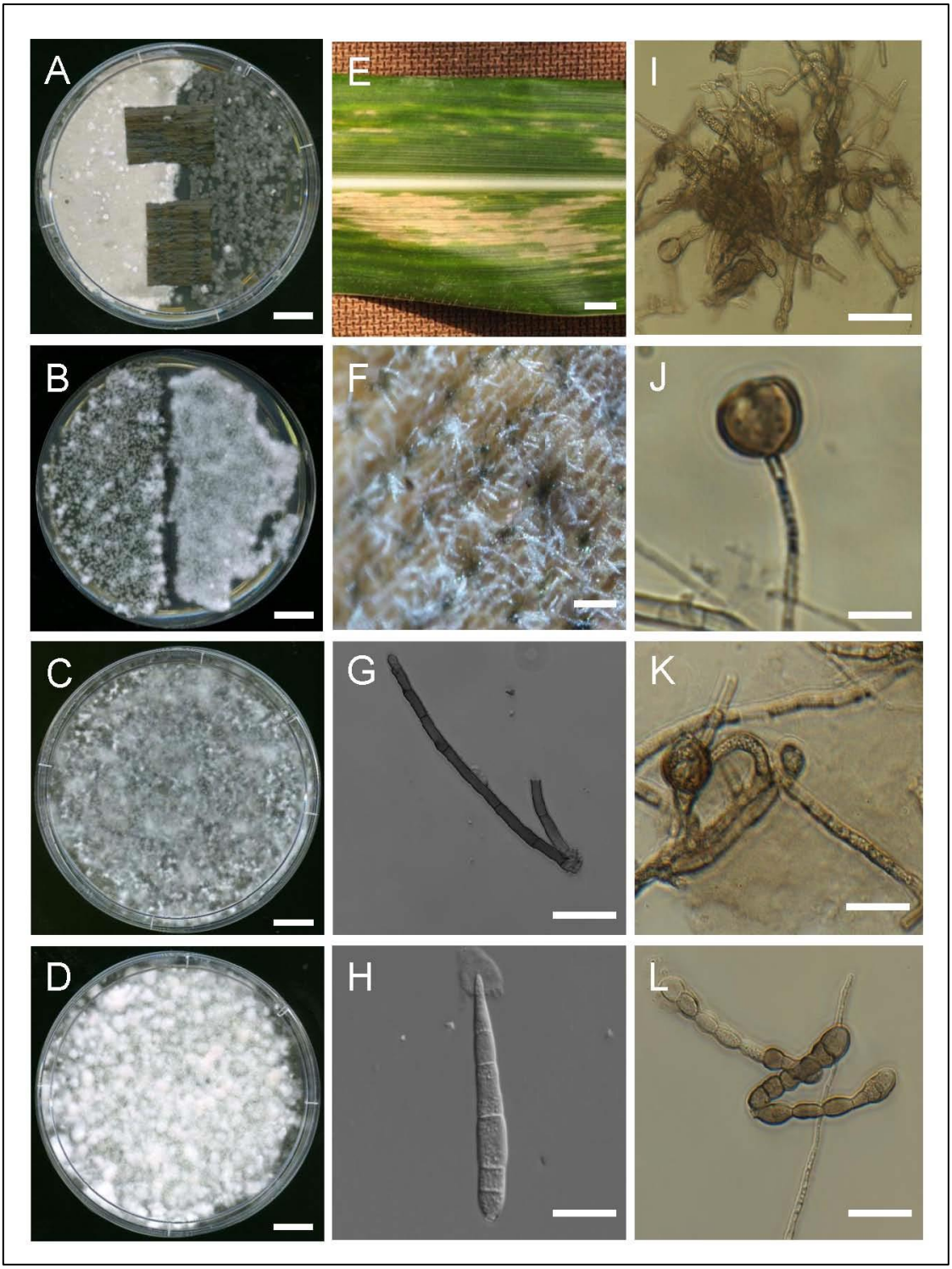


Figure 4.6: Structures observed from the *Cercospora zeina* mating type crosses: A and B: Zones of compatibility barriers based on MAT1 and MAT2 crosses in the presence of a substrate (A) and without substrate (B) with no mating type growing into each other. C and D: Cultures after crossing isolates of the same mating type together with no zones of compatibility barriers. E: Lesions from the mating type crosses. F: *C. zeina* conidia on top of the lesions after successful colonization of the maize leaves by the mixed mating types. G: *C. zeina* conidiophore. H: *C. zeina* conidium. I to K: Chlamydospores developed from the different mating combinations of MAT1 and MAT2. L: Mycelial structures from some mating combinations. In all crosses, there were no sexual structures observed. Scale bars: A to D = 0.5 cm. E = 2 cm. F = 100 µm. G to L = 10 µm.

4.5.3 High genotypic diversity among *Cercospora zeina* populations

The observed high genotypic diversity among all populations is very likely due to sexual recombination, consistent with the previous reports on the pathogen (Muller *et al.*, 2016; Nsibo *et al.*, 2019). Similar results exist in other *Cercospora* species that are predominantly asexual in nature and conclusions of cryptic sexual recombination were reached (Groenewald *et al.*, 2006; Kim *et al.*, 2013). Such high levels of genotypic diversity are also common and comparable to other cereal fungi with frequent incidences of sexual recombination, particularly the wheat pathogens *Phaeosphaeria nodorum* (Sommerhalder *et al.*, 2010; Sommerhalder *et al.*, 2006) and *Z. tritici* (Zhan *et al.*, 2003). Sexual recombination is reported to breed novel recombinants with decreased effects from linked deleterious alleles. It allows the maintenance of high gene diversity through the process of intragenic recombination among sexually reproducing plant pathogens (Taylor *et al.*, 2017; Zhan *et al.*, 2003). Thus, presence of sexual recombination among *C. zeina* populations may result into more adaptable genotypes to its host and the environment as well as acquisition of more virulence to overcome several levels of host resistance, specifically in the absence of effective management and monitoring.

4.5.4 Low genetic differentiation among *Cercospora zeina* mating types

When populations were grouped into two sub-populations comprising each mating type, the genetic differentiation between the two groups was very low, suggesting occurrence of a high degree of genetic exchange. Pooling isolates between fields to form a country population also revealed very low population differentiation among mating types. In contrast, four populations, Chinhoyi, Kisumu, Siaya and Vihiga revealed moderate to high population differentiation. This may be attributed to sampling bias as very few samples were analyzed from these populations. To further test for the occurrence of population differentiation, the phylogenetic analyses showed no genetic structure between the two sub-populations and no clones carried different mating types. These results together with other population genetic analyses particularly, genotypic diversity, equal mating type frequencies and distribution, gametic equilibrium can be interpreted as evidence for a history of sexual recombination among *C. zeina* as has been

shown in other *Cercospora* species, particularly *C. beticola* (Groenewald *et al.*, 2008; Knight *et al.*, 2019; Vaghefi, Nelson*, et al.*, 2017).

4.5.5 No sexual stage observed in both glasshouse and laboratory conditions.

The induction of sexual structures was unsuccessful following several mating type combinations under a relatively wide range of conditions. This may be attributed to several biological, environmental, and ecological factors that are crucial to the sexual induction process (Nel *et al.*, 2018). In addition to the presence of mating type genes, several other factors are needed for the induction of sexual reproduction. Signaling pathways including striatin-interacting phosphate and kinase (STRIPAK) and COP9 signalosome (CSN) complexes play an integral role in the induction of sexual reproduction. Extensive studies of their role in regulating ascomata production have been conducted in *Aspergillus nudilans* and *Sordaria macrospora* (Busch *et al.*, 2003; Teichert *et al.*, 2014). Other factors implicated include several environmental factors such as light and its perception, expression of genes that regulate reactive oxygen species production, and the availability of pheromones (Wilson *et al.*, 2019). Since the draft genome of *C. zeina* is available (Wingfield *et al.*, 2017), these processes need to be explored to confirm their functional relevance in sexual reproduction in this fungus and how they can be manipulated to allow for the development of sexual structures. Additionally, nutrient requirements such as sugars, amino acids and minerals are also important (Wilson *et al.*, 2019). Absence of many of these factors may therefore hinder the induction of sex and the production of sexual structures. Their further manipulation such as the use of fertilizing agents such as spermatia may ultimately allow the induction of sexual structures in *C. zeina.* The observation of chlamydospores is indicative of *C. zeina*'s ability to develop structures that can persist for a long time in the absence of favorable conditions for growth. This survival mechanism could explain its ability to remain viable for a long period on media whose nutrients have depleted. Chlamydospores have also been reported in other

Cercospora species as survival structures that are observed during the overwintering stage of these pathogens (Adams *et al.*, 1995; Nuesry, 1981; Price III, 2013).

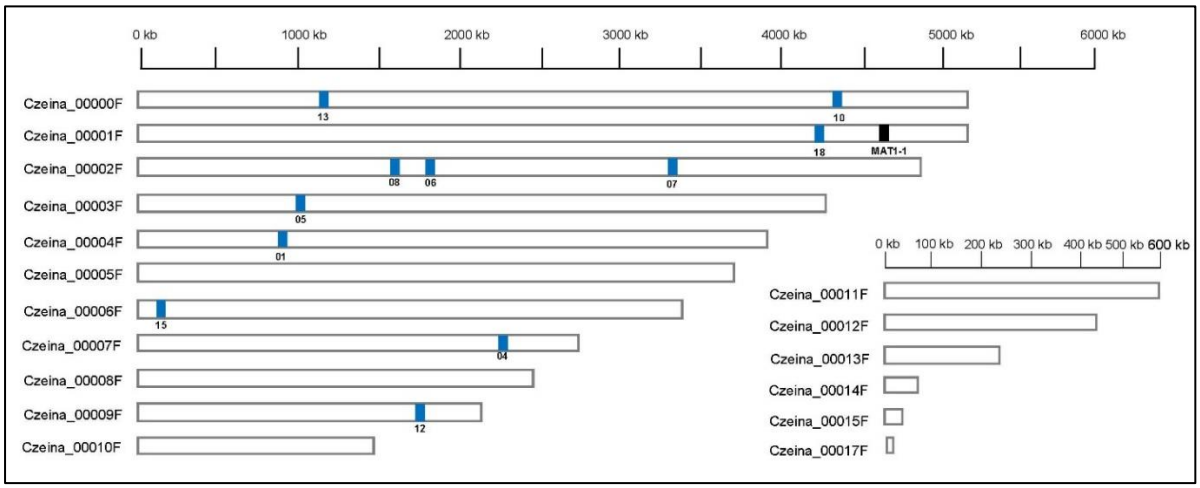


Figure 4.7: Overview of the microsatellite markers and *MAT1-1* gene mapped to the PacBio re-sequenced *Cercospora zeina* CMW25467 genome: Schematic representation of the 17 contigs (gray open bars) in the recently re-sequenced genome assembly of *C. zeina*. A majority of the markers are well spaced within the genome with no overlaps. The *MAT1-1* gene is closely linked to microsatellite CzSSR18 in the genome. Czeina_00000F-Czeina00017F are the contig numbers. Blue bars represent the different microsatellite markers (given in numbers) and their locations on the different contigs. Black bars represent the *MAT1-1* gene and its location on the genome. Different scale bars (kb) are shown above contigs Czeina_00000F to Czeina_00010F; and contigs Czeina_00011F to Czeina_00017F

4.5.6 Mapping of the microsatellite markers and *MAT1-1* gene to the *Cercospora zeina* genome

Microsatellite markers have been characterized in various *Cercospora* species (Cai & Schneider, 2008; Kim *et al.*, 2013; Vaghefi, Kikkert*, et al.*, 2017), but this is the first study in which they have been mapped on the genome and more specifically in *C. zeina*. Although all microsatellite markers were previously confirmed to be polymorphic and have been used in understanding the population structure of the *C. zeina* populations (Muller *et al.*, 2016), their positions on the genome were not known. This study has precisely determined their positions and distribution, revealing that they are well distributed among the major contigs of the genome, thus, confirming that they are unlinked and are randomly segregating. Interestingly, marker CzSSR18 is in proximity to the *MAT1*locus, which may suggest that there could be more markers that are located close or within putative gene regions. However, no further

analysis was conducted to prove that this marker is within an open reading frame. This study is therefore a foundation to further studies that will determine locations of these markers in relation to noncoding, intronic and coding regions. Coding regions are shown to exhibit some similarity between species (Metzgar *et al.*, 2000). Although all these markers have been tested on both *C. zeina* and *C. zeae-maydis* and have showed specificity to *C. zeina* (Muller *et al.*, 2016), tests of any of these markers that lie within the coding regions need to be conducted on other *Cercospora* species to further confirm species specificity or select markers that would be used to differentiate between several other *Cercospora* species. A similar study has been conducted in *C. fimbriata* where microsatellite markers were mapped to its genome and results showed that ten newly developed markers localized within putative genes. These markers successfully differentiated between several species in the *C. fimbriata* s.l. complex (Simpson *et al.*, 2013).

4.6 CONCLUSION

Upon combining our results of high genotypic diversity, random association among alleles across the 11 microsatellite markers, equal mating type frequencies, as well as the lack of population differentiation among mating types, we conclude that *C. zeina* likely undergoes regular sexual cycles in nature. With sexual recombination, *C. zeina* can acquire novel strains which are more virulent. Therefore, consistent monitoring of the disease and a systematic search for the sexual stage in nature and laboratory conditions is needed to further shed light on the biology of the pathogen. Occurrence and the biology of the sexual stage of *C. zeina* remain unclear. Therefore, its discovery will provide new knowledge of the complete life cycle of the pathogen. This can be incorporated into epidemiological and yield loss models of GLS to allow predictions of potential epidemics and yield losses and as such foster improved management strategies that limit its spread and impact on maize.

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4.8 REFERENCES

Adams, H., Schäufele, W., & Märländer, B. (1995). A method for the artificial inoculation of sugarbeet with *Cercospora beticola* under field conditions/Eine Methode zur künstlichen Inokulation von Zuckerrüben mit *Cercospora beticola* unter Freilandbedingungen. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection*, 320-322.

Agapow, P. M., & Burt, A. (2001). Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes, 1*(1-2), 101-102.

Ali, S., Gladieux, P., Leconte, M., Gautier, A., Justesen, A. F., Hovmøller, M. S., & de Vallavieille-Pope, C. (2014). Origin, migration routes and worldwide population genetic structure of the wheat yellow rust pathogen *Puccinia striiformis* f. sp. *tritici*. *PLoS Pathogens, 10*(1), e1003903.

Ali, S., Leconte, M., Walker, A.-S., Enjalbert, J., & de Vallavieille-Pope, C. (2010). Reduction in the sex ability of worldwide clonal populations of *Puccinia striiformis* f. sp. *tritici*. *Fungal Genetics and Biology, 47*(10), 828-838.

Bihon, W., Burgess, T., Slippers, B., Wingfield, M. J., & Wingfield, B. D. (2012). High levels of genetic diversity and cryptic recombination is widespread in introduced *Diplodia pinea* populations. *Australasian Plant Pathology, 41*(1), 41-46.

Bihon, W., Wingfield, M. J., Slippers, B., Duong, T. A., & Wingfield, B. D. (2014). *MAT* gene idiomorphs suggest a heterothallic sexual cycle in a predominantly asexual and important pine pathogen. *Fungal Genetics and Biology, 62*, 55-61.

Birky, C. W. (1996). Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. *Genetics, 144*(1), 427-437.

Bolton, M. D., Secor, G. A., Rivera, V., Weiland, J. J., Rudolph, K., Birla, K., & Campbell, L. G. (2012). Evaluation of the potential for sexual reproduction in field populations of *Cercospora beticola* from USA. *Fungal Biology, 116*(4), 511-521.

Busch, S., Eckert, S. E., Krappmann, S., & Braus, G. H. (2003). The COP9 signalosome is an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. *Molecular Microbiology, 49*(3), 717-730.

Cai, G., & Schneider, R. (2008). Population structure of *Cercospora kikuchii*, the causal agent of *Cercospora* leaf blight and purple seed stain in soybean. *Phytopathology, 98*(7), 823-829.

Chen, R.-S., & McDonald, B. A. (1996). Sexual reproduction plays a major role in the genetic structure of populations of the fungus *Mycosphaerella graminicola*. *Genetics, 142*(4), 1119-1127.

Coppin, E., Debuchy, R., Arnaise, S., & Picard, M. (1997). Mating types and sexual development in filamentous ascomycetes. *Microbiology and Molecular Biology Reviews, 61*(4), 411-428.

Couch, B. C., Fudal, I., Lebrun, M.-H., Tharreau, D., Valent, B., Van Kim, P., Kohn, L. M. (2005). Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. *Genetics, 170*(2), 613-630.

Crous, P. W., & Braun, U. (2003). *Mycosphaerella and its anamorphs: 1. Names published in Cercospora and Passalora*: Centraalbureau voor Schimmelcultures (CBS).

Crous, P. W., Kang, J.-C., & Braun, U. (2001). A phylogenetic redefinition of anamorph genera in *Mycosphaerella* based on ITS rDNA sequence and morphology. *Mycologia, 93*(6), 1081-1101.

Debuchy, R., & Turgeon, B. (2006). Mating-type structure, evolution, and function in Euascomycetes. In: Kües U., Fischer R. (eds) growth, differentiation and sexuality. *The Mycota* (A comprehensive treatise on fungi as experimental systems for basic and applied research), vol 1. Springer, Berlin, Heidelberg.

Dyer, P. S., & O'Gorman, C. M. (2011). A fungal sexual revolution: *Aspergillus* and *Penicillium* show the way. *Current Opinion in Microbiology, 14*(6), 649-654.

Ellegren, H., & Parsch, J. (2007). The evolution of sex-biased genes and sex-biased gene expression. *Nature Reviews Genetics, 8*(9), 689.

Everitt, B. S. (1992). *The analysis of contingency tables*: Chapman and Hall/CRC.

Giraud, T., Enjalbert, J., Fournier, E., Delmotte, F., & Dutech, C. (2008). Population genetics of fungal diseases of plants. *Parasite, 15*, 449-454.

Goodwin, S. B., Dunkle, L. D., & Zismann, V. L. (2001). Phylogenetic analysis of *Cercospora* and *Mycosphaerella* based on the internal transcribed spacer region of ribosomal DNA. *Phytopathology, 91*(7), 648-658.

Goyeau, H., Halkett, F., Zapater, M.-F., Carlier, J., & Lannou, C. (2007). Clonality and host selection in the wheat pathogenic fungus *Puccinia triticina*. *Fungal Genetics and Biology, 44*(6), 474-483.

Groenewald, M., Groenewald, J. Z., Harrington, T. C., Abeln, E. C., & Crous, P. W. (2006). Mating type gene analysis in apparently asexual *Cercospora* species is suggestive of cryptic sex. *Fungal Genetics and Biology, 43*(12), 813-825.

Groenewald, M., Linde, C., Groenewald, J. Z., & Crous, P. W. (2008). Indirect evidence for sexual reproduction in *Cercospora beticola* populations from sugar beet. *Plant Pathology, 57*(1), 25-32.

Gross, A., Zaffarano, P. L., Duo, A., & Grünig, C. (2012). Reproductive mode and life cycle of the ash dieback pathogen *Hymenoscyphus pseudoalbidus*. *Fungal Genetics and Biology, 49*(12), 977-986.

Halama, P. (1996). The occurrence of *Mycosphaerella graminicola*, teleomorph of *Septoria tritici* in France. *Plant Pathology, 45*(1), 135-138.

Houbraken, J., & Dyer, P. S. (2015). Induction of the sexual cycle in filamentous ascomycetes. In: van den Berg M., Maruthachalam K. (eds) *Genetic Transformation Systems in Fungi*, Volume 2 (pp. 23-46). Fungal Biology. Springer, Cham.

Jin, Y., Szabo, L. J., & Carson, M. (2010). Century-old mystery of *Puccinia striiformis* life history solved with the identification of Berberis as an alternate host. *Phytopathology, 100*(5), 432-435.
Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ, 2*, e281.
Kim, H., Newell, A. D., Cota-Sieckmeyer, R. G., Rupe, J. C., Fakhoury, A. M., & Bluhm, B. H. (2013). Mating-type distribution and genetic diversity of *Cercospora sojina* populations on soybean from Arkansas: evidence for potential sexual reproduction. *Phytopathology, 103*(10), 1045-1051.
Knight, N. L., Vaghefi, N., Kikkert, J. R., Bolton, M. D., Secor, G. A., Rivera, V. V., Pethybridge, S. J. (2019). Genetic diversity and structure in regional *Cercospora beticola* populations from *Beta vulgaris* subsp*. vulgaris* suggest two clusters of separate origin. *Phytopathology*, PHYTO-07-18-0264-R.
Kronstad, J., & Staben, C. (1997). Mating type in filamentous fungi. *Annual Review of Genetics, 31*(1), 245-276.
Kück, U., & Pöggeler, S. (2009). Cryptic sex in fungi. *Fungal Biology Reviews, 23*(3), 86-90.
Lee, S., Milgroom, M., & Taylor, J. (1988). A rapid, high yield mini-prep method for isolation of total genomic DNA from fungi. *Fungal Genetics Reports, 35*(1), 23.
Lee, S. C., Ni, M., Li, W., Shertz, C., & Heitman, J. (2010). The evolution of sex: a perspective from the fungal kingdom. *Microbiology and Molecular Biology Reviews, 74*(2), 298-340.
Lin, X., & Heitman, J. (2007). Mechanisms of homothallism in fungi and transitions between heterothallism and homothallism *Sex in fungi* (pp. 35-57): American Society of Microbiology.
Lin, X., Hull, C. M., & Heitman, J. (2005). Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. *Nature, 434*(7036), 1017.
Linde, C. C., Zala, M., Ceccarelli, S., & McDonald, B. A. (2003). Further evidence for sexual reproduction in *Rhynchosporium secalis* based on distribution and frequency of mating-type alleles. *Fungal Genetics and Biology, 40*(2), 115-125.
Martin, S. H., Wingfield, B. D., Wingfield, M. J., & Steenkamp, E. T. (2011). Structure and evolution of the *Fusarium* mating type locus: new insights from the *Gibberella fujikuroi* complex. *Fungal Genetics and Biology, 48*(7), 731-740.
May, G., Shaw, F., Badrane, H., & Vekemans, X. (1999). The signature of balancing selection: fungal mating compatibility gene evolution. *Proceedings of the National Academy of Sciences, 96*(16), 9172-9177.
McDonald, B. A., & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology, 40*(1), 349-379.
Meng, J.-W., Zhu, W., He, M.-H., Wu, E.-J., Duan, G.-H., Xie, Y.-K., Zhan, J. (2015). Population genetic analysis reveals cryptic sex in the phytopathogenic fungus *Alternaria alternata*. *Scientific Reports, 5*, 18250.
Metzenberg, R. L., & Glass, N. L. (1990). Mating type and mating strategies in *Neurospora*. *Bioessays, 12*(2), 53-59.
Metzgar, D., Bytof, J., & Wills, C. (2000). Selection against frameshift mutations limits microsatellite expansion in coding DNA. *Genome Research, 10*(1), 72-80.
Milgroom, M. G. (1996). Recombination and the multilocus structure of fungal populations. *Annual Review of Phytopathology, 34*(1), 457-477.
Möller, M., & Stukenbrock, E. H. (2017). Evolution and genome architecture in fungal plant pathogens. *Nature Reviews Microbiology, 15*(12), 756.
Muller, M. F., Barnes, I., Kunene, N. T., Crampton, B. G., Bluhm, B. H., Phillips, S. M., Berger, D. K. (2016). *Cercospora zeina* from maize in South Africa exhibits high genetic diversity and lack of regional population differentiation. *Phytopathology, 106*(10), 1194-1205.
Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics, 89*(3), 583-590.

Nel, W., Duong, T., Wingfield, M., Wingfield, B., Hammerbacher, A., & de Beer, Z. (2018). Heterothallism revealed in the root rot fungi *Berkeleyomyces basicola* and *B. rouxiae*. *Fungal Biology, 122*(11), 1031-1040.

Nsibo, D. L., Barnes, I., Kunene, N. T., & Berger, D. K. (2019). Influence of farming practices on the population genetics of the maize pathogen *Cercospora zeina* in South Africa. *Fungal Genetics and Biology, 125*, 36-44.

Nuesry, S. M. (1981). *Survival of Cercospora arachidicola, Cercosporidium personatum, and primary infection of peanuts in Oklahoma.* Oklahoma State University.

O'Gorman, C. M., Fuller, H. T., & Dyer, P. S. (2009). Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Nature, 457*(7228), 471.

Peakall, R., & Smouse, P. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and researchd-an update. *Bioinformatics, 28*, 2537-2539.

Price III, P. P. (2013). Sensitivity and resistance of *Cercospora kikuchii*, causal agent of *Cercospora* leaf blight and purple seed stain of soybean, to selected fungicides. (Doctoral dissertation), Louisiana Tech University.

Rambaut, A. (2009). FigTree v1. 4 2012–2014: Tree Figure Drawing Tool. *Recuperado de:* http://tree. bio. ed. ac. uk/software/figtree.

Richman, A. (2000). Evolution of balanced genetic polymorphism. *Molecular Ecology, 9*(12), 1953-1963.

Saleh, D., Xu, P., Shen, Y., Li, C., Adreit, H., Milazzo, J., & Fournier, E. (2012). Sex at the origin: an Asian population of the rice blast fungus *Magnaporthe oryzae* reproduces sexually. *Molecular Ecology, 21*(6), 1330-1344.

Shannon, C. E. (2001). A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing and Communications Review, 5*(1), 3-55.

Simpson, M. C., Wilken, P. M., Coetzee, M. P., Wingfield, M. J., & Wingfield, B. D. (2013). Analysis of microsatellite markers in the genome of the plant pathogen *Ceratocystis fimbriata*. *Fungal Biology, 117*(7-8), 545-555.

Sommerhalder, R. J., McDonald, B. A., Mascher, F., & Zhan, J. (2010). Sexual recombinants make a significant contribution to epidemics caused by the wheat pathogen *Phaeosphaeria nodorum*. *Phytopathology, 100*(9), 855-862.

Sommerhalder, R. J., McDonald, B. A., & Zhan, J. (2006). The frequencies and spatial distribution of mating types in *Stagonospora nodorum* are consistent with recurring sexual reproduction. *Phytopathology, 96*(3), 234-239.

Sugui, J. A., Losada, L., Wang, W., Varga, J., Ngamskulrungroj, P., Abu-Asab, M., & Nierman, W. C. (2011). Identification and characterization of an *Aspergillus fumigatus* "supermater" pair. *Microbiology, 2*(6), e00234-00211.

Taylor, J., Jacobson, D., & Fisher, M. (1999). The evolution of asexual fungi: reproduction, speciation and classification. *Annual Review of Phytopathology, 37*(1), 197-246.

Taylor, J. W., Branco, S., Gao, C., Hann-Soden, C., Montoya, L., Sylvain, I., & Gladieux, P. (2017). Sources of fungal genetic variation and associating it with phenotypic diversity. *Gene, 18*, 19.

Teichert, I., Nowrousian, M., Pöggeler, S., & Kück, U. (2014). The filamentous fungus *Sordaria macrospora* as a genetic model to study fruiting body development *Advances in Genetics* 87, 199-244.

Thorvaldsdóttir, H., Robinson, J. T., & Mesirov, J. P. (2013). Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Briefings in Bioinformatics, 14*(2), 178-192.

Turgeon, B. G. (1998). Application of mating type gene technology to problems in fungal biology. *Annual Review of Phytopathology, 36*(1), 115-137.

Turgeon, B. G., & Yoder, O. (2000). Proposed nomenclature for mating type genes of filamentous ascomycetes. *Fungal Genetics and Biology, 31*(1), 1-5.

Vaghefi, N., Kikkert, J. R., Bolton, M. D., Hanson, L. E., Secor, G. A., & Pethybridge, S. J. (2017). De novo genome assembly of *Cercospora beticola* for microsatellite marker development and validation. *Fungal Ecology, 26*, 125-134.

Vaghefi, N., Nelson, S. C., Kikkert, J. R., & Pethybridge, S. J. (2017). Genetic structure of *Cercospora beticola* populations on *Beta vulgaris* in New York and Hawaii. *Scientific Reports, 7*(1), 1726.

Ware, S. B., Verstappen, E. C., Breeden, J., Cavaletto, J. R., Goodwin, S. B., Waalwijk, C., & Kema, G. H. (2007). Discovery of a functional *Mycosphaerella* teleomorph in the presumed asexual barley pathogen *Septoria passerinii*. *Fungal Genetics and Biology, 44*(5), 389-397.

Whitlock, M. C. (2000). Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles, and sexual selection. *Evolution, 54*(6), 1855-1861.

Wilken, P. M., Steenkamp, E. T., Wingfield, M. J., De Beer, Z. W., & Wingfield, B. D. (2014). DNA loss at the *Ceratocystis fimbriata* mating locus results in self-sterility. *PLoS One, 9*(3), e92180.

Wilson, A. M., Godlonton, T., Van der Nest, M. A., Wilken, P. M., Wingfield, M. J., & Wingfield, B. D. (2015). Unisexual reproduction in *Huntiella moniliformis*. *Fungal Genetics and Biology, 80*, 1-9.

Wilson, A. M., Wilken, P. M., van der Nest, M. A., Wingfield, M. J., & Wingfield, B. D. (2019). It's All in the Genes: The regulatory pathways of sexual reproduction in filamentous ascomycetes. *Genes, 10*(5), 330.

Wingfield, B. D., Berger, D. K., Steenkamp, E. T., Lim, H.-J., Duong, T. A., Bluhm, B. H., Wingfield, M. J. (2017). Draft genome of *Cercospora zeina*, *Fusarium pininemorale, Hawksworthiomyces lignivorus*, *Huntiella decipiens* and *Ophiostoma ips*. *IMA Fungus, 8*(2), 385-396.

Zeller, K. A., Bowden, R. L., & Leslie, J. F. (2004). Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from the United States. *Molecular Ecology, 13*(3), 563-571.

Zhan, J., Kema, G. H., Waalwijk, C., & McDonald, B. A. (2002). Distribution of mating type alleles in the wheat pathogen *Mycosphaerella graminicola* over spatial scales from lesions to continents. *Fungal Genetics and Biology, 36*(2), 128-136.

Zhan, J., Pettway, R. E., & McDonald, B. A. (2003). The global genetic structure of the wheat pathogen *Mycosphaerella graminicola* is characterized by high nuclear diversity, low mitochondrial diversity, regular recombination, and gene flow. *Fungal Genetics and Biology, 38*(3), 286-297.

Zhou, X., O'Donnell, K., Kim, H.-S., Proctor, R. H., Doehring, G., & Cao, Z.-M. (2018). Heterothallic sexual reproduction in three canker-inducing tree pathogens within the *Fusarium torreyae* species complex. *Mycologia, 110*(4), 710-725.

CHAPTER 5

General discussion and future prospects

5.1 INTRODUCTION

By 2050, the global and sub-Saharan Africa (SSA) food demands are estimated to reach 60 and 300 % of the current production, respectively, owing to the fast-growing human population (Alexandratos & Bruinsma, 2012; Van Ittersum *et al.*, 2016). Thus, there is a greater necessity to upscale the current food production by 70 % (Tripathi *et al.*, 2019). The annual cereal production alone must raise from the current 2.1 billion tons to more than 3 billion tons to meet the required global food security demands (FAO, 2019). Maize is the most widely cultivated and major security crop in SSA (Tesfaye *et al.*, 2015) and yet formidable abiotic and biotic stresses, particularly, low soil fertility, drought, weeds, pests, and diseases, continue to threaten its production (Shiferaw *et al.*, 2011; ten Berge *et al.*, 2019).

Gray leaf spot (GLS) is one of the most notorious fungal foliar diseases that threaten maize production and is implicated in causing 20 to 80 % field-specific yield losses (Latterell & Rossi, 1983; Manandhar *et al.*, 2011; Ward *et al.*, 1999), either singly or in combination with other fungal foliar diseases. Gray leaf spot disease is caused by two sibling species, *Cercospora zeina* Crous & U. Braun (Crous & Braun, 2003) which was originally designated *Cercospora zeae-maydis* type II, and *C. zeae-maydis* Tehon & E.Y Daniels (Tehon & Daniels, 1925), of which the latter has not been reported in Africa (Crous *et al.*, 2006; Dunkle & Levy, 2000; Wang *et al.*, 1998). The few available population genetic studies on *C. zeina* based on small numbers of isolates have proved that the fungus is genetically diverse, and its diversity is as a result of both genetic recombination and gene flow (Meisel *et al.*, 2009; Muller *et al.*, 2016; Okori *et al.*, 2003; Okori *et al.*, 2015).

Following the sequencing of the *C. zeina* genome (Wingfield *et al.*, 2017) and the characterization of microsatellite and mating type markers (Muller *et al.*, 2016), we set out to conduct a larger geographical scale population genetics study of *C. zeina* from five SSA countries. We posed four major hypotheses including: (1) *C. zeina* was the predominant causal pathogen of GLS in Africa with no *C. zeae-maydis*; (2) *C. zeina* was a highly diverse

pathogen whose population structure was influenced by sexual recombination, gene flow and human activities such as the farming systems; (3) *C. zeina* was a sexually reproducing pathogen and its sexual stage would be induced under laboratory and greenhouse conditions: and 4) Durban harbor is *C. zeina*'s point of entry into Africa. To test these hypotheses, this study had the following specific objectives: (i) to confirm that *C. zeina* is the causal pathogen of GLS in the rest of Africa; (ii) to determine the influence of farming practices on the population structure of *C. zeina*; (iii) to determine the genetic diversity and population structure of *C. zeina* in other SSA countries; (iv) to understand the role sexual recombination and migration have on its evolutionary history. In total, 1174 *C. zeina* isolates were used in this study: 835 from smallholder farms collected during this study and a dataset of 239 isolates from Muller *et al.* (2016). The latter dataset was used in a population genetics comparative study between commercial and smallholder farms in South Africa. All isolates were subjected to microsatellite and mating-type (MAT)-specific PCR assays and several population genetic analyses.

5.2 CO-OCCURRENCE OF *CERCOSPORA ZEINA*, *E. TURCICUM*, AND *B. MAYDIS* ON MAIZE

Cercospora zeina is one of the most notorious fungal pathogens second to *Exserohilum turcicum*, the causal pathogen of northern corn leaf blight and before *Bipolaris maydis*, the causal pathogen of southern corn leaf blight. These pathogens have been reported to cause over 1 % of the global yield losses and up to 80 % of field-specific yield losses of maize (Bruns, 2017; Jindal *et al.*, 2019; Manandhar *et al.*, 2011; Savary *et al.*, 2019). This study provided the first comprehensive review of the recent advances in understanding of their population biology and management in the African context. It revealed that all three pathogens exist in Africa and thrive under similar environmental conditions. They are highly diverse pathogens with the existence of population structure among their populations, owing to cryptic sexual recombination and gene flow. Except for *E. turcicum* and *C. zeina*, *B. maydis* is rarer on the continent and is air-, splash- and seed-borne, making it a phytosanitary threat to countries where it has not been reported. Thus far, no study has been conducted to assess the impact

of their co-occurrence on maize. Given that *C. zeina* and *B. maydis* colonize their host intercellularly, in the event of co-infection, we speculate the possibility of direct competition for resources from both pathogens which might positively or negatively impact on their virulence (Alizon *et al.*, 2013; Bose *et al.*, 2016; Bull & Lauring, 2014; Staves & Knell, 2010). This has extensively been studied in anther smut pathogens with results revealing direct competition and signatures of potential hybridization among co-infecting species and genotypes (Petit *et al.*, 2017). Furthermore, viruses that co-infect kill their host faster than in single infections (Leggett *et al.*, 2013). We also predict the possibility of *C. zeina* and *B. maydis* to each co-operate with *E. turcicum* which is known to colonize its host through the xylem. This may likely increase each pathogen's fitness to cause more damage without any form of competition, especially if the co-operation occurs frequently, thus, causing more damage (Bose *et al.*, 2016). The ecological effect of the co-occurrence of these three pathogens in one niche, therefore, needs to be investigated through co-infection assays and genome comparative studies.

5.3 *CERCOSPORA ZEINA* IS THE PREDOMINANT CAUSAL PATHOGEN OF GLS IN AFRICA

Our study set out to determine which *Cercospora* species associated with GLS is predominant in Africa, with the hypothesis that *C. zeina* is the causal fungus of GLS in Africa (Crous *et al.*, 2006; Dunkle & Levy, 2000; Kinyua *et al.*, 2010; Meisel *et al.*, 2009). We confirmed that *C. zeina* was the predominant pathogen in the studied African countries, with no reports of *C. zeae-maydis*, its sibling species yet. Two lines of evidence supported this conclusion. Firstly, the morphologies of the conidia, conidiophores, and colonies were similar to the *C. zeina* described by Crous *et al.* (2006) and Meisel *et al.* (2009). Secondly, the amplification of the *CTB-7* gene region confirmed all isolates studied to have a fragment of 618 bp of the gene, diagnostic of *C. zeina*.

The absence of *C. zeae-maydis* in Africa may be attributed to a lack of the favorable environmental conditions for its establishment on the continent. Temperate and cooler conditions favor *C. zeae-maydis* growth and establishment (Wang *et al.*, 1998), conditions which are not common to Africa and other areas where *C. zeina* has been reported. Some studies have reported *C. zeae-maydis* to be the causal pathogen of GLS in Ethiopia even though no molecular confirmations exist (Bekeko *et al.*, 2018; Nega *et al.*, 2016; Wegary *et al.*, 2001). Therefore, the hypothesis of the existence of *C. zeae-maydis* on the continent still needs to be tested through more comprehensive surveys and collection of GLS isolates from all maize-producing countries. This can be followed by molecular identification of the causal pathogen using the available diagnostic tools (Crous *et al.*, 2006; Korsman *et al.*, 2012; Meisel *et al.*, 2009; Swart *et al.*, 2017).

Single isolates of two other species, *Cercospora sorghi* var. *maydis* and *Cercospora* sp. CPC 12062 were previously isolated from GLS lesions in Kenya and South Africa, respectively (Crous *et al.*, 2006; Kinyua *et al.*, 2010), but none were identified during this study. Their absence in more than 1000 isolates collected from five African countries suggests that these two are very rare. This is consistent with a small number of *C. sorghi* var. *maydis* isolates that were previously retrieved from maize fields in Brazil (Neves *et al.*, 2015). Additionally, these two species may exist as saprophytes that occasionally thrive on GLS lesions and dead plant material but are not plant pathogens. A similar conclusion was reached about *Botrytis pyriformis* sp. nov., a novel species of *Botrytis* which together with *Sclerotinia nivalis*, is associated with the white mold disease that infects leaves and stems of *Sedum sarmentosum* (gold moss stonecrop) (Zhang *et al.*, 2016). On testing the pathogenicity of *B. pyriformis* on *S. sarmentosum*, there was a negligible infection caused and it was concluded that it existed as an epiphytic saprophyte or an endophyte rather than a pathogen (Zhang *et al.*, 2016). Pathogenicity tests of *C. sorghi* var. *maydis* and *Cercospora* sp. CPC12062. on maize could substantiate this hypothesis.

Studies in *Fusarium*, *Alternaria*, and *Bipolaris* species have demonstrated that fungal pathogenicity evolved from saprotrophic ancestry through the acquisition of pathogenicity factors such as host-specific phytotoxins (Ma *et al.*, 2010; Thomma, 2003; Turgeon & Lu, 2000). To our knowledge, no saprophytic *Cercospora* species have been reported. From these lines of evidence, therefore, testing of the hypothesis of either of the two *Cercospora* species existing as a saprophytic ancestor of *C. zeina* is needed. This can be achieved through genome comparative studies to trace any lineage-specific genomic regions with distinct evolutionary profiles and the indicators of horizontal gene acquisition that may exist between these pathogens. Another plausible explanation for these two species existing as saprophytes is the pogo stick hypothesis as has been proposed in *Mycosphaerella* species (Crous & Groenewald, 2005). This suggests that pathogens can jump to a host on which they are not primary pathogens, but that is infected with a related pathogen such as *C. zeina*, and use it as a base to produce enough inoculum that will enable their further dispersal in search for their natural hosts (Crous *et al.*, 2006).

5.4 INSIGHTS INTO THE POPULATION GENETICS AND MIGRATION PATTERNS OF *CERCOSPORA ZEINA*

Past studies analyzed the genetic variation of *C. zeina* at a country and regional level based on amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) and microsatellite markers (Dunkle & Levy, 2000; Muller *et al.*, 2016; Okori *et al.*, 2015). They all revealed that the pathogen was highly diverse with low signatures of population differentiation. In our study, a set of neutral microsatellite markers (Muller *et al.*, 2016) was employed to evaluate genetic diversity patterns in five SSA countries (Kenya, South Africa, Uganda, Zambia, and Zimbabwe) and determine the evolutionary processes involved. Our results showed that *C. zeina* is a highly diverse pathogen in SSA with populations from smallholder farms exhibiting a higher genetic diversity than those from commercial farms. Also, populations from commercial maize farms exhibited higher clonality than smallholder populations, most likely due to host resistance and selection pressure from fungicide usage

(Nsibo *et al.*, 2019). In contrast to the findings by Okori *et al.* (2003) of low to no population differentiation among *C. zeina* populations, our results revealed moderate to high population differentiation among populations (i.e. samples from either a farming system or country comprised of a population). Remarkably, the Zambia population was distinct, having a lower genetic diversity, higher clonality, and a unique population structure from the rest of the studied populations.

These findings are comparable to those of other studied cereal pathogens which have a very high genetic diversity and are defined by the existence of population structure such as *Zymoseptoria tritici* (Abrinbana *et al.*, 2010; Boukef *et al.*, 2012; Grandaubert *et al.*, 2017; Siah *et al.*, 2018) and *Rhynchosporium commune* (Linde *et al.*, 2009; Salamati *et al.*, 2000). Siah *et al.* (2018) further studied the genetic diversity of *Z. tritici* at a more micro-scale (plant, and different leaf layers) and noted that even at such levels, the pathogen was highly diverse. Although our study unveiled the genetic diversity of *C. zeina* at a cropping system level and a larger geographical scale, no study has been conducted at a smaller or local spatial scale. There is, therefore, room to explore the genetic variation of this fungus at a micro-scale particularly between lesions, leaves, and cultivars to further understand its overall genetic patterns at a global, regional, field, and plant scale.

The existence of a very high genetic diversity implies that *C. zeina* has stably established itself on the continent and has had more time to acquire its genetic identity than would be for a very recent invasion of a species. From our findings, we speculate that if *C. zeina* originated from out of Africa, its entry into the continent was in the sixteenth century, the time of entry of maize into Africa (McCann, 2001; Stanton & Willett, 1963). We further speculate that *C. zeina* could have jumped from a native host such as a grass in Africa to maize, like previously proposed by Crous *et al.* (2006). To test these hypotheses of entry and origin, more *C. zeina* sampling is needed especially from all other entry regions of maize into Africa particularly the West African regions and other African countries where GLS exists (McCann, 2001; Stanton &

Willett, 1963). These isolates can be subjected to comparative population genetic studies with *C. zeina* isolates from different continents.

Results from the principal coordinate analysis, Bayesian clustering, and population differentiation analyses in our study provided evidence that *C. zeina* in SSA is not part of a single panmictic population but rather, is structured based on different farming systems (Nsibo *et al.*, 2019) and geographical regions. This population structure could in part be explained by selection pressures from the different country-specific maize genotypes (Access to Seeds Foundation, 2017). Following previous studies, different banana plants and cultivars have influenced the observed high genetic variability observed among *Mycosphaerella fijiensis* isolates from Nigeria (Müller *et al.*, 1997). It is therefore possible that maize genotypes define the observed *C. zeina* population structure in the different parts of Africa. The role of maize genotypes on the population structure of *C. zeina* was not tested in our study because it was difficult to obtain such information from the smallholder farmers. Experiments including infection assays, population genetic analyses, and genome-wide association analyses can be conducted to understand the role of different maize genotypes on the *C. zeina* genetic variability. Ultimately, this will guide the breeding programs against the fungus.

While population structure was observed among the studied populations, significant gene flow and migration of genotypes were evident. South Africa and Zambia were the highest donor and recipient of historical migrants, respectively (Banke & McDonald, 2005). Following the first report of GLS in Africa (Ward *et al.*, 1997), our migration analysis could not confirm that South Africa was the ancestral origin of *C. zeina*, thus rejecting the hypothesis of Durban harbor being the entry point of *C. zeina* into Africa. Also, Zambia's lower genetic diversity, high clonality, low effective population size, unique population structure and migration pattern from the rest of the populations suggest that it is a younger population whose source population is none of the studied populations. Given these limitations, a wider sampling from all maize growing countries where GLS is endemic is advisable. These can then be subjected to

approximate Bayesian computations to compare different scenarios that may explain the ancestral relationship of the different *C. zeina* populations.

Microsatellite markers are the most widely used for population genetic analysis. They are highly polymorphic, presumably neutral (i.e. gene variants detected have no direct effect on fitness), are relatively easy to characterize, and are randomly distributed within the genome (Kirk & Freeland, 2011). Additionally, microsatellite markers can estimate random processes such as mutation, genetic drift, and gene flow without any bias (Kirk & Freeland, 2011). They proved to be reliable in this study because of their ability to resolve the population genetic structure of *C. zeina* at both micro (field and country-level) and continental scale. They inferred stronger evidence for population differentiation among *C. zeina* populations than previously revealed by dominant DNA markers (Dunkle & Levy, 2000; Okori *et al.*, 2003; Okori *et al.*, 2015; Wang *et al.*, 1998), thus highlighting that they are more efficient. However, microsatellites are known to underestimate diversity since they are based on fragment size and not sequence data and probably, they are not representative of the true genetic variability of the whole *C. zeina* genome. Given this limitation, whole-genome scanning for alternative biallelic markers such as single nucleotide polymorphism (SNPs) could be an option for future population genetic studies and other functional gene analyses.

5.5 STRONG EVIDENCE FOR THE OCCURRENCE OF SEXUAL RECOMBINATION AMONG *CERCOSPORA ZEINA* POPULATIONS

Understanding the role of sexual recombination and the frequency of mating types gives insights into the biology, and the population genetics of pathogens (Conde-Ferráez *et al.*, 2007). In our study, we employed mating type and microsatellite loci to determine the role of sexual recombination in driving the population structure of *C. zeina*. Except for a few populations, both mating types were present in fields and at equal frequencies (1:1 ratio). For the populations that deviated from the 1:1 ratio, there was no specific pattern of dominance of

one MAT idiomorph over the other. Results also revealed high genotypic diversities and low linkage disequilibrium, indicative of outbreeding sexual recombination occurring or having occurred in the past. Induction of the sexual stage of this fungus under laboratory and greenhouse conditions was unsuccessful which does place some limitation on our study. This was attributed to the lack of knowledge of the biological, ecological, and environmental factors that are crucial for the process (Nel *et al.*, 2018; Wilson *et al.*, 2019). To address this limitation, the search for the sexual stage in nature on overwintering maize residues from heavily infested fields and other native grasses is advisable.

Indeed, evidence for sexual recombination has already been inferred among *C. zeina* populations in South Africa's commercial farms including at a more finer scale (i.e. both mating types were recovered from a single lesion) (Muller *et al.*, 2016). Cryptic sexual recombination has also been demonstrated to occur in other *Cercospora* species that have previously been designated as asexual in nature even though their sexual stages are still unknown (Groenewald *et al.*, 2008; Knight *et al.*, 2019; Vaghefi *et al.*, 2017). The only sexual stages reported among the *Cercospora* species exist in *Mycosphaerella* (Goodwin et al., 2001). It is possible that the sexual stage of *C. zeina* also exists in this genus as has been proposed for other *Cercospora* species (Bolton *et al.*, 2012; Groenewald *et al.*, 2008).

From these findings, we concluded that *C. zeina* possesses a very high evolutionary potential. As a result of recombination, the pathogen may undergo genetic changes that enhance its fitness for survival in new and changing environments and equips it to withstand several management strategies and to become more virulent (Ellwood *et al.*, 2019; Liu *et al.*, 2019; McDonald *et al.*, 2019).

Genome-wide association studies of the most variable genotypes are thus necessary to further understand the evolutionary factors driving the observed *C. zeina* genetic variability. Also, fungicide sensitivity assays followed by sequencing to confirm the presence of the already known fungicide resistance mutations or whole-genome sequencing for discovery of novel fungicide resistance mutations can be performed to predict any genetic basis for resistance.

Fungicide resistance has been shown to exist in other *Cercospora* species including *C. sojina*, *C. beticola* and *C. kikuchii*, against quinone outside inhibitor (QoI; also known as strobilurin) fungicides, owing to a mutation within the cytochrome b gene (Bolton *et al.*, 2013; Price III *et al.*, 2015; Zeng *et al.*, 2015; Zhang *et al.*, 2012). Conducting similar studies in *C. zeina* will not only facilitate the prediction of acquisition of such resistance mutations but also guide in the development of more efficient management strategies. This study further presents a basis to characterize the *MAT1-1* and *MAT1-2* genes to better understand their role in sexual recombination. Also, it will allow further exploration of the *C. zeina* genome to ascertain the presence or absence of any other genes associated with sexual reproduction as has been shown in other fungi (Wilson *et al.*, 2019). Such genes may include those involved in signaling pathways, meiosis, fruiting body development, and pheromone synthesis. Functional characterization of such genes can be a source of experimental evidence for sexuality in *C. zeina*.

5.6 GENERAL CONCLUSION

There is cumulative evidence based on population genetic studies that *C. zeina* is highly diverse, owing to cryptic sexual recombination, migration and gene flow. Due to its high evolutionary potential, *C. zeina* is likely to become one of the most prevalent airborne and splash-dispersed foliar pathogens of maize in addition to *E. turcicum* and *B. maydis*, causing severe epidemics in Africa and globally. For its effective management, therefore, we recommend the deployment of a mixture of resistant maize genotypes and the usage of fungicides in either varying combinations or in rotations depending on their mechanisms of action. The former management strategy applies to both smallholder (who cannot afford fungicides) and commercial farming systems while the latter applies to large-scale commercial farming systems. These management strategies need to be augmented with several cultural management practices such as occasional deep plowing, fallows, and crop rotation schemes that are aimed at delaying disease establishment. This will limit the movement of fungicide resistance traits and highly virulent strains. Ultimately, these findings and future investigations

into the relationship between molecular and pathogenicity variability will aid in a comprehensive understanding of the microevolution, epidemiology, and biology of *C. zeina*.

5.7 REFERENCES

Abrinbana, M., Mozafari, J., Shams-Bakhsh, M., & Mehrabi, R. (2010). Genetic structure of *Mycosphaerella graminicola* populations in Iran. *Plant Pathology, 59*(5), 829-838.

Access to Seeds Foundation. (2017). Identifying leading seed companies in Eastern and Southern Africa. https://www.accesstoseeds.org/app/uploads/2018/2003/Leading-Seed-Companies-in-Eastern-and-Southern-Africa_DEF.pdf (Accessed January 2019).

Alexandratos, N., & Bruinsma, J. (2012). World agriculture towards 2030/2050: the 2012 revision. http://www.fao.org/3/a-ap106e.pdf .

Alizon, S., de Roode, J. C., & Michalakis, Y. (2013). Multiple infections and the evolution of virulence. *Ecology Letters, 16*(4), 556-567.

Banke, S., & McDonald, B. (2005). Migration patterns among global populations of the pathogenic fungus *Mycosphaerella graminicola*. *Molecular Ecology, 14*(7), 1881-1896.

Bekeko, Z., Fininsa, C., Wegary, D., Hussien, T., Hussien, S., & Asalf, B. (2018). Combining ability and nature of gene action in maize (*Zea mays* L) inbred lines for resistance to gray leaf spot disease (*Cercospora zeae maydis*) in Ethiopia. *Crop Protection, 112*, 39-48.

Bolton, M. D., Rivera, V., & Secor, G. (2013). Identification of the G143A mutation associated with QoI resistance in *Cercospora beticola* field isolates from Michigan, United States. *Pest Management Science, 69*(1), 35-39.

Bolton, M. D., Secor, G. A., Rivera, V., Weiland, J. J., Rudolph, K., Birla, K., & Campbell, L. G. (2012). Evaluation of the potential for sexual reproduction in field populations of *Cercospora beticola* from USA. *Fungal Biology, 116*(4), 511-521.

Bose, J., Kloesener, M. H., & Schulte, R. D. (2016). Multiple-genotype infections and their complex effect on virulence. *Zoology, 119*(4), 339-349.

Boukef, S., McDonald, B. A., Yahyaoui, A., Rezgui, S., & Brunner, P. C. (2012). Frequency of mutations associated with fungicide resistance and population structure of *Mycosphaerella graminicola* in Tunisia. *European Journal of Plant Pathology, 132*(1), 111-122.

Bruns, H. A. (2017). Southern corn leaf blight: a story worth retelling. *Agronomy Journal, 109*(4), 1218-1224.

Bull, J. J., & Lauring, A. S. (2014). Theory and empiricism in virulence evolution. *PLoS Pathogens, 10*(10), e1004387.

Conde-Ferráez, L., Waalwijk, C., Canto-Canché, B. B., Kema, G. H., Crous, P. W., James, A. C., & Abeln, E. C. (2007). Isolation and characterization of the mating type locus of *Mycosphaerella fijiensis*, the causal agent of black leaf streak disease of banana. *Molecular Plant Pathology, 8*(1), 111-120.

Crous, P., & Groenewald, J. (2005). Hosts, species and genotypes: opinions versus data. *Australasian Plant Pathology, 34*(4), 463-470.

Crous, P. W., & Braun, U. (2003). *Mycosphaerella and its anamorphs: 1. Names published in Cercospora and Passalora*: Centraalbureau voor Schimmelcultures (CBS).

Crous, P. W., Groenewald, J. Z., Groenewald, M., Caldwell, P., Braun, U., & Harrington, T. C. (2006). Species of *Cercospora* associated with grey leaf spot of maize. *Studies in Mycology, 55*, 189-197.

Dunkle, L. D., & Levy, M. (2000). Genetic relatedness of African and United States populations of *Cercospora zeae-maydis*. *Phytopathology, 90*(5), 486-490.

Ellwood, S. R., Piscetek, V., Mair, W. J., Lawrence, J. A., Lopez-Ruiz, F. J., & Rawlinson, C. (2019). Genetic variation of *Pyrenophora teres f. teres* isolates in Western Australia

and emergence of a *Cyp51A* fungicide resistance mutation. *Plant Pathology, 68*(1), 135-142.
FAO. (2019). Food and Agriculture Organization, United Nations of Organization 2019-2020. www.fao.org/worldfoodsituation/csdb/en/, (Accessed July 2019).
Goodwin, S. B., Dunkle, L. D., & Zismann, V. L. (2001). Phylogenetic analysis of *Cercospora* and *Mycosphaerella* based on the internal transcribed spacer region of ribosomal DNA. *Phytopathology, 91*(7), 648-658.
Grandaubert, J., Dutheil, J., & Stukenbrock, E. (2017). The genomic rate of adaptation in the fungal wheat pathogen *Zymoseptoria tritici*. *bioRxiv*. http://doi.org/10.1101/176727.
Groenewald, M., Linde, C., Groenewald, J. Z., & Crous, P. W. (2008). Indirect evidence for sexual reproduction in *Cercospora beticola* populations from sugar beet. *Plant Pathology, 57*(1), 25-32.
Jindal, K. K., Tenuta, A. U., Woldemariam, T., Zhu, X., Hooker, D. C., & Reid, L. M. (2019). Occurrence and distribution of physiological races of *Exserohilum turcicum* in Ontario, Canada. *Plant Disease, 103*, 1450-1457.
Kinyua, Z., Smith, J., Kibata, G., Simons, S., & Langat, B. (2010). Status of grey leaf spot disease in Kenyan maize production ecosystems. *African Crop Science Journal, 18*(4), 183 - 194.
Kirk, H., & Freeland, J. R. (2011). Applications and implications of neutral versus non-neutral markers in molecular ecology. *International Journal of Molecular Sciences, 12*(6), 3966-3988.
Knight, N. L., Vaghefi, N., Kikkert, J. R., Bolton, M. D., Secor, G. A., Rivera, V. V., & Pethybridge, S. J. (2019). Genetic diversity and structure in regional *Cercospora beticola* populations from *Beta vulgaris subsp. vulgaris* suggest two clusters of separate origin. *Phytopathology*, PHYTO-07-18-0264-R.
Korsman, J., Meisel, B., Kloppers, F. J., Crampton, B. G., & Berger, D. K. (2012). Quantitative phenotyping of grey leaf spot disease in maize using real-time PCR. *European Journal of Plant Pathology, 133*(2), 461-471.
Latterell, F. M., & Rossi, A. E. (1983). Gray leaf spot of corn: a disease on the move. *Plant Disease, 67*(8), 842-847.
Leggett, H. C., Benmayor, R., Hodgson, D. J., & Buckling, A. (2013). Experimental evolution of adaptive phenotypic plasticity in a parasite. *Current Biology, 23*(2), 139-142.
Linde, C. C., Zala, M., & McDonald, B. A. (2009). Molecular evidence for recent founder populations and human-mediated migration in the barley scald pathogen *Rhynchosporium secalis*. *Molecular Phylogenetics and Evolution, 51*(3), 454-464.
Liu, S., Fu, L., Wang, S., Chen, J., Jiang, J., Che, Z., & Chen, G. (2019). Carbendazim resistance of *Fusarium graminearum* from Henan wheat. *Plant Disease*103:2536-2540.
Ma, L.-J., Van Der Does, H. C., Borkovich, K. A., Coleman, J. J., Daboussi, M.-J., Di Pietro, A., & Henrissat, B. (2010). Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature, 464*(7287), 367.
Manandhar, G., Ferrara, G., Tiwari, T., Baidya, S., Bajracharya, A., Khadge, B., & Narro, L. (2011). Response of maize genotypes to gray leaf spot disease (*Cercospora zeae-maydis*) in the hills of Nepal. *Agronomy Journal of Nepal, 2*, 93-101.
McCann, J. (2001). Maize and grace: history, corn, and Africa's new landscapes, 1500–1999. *Comparative Studies in Society and History, 43*(2), 246-272.
McDonald, M. C., Renkin, M., Spackman, M., Orchard, B., Croll, D., Solomon, P. S., & Milgate, A. (2019). Rapid parallel evolution of azole fungicide resistance in Australian populations of the wheat pathogen *Zymoseptoria tritici*. *Applied and Environmental Microbiology, 85*(4), e01908-01918.
Meisel, B., Korsman, J., Kloppers, F. J., & Berger, D. K. (2009). *Cercospora zeina* is the causal agent of grey leaf spot disease of maize in Southern Africa. *European Journal of Plant Pathology, 124*(4), 577-583.
Muller, M. F., Barnes, I., Kunene, N. T., Crampton, B. G., Bluhm, B. H., Phillips, S. M., & Berger, D. K. (2016). *Cercospora zeina* from maize in South Africa exhibits high

genetic diversity and lack of regional population differentiation. *Phytopathology, 106*(10), 1194-1205.
Müller, R., Pasberg-Gauhl, C., Gauhl, F., Ramser, J., & Kahl, G. (1997). Oligonucleotide fingerprinting detects genetic variability at different levels in Nigerian *Mycosphaerella fijiensis*. *Journal of Phytopathology, 145*(1), 25-30.
Nega, A., Lemessa, F., & Berecha, G. (2016). Distribution and importance of maize gray leaf spot *Cercospora zeae-maydis* (Tehon and Daniels) in south and southwest Ethiopia. *Journal of Plant Pathology & Microbiology, 7*(362), 2.
Nel, W., Duong, T., Wingfield, M., Wingfield, B., Hammerbacher, A., & de Beer, Z. (2018). Heterothallism revealed in the root rot fungi *Berkeleyomyces basicola* and *B. rouxiae*. *Fungal Biology, 122*(11), 1031-1040.
Neves, D. L., Silva, C. N., Pereira, C. B., Campos, H. D., & Tessmann, D. J. (2015). *Cercospora zeina* is the main species causing gray leaf spot in southern and central Brazilian maize regions. *Tropical Plant Pathology, 40*(6), 368-374.
Nsibo, D. L., Barnes, I., Kunene, N. T., & Berger, D. K. (2019). Influence of farming practices on the population genetics of the maize pathogen *Cercospora zeina* in South Africa. *Fungal Genetics and Biology, 125*, 36-44.
Okori, P., Fahleson, J., Rubaihayo, P., Adipala, E., & Dixelius, C. (2003). Assessment of genetic variation among East African *Cercospora zeae-maydis*. *African Crop Science Journal, 11*(2), 75-85.
Okori, P., Rubaihayo, P., Adipala, E., Fahieson, J., & Dixelius, C. (2015). Dynamics of *Cercospora zeina* populations in maize-based agro-ecologies of Uganda. *African Crop Science Journal, 23*(1), 45-57.
Petit, E., Silver, C., Cornille, A., Gladieux, P., Rosenthal, L., Bruns, E., & Hood, M. E. (2017). Co-occurrence and hybridization of anther-smut pathogens specialized on *Dianthus* hosts. *Molecular Ecology, 26*(7), 1877-1890.
Price III, P. P., Purvis, M. A., Cai, G., Padgett, G. B., Robertson, C. L., Schneider, R. W., & Albu, S. (2015). Fungicide resistance in *Cercospora kikuchii*, a soybean pathogen. *Plant Disease, 99*(11), 1596-1603.
Salamati, S., Zhan, J., Burdon, J. J., & McDonald, B. A. (2000). The genetic structure of field populations of *Rhynchosporium secalis* from three continents suggests moderate gene flow and regular recombination. *Phytopathology, 90*(8), 901-908.
Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, 1.
Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security, 3*(3), 307.
Siah, A., Bomble, M., Tisserant, B., Cadalen, T., Holvoet, M., Hilbert, J.-L., & Reignault, P. (2018). Genetic structure of *Zymoseptoria tritici* in Northern France at region, field, plant, and leaf layer scales. *Phytopathology, 108*(9), 1114-1123.
Stanton, W., & Willett, F. (1963). 150. Archaeological evidence for changes in maize type in West Africa: An experiment in technique. *Man*, 117-123.
Staves, P. A., & Knell, R. J. (2010). Virulence and competitiveness: testing the relationship during inter-and intraspecific mixed infections. *Evolution: International Journal of Organic Evolution, 64*(9), 2643-2652.
Swart, V., Crampton, B. G., Ridenour, J. B., Bluhm, B. H., Olivier, N. A., Meyer, J. M., & Berger, D. K. (2017). Complementation of *CTB7* in the maize pathogen *Cercospora zeina* overcomes the lack of *in vitro* cercosporin production. *Molecular Plant-Microbe Interactions, 30*(9), 710-724.
Tehon, L., & Daniels, E. (1925). Notes on the parasitic fungi of Illinois—II. *Mycologia, 17*(6), 240-249.
ten Berge, H. F. M., Hijbeek, R., van Loon, M. P., Rurinda, J., Tesfaye, K., Zingore, S., & van Ittersum, M. K. (2019). Maize crop nutrient input requirements for food security in sub-Saharan Africa. *Global Food Security, 23*, 9-21.

Tesfaye, K., Gbegbelegbe, S., Cairns, J. E., Shiferaw, B., Prasanna, B. M., Sonder, K., & Robertson, R. (2015). Maize systems under climate change in sub-Saharan Africa: potential impacts on production and food security. *International Journal of Climate Change Strategies and Management, 7*(3), 247-271.

Thomma, B. P. (2003). *Alternaria* spp: from general saprophyte to specific parasite. *Molecular Plant Pathology, 4*(4), 225-236.

Tripathi, A. D., Mishra, R., Maurya, K. K., Singh, R. B., & Wilson, D. W. (2019). Estimates for world population and global food availability for global health *The Role of Functional Food Security in Global Health* (pp. 3-24): Elsevier.

Turgeon, B. G., & Lu, S.-W. (2000). Evolution of host specific virulence in *Cochliobolus heterostrophus. Fungal Pathology* (pp. 93-126): Springer.

Vaghefi, N., Nelson, S. C., Kikkert, J. R., & Pethybridge, S. J. (2017). Genetic structure of *Cercospora beticola* populations on *Beta vulgaris* in New York and Hawaii. *Scientific Reports, 7*(1), 1726.

Van Ittersum, M. K., Van Bussel, L. G., Wolf, J., Grassini, P., Van Wart, J., Guilpart, N., & Mason-D'Croz, D. (2016). Can sub-Saharan Africa feed itself? *Proceedings of the National Academy of Sciences, 113*(52), 14964-14969.

Wang, J., Levy, M., & Dunkle, L. D. (1998). Sibling species of *Cercospora* associated with gray leaf spot of maize. *Phytopathology, 88*(12), 1269-1275.

Ward, J. M., Laing, M., & Rijkenberg, F. (1997). Frequency and timing of fungicide applications for the control of gray leaf spot in maize. *Plant Disease, 81*(1), 41-48.

Ward, J. M., Stromberg, E. L., Nowell, D. C., & Nutter Jr, F. W. (1999). Gray leaf spot: a disease of global importance in maize production. *Plant Disease, 83*(10), 884-895.

Wegary, D., Abebe, F., Wolde, L., & Keneni, G. (2001). Grey leaf spot disease: a potential threat to maize production in Ethiopia. *Sebil (Ethiopia)*. *CSSE* 9, 147-157.

Wilson, A. M., Wilken, P. M., van der Nest, M. A., Wingfield, M. J., & Wingfield, B. D. (2019). It's All in the Genes: The regulatory pathways of sexual reproduction in filamentous ascomycetes. *Genes, 10*(5), 330.

Wingfield, B. D., Berger, D. K., Steenkamp, E. T., Lim, H.-J., Duong, T. A., Bluhm, B. H., Wingfield, M. J. (2017). Draft genome of *Cercospora zeina*, *Fusarium pininemorale, Hawksworthiomyces lignivorus*, *Huntiella decipiens* and *Ophiostoma ips*. *IMA Fungus, 8*(2), 385-396.

Zeng, F., Arnao, E., Zhang, G., Olaya, G., Wullschleger, J., Sierotzki, H., & Fakhoury, A. (2015). Characterization of quinone outside inhibitor fungicide resistance in *Cercospora sojina* and development of diagnostic tools for its identification. *Plant Disease, 99*(4), 544-550.

Zhang, G., Newman, M., & Bradley, C. (2012). First report of the soybean frogeye leaf spot fungus (*Cercospora sojina*) resistant to quinone outside inhibitor fungicides in North America. *Plant Disease, 96*(5), 767-767.

Zhang, J., Yang, H., Yu, Q., Wu, M., Yang, L., Zhuang, W., & Li, G. (2016). *Botrytis pyriformis sp. nov*., a novel and likely saprophytic species of *Botrytis*. *Mycologia, 108*(4), 682-696.

APPENDICES

Appendix A: Microsatellite markers alleles (numbers 01 to 18) and mating types (*MAT1-1* and *MAT1-2*) for *Cercospora zeina* Isolates from smallholder (SF) and commercial (CF) farms in KwaZulu-Natal, South Africa.

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2012.ZA.BNF.026	Baynesfield	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-1*
2013.ZA.BNF.001	Baynesfield	158	345	462	242	236	209	199	191	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.BNF.002	Baynesfield	158	345	462	242	236	209	199	191	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.BNF.003	Baynesfield	158	345	462	242	236	230	199	191	244	329	251	474	134	CF	*MAT1-1*
2013.ZA.BNF.004	Baynesfield	158	345	465	242	236	230	199	191	244	329	251	474	134	CF	*MAT1-1*
2013.ZA.BNF.005	Baynesfield	155	345	465	245	236	230	196	175	244	329	245	480	134	CF	*MAT1-1*
2013.ZA.BNF.006	Baynesfield	155	345	465	245	236	230	196	175	244	329	245	480	134	CF	*MAT1-1*
2013.ZA.BNF.007	Baynesfield	158	345	462	242	223	230	196	175	240	329	251	474	134	CF	*MAT1-1*
2013.ZA.BNF.008	Baynesfield	158	345	465	248	223	230	196	175	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.BNF.009	Baynesfield	158	348	462	242	236	226	199	175	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.BNF.010	Baynesfield	158	348	465	242	236	209	199	175	240	329	245	474	137	CF	*MAT1-1*
2012.ZA.BNF.001	Baynesfield	155	345	465	242	223	236	199	175	240	329	245	480	134	CF	*MAT1-2*
2012.ZA.BNF.002	Baynesfield	155	345	465	245	236	230	196	175	240	329	251	480	134	CF	*MAT1-2*
2012.ZA.BNF.003	Baynesfield	155	345	462	242	223	230	199	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.BNF.004	Baynesfield	155	345	462	242	223	209	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.BNF.005	Baynesfield	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.BNF.006	Baynesfield	161	345	465	242	236	209	199	175	236	329	251	474	137	CF	*MAT1-2*
2012.ZA.BNF.007	Baynesfield	155	345	465	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.BNF.008	Baynesfield	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.BNF.009	Baynesfield	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.BNF.010	Baynesfield	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.BNF.011	Baynesfield	158	345	462	242	223	230	196	175	240	329	245	480	137	CF	*MAT1-2*
2012.ZA.BNF.012	Baynesfield	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.BNF.013	Baynesfield	155	345	468	242	236	230	196	175	240	329	251	480	134	CF	*MAT1-2*
2012.ZA.BNF.014	Baynesfield	158	345	462	242	236	209	196	175	240	329	251	474	137	CF	*MAT1-2*
2012.ZA.BNF.015	Baynesfield	155	345	465	242	223	230	199	175	244	329	245	480	134	CF	*MAT1-2*
2012.ZA.BNF.016	Baynesfield	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.BNF.017	Baynesfield	155	345	465	242	223	230	199	175	240	329	251	480	137	CF	*MAT1-2*
2012.ZA.BNF.018	Baynesfield	152	345	465	242	223	212	196	175	236	329	245	474	137	CF	*MAT1-2*
2012.ZA.BNF.019	Baynesfield	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.BNF.020	Baynesfield	155	345	462	242	223	230	196	175	240	329	245	474	134	CF	*MAT1-2*

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2012.ZA.BNF.021	Baynesfield	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.BNF.022	Baynesfield	155	345	465	242	223	209	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.BNF.023	Baynesfield	155	345	462	245	236	209	199	175	240	329	245	480	134	CF	*MAT1-2*
2012.ZA.BNF.024	Baynesfield	158	345	465	245	236	230	199	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.BNF.025	Baynesfield	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2013.ZA.CED.007	Cedara	155	345	465	242	236	230	196	175	240	329	251	480	137	CF	*MAT1-1*
2013.ZA.CED.008	Cedara	158	345	465	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-1*
2013.ZA.CED.009	Cedara	158	345	465	242	223	230	196	175	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.CED.010	Cedara	155	345	468	242	236	230	196	175	236	329	245	474	134	CF	*MAT1-1*
2013.ZA.CED.011	Cedara	155	345	468	242	236	230	196	175	236	329	245	474	134	CF	*MAT1-1*
2013.ZA.CED.012	Cedara	158	345	465	245	236	230	196	175	240	329	251	480	137	CF	*MAT1-1*
2013.ZA.CED.013	Cedara	155	345	465	242	236	230	196	191	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.CED.014	Cedara	155	345	462	242	236	230	196	175	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.CED.015	Cedara	155	345	465	245	236	230	196	191	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.CED.016	Cedara	155	345	462	242	236	230	196	175	240	329	251	474	137	CF	*MAT1-1*
2013.ZA.CED.017	Cedara	155	345	462	242	236	230	196	175	240	329	251	474	137	CF	*MAT1-1*
2013.ZA.CED.018	Cedara	158	345	465	242	223	209	196	175	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.CED.019	Cedara	155	345	462	242	236	230	196	175	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.CED.020	Cedara	155	345	465	248	236	230	196	175	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.CED.021	Cedara	155	345	465	245	236	230	196	191	244	329	245	474	134	CF	*MAT1-1*
2013.ZA.CED.022	Cedara	155	345	465	245	236	230	196	175	240	329	251	474	137	CF	*MAT1-1*
2013.ZA.CED.023	Cedara	155	345	465	245	236	230	196	191	240	329	251	474	137	CF	*MAT1-1*
2013.ZA.CED.024	Cedara	155	345	462	245	236	230	196	191	240	329	251	474	137	CF	*MAT1-1*
2013.ZA.CED.025	Cedara	155	345	462	242	236	230	196	175	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.CED.026	Cedara	155	345	462	242	236	230	196	191	240	329	251	474	134	CF	*MAT1-1*
2013.ZA.CED.027	Cedara	155	345	462	245	236	230	196	175	240	329	245	480	137	CF	*MAT1-1*
2013.ZA.CED.028	Cedara	155	345	462	245	236	230	196	175	240	329	251	474	134	CF	*MAT1-1*
2013.ZA.CED.029	Cedara	155	345	465	245	236	230	196	175	240	329	245	480	134	CF	*MAT1-1*
2011.ZA.CED.001	Cedara	155	345	462	245	236	230	196	191	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.CED.002	Cedara	155	345	462	245	236	230	196	191	240	329	245	474	134	CF	*MAT1-2*
2011.ZA.CED.003	Cedara	155	345	462	242	236	230	196	175	240	329	251	480	134	CF	*MAT1-2*
2011.ZA.CED.004	Cedara	158	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2011.ZA.CED.005	Cedara	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.CED.006	Cedara	155	345	462	242	223	209	199	191	240	329	245	474	134	CF	*MAT1-2*
2011.ZA.CED.007	Cedara	155	345	462	242	223	230	199	175	240	329	245	474	134	CF	*MAT1-2*

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2011.ZA.CED.008	Cedara	155	345	462	245	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2011.ZA.CED.009	Cedara	155	345	462	245	236	230	196	175	240	329	251	480	134	CF	*MAT1-2*
2011.ZA.CED.010	Cedara	155	345	462	245	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.CED.011	Cedara	158	345	465	242	223	209	199	175	244	329	251	474	137	CF	*MAT1-2*
2011.ZA.CED.012	Cedara	158	345	465	242	236	209	199	175	240	329	251	480	134	CF	*MAT1-2*
2011.ZA.CED.013	Cedara	155	345	465	242	236	209	196	194	240	329	251	474	137	CF	*MAT1-2*
2011.ZA.CED.014	Cedara	155	345	462	245	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.CED.015	Cedara	155	345	462	245	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.CED.001	Cedara	155	345	462	242	236	209	199	175	240	329	251	480	134	CF	*MAT1-2*
2012.ZA.CED.002	Cedara	155	345	462	242	236	209	199	175	240	329	251	480	134	CF	*MAT1-2*
2012.ZA.CED.003	Cedara	161	345	462	245	236	233	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.CED.004	Cedara	155	345	468	242	223	209	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.CED.005	Cedara	155	345	462	242	236	209	199	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.CED.006	Cedara	158	345	465	242	223	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.CED.007	Cedara	158	345	465	242	223	230	196	191	248	329	245	474	137	CF	*MAT1-2*
2012.ZA.CED.008	Cedara	155	345	468	245	223	230	199	175	240	329	251	480	134	CF	*MAT1-2*
2012.ZA.CED.009	Cedara	155	345	465	242	236	209	199	175	248	329	245	474	137	CF	*MAT1-2*
2012.ZA.CED.010	Cedara	161	345	462	245	236	233	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.CED.011	Cedara	155	345	468	245	223	230	199	175	240	329	251	480	134	CF	*MAT1-2*
2013.ZA.CED.001	Cedara	158	345	465	242	236	209	196	194	240	329	245	474	134	CF	*MAT1-2*
2013.ZA.CED.002	Cedara	155	345	465	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2013.ZA.CED.003	Cedara	158	345	465	248	223	209	196	175	240	329	245	480	134	CF	*MAT1-2*
2013.ZA.CED.004	Cedara	158	345	465	242	236	230	196	175	240	329	251	474	137	CF	*MAT1-2*
2013.ZA.CED.005	Cedara	155	345	462	242	236	230	196	175	240	329	251	474	137	CF	*MAT1-2*
2013.ZA.CED.006	Cedara	155	345	465	242	236	230	196	191	240	329	251	480	137	CF	*MAT1-2*
2012.ZA.GYT.051	Graytown	158	345	462	245	236	230	196	191	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.GYT.052	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.GYT.053	Graytown	155	345	462	242	236	230	196	191	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.GYT.054	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.GYT.055	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.GYT.056	Graytown	158	345	465	242	236	209	196	191	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.GYT.057	Graytown	158	345	465	242	236	230	199	191	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.GYT.058	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	137	CF	*MAT1-1*
2012.ZA.GYT.059	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	137	CF	*MAT1-1*
2012.ZA.GYT.060	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	137	CF	*MAT1-1*

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2012.ZA.GYT.061	Graytown	155	345	462	245	236	230	196	175	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.GYT.062	Graytown	155	345	462	245	236	230	196	175	240	329	245	480	137	CF	*MAT1-1*
2012.ZA.GYT.063	Graytown	158	345	465	245	236	230	196	194	240	329	245	480	134	CF	*MAT1-1*
2012.ZA.GYT.064	Graytown	155	345	465	245	236	209	196	191	240	329	251	480	134	CF	*MAT1-1*
2012.ZA.GYT.065	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.GYT.066	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.GYT.067	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.GYT.001	Graytown	158	345	465	245	223	230	199	191	240	329	251	480	137	CF	*MAT1-1*
2013.ZA.GYT.002	Graytown	158	345	465	245	236	230	196	175	240	329	251	480	134	CF	*MAT1-1*
2013.ZA.GYT.003	Graytown	158	345	465	245	223	212	199	191	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.GYT.004	Graytown	158	345	462	245	223	209	196	191	240	329	251	480	134	CF	*MAT1-1*
2013.ZA.GYT.005	Graytown	155	345	468	245	236	209	199	191	240	329	251	480	137	CF	*MAT1-1*
2013.ZA.GYT.006	Graytown	155	345	465	245	236	230	199	175	240	329	251	480	137	CF	*MAT1-1*
2013.ZA.GYT.007	Graytown	158	345	465	242	223	209	196	191	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.GYT.008	Graytown	158	345	465	245	223	230	199	175	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.GYT.009	Graytown	158	345	465	245	223	209	196	175	240	329	251	480	137	CF	*MAT1-1*
2013.ZA.GYT.010	Graytown	155	345	462	245	236	233	199	175	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.GYT.011	Graytown	155	345	462	245	236	233	199	175	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.GYT.012	Graytown	155	345	462	245	236	209	196	175	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.GYT.013	Graytown	158	345	462	242	223	230	196	191	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.GYT.014	Graytown	155	345	462	245	236	209	196	175	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.GYT.015	Graytown	158	345	465	242	236	209	199	175	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.GYT.016	Graytown	158	345	462	245	236	209	199	191	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.GYT.017	Graytown	155	345	465	245	236	209	199	175	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.GYT.018	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.GYT.019	Graytown	158	345	462	245	223	230	196	191	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.GYT.020	Graytown	155	345	465	242	223	209	196	175	240	329	251	474	137	CF	*MAT1-1*
2013.ZA.GYT.021	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.GYT.022	Graytown	158	345	465	242	223	212	199	191	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.GYT.023	Graytown	158	345	462	242	223	209	196	191	240	329	245	474	134	CF	*MAT1-1*
2013.ZA.GYT.024	Graytown	158	345	462	242	223	209	196	175	240	329	251	474	134	CF	*MAT1-1*
2013.ZA.GYT.025	Graytown	155	345	465	242	236	230	196	175	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.GYT.026	Graytown	155	345	465	242	236	209	199	175	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.GYT.027	Graytown	158	345	468	242	223	206	199	191	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.GYT.028	Graytown	155	345	465	242	223	230	196	175	240	329	245	480	134	CF	*MAT1-1*

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2013.ZA.GYT.029	Graytown	155	345	465	242	223	230	196	175	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.GYT.030	Graytown	155	345	465	242	223	230	196	175	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.GYT.031	Graytown	155	345	465	245	236	230	199	191	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.GYT.032	Graytown	155	345	465	242	236	230	196	175	240	329	245	474	137	CF	*MAT1-1*
2013.ZA.GYT.033	Graytown	158	345	462	245	236	230	196	175	240	329	245	480	137	CF	*MAT1-1*
2013.ZA.GYT.034	Graytown	155	345	462	245	236	209	196	175	240	329	245	480	134	CF	*MAT1-1*
2013.ZA.GYT.035	Graytown	158	345	465	242	236	209	199	175	240	329	245	480	134	CF	*MAT1-1*
2011.ZA.GYT.001	Graytown	155	345	465	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.002	Graytown	158	345	465	245	236	209	196	191	240	329	251	480	137	CF	*MAT1-2*
2011.ZA.GYT.003	Graytown	155	345	462	245	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.004	Graytown	155	345	462	245	236	230	196	175	240	329	251	480	134	CF	*MAT1-2*
2011.ZA.GYT.005	Graytown	155	345	462	245	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.006	Graytown	155	345	462	245	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.007	Graytown	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.008	Graytown	155	345	465	242	236	230	196	175	240	329	245	474	137	CF	*MAT1-2*
2011.ZA.GYT.009	Graytown	155	345	465	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.010	Graytown	158	345	462	242	236	209	196	175	240	329	251	474	137	CF	*MAT1-2*
2011.ZA.GYT.011	Graytown	155	345	462	248	223	209	199	191	240	329	245	474	137	CF	*MAT1-2*
2011.ZA.GYT.012	Graytown	155	345	468	245	223	230	196	191	240	329	245	480	134	CF	*MAT1-2*
2011.ZA.GYT.013	Graytown	155	345	468	245	223	230	196	191	240	329	245	480	134	CF	*MAT1-2*
2011.ZA.GYT.014	Graytown	155	345	462	242	236	209	196	175	240	329	245	474	134	CF	*MAT1-2*
2011.ZA.GYT.015	Graytown	155	345	462	245	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.016	Graytown	155	345	459	242	223	230	199	194	240	329	245	480	134	CF	*MAT1-2*
2011.ZA.GYT.017	Graytown	155	345	462	242	236	230	196	175	240	329	245	480	134	CF	*MAT1-2*
2011.ZA.GYT.018	Graytown	155	345	462	245	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2011.ZA.GYT.019	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2011.ZA.GYT.020	Graytown	155	345	462	245	236	230	196	175	240	329	251	480	134	CF	*MAT1-2*
2011.ZA.GYT.021	Graytown	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.022	Graytown	155	345	462	245	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.023	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2011.ZA.GYT.024	Graytown	155	345	462	245	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.025	Graytown	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2011.ZA.GYT.026	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2011.ZA.GYT.027	Graytown	155	345	462	242	236	230	196	175	240	329	251	480	134	CF	*MAT1-2*
2011.ZA.GYT.028	Graytown	155	345	462	242	236	230	196	175	240	329	251	480	134	CF	*MAT1-2*

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2011.ZA.GYT.029	Graytown	155	345	465	242	236	209	196	194	240	329	251	474	137	CF	*MAT1-2*
2012.ZA.GYT.001	Graytown	155	345	462	242	236	209	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.GYT.002	Graytown	158	345	462	245	223	230	199	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.003	Graytown	158	345	462	245	223	230	199	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.004	Graytown	158	345	462	242	223	230	199	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.005	Graytown	158	345	462	245	223	230	199	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.006	Graytown	158	345	465	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.007	Graytown	158	345	462	245	223	230	199	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.008	Graytown	158	345	462	245	223	209	199	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.009	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.010	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.011	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.012	Graytown	158	345	465	245	236	209	196	191	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.GYT.013	Graytown	155	345	462	242	236	230	199	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.014	Graytown	158	345	462	245	236	230	196	175	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.GYT.015	Graytown	158	345	465	242	236	230	196	191	240	329	245	480	137	CF	*MAT1-2*
2012.ZA.GYT.016	Graytown	158	345	465	242	223	209	199	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.GYT.017	Graytown	155	345	465	245	223	209	196	191	240	329	245	480	137	CF	*MAT1-2*
2012.ZA.GYT.018	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.019	Graytown	155	345	465	245	223	209	196	191	240	329	245	480	137	CF	*MAT1-2*
2012.ZA.GYT.020	Graytown	158	345	465	242	223	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.021	Graytown	161	345	465	245	236	209	196	191	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.GYT.022	Graytown	158	345	465	245	223	209	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.GYT.023	Graytown	152	345	462	245	236	230	199	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.024	Graytown	155	345	465	242	236	233	196	175	240	329	245	480	137	CF	*MAT1-2*
2012.ZA.GYT.025	Graytown	155	345	465	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.026	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.027	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.028	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.029	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.030	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.031	Graytown	158	345	465	245	236	233	199	191	240	329	251	480	134	CF	*MAT1-2*
2012.ZA.GYT.032	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.033	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.034	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2012.ZA.GYT.035	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.036	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.037	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.041	Graytown	155	345	462	242	223	209	196	175	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.GYT.042	Graytown	155	345	462	242	223	209	196	175	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.GYT.043	Graytown	155	345	462	242	236	230	196	175	240	329	245	480	134	CF	*MAT1-2*
2012.ZA.GYT.044	Graytown	155	345	465	242	236	209	196	175	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.GYT.045	Graytown	158	345	465	242	236	230	196	191	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.046	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.GYT.047	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.048	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.GYT.049	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.GYT.050	Graytown	155	345	462	242	236	230	196	175	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.BRG.008	Winterton	158	345	462	242	223	230	196	175	240	329	245	480	134	CF	*MAT1-1*
2012.ZA.WNT.007	Winterton	158	345	468	242	223	209	196	175	240	329	245	474	134	CF	*MAT1-1*
2012.ZA.WNT.011	Winterton	155	345	465	245	236	230	196	175	240	329	245	480	137	CF	*MAT1-1*
2012.ZA.WNT.012	Winterton	158	345	468	242	223	230	199	175	240	329	251	480	134	CF	*MAT1-1*
2012.ZA.BRG.003	Winterton	155	345	462	245	236	230	196	175	240	329	251	480	134	CF	*MAT1-1*
2012.ZA.BRG.004	Winterton	155	345	462	245	223	230	199	175	240	329	251	480	134	CF	*MAT1-1*
2012.ZA.BRG.006	Winterton	155	345	465	245	223	209	196	175	240	329	245	480	134	CF	*MAT1-1*
2012.ZA.BRG.007	Winterton	155	345	465	242	223	209	196	175	240	329	245	480	134	CF	*MAT1-1*
2012.ZA.WNT.001	Winterton	158	345	465	242	236	230	199	175	240	329	245	474	137	CF	*MAT1-1*
2012.ZA.WNT.006	Winterton	158	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-1*
2012.ZA.WNT.009	Winterton	158	345	462	242	236	209	196	175	240	329	245	474	134	CF	*MAT1-1*
2007.ZA.WNT.001	Winterton	158	345	462	242	236	230	199	175	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.WNT.010	Winterton	155	345	462	242	236	230	196	175	240	329	251	480	134	CF	*MAT1-2*
2012.ZA.WNT.002	Winterton	158	345	468	242	223	230	199	191	240	329	251	480	134	CF	*MAT1-2*
2012.ZA.WNT.003	Winterton	158	345	462	245	223	230	196	191	240	329	245	474	137	CF	*MAT1-2*
2012.ZA.WNT.004	Winterton	155	345	462	242	236	230	196	175	240	329	251	474	134	CF	*MAT1-2*
2012.ZA.WNT.005	Winterton	158	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.WNT.008	Winterton	158	345	462	242	236	230	196	175	240	329	245	474	134	CF	*MAT1-2*
2012.ZA.BRG.001	Winterton	155	345	465	245	236	209	199	175	240	329	251	480	134	CF	*MAT1-2*
2012.ZA.BRG.002	Winterton	155	345	465	245	236	230	196	175	240	329	245	480	134	CF	*MAT1-2*
2015.ZA.CRG.069	Hlanganani	155	345	462	242	223	233	196	175	244	329	251	480	134	SF	*MAT1-1*
2015.ZA.CRG.070	Hlanganani	155	345	462	242	223	233	196	175	244	329	251	480	134	SF	*MAT1-1*

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2015.ZA.CRG.071	Hlanganani	155	345	474	245	223	230	199	191	240	329	251	480	134	SF	*MAT1-1*
2015.ZA.CRG.072	Hlanganani	158	345	465	245	236	209	199	194	240	329	245	480	134	SF	*MAT1-1*
2015.ZA.CRG.073	Hlanganani	158	345	465	245	236	209	199	194	240	329	245	480	137	SF	*MAT1-1*
2015.ZA.CRG.074	Hlanganani	158	345	462	242	223	190	199	175	240	329	245	474	134	SF	*MAT1-1*
2015.ZA.CRG.075	Hlanganani	155	345	462	242	236	230	199	191	244	329	245	480	137	SF	*MAT1-1*
2015.ZA.CRG.076	Hlanganani	158	345	462	242	223	190	199	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.CRG.077	Hlanganani	158	345	465	245	223	230	199	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.CRG.078	Hlanganani	155	345	465	242	223	233	196	191	240	329	242	474	137	SF	*MAT1-1*
2015.ZA.CRG.079	Hlanganani	158	345	462	242	223	226	196	191	240	329	245	480	137	SF	*MAT1-1*
2015.ZA.CRG.080	Hlanganani	155	345	465	245	223	197	196	191	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.CRG.081	Hlanganani	155	345	465	245	236	230	196	191	252	329	245	480	137	SF	*MAT1-1*
2015.ZA.CRG.082	Hlanganani	158	345	465	242	236	209	199	191	248	329	245	480	134	SF	*MAT1-1*
2015.ZA.CRG.084	Hlanganani	158	345	465	245	223	230	199	191	244	329	251	474	137	SF	*MAT1-1*
2015.ZA.CRG.085	Hlanganani	155	345	465	245	236	230	196	175	240	329	245	480	134	SF	*MAT1-1*
2015.ZA.CRG.086	Hlanganani	158	345	465	245	236	209	196	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.CRG.087	Hlanganani	155	345	474	245	223	230	199	191	240	329	251	480	134	SF	*MAT1-1*
2015.ZA.CRG.088	Hlanganani	155	345	468	245	223	230	196	191	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.CRG.089	Hlanganani	155	345	468	245	223	230	196	191	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.CRG.094	Hlanganani	155	345	465	245	236	230	196	175	240	329	245	474	134	SF	*MAT1-1*
2015.ZA.CRG.095	Hlanganani	158	345	465	245	236	230	199	191	240	329	245	480	134	SF	*MAT1-1*
2015.ZA.CRG.096	Hlanganani	158	345	462	242	223	230	199	175	240	329	242	480	134	SF	*MAT1-1*
2015.ZA.CRG.097	Hlanganani	152	345	465	242	236	209	196	175	240	329	239	474	134	SF	*MAT1-1*
2015.ZA.CRG.099	Hlanganani	158	345	462	242	236	230	196	191	244	329	242	474	137	SF	*MAT1-1*
2015.ZA.CRG.100	Hlanganani	155	345	465	245	236	197	196	175	240	329	251	474	134	SF	*MAT1-1*
2015.ZA.CRG.101	Hlanganani	155	345	462	242	236	230	199	191	244	329	245	480	134	SF	*MAT1-1*
2015.ZA.CRG.102	Hlanganani	158	345	465	245	223	209	199	191	240	329	245	474	134	SF	*MAT1-1*
2015.ZA.CRG.103	Hlanganani	158	345	462	242	223	209	199	191	244	329	251	480	137	SF	*MAT1-1*
2015.ZA.CRG.104	Hlanganani	155	345	465	245	223	230	199	191	240	329	245	480	137	SF	*MAT1-1*
2015.ZA.CRG.002	Hlanganani	155	345	462	245	223	230	199	175	240	329	245	474	137	SF	*MAT1-2*
2015.ZA.CRG.006	Hlanganani	155	345	462	242	223	233	196	175	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.009	Hlanganani	158	345	462	245	223	230	199	191	240	329	245	480	134	SF	*MAT1-2*
2015.ZA.CRG.014	Hlanganani	155	345	465	242	223	209	196	191	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.015	Hlanganani	158	345	465	245	223	230	199	175	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.016	Hlanganani	158	345	462	245	223	212	196	191	260	329	245	480	134	SF	*MAT1-2*
2015.ZA.CRG.017	Hlanganani	158	345	465	239	223	230	196	175	240	329	251	474	134	SF	*MAT1-2*

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2015.ZA.CRG.018	Hlanganani	158	345	462	245	223	209	199	175	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.020	Hlanganani	155	345	465	245	236	230	196	175	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.021	Hlanganani	158	345	462	245	223	212	196	191	240	329	245	480	134	SF	*MAT1-2*
2015.ZA.CRG.024	Hlanganani	158	345	465	245	236	209	199	194	240	329	245	480	137	SF	*MAT1-2*
2015.ZA.CRG.025	Hlanganani	158	345	465	242	236	209	199	191	248	329	245	480	134	SF	*MAT1-2*
2015.ZA.CRG.027	Hlanganani	155	345	465	242	223	230	199	175	240	329	251	480	134	SF	*MAT1-2*
2015.ZA.CRG.028	Hlanganani	155	345	474	242	223	230	199	191	240	329	251	480	134	SF	*MAT1-2*
2015.ZA.CRG.029	Hlanganani	155	345	462	242	223	209	199	191	248	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.032	Hlanganani	158	345	465	242	223	209	196	175	240	329	245	480	137	SF	*MAT1-2*
2015.ZA.CRG.033	Hlanganani	158	345	462	245	223	230	196	191	240	329	230	480	137	SF	*MAT1-2*
2015.ZA.CRG.034	Hlanganani	155	345	462	245	223	230	199	175	240	329	251	474	137	SF	*MAT1-2*
2015.ZA.CRG.035	Hlanganani	155	345	462	242	236	226	199	175	240	329	245	480	137	SF	*MAT1-2*
2015.ZA.CRG.038	Hlanganani	158	345	471	245	223	230	196	175	240	329	245	474	137	SF	*MAT1-2*
2015.ZA.CRG.039	Hlanganani	155	345	462	242	223	233	196	175	244	329	251	480	134	SF	*MAT1-2*
2015.ZA.CRG.040	Hlanganani	158	345	462	245	223	212	196	191	264	329	245	480	137	SF	*MAT1-2*
2015.ZA.CRG.041	Hlanganani	158	345	462	242	236	230	196	191	244	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.042	Hlanganani	158	345	465	245	236	230	199	191	240	329	251	480	134	SF	*MAT1-2*
2015.ZA.CRG.043	Hlanganani	155	345	465	248	223	230	196	175	240	329	239	474	134	SF	*MAT1-2*
2015.ZA.CRG.048	Hlanganani	155	345	462	245	223	233	196	175	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.049	Hlanganani	155	345	465	242	223	230	199	175	240	329	251	480	137	SF	*MAT1-2*
2015.ZA.CRG.050	Hlanganani	158	345	462	245	236	230	199	187	240	329	239	480	134	SF	*MAT1-2*
2015.ZA.CRG.051	Hlanganani	155	345	465	242	223	230	199	175	240	329	251	480	137	SF	*MAT1-2*
2015.ZA.CRG.054	Hlanganani	158	345	462	242	223	230	196	191	240	329	242	480	137	SF	*MAT1-2*
2015.ZA.CRG.055	Hlanganani	158	345	462	245	236	230	199	187	240	329	245	480	137	SF	*MAT1-2*
2015.ZA.CRG.056	Hlanganani	155	345	462	242	223	230	199	175	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.057	Hlanganani	158	345	465	242	223	209	199	175	240	329	245	474	134	SF	*MAT1-2*
2015.ZA.CRG.058	Hlanganani	158	345	462	245	223	209	199	191	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.059	Hlanganani	158	345	465	245	245	223	196	175	240	329	251	474	137	SF	*MAT1-2*
2015.ZA.CRG.060	Hlanganani	155	345	465	245	223	209	196	191	240	329	245	474	134	SF	*MAT1-2*
2015.ZA.CRG.062	Hlanganani	155	345	462	242	236	209	202	191	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.063	Hlanganani	158	345	465	245	223	209	199	191	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.CRG.064	Hlanganani	158	345	465	242	223	209	199	191	240	329	245	474	134	SF	*MAT1-2*
2015.ZA.CRG.065	Hlanganani	155	345	465	245	223	209	196	191	240	329	245	474	134	SF	*MAT1-2*
2015.ZA.CRG.067	Hlanganani	158	345	465	245	223	209	199	175	240	329	245	480	137	SF	*MAT1-2*
2015.ZA.CRG.068	Hlanganani	155	345	462	242	236	233	196	175	240	329	251	480	137	SF	*MAT1-2*

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2015.ZA.NXM.069	KwaNxamalala	158	345	462	245	223	209	199	191	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.NXM.071	KwaNxamalala	158	345	465	245	223	230	199	191	240	329	245	480	134	SF	*MAT1-1*
2015.ZA.NXM.073	KwaNxamalala	158	345	462	242	236	209	199	191	240	329	251	480	137	SF	*MAT1-1*
2015.ZA.NXM.074	KwaNxamalala	158	345	462	245	223	230	196	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.NXM.076	KwaNxamalala	155	345	459	245	223	209	196	175	248	329	245	480	137	SF	*MAT1-1*
2015.ZA.NXM.080	KwaNxamalala	158	345	465	242	223	209	202	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.NXM.087	KwaNxamalala	158	345	462	242	223	230	199	191	236	329	245	474	137	SF	*MAT1-1*
2015.ZA.NXM.093	KwaNxamalala	158	345	465	245	223	209	196	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.NXM.094	KwaNxamalala	158	345	465	245	223	209	196	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.NXM.095	KwaNxamalala	155	345	465	245	223	226	199	175	240	329	245	474	134	SF	*MAT1-1*
2015.ZA.NXM.097	KwaNxamalala	164	345	462	242	223	209	196	191	244	329	245	480	137	SF	*MAT1-1*
2015.ZA.NXM.101	KwaNxamalala	155	345	465	245	223	233	199	175	240	329	245	480	134	SF	*MAT1-1*
2015.ZA.NXM.103	KwaNxamalala	152	345	465	242	223	230	202	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.NXM.105	KwaNxamalala	155	345	465	245	223	226	199	175	240	329	245	480	134	SF	*MAT1-1*
2015.ZA.NXM.106	KwaNxamalala	158	345	465	245	236	230	199	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.NXM.107	KwaNxamalala	158	345	465	245	236	230	199	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.NXM.108	KwaNxamalala	158	345	462	245	223	209	196	175	240	329	245	474	134	SF	*MAT1-1*
2015.ZA.NXM.109	KwaNxamalala	158	345	462	245	236	230	196	194	240	329	245	474	134	SF	*MAT1-1*
2015.ZA.NXM.110	KwaNxamalala	158	345	465	242	236	209	202	191	244	329	245	480	137	SF	*MAT1-1*
2015.ZA.NXM.112	KwaNxamalala	155	345	465	245	223	209	196	191	240	329	245	480	134	SF	*MAT1-1*
2015.ZA.NXM.113	KwaNxamalala	158	345	465	245	223	230	199	175	240	329	245	480	134	SF	*MAT1-1*
2015.ZA.NXM.116	KwaNxamalala	158	345	465	245	223	230	196	175	240	329	245	480	134	SF	*MAT1-1*
2015.ZA.NXM.117	KwaNxamalala	158	345	465	242	236	230	196	175	240	329	245	474	137	SF	*MAT1-1*
2015.ZA.NXM.119	KwaNxamalala	155	345	465	245	223	209	199	175	240	329	245	480	137	SF	*MAT1-1*
2015.ZA.NXM.048	KwaNxamalala	158	345	462	245	236	230	199	191	240	329	251	480	137	SF	*MAT1-2*
2015.ZA.NXM.051	KwaNxamalala	158	345	462	245	223	230	196	191	236	329	251	474	134	SF	*MAT1-2*
2015.ZA.NXM.052	KwaNxamalala	158	345	462	242	223	230	199	191	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.NXM.053	KwaNxamalala	158	345	462	242	236	209	196	191	248	329	245	480	134	SF	*MAT1-2*
2015.ZA.NXM.054	KwaNxamalala	158	345	462	242	223	230	199	191	236	329	245	474	137	SF	*MAT1-2*
2015.ZA.NXM.055	KwaNxamalala	155	345	462	245	223	230	196	191	240	329	251	480	137	SF	*MAT1-2*
2015.ZA.NXM.056	KwaNxamalala	155	345	462	245	223	230	196	191	240	329	245	480	137	SF	*MAT1-2*
2015.ZA.NXM.059	KwaNxamalala	158	345	462	245	236	230	196	175	240	329	245	480	134	SF	*MAT1-2*
2015.ZA.NXM.061	KwaNxamalala	155	345	462	245	236	209	196	175	240	329	245	474	134	SF	*MAT1-2*
2015.ZA.NXM.062	KwaNxamalala	158	345	465	245	236	209	196	191	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.NXM.064	KwaNxamalala	158	345	462	242	223	230	199	175	240	329	251	474	134	SF	*MAT1-2*

Isolate	Population	01	02	04	05	06	07	08	10	12	13	15	17	18	Farming system	Mating type
2015.ZA.NXM.067	KwaNxamalala	158	345	465	245	223	230	196	191	240	329	245	480	134	SF	*MAT1-2*
2015.ZA.NXM.068	KwaNxamalala	158	345	465	245	223	230	196	191	240	329	245	480	134	SF	*MAT1-2*
2015.ZA.EST.012	Ntabamhlophe	158	345	462	242	236	209	199	175	240	329	242	474	137	SF	*MAT1-1*
2015.ZA.EST.013	Ntabamhlophe	155	345	462	245	223	220	196	175	236	329	251	480	137	SF	*MAT1-1*
2015.ZA.EST.014	Ntabamhlophe	155	345	465	245	223	209	196	175	244	329	245	474	137	SF	*MAT1-1*
2015.ZA.EST.015	Ntabamhlophe	158	345	462	242	236	209	202	175	244	329	245	480	137	SF	*MAT1-1*
2015.ZA.EST.016	Ntabamhlophe	158	345	468	248	236	230	199	175	240	329	248	474	134	SF	*MAT1-1*
2015.ZA.EST.017	Ntabamhlophe	158	345	465	245	236	209	196	191	236	329	251	474	137	SF	*MAT1-1*
2015.ZA.EST.018	Ntabamhlophe	155	345	465	245	223	209	196	175	244	329	245	474	137	SF	*MAT1-1*
2015.ZA.EST.019	Ntabamhlophe	155	345	465	242	223	230	196	175	240	329	245	480	134	SF	*MAT1-1*
2015.ZA.EST.020	Ntabamhlophe	155	345	465	245	223	220	196	175	236	329	251	480	137	SF	*MAT1-1*
2015.ZA.EST.021	Ntabamhlophe	158	345	465	245	236	209	196	191	244	329	245	474	134	SF	*MAT1-2*
2015.ZA.EST.001	Ntabamhlophe	158	345	465	248	236	230	199	175	240	329	251	474	134	SF	*MAT1-2*
2015.ZA.EST.002	Ntabamhlophe	155	345	465	242	223	230	196	175	240	329	245	480	134	SF	*MAT1-2*
2015.ZA.EST.003	Ntabamhlophe	155	345	465	242	223	230	196	175	240	329	239	480	134	SF	*MAT1-2*
2015.ZA.EST.004	Ntabamhlophe	158	345	465	245	236	209	196	191	236	329	251	474	137	SF	*MAT1-2*
2015.ZA.EST.005	Ntabamhlophe	158	345	462	242	223	230	199	175	244	329	242	480	137	SF	*MAT1-2*
2015.ZA.EST.006	Ntabamhlophe	158	345	462	242	223	230	199	175	240	329	242	480	137	SF	*MAT1-2*
2015.ZA.EST.007	Ntabamhlophe	158	345	465	245	236	209	196	191	240	329	245	474	134	SF	*MAT1-2*
2015.ZA.EST.009	Ntabamhlophe	155	345	465	245	223	220	196	175	236	329	245	480	137	SF	*MAT1-2*
2015.ZA.EST.010	Ntabamhlophe	155	345	465	245	223	220	196	175	236	329	251	480	137	SF	*MAT1-2*
2015.ZA.EST.011	Ntabamhlophe	158	345	465	245	236	209	196	191	236	329	251	474	137	SF	*MAT1-2*

Appendix B: Microsatellite markers alleles (numbers 01 to 18) and mating types (*MAT1-1* and *MAT1-2*) for *Cercospora zeina* Isolates from Kenya, South Africa, Uganda, Zambia and Zimbabwe.

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2016.KE.BUN.382	Kenya	Bungoma	158	465	242	223	197	196	175	240	329	161	134	*MAT1-2*
2016.KE.BUN.383	Kenya	Bungoma	158	462	245	223	200	199	175	244	329	245	134	*MAT1-1*
2016.KE.BUN.384	Kenya	Bungoma	158	462	245	223	226	199	175	240	329	245	134	*MAT1-2*
2016.KE.BUN.385	Kenya	Bungoma	158	462	248	223	197	196	175	240	329	173	137	*MAT1-1*
2016.KE.BUN.388	Kenya	Bungoma	158	465	245	223	200	199	175	240	329	176	134	*MAT1-1*
2016.KE.BUN.390	Kenya	Bungoma	158	462	245	223	200	199	175	240	329	161	137	*MAT1-1*
2016.KE.BUN.393	Kenya	Bungoma	158	462	245	236	209	196	175	236	329	245	134	*MAT1-1*
2016.KE.BUN.395	Kenya	Bungoma	158	462	248	223	209	196	175	240	329	245	134	*MAT1-2*
2016.KE.BUN.399	Kenya	Bungoma	158	462	245	236	209	199	175	240	329	245	134	*MAT1-1*
2016.KE.BUN.400	Kenya	Bungoma	172	462	245	223	197	196	175	240	329	173	137	*MAT1-1*
2016.KE.BUN.404	Kenya	Bungoma	158	462	245	223	209	196	175	240	329	245	137	*MAT1-1*
2016.KE.BUN.422	Kenya	Bungoma	158	462	248	223	209	196	175	240	329	248	134	*MAT1-1*
2016.KE.KAK.229	Kenya	Kakamega	158	462	245	223	200	199	175	240	329	245	137	*MAT1-1*
2016.KE.KAK.292	Kenya	Kakamega	118	462	242	223	200	199	175	240	329	242	134	*MAT1-1*
2016.KE.KAK.319	Kenya	Kakamega	161	459	242	223	209	196	175	240	329	245	134	*MAT1-2*
2016.KE.KAK.321	Kenya	Kakamega	158	465	245	223	197	196	191	236	329	245	134	*MAT1-1*
2016.KE.KAK.329	Kenya	Kakamega	158	462	248	223	209	196	175	240	329	176	137	*MAT1-2*
2016.KE.KAK.332	Kenya	Kakamega	158	462	245	223	197	196	175	244	329	176	134	*MAT1-2*
2016.KE.KAK.335	Kenya	Kakamega	158	462	242	223	197	196	175	240	329	245	134	*MAT1-1*
2016.KE.KAK.342	Kenya	Kakamega	158	462	245	223	197	196	175	240	329	245	134	*MAT1-2*
2016.KE.KAK.344	Kenya	Kakamega	118	462	245	223	203	202	175	244	329	179	134	*MAT1-1*
2016.KE.KAK.347	Kenya	Kakamega	158	462	245	223	197	196	175	236	329	245	137	*MAT1-1*
2016.KE.KAK.349	Kenya	Kakamega	172	465	245	223	200	199	191	244	329	245	137	*MAT1-1*
2016.KE.KAK.354	Kenya	Kakamega	172	462	245	223	197	196	175	244	329	245	134	*MAT1-2*
2016.KE.KAK.358	Kenya	Kakamega	155	462	245	223	209	196	175	244	329	245	137	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2016.KE.KAK.360	Kenya	Kakamega	158	462	245	223	200	199	175	244	329	176	137	*MAT1-2*
2016.KE.KAK.367	Kenya	Kakamega	155	462	245	236	197	196	175	240	329	245	137	*MAT1-1*
2016.KE.KAK.368	Kenya	Kakamega	158	462	245	223	197	196	175	240	329	245	134	*MAT1-2*
2016.KE.KAK.372	Kenya	Kakamega	158	462	245	223	209	199	175	240	329	176	137	*MAT1-2*
2016.KE.KAK.374	Kenya	Kakamega	158	462	248	223	209	199	175	240	329	245	134	*MAT1-2*
2016.KE.KAK.392	Kenya	Kakamega	158	465	245	223	209	199	175	240	329	245	134	*MAT1-2*
2016.KE.KAK.426	Kenya	Kakamega	158	465	248	223	230	196	175	236	329	251	134	*MAT1-1*
2016.KE.KAK.432	Kenya	Kakamega	161	462	242	119	200	196	175	240	329	245	134	*MAT1-2*
2016.KE.KAK.433	Kenya	Kakamega	158	462	242	223	197	196	175	240	329	245	134	*MAT1-1*
2016.KE.KIS.232	Kenya	Kisumu	155	462	251	223	191	196	175	244	329	239	134	*MAT1-1*
2016.KE.KIS.243	Kenya	Kisumu	158	462	245	223	197	196	175	244	329	176	134	*MAT1-2*
2016.KE.KIS.246	Kenya	Kisumu	158	462	245	223	197	196	175	244	329	245	134	*MAT1-2*
2016.KE.KIS.252	Kenya	Kisumu	158	465	248	223	230	181	175	240	329	245	134	*MAT1-1*
2016.KE.KIS.253	Kenya	Kisumu	155	462	245	223	209	193	175	244	329	245	134	*MAT1-1*
2016.KE.KIS.259	Kenya	Kisumu	158	462	245	223	197	196	175	244	329	176	134	*MAT1-2*
2016.KE.KIS.262	Kenya	Kisumu	155	462	251	223	209	196	175	236	329	176	134	*MAT1-1*
2016.KE.KIS.401	Kenya	Kisumu	158	462	245	223	230	196	175	240	329	245	134	*MAT1-1*
2016.KE.SIA.002	Kenya	Siaya	161	462	245	223	230	205	157	244	329	245	134	*MAT1-2*
2016.KE.SIA.077	Kenya	Siaya	158	462	245	223	209	196	175	244	329	245	134	*MAT1-2*
2016.KE.SIA.081	Kenya	Siaya	158	465	245	223	200	199	175	240	329	245	134	*MAT1-1*
2016.KE.SIA.250	Kenya	Siaya	155	462	245	223	209	196	175	240	329	245	137	*MAT1-1*
2016.KE.SIA.254	Kenya	Siaya	155	462	245	223	200	199	175	240	329	245	137	*MAT1-2*
2016.KE.SIA.288	Kenya	Siaya	158	465	248	223	223	181	175	240	329	245	134	*MAT1-1*
2016.KE.VHG.140	Kenya	Vihiga	155	465	242	223	230	199	175	240	329	176	134	*MAT1-2*
2016.KE.VHG.144	Kenya	Vihiga	158	462	242	223	179	196	175	240	329	242	134	*MAT1-2*
2016.KE.VHG.146	Kenya	Vihiga	158	465	245	236	197	196	178	244	329	245	134	*MAT1-2*
2016.KE.VHG.156	Kenya	Vihiga	158	465	245	236	230	196	178	240	329	245	134	*MAT1-2*
2016.KE.VHG.157	Kenya	Vihiga	161	462	245	223	197	196	175	244	329	245	134	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2016.KE.VHG.167	Kenya	Vihiga	158	465	245	223	209	193	175	244	329	176	137	*MAT1-2*
2016.KE.VHG.184	Kenya	Vihiga	158	462	245	223	197	196	175	240	329	245	137	*MAT1-2*
2017.KE.KER.441	Kenya	Kericho	158	462	245	223	197	196	175	240	329	245	140	*MAT1-1*
2017.KE.KER.445	Kenya	Kericho	158	462	242	223	194	193	175	240	329	251	134	*MAT1-2*
2017.KE.KER.446	Kenya	Kericho	158	462	245	223	197	196	175	240	329	239	134	*MAT1-1*
2017.KE.KER.448	Kenya	Kericho	158	462	242	223	200	199	178	240	329	245	134	*MAT1-1*
2017.KE.KER.451	Kenya	Kericho	158	462	245	223	245	196	175	257	329	245	134	*MAT1-1*
2017.KE.KER.452	Kenya	Kericho	158	462	245	223	209	196	175	240	329	245	134	*MAT1-2*
2017.KE.KER.453	Kenya	Kericho	158	462	242	223	197	193	191	244	329	242	140	*MAT1-2*
2017.KE.KER.454	Kenya	Kericho	158	465	245	223	197	196	175	240	329	245	134	*MAT1-2*
2017.KE.KER.458	Kenya	Kericho	158	462	242	223	197	196	175	240	329	242	140	*MAT1-1*
2017.KE.KER.459	Kenya	Kericho	158	462	242	223	197	196	175	240	329	242	137	*MAT1-1*
2017.KE.KER.460	Kenya	Kericho	158	465	245	223	197	196	175	240	329	245	134	*MAT1-2*
2017.KE.KER.463	Kenya	Kericho	161	465	245	223	197	196	175	240	329	245	137	*MAT1-2*
2017.KE.KER.464	Kenya	Kericho	158	462	242	223	197	196	175	240	329	245	134	*MAT1-2*
2017.KE.KER.466	Kenya	Kericho	158	462	245	223	197	196	191	244	329	245	137	*MAT1-2*
2017.KE.KER.467	Kenya	Kericho	158	462	245	223	209	196	191	240	329	245	134	*MAT1-1*
2017.KE.KER.468	Kenya	Kericho	172	462	245	223	197	196	191	244	329	245	134	*MAT1-1*
2017.KE.KER.471	Kenya	Kericho	158	462	242	223	209	196	175	240	329	242	137	*MAT1-1*
2017.KE.KER.473	Kenya	Kericho	158	462	242	223	200	199	175	240	329	245	140	*MAT1-2*
2017.KE.KER.474	Kenya	Kericho	158	462	245	223	197	196	175	240	329	245	134	*MAT1-2*
2017.KE.KER.476	Kenya	Kericho	158	465	245	223	209	196	175	240	329	245	134	*MAT1-1*
2017.KE.KER.479	Kenya	Kericho	161	462	248	223	197	196	175	240	329	245	134	*MAT1-1*
2017.KE.KER.483	Kenya	Kericho	161	462	248	223	230	196	175	240	329	245	134	*MAT1-1*
2017.KE.KER.485	Kenya	Kericho	158	465	245	223	230	196	175	240	329	245	134	*MAT1-1*
2017.KE.KER.487	Kenya	Kericho	158	465	245	223	197	196	175	240	329	245	137	*MAT1-1*
2017.KE.KER.488	Kenya	Kericho	161	462	245	223	197	196	175	236	329	176	134	*MAT1-1*
2017.KE.KER.490	Kenya	Kericho	158	465	245	223	209	196	175	240	329	245	134	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.KE.KER.494	Kenya	Kericho	158	462	245	223	209	199	175	236	329	245	137	*MAT1-2*
2017.KE.KER.499	Kenya	Kericho	158	465	245	223	236	196	175	240	329	245	137	*MAT1-1*
2017.KE.KER.502	Kenya	Kericho	155	462	245	223	209	199	191	240	329	245	134	*MAT1-1*
2017.KE.KER.503	Kenya	Kericho	158	462	245	223	197	196	175	240	329	245	134	*MAT1-1*
2017.KE.KER.507	Kenya	Kericho	155	462	245	223	197	196	175	236	329	176	137	*MAT1-1*
2017.KE.KER.511	Kenya	Kericho	158	465	248	223	197	196	175	232	329	176	134	*MAT1-1*
2017.KE.KER.512	Kenya	Kericho	158	465	245	223	197	196	175	236	329	245	134	*MAT1-2*
2017.KE.KER.515	Kenya	Kericho	158	459	245	223	197	196	175	240	329	245	134	*MAT1-2*
2017.KE.KER.522	Kenya	Kericho	158	462	245	223	197	196	191	240	329	245	134	*MAT1-1*
2017.KE.KER.535	Kenya	Kericho	155	465	242	223	200	199	175	236	329	176	134	*MAT1-2*
2017.KE.KER.538	Kenya	Kericho	158	465	245	223	197	196	175	240	329	245	134	*MAT1-1*
2017.KE.KER.616	Kenya	Kericho	158	462	242	223	206	199	175	240	329	245	134	*MAT1-1*
2017.KE.KER.632	Kenya	Kericho	158	465	245	223	209	193	175	240	329	245	134	*MAT1-2*
2017.KE.KER.650	Kenya	Kericho	158	462	245	236	197	196	175	202	329	245	137	*MAT1-2*
2017.KE.KER.679	Kenya	Kericho	172	465	239	223	197	196	178	240	329	242	137	*MAT1-2*
2017.KE.KIT.613	Kenya	Kitale	152	394	245	223	209	196	175	240	329	239	134	*MAT1-1*
2017.KE.KIT.618	Kenya	Kitale	118	465	242	223	200	199	175	240	329	245	137	*MAT1-2*
2017.KE.KIT.625	Kenya	Kitale	158	465	245	223	200	199	175	240	329	245	134	*MAT1-1*
2017.KE.KIT.707	Kenya	Kitale	158	462	245	223	197	196	175	244	329	245	134	*MAT1-2*
2017.KE.KIT.712	Kenya	Kitale	158	462	245	223	209	193	175	240	329	245	134	*MAT1-1*
2017.KE.KIT.715	Kenya	Kitale	158	465	245	223	230	196	175	248	329	245	134	*MAT1-1*
2017.KE.KIT.719	Kenya	Kitale	155	465	245	223	209	199	175	240	329	245	137	*MAT1-1*
2017.KE.KIT.721	Kenya	Kitale	158	465	245	223	209	196	175	240	329	245	137	*MAT1-1*
2017.KE.KIT.725	Kenya	Kitale	158	459	245	223	209	202	175	236	329	245	140	*MAT1-2*
2017.KE.KIT.728	Kenya	Kitale	118	462	245	223	197	196	175	240	329	245	140	*MAT1-1*
2017.KE.KIT.731	Kenya	Kitale	155	459	245	223	209	196	191	240	329	245	134	*MAT1-2*
2017.KE.KIT.733	Kenya	Kitale	155	465	245	223	209	202	175	240	329	251	134	*MAT1-1*
2017.KE.KIT.734	Kenya	Kitale	158	462	242	223	197	196	175	205	329	245	134	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.KE.KIT.735	Kenya	Kitale	158	468	245	223	209	196	175	240	329	245	134	*MAT1-1*
2017.KE.KIT.737	Kenya	Kitale	158	465	245	223	209	199	175	236	329	245	134	*MAT1-2*
2017.KE.KIT.739	Kenya	Kitale	155	465	245	223	206	196	175	232	329	245	134	*MAT1-1*
2017.KE.KIT.740	Kenya	Kitale	118	465	245	223	197	196	175	244	329	245	137	*MAT1-1*
2017.KE.KIT.741	Kenya	Kitale	158	462	245	223	197	196	175	244	329	245	134	*MAT1-2*
2017.KE.KIT.742	Kenya	Kitale	158	465	245	223	200	199	191	240	329	191	134	*MAT1-2*
2017.KE.KIT.758	Kenya	Kitale	118	465	245	223	197	196	175	240	329	245	137	*MAT1-1*
2017.KE.KIT.762	Kenya	Kitale	155	462	242	223	197	196	175	244	329	245	134	*MAT1-2*
2017.KE.KIT.763	Kenya	Kitale	158	462	245	223	197	196	178	236	329	179	137	*MAT1-2*
2017.KE.KIT.764	Kenya	Kitale	158	462	245	223	200	199	175	244	329	245	134	*MAT1-1*
2017.KE.KIT.768	Kenya	Kitale	158	459	242	223	209	196	175	240	329	245	137	*MAT1-1*
2017.KE.TRS.516	Kenya	Transzoia	158	465	245	215	197	196	175	244	329	245	134	*MAT1-2*
2017.KE.TRS.520	Kenya	Transzoia	161	465	242	223	200	199	175	240	329	176	140	*MAT1-2*
2017.KE.TRS.524	Kenya	Transzoia	118	462	245	223	197	196	175	244	329	245	137	*MAT1-1*
2017.KE.TRS.536	Kenya	Transzoia	161	462	248	223	230	196	175	240	329	245	134	*MAT1-1*
2017.KE.TRS.541	Kenya	Transzoia	172	465	245	223	179	196	191	240	329	191	137	*MAT1-2*
2017.KE.TRS.544	Kenya	Transzoia	118	465	245	223	194	193	175	244	329	245	134	*MAT1-2*
2017.KE.TRS.547	Kenya	Transzoia	158	462	245	223	197	196	175	240	329	173	134	*MAT1-1*
2017.KE.TRS.555	Kenya	Transzoia	158	459	245	223	197	196	175	240	329	176	134	*MAT1-1*
2017.KE.TRS.556	Kenya	Transzoia	158	462	245	223	209	196	191	244	329	245	137	*MAT1-2*
2017.KE.TRS.558	Kenya	Transzoia	158	462	242	223	197	196	175	240	329	176	134	*MAT1-1*
2017.KE.TRS.559	Kenya	Transzoia	118	462	245	223	200	199	175	244	329	245	140	*MAT1-2*
2017.KE.TRS.565	Kenya	Transzoia	158	462	245	223	197	196	175	240	329	245	134	*MAT1-2*
2017.KE.TRS.566	Kenya	Transzoia	158	465	245	223	209	196	175	240	329	245	137	*MAT1-1*
2017.KE.TRS.568	Kenya	Transzoia	155	462	248	223	200	199	175	232	329	251	134	*MAT1-1*
2017.KE.TRS.570	Kenya	Transzoia	158	462	245	223	197	196	175	240	329	245	137	*MAT1-2*
2017.KE.TRS.571	Kenya	Transzoia	158	462	245	236	209	196	175	232	329	245	137	*MAT1-2*
2017.KE.TRS.579	Kenya	Transzoia	158	462	245	223	209	196	175	240	329	176	134	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.KE.TRS.580	Kenya	Transzoia	158	462	245	223	209	196	175	240	329	245	134	*MAT1-1*
2017.KE.TRS.582	Kenya	Transzoia	158	465	248	223	209	196	175	240	329	245	134	*MAT1-2*
2017.KE.TRS.593	Kenya	Transzoia	158	462	245	223	200	199	175	236	329	245	134	*MAT1-1*
2017.KE.TRS.599	Kenya	Transzoia	158	462	242	223	209	196	175	240	329	245	137	*MAT1-1*
2017.KE.TRS.608	Kenya	Transzoia	158	462	242	223	197	196	175	240	329	242	134	*MAT1-2*
2017.KE.TRS.617	Kenya	Transzoia	158	468	245	223	209	196	175	240	329	245	134	*MAT1-2*
2017.KE.TRS.621	Kenya	Transzoia	158	462	245	223	197	196	175	240	329	176	134	*MAT1-2*
2017.KE.TRS.736	Kenya	Transzoia	158	465	242	223	200	199	175	240	329	242	134	*MAT1-2*
2017.KE.TRS.767	Kenya	Transzoia	158	462	248	223	230	199	175	244	329	245	137	*MAT1-1*
2015.ZA.CRG.002	South Africa	Hlanganani	155.155	462	245	223	230	199	175	240	329	245	137	*MAT1-2*
2015.ZA.CRG.006	South Africa	Hlanganani	155	462	242	223	233	196	175	240	329	251	134	*MAT1-1*
2015.ZA.CRG.009	South Africa	Hlanganani	158	462	245	223	230	199	191	240	329	245	134	*MAT1-1*
2015.ZA.CRG.014	South Africa	Hlanganani	155	465	242	223	209	196	191	240	329	251	134	*MAT1-2*
2015.ZA.CRG.015	South Africa	Hlanganani	158	465	245	223	230	199	175	240	329	251	134	*MAT1-1*
2015.ZA.CRG.016	South Africa	Hlanganani	158	462	245	223	212	196	191	260	329	245	134	*MAT1-1*
2015.ZA.CRG.017	South Africa	Hlanganani	158	465	239	223	230	196	175	240	329	251	134	*MAT1-2*
2015.ZA.CRG.018	South Africa	Hlanganani	158	462	245	223	209	199	175	240	329	251	134	*MAT1-2*
2015.ZA.CRG.020	South Africa	Hlanganani	155	465	245	236	230	196	175	240	329	251	134	*MAT1-1*
2015.ZA.CRG.021	South Africa	Hlanganani	158	462	245	223	212	196	191	240	329	245	134	*MAT1-2*
2015.ZA.CRG.024	South Africa	Hlanganani	158	465	245	236	209	199	194	240	329	245	137	*MAT1-2*
2015.ZA.CRG.025	South Africa	Hlanganani	158	465	242	236	209	199	191	248	329	245	134	*MAT1-2*
2015.ZA.CRG.027	South Africa	Hlanganani	155	465	242	223	230	199	175	240	329	251	134	*MAT1-1*
2015.ZA.CRG.028	South Africa	Hlanganani	155	474	242	223	230	199	191	240	329	251	134	*MAT1-2*
2015.ZA.CRG.029	South Africa	Hlanganani	155	462	242	223	209	199	191	248	329	251	134	*MAT1-2*
2015.ZA.CRG.032	South Africa	Hlanganani	158	465	242	223	209	196	175	240	329	245	137	*MAT1-2*
2015.ZA.CRG.033	South Africa	Hlanganani	158	462	245	223	230	196	191	240	329	230	137	*MAT1-1*
2015.ZA.CRG.034	South Africa	Hlanganani	155	462	245	223	230	199	175	240	329	251	137	*MAT1-2*
2015.ZA.CRG.035	South Africa	Hlanganani	155	462	242	236	226	199	175	240	329	245	137	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZA.CRG.038	South Africa	Hlanganani	158	471	245	223	230	196	175	240	329	245	137	*MAT1-2*
2015.ZA.CRG.039	South Africa	Hlanganani	155	462	242	223	233	196	175	244	329	251	134	*MAT1-2*
2015.ZA.CRG.040	South Africa	Hlanganani	158	462	245	223	212	196	191	264	329	245	137	*MAT1-2*
2015.ZA.CRG.041	South Africa	Hlanganani	158	462	242	236	230	196	191	244	329	251	134	*MAT1-1*
2015.ZA.CRG.042	South Africa	Hlanganani	158	465	245	236	230	199	191	240	329	251	134	*MAT1-2*
2015.ZA.CRG.043	South Africa	Hlanganani	155	465	248	223	230	196	175	240	329	239	134	*MAT1-2*
2015.ZA.CRG.048	South Africa	Hlanganani	155	462	245	223	233	196	175	240	329	251	134	*MAT1-1*
2015.ZA.CRG.049	South Africa	Hlanganani	155	465	242	223	230	199	175	240	329	251	137	*MAT1-2*
2015.ZA.CRG.050	South Africa	Hlanganani	158	462	245	236	230	199	187	240	329	239	134	*MAT1-1*
2015.ZA.CRG.051	South Africa	Hlanganani	155	465	242	223	230	199	175	240	329	251	137	*MAT1-1*
2015.ZA.CRG.054	South Africa	Hlanganani	158	462	242	223	230	196	191	240	329	242	137	*MAT1-1*
2015.ZA.CRG.055	South Africa	Hlanganani	158	462	245	236	230	199	187	240	329	245	137	*MAT1-1*
2015.ZA.CRG.056	South Africa	Hlanganani	155	462	242	223	230	199	175	240	329	251	134	*MAT1-2*
2015.ZA.CRG.057	South Africa	Hlanganani	158	465	242	223	209	199	175	240	329	245	134	*MAT1-1*
2015.ZA.CRG.058	South Africa	Hlanganani	158	462	245	223	209	199	191	240	329	251	134	*MAT1-2*
2015.ZA.CRG.059	South Africa	Hlanganani	158	465	245	245	223	196	175	240	329	251	137	*MAT1-2*
2015.ZA.CRG.060	South Africa	Hlanganani	155	465	245	223	209	196	191	240	329	245	134	*MAT1-2*
2015.ZA.CRG.062	South Africa	Hlanganani	155	462	242	236	209	202	191	240	329	251	134	*MAT1-2*
2015.ZA.CRG.063	South Africa	Hlanganani	158	465	245	223	209	199	191	240	329	251	134	*MAT1-2*
2015.ZA.CRG.064	South Africa	Hlanganani	158	465	242	223	209	199	191	240	329	245	134	*MAT1-2*
2015.ZA.CRG.065	South Africa	Hlanganani	155	465	245	223	209	196	191	240	329	245	134	*MAT1-2*
2015.ZA.CRG.067	South Africa	Hlanganani	158	465	245	223	209	199	175	240	329	245	137	*MAT1-2*
2015.ZA.CRG.068	South Africa	Hlanganani	155	462	242	236	233	196	175	240	329	251	137	*MAT1-1*
2015.ZA.CRG.069	South Africa	Hlanganani	155	462	242	223	233	196	175	244	329	251	134	*MAT1-2*
2015.ZA.CRG.070	South Africa	Hlanganani	155	462	242	223	233	196	175	244	329	251	134	*MAT1-2*
2015.ZA.CRG.071	South Africa	Hlanganani	155	474	245	223	230	199	191	240	329	251	134	*MAT1-2*
2015.ZA.CRG.072	South Africa	Hlanganani	158	465	245	236	209	199	194	240	329	245	134	*MAT1-1*
2015.ZA.CRG.073	South Africa	Hlanganani	158	465	245	236	209	199	194	240	329	245	137	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZA.CRG.074	South Africa	Hlanganani	158	462	242	223	190	199	175	240	329	245	134	*MAT1-1*
2015.ZA.CRG.075	South Africa	Hlanganani	155	462	242	236	230	199	191	244	329	245	137	*MAT1-1*
2015.ZA.CRG.076	South Africa	Hlanganani	158	462	242	223	190	199	175	240	329	245	137	*MAT1-1*
2015.ZA.CRG.077	South Africa	Hlanganani	158	465	245	223	230	199	175	240	329	245	137	*MAT1-2*
2015.ZA.CRG.078	South Africa	Hlanganani	155	465	242	223	233	196	191	240	329	242	137	*MAT1-1*
2015.ZA.CRG.079	South Africa	Hlanganani	158	462	242	223	226	196	191	240	329	245	137	*MAT1-1*
2015.ZA.CRG.080	South Africa	Hlanganani	155	465	245	223	197	196	191	240	329	245	137	*MAT1-1*
2015.ZA.CRG.081	South Africa	Hlanganani	155	465	245	236	230	196	191	252	329	245	137	*MAT1-1*
2015.ZA.CRG.082	South Africa	Hlanganani	158	465	242	236	209	199	191	248	329	245	134	*MAT1-2*
2015.ZA.CRG.084	South Africa	Hlanganani	158	465	245	223	230	199	191	244	329	251	137	*MAT1-2*
2015.ZA.CRG.085	South Africa	Hlanganani	155	465	245	236	230	196	175	240	329	245	134	*MAT1-1*
2015.ZA.CRG.086	South Africa	Hlanganani	158	465	245	236	209	196	175	240	329	245	137	*MAT1-1*
2015.ZA.CRG.087	South Africa	Hlanganani	155	474	245	223	230	199	191	240	329	251	134	*MAT1-2*
2015.ZA.CRG.088	South Africa	Hlanganani	155	468	245	223	230	196	191	240	329	245	137	*MAT1-2*
2015.ZA.CRG.089	South Africa	Hlanganani	155	468	245	223	230	196	191	240	329	245	137	*MAT1-2*
2015.ZA.CRG.094	South Africa	Hlanganani	155	465	245	236	230	196	175	240	329	245	134	*MAT1-1*
2015.ZA.CRG.095	South Africa	Hlanganani	158	465	245	236	230	199	191	240	329	245	134	*MAT1-2*
2015.ZA.CRG.096	South Africa	Hlanganani	158	462	242	223	230	199	175	240	329	242	134	*MAT1-2*
2015.ZA.CRG.097	South Africa	Hlanganani	152	465	242	236	209	196	175	240	329	239	134	*MAT1-2*
2015.ZA.CRG.099	South Africa	Hlanganani	158	462	242	236	230	196	191	244	329	242	137	*MAT1-1*
2015.ZA.CRG.100	South Africa	Hlanganani	155	465	245	236	197	196	175	240	329	251	134	*MAT1-1*
2015.ZA.CRG.101	South Africa	Hlanganani	155	462	242	236	230	199	191	244	329	245	134	*MAT1-1*
2015.ZA.CRG.102	South Africa	Hlanganani	158	465	245	223	209	199	191	240	329	245	134	*MAT1-2*
2015.ZA.CRG.103	South Africa	Hlanganani	158	462	242	223	209	199	191	244	329	251	137	*MAT1-2*
2015.ZA.CRG.104	South Africa	Hlanganani	155	465	245	223	230	199	191	240	329	245	137	*MAT1-2*
2015.ZA.EST.001	South Africa	Ntabamhlophe	158	465	248	236	230	199	175	240	329	251	134	*MAT1-1*
2015.ZA.EST.002	South Africa	Ntabamhlophe	155	465	242	223	230	196	175	240	329	245	134	*MAT1-2*
2015.ZA.EST.003	South Africa	Ntabamhlophe	155	465	242	223	230	196	175	240	329	239	134	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZA.EST.004	South Africa	Ntabamhlophe	158	465	245	236	209	196	191	236	329	251	137	*MAT1-2*
2015.ZA.EST.005	South Africa	Ntabamhlophe	158	462	242	223	230	199	175	244	329	242	137	*MAT1-2*
2015.ZA.EST.006	South Africa	Ntabamhlophe	158	462	242	223	230	199	175	240	329	242	137	*MAT1-2*
2015.ZA.EST.007	South Africa	Ntabamhlophe	158	465	245	236	209	196	191	240	329	245	134	*MAT1-1*
2015.ZA.EST.009	South Africa	Ntabamhlophe	155	465	245	223	220	196	175	236	329	245	137	*MAT1-1*
2015.ZA.EST.010	South Africa	Ntabamhlophe	155	465	245	223	220	196	175	236	329	251	137	*MAT1-2*
2015.ZA.EST.011	South Africa	Ntabamhlophe	158	465	245	236	209	196	191	236	329	251	137	*MAT1-2*
2015.ZA.EST.012	South Africa	Ntabamhlophe	158	462	242	236	209	199	175	240	329	242	137	*MAT1-2*
2015.ZA.EST.013	South Africa	Ntabamhlophe	155	462	245	223	220	196	175	236	329	251	137	*MAT1-1*
2015.ZA.EST.014	South Africa	Ntabamhlophe	155	465	245	223	209	196	175	244	329	245	137	*MAT1-1*
2015.ZA.EST.015	South Africa	Ntabamhlophe	158	462	242	236	209	202	175	244	329	245	137	*MAT1-2*
2015.ZA.EST.016	South Africa	Ntabamhlophe	158	468	248	236	230	199	175	240	329	248	134	*MAT1-1*
2015.ZA.EST.017	South Africa	Ntabamhlophe	158	465	245	236	209	196	191	236	329	251	137	*MAT1-2*
2015.ZA.EST.018	South Africa	Ntabamhlophe	155	465	245	223	209	196	175	244	329	245	137	*MAT1-2*
2015.ZA.EST.019	South Africa	Ntabamhlophe	155	465	242	223	230	196	175	240	329	245	134	*MAT1-1*
2015.ZA.EST.020	South Africa	Ntabamhlophe	155	465	245	223	220	196	175	236	329	251	137	*MAT1-1*
2015.ZA.EST.021	South Africa	Ntabamhlophe	158	465	245	236	209	196	191	244	329	245	134	*MAT1-2*
2015.ZA.NXM.048	South Africa	KwaNxamalala	158	462	245	236	230	199	191	240	329	251	137	*MAT1-1*
2015.ZA.NXM.051	South Africa	KwaNxamalala	158	462	245	223	230	196	191	236	329	251	134	*MAT1-1*
2015.ZA.NXM.052	South Africa	KwaNxamalala	158	462	242	223	230	199	191	240	329	251	134	*MAT1-1*
2015.ZA.NXM.053	South Africa	KwaNxamalala	158	462	242	236	209	196	191	248	329	245	134	*MAT1-1*
2015.ZA.NXM.054	South Africa	KwaNxamalala	158	462	242	223	230	199	191	236	329	245	137	*MAT1-2*
2015.ZA.NXM.055	South Africa	KwaNxamalala	155	462	245	223	230	196	191	240	329	251	137	*MAT1-2*
2015.ZA.NXM.056	South Africa	KwaNxamalala	155	462	245	223	230	196	191	240	329	245	137	*MAT1-2*
2015.ZA.NXM.059	South Africa	KwaNxamalala	158	462	245	236	230	196	175	240	329	245	134	*MAT1-1*
2015.ZA.NXM.061	South Africa	KwaNxamalala	155	462	245	236	209	196	175	240	329	245	134	*MAT1-2*
2015.ZA.NXM.062	South Africa	KwaNxamalala	158	465	245	236	209	196	191	240	329	251	134	*MAT1-1*
2015.ZA.NXM.064	South Africa	KwaNxamalala	158	462	242	223	230	199	175	240	329	251	134	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZA.NXM.067	South Africa	KwaNxamalala	158	465	245	223	230	196	191	240	329	245	134	*MAT1-1*
2015.ZA.NXM.068	South Africa	KwaNxamalala	158	465	245	223	230	196	191	240	329	245	134	*MAT1-2*
2015.ZA.NXM.069	South Africa	KwaNxamalala	158	462	245	223	209	199	191	240	329	245	137	*MAT1-2*
2015.ZA.NXM.071	South Africa	KwaNxamalala	158	465	245	223	230	199	191	240	329	245	134	*MAT1-1*
2015.ZA.NXM.073	South Africa	KwaNxamalala	158	462	242	236	209	199	191	240	329	251	137	*MAT1-1*
2015.ZA.NXM.074	South Africa	KwaNxamalala	158	462	245	223	230	196	175	240	329	245	137	*MAT1-1*
2015.ZA.NXM.076	South Africa	KwaNxamalala	155	459	245	223	209	196	175	248	329	245	137	*MAT1-1*
2015.ZA.NXM.080	South Africa	KwaNxamalala	158	465	242	223	209	202	175	240	329	245	137	*MAT1-1*
2015.ZA.NXM.087	South Africa	KwaNxamalala	158	462	242	223	230	199	191	236	329	245	137	*MAT1-1*
2015.ZA.NXM.093	South Africa	KwaNxamalala	158	465	245	223	209	196	175	240	329	245	137	*MAT1-2*
2015.ZA.NXM.094	South Africa	KwaNxamalala	158	465	245	223	209	196	175	240	329	245	137	*MAT1-2*
2015.ZA.NXM.095	South Africa	KwaNxamalala	155	465	245	223	226	199	175	240	329	245	134	*MAT1-1*
2015.ZA.NXM.097	South Africa	KwaNxamalala	164	462	242	223	209	196	191	244	329	245	137	*MAT1-1*
2015.ZA.NXM.101	South Africa	KwaNxamalala	155	465	245	223	233	199	175	240	329	245	134	*MAT1-2*
2015.ZA.NXM.103	South Africa	KwaNxamalala	152	465	242	223	230	202	175	240	329	245	137	*MAT1-2*
2015.ZA.NXM.105	South Africa	KwaNxamalala	155	465	245	223	226	199	175	240	329	245	134	*MAT1-1*
2015.ZA.NXM.106	South Africa	KwaNxamalala	158	465	245	236	230	199	175	240	329	245	137	*MAT1-2*
2015.ZA.NXM.107	South Africa	KwaNxamalala	158	465	245	236	230	199	175	240	329	245	137	*MAT1-1*
2015.ZA.NXM.108	South Africa	KwaNxamalala	158	462	245	223	209	196	175	240	329	245	134	*MAT1-2*
2015.ZA.NXM.109	South Africa	KwaNxamalala	158	462	245	236	230	196	194	240	329	245	134	*MAT1-1*
2015.ZA.NXM.110	South Africa	KwaNxamalala	158	465	242	236	209	202	191	244	329	245	137	*MAT1-1*
2015.ZA.NXM.112	South Africa	KwaNxamalala	155	465	245	223	209	196	191	240	329	245	134	*MAT1-1*
2015.ZA.NXM.113	South Africa	KwaNxamalala	158	465	245	223	230	199	175	240	329	245	134	*MAT1-1*
2015.ZA.NXM.116	South Africa	KwaNxamalala	158	465	245	223	230	196	175	240	329	245	134	*MAT1-1*
2015.ZA.NXM.117	South Africa	KwaNxamalala	158	465	242	236	230	196	175	240	329	245	137	*MAT1-2*
2015.ZA.NXM.119	South Africa	KwaNxamalala	155	465	245	223	209	199	175	240	329	245	137	*MAT1-2*
2015.ZA.BZN.001	South Africa	Mbizana	158	465	242	236	230	202	191	240	329	245	134	*MAT1-1*
2015.ZA.BZN.007	South Africa	Mbizana	155	465	248	223	209	199	175	248	329	245	137	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZA.BZN.009	South Africa	Mbizana	155	468	245	220	209	199	191	236	329	245	134	*MAT1-1*
2015.ZA.BZN.011	South Africa	Mbizana	155	465	242	236	230	196	175	244	329	242	137	*MAT1-2*
2015.ZA.BZN.014	South Africa	Mbizana	155	465	242	223	230	196	175	244	329	242	137	*MAT1-2*
2015.ZA.BZN.015	South Africa	Mbizana	155	462	242	236	233	199	175	244	329	245	134	*MAT1-1*
2015.ZA.BZN.017	South Africa	Mbizana	158	462	242	223	233	196	175	240	329	242	134	*MAT1-2*
2015.ZA.BZN.018	South Africa	Mbizana	155	462	242	223	209	199	175	240	329	251	134	*MAT1-1*
2015.ZA.BZN.020	South Africa	Mbizana	158	465	245	236	248	196	187	244	329	245	134	*MAT1-2*
2015.ZA.BZN.021	South Africa	Mbizana	158	465	245	236	248	196	187	240	329	251	134	*MAT1-2*
2015.ZA.BZN.023	South Africa	Mbizana	155	462	242	223	230	196	191	240	329	245	134	*MAT1-2*
2015.ZA.BZN.025	South Africa	Mbizana	155	462	242	223	212	199	191	244	329	245	134	*MAT1-1*
2015.ZA.BZN.027	South Africa	Mbizana	155	462	242	223	212	199	191	240	329	245	134	*MAT1-1*
2015.ZA.BZN.028	South Africa	Mbizana	158	462	245	236	230	196	194	240	329	245	134	*MAT1-1*
2015.ZA.BZN.029	South Africa	Mbizana	158	462	242	223	212	199	175	240	329	242	134	*MAT1-1*
2015.ZA.BZN.031	South Africa	Mbizana	155	462	242	223	230	199	175	240	329	242	134	*MAT1-2*
2015.ZA.BZN.032	South Africa	Mbizana	155	462	242	223	230	199	175	240	329	242	134	*MAT1-2*
2015.ZA.BZN.033	South Africa	Mbizana	158	462	242	223	230	196	191	240	329	242	134	*MAT1-2*
2015.ZA.BZN.035	South Africa	Mbizana	155	462	242	223	209	199	191	244	329	251	134	*MAT1-2*
2015.ZA.BZN.036	South Africa	Mbizana	158	462	245	236	209	208	191	240	329	245	134	*MAT1-2*
2015.ZA.BZN.040	South Africa	Mbizana	155	462	245	223	233	196	191	244	329	245	137	*MAT1-1*
2015.ZA.BZN.044	South Africa	Mbizana	155	465	245	236	230	196	191	240	329	251	134	*MAT1-2*
2015.ZA.BZN.046	South Africa	Mbizana	155	462	245	236	233	199	191	240	329	245	134	*MAT1-2*
2015.ZA.BZN.048	South Africa	Mbizana	158	462	242	236	197	196	191	244	329	251	134	*MAT1-1*
2015.ZA.BZN.052	South Africa	Mbizana	155	465	242	236	233	196	175	240	329	245	134	*MAT1-2*
2015.ZA.BZN.053	South Africa	Mbizana	155	465	242	236	233	196	175	240	329	245	134	*MAT1-2*
2015.ZA.BZN.055	South Africa	Mbizana	155	465	242	236	230	196	175	268	329	242	134	*MAT1-1*
2015.ZA.BZN.057	South Africa	Mbizana	158	462	245	236	230	196	175	240	329	245	137	*MAT1-1*
2015.ZA.BZN.059	South Africa	Mbizana	158	468	245	236	230	196	172	244	329	245	134	*MAT1-1*
2015.ZA.BZN.061	South Africa	Mbizana	155	465	245	239	233	199	175	240	329	245	137	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZA.BZN.062	South Africa	Mbizana	158	465	242	223	209	196	175	240	329	251	137	*MAT1-2*
2015.ZA.BZN.065	South Africa	Mbizana	158	465	242	236	230	196	175	240	329	245	134	*MAT1-1*
2015.ZA.BZN.066	South Africa	Mbizana	155	465	242	236	230	196	175	244	329	251	134	*MAT1-1*
2015.ZA.BZN.067	South Africa	Mbizana	155	465	242	236	230	196	175	244	329	251	134	*MAT1-1*
2015.ZA.BZN.070	South Africa	Mbizana	155	462	242	236	217	202	175	240	329	251	134	*MAT1-1*
2015.ZA.BZN.072	South Africa	Mbizana	155	465	242	236	209	199	191	240	329	245	137	*MAT1-1*
2015.ZA.BZN.073	South Africa	Mbizana	155	465	245	236	209	199	191	240	329	245	134	*MAT1-2*
2015.ZA.BZN.075	South Africa	Mbizana	158	465	245	236	209	196	191	244	329	245	137	*MAT1-2*
2015.ZA.BZN.076	South Africa	Mbizana	158	465	245	236	209	196	191	244	329	245	137	*MAT1-2*
2015.ZA.BZN.078	South Africa	Mbizana	158	465	242	236	230	199	175	240	329	251	137	*MAT1-1*
2015.ZA.BZN.081	South Africa	Mbizana	158	462	242	223	230	196	175	240	329	251	134	*MAT1-2*
2015.ZA.BZN.F2.002	South Africa	Mbizana	158	465	245	236	230	199	175	244	329	245	134	*MAT1-2*
2015.ZA.BZN.F2.004	South Africa	Mbizana	158	468	245	223	233	202	175	244	329	251	137	*MAT1-1*
2015.ZA.BZN.F2.005	South Africa	Mbizana	158	459	245	236	209	196	175	236	329	245	137	*MAT1-1*
2015.ZA.BZN.F2.007	South Africa	Mbizana	158	462	242	236	230	199	175	244	329	245	134	*MAT1-2*
2015.ZA.BZN.F2.008	South Africa	Mbizana	158	462	242	236	230	199	175	240	329	251	137	*MAT1-1*
2015.ZA.BZN.F2.009	South Africa	Mbizana	158	462	245	223	230	196	191	240	329	245	134	*MAT1-1*
2015.ZA.BZN.F2.011	South Africa	Mbizana	155	465	242	223	230	199	194	240	329	251	137	*MAT1-1*
2015.ZA.BZN.F2.013	South Africa	Mbizana	158	465	242	223	209	199	175	240	329	251	137	*MAT1-1*
2015.ZA.BZN.F2.014	South Africa	Mbizana	155	465	242	223	236	196	191	244	329	251	134	*MAT1-1*
2015.ZA.BZN.F2.016	South Africa	Mbizana	155	462	242	236	209	196	175	240	329	245	134	*MAT1-2*
2015.ZA.BZN.F2.017	South Africa	Mbizana	155	462	245	236	209	196	191	240	329	251	134	*MAT1-2*
2015.ZA.BZN.F2.019	South Africa	Mbizana	155	462	242	236	209	196	191	244	329	245	134	*MAT1-1*
2015.ZA.BZN.F2.020	South Africa	Mbizana	155	462	245	236	209	196	191	244	329	245	134	*MAT1-2*
2015.ZA.BZN.F2.021	South Africa	Mbizana	155	462	245	236	197	196	191	240	329	245	134	*MAT1-2*
2015.ZA.BZN.F2.023	South Africa	Mbizana	155	462	242	236	209	199	191	240	329	245	134	*MAT1-2*
2015.ZA.BZN.F2.024	South Africa	Mbizana	158	462	242	236	209	199	191	240	329	245	134	*MAT1-2*
2015.ZA.BZN.F2.025	South Africa	Mbizana	158	462	245	236	209	199	191	244	329	245	134	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZA.BZN.F2.026	South Africa	Mbizana	155	462	242	223	230	199	175	240	329	251	134	*MAT1-2*
2015.ZA.BZN.F2.029	South Africa	Mbizana	158	465	245	236	200	199	191	240	329	251	134	*MAT1-2*
2015.ZA.BZN.F2.030	South Africa	Mbizana	155	462	245	236	230	199	191	240	329	251	134	*MAT1-2*
2015.ZA.BZN.F2.031	South Africa	Mbizana	155	462	242	236	230	199	175	240	329	245	137	*MAT1-1*
2015.ZA.BZN.F2.033	South Africa	Mbizana	155	465	242	236	209	196	191	240	329	251	134	*MAT1-2*
2015.ZA.BZN.F2.034	South Africa	Mbizana	155	465	242	236	209	199	191	240	329	251	134	*MAT1-2*
2015.ZA.BZN.F2.035	South Africa	Mbizana	155	465	242	236	209	199	175	244	329	245	134	*MAT1-2*
2015.ZA.BZN.F2.037	South Africa	Mbizana	155	465	245	236	209	199	175	240	329	251	137	*MAT1-2*
2015.ZA.BZN.F2.041	South Africa	Mbizana	158	465	245	223	230	199	191	240	329	251	134	*MAT1-1*
2015.ZA.BZN.F2.042	South Africa	Mbizana	155	462	245	223	233	199	191	244	329	245	134	*MAT1-2*
2015.ZA.BZN.F2.044	South Africa	Mbizana	161	462	245	223	209	199	175	240	329	245	137	*MAT1-1*
2015.ZA.BZN.F2.045	South Africa	Mbizana	155	462	245	236	230	199	191	240	329	251	134	*MAT1-1*
2015.ZA.BZN.F2.047	South Africa	Mbizana	158	465	245	223	209	199	175	240	329	248	134	*MAT1-2*
2015.ZA.BZN.F2.049	South Africa	Mbizana	158	465	242	236	209	196	175	240	329	245	134	*MAT1-1*
2015.ZA.BZN.F2.051	South Africa	Mbizana	158	465	239	236	209	199	175	244	329	245	134	*MAT1-1*
2015.ZA.BZN.F2.052	South Africa	Mbizana	158	465	239	236	209	199	175	236	329	245	134	*MAT1-1*
2015.ZA.BZN.F2.053	South Africa	Mbizana	155	465	245	223	209	196	175	244	329	251	137	*MAT1-1*
2015.ZA.BZN.F2.054	South Africa	Mbizana	158	462	245	236	230	196	175	240	329	245	134	*MAT1-1*
2015.ZA.BZN.F2.057	South Africa	Mbizana	158	465	242	236	209	199	175	240	329	245	134	*MAT1-2*
2015.ZA.BZN.F2.058	South Africa	Mbizana	158	462	242	223	233	199	175	240	329	245	134	*MAT1-1*
2015.ZA.BZN.F2.060	South Africa	Mbizana	155	462	245	223	209	199	175	240	329	251	134	*MAT1-2*
2015.ZA.BZN.F2.061	South Africa	Mbizana	155	462	245	223	209	199	175	240	329	251	134	*MAT1-2*
2015.ZA.NTB.002	South Africa	Ntabankulu	155	465	245	223	230	196	191	244	329	251	137	*MAT1-2*
2015.ZA.NTB.004	South Africa	Ntabankulu	158	465	245	223	230	196	187	244	329	245	137	*MAT1-1*
2015.ZA.NTB.007	South Africa	Ntabankulu	158	462	242	223	230	196	175	240	329	251	134	*MAT1-2*
2015.ZA.NTB.008	South Africa	Ntabankulu	158	465	245	223	230	202	175	244	329	245	134	*MAT1-2*
2015.ZA.NTB.013	South Africa	Ntabankulu	158	462	245	236	233	199	175	244	329	245	134	*MAT1-1*
2015.ZA.NTB.014	South Africa	Ntabankulu	158	462	245	236	233	199	175	244	329	245	134	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZA.NTB.017	South Africa	Ntabankulu	155	465	245	223	209	199	175	240	329	251	134	*MAT1-1*
2015.ZA.NTB.018	South Africa	Ntabankulu	155	462	245	223	209	199	175	240	329	251	134	*MAT1-1*
2015.ZA.NTB.020	South Africa	Ntabankulu	155	459	239	223	248	196	191	240	329	251	134	*MAT1-1*
2015.ZA.NTB.022	South Africa	Ntabankulu	155	465	242	236	230	202	191	244	329	251	134	*MAT1-2*
2015.ZA.NTB.024	South Africa	Ntabankulu	155	468	245	223	230	196	191	240	329	245	134	*MAT1-2*
2015.ZA.NTB.025	South Africa	Ntabankulu	158	462	242	223	230	196	175	244	329	245	134	*MAT1-1*
2015.ZA.NTB.029	South Africa	Ntabankulu	158	462	245	236	209	196	175	244	329	245	137	*MAT1-2*
2015.ZA.NTB.031	South Africa	Ntabankulu	155	462	242	223	230	196	175	240	329	251	137	*MAT1-2*
2015.ZA.NTB.033	South Africa	Ntabankulu	158	468	245	223	230	196	175	240	329	245	137	*MAT1-2*
2015.ZA.NTB.034	South Africa	Ntabankulu	158	468	245	223	230	196	175	240	329	245	134	*MAT1-1*
2015.ZA.NTB.035	South Africa	Ntabankulu	155	465	245	236	209	196	191	240	329	251	137	*MAT1-1*
2015.ZA.NTB.037	South Africa	Ntabankulu	158	465	245	223	209	199	191	240	329	251	137	*MAT1-2*
2015.ZA.NTB.039	South Africa	Ntabankulu	158	465	242	223	209	199	175	240	329	245	134	*MAT1-1*
2015.ZA.NTB.040	South Africa	Ntabankulu	158	465	245	236	209	199	175	240	329	251	134	*MAT1-1*
2015.ZA.NTB.042	South Africa	Ntabankulu	158	465	245	223	209	196	175	240	329	251	134	*MAT1-2*
2015.ZA.NTB.043	South Africa	Ntabankulu	158	465	245	236	230	196	175	248	329	245	134	*MAT1-2*
2015.ZA.NTB.045	South Africa	Ntabankulu	155	462	245	223	230	196	175	240	329	245	134	*MAT1-2*
2015.ZA.NTB.046	South Africa	Ntabankulu	155	462	245	223	209	199	175	240	329	245	134	*MAT1-2*
2015.ZA.NTB.048	South Africa	Ntabankulu	158	462	242	236	209	196	191	240	329	245	137	*MAT1-1*
2015.ZA.NTB.049	South Africa	Ntabankulu	158	465	242	236	209	196	191	240	329	251	134	*MAT1-2*
2015.ZA.NTB.051	South Africa	Ntabankulu	158	465	242	223	230	196	191	244	329	245	134	*MAT1-1*
2015.ZA.NTB.054	South Africa	Ntabankulu	158	462	245	236	226	199	175	240	329	251	137	*MAT1-1*
2015.ZA.NTB.055	South Africa	Ntabankulu	158	465	245	236	193	196	191	240	329	251	134	*MAT1-1*
2015.ZA.NTB.058	South Africa	Ntabankulu	155	465	242	236	230	199	191	240	329	251	134	*MAT1-2*
2015.ZA.NTB.059	South Africa	Ntabankulu	158	465	245	236	209	202	191	248	329	251	134	*MAT1-2*
2015.ZA.NTB.060	South Africa	Ntabankulu	158	465	242	236	209	202	191	248	329	251	134	*MAT1-2*
2015.ZA.NTB.062	South Africa	Ntabankulu	158	468	242	236	230	196	191	240	329	251	134	*MAT1-1*
2015.ZA.NTB.063	South Africa	Ntabankulu	155	462	245	236	230	199	191	244	329	245	137	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZA.NTB.065	South Africa	Ntabankulu	158	462	242	223	230	196	175	240	329	251	134	*MAT1-1*
2015.ZA.NTB.066	South Africa	Ntabankulu	155	465	242	223	209	199	175	240	329	245	134	*MAT1-2*
2015.ZA.NTB.068	South Africa	Ntabankulu	158	465	245	236	209	199	175	240	329	245	134	*MAT1-2*
2015.ZA.NTB.069	South Africa	Ntabankulu	134	462	245	236	209	193	191	240	329	245	137	*MAT1-2*
2015.ZA.NTB.070	South Africa	Ntabankulu	158	465	242	236	212	196	175	240	329	251	134	*MAT1-1*
2015.ZA.NTB.072	South Africa	Ntabankulu	155	459	242	236	230	199	191	240	329	251	137	*MAT1-2*
2015.ZA.NTB.074	South Africa	Ntabankulu	155	465	242	223	209	196	191	244	329	245	137	*MAT1-2*
2015.ZA.NTB.076	South Africa	Ntabankulu	158	462	245	223	206	196	191	240	329	251	137	*MAT1-1*
2015.ZA.NTB.077	South Africa	Ntabankulu	155	465	245	223	209	196	175	240	329	245	137	*MAT1-2*
2015.ZA.NTB.078	South Africa	Ntabankulu	158	465	245	236	209	196	175	240	329	251	137	*MAT1-1*
2015.ZA.NTB.080	South Africa	Ntabankulu	155	465	245	223	230	196	191	240	329	245	134	*MAT1-1*
2016.ZA.UMT.001	South Africa	Mthatha	158	465	242	223	209	196	191	244	338	245	137	*MAT1-1*
2016.ZA.UMT.002	South Africa	Mthatha	158	462	242	223	230	196	175	244	329	245	134	*MAT1-1*
2016.ZA.UMT.003	South Africa	Mthatha	158	462	242	236	209	199	197	244	329	251	137	*MAT1-1*
2016.ZA.UMT.004	South Africa	Mthatha	158	462	242	223	226	199	191	240	329	245	137	*MAT1-1*
2016.ZA.UMT.007	South Africa	Mthatha	152	465	242	223	209	199	175	240	329	251	137	*MAT1-1*
2016.ZA.UMT.008	South Africa	Mthatha	155	462	242	236	230	196	191	240	329	251	134	*MAT1-1*
2016.ZA.UMT.009	South Africa	Mthatha	158	462	242	223	226	199	191	240	329	245	137	*MAT1-1*
2016.ZA.UMT.010	South Africa	Mthatha	155	465	245	223	209	196	191	240	329	245	134	*MAT1-1*
2016.ZA.UMT.011	South Africa	Mthatha	155	462	242	236	230	196	175	240	329	251	134	*MAT1-1*
2016.ZA.UMT.013	South Africa	Mthatha	155	465	245	236	209	199	191	248	329	245	137	*MAT1-1*
2016.ZA.UMT.014	South Africa	Mthatha	158	462	245	236	230	199	175	240	329	245	137	*MAT1-1*
2016.ZA.UMT.015	South Africa	Mthatha	155	462	245	236	209	196	175	240	329	245	137	*MAT1-1*
2016.ZA.UMT.016	South Africa	Mthatha	158	462	242	223	230	199	175	240	329	251	134	*MAT1-1*
2016.ZA.UMT.017	South Africa	Mthatha	155	459	248	236	230	196	175	240	329	251	134	*MAT1-1*
2016.ZA.UMT.018	South Africa	Mthatha	155	465	242	223	230	196	191	240	329	251	134	*MAT1-1*
2016.ZA.UMT.019	South Africa	Mthatha	155	462	242	223	230	196	191	240	329	245	134	*MAT1-1*
2016.ZA.UMT.020	South Africa	Mthatha	158	465	242	236	223	199	191	240	329	251	137	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2016.ZA.UMT.021	South Africa	Mthatha	158	465	245	223	230	193	175	240	329	245	137	*MAT1-2*
2016.ZA.UMT.022	South Africa	Mthatha	158	465	245	223	209	196	194	240	329	251	137	*MAT1-2*
2016.ZA.UMT.023	South Africa	Mthatha	158	465	242	236	209	196	191	244	338	245	137	*MAT1-2*
2016.ZA.UMT.025	South Africa	Mthatha	158	465	242	223	230	199	175	240	329	245	134	*MAT1-2*
2016.ZA.UMT.049	South Africa	Mthatha	158	462	242	236	230	199	191	240	329	251	134	*MAT1-2*
2016.ZA.UMT.050	South Africa	Mthatha	158	465	242	223	230	202	175	240	329	251	137	*MAT1-2*
2016.ZA.UMT.051	South Africa	Mthatha	155	462	242	223	209	196	191	240	329	245	137	*MAT1-2*
2016.ZA.UMT.052	South Africa	Mthatha	155	462	245	223	209	196	175	240	329	245	134	*MAT1-2*
2016.ZA.UMT.053	South Africa	Mthatha	158	465	242	236	212	196	191	240	329	251	134	*MAT1-2*
2016.ZA.UMT.054	South Africa	Mthatha	155	465	245	236	233	199	191	240	329	251	134	*MAT1-2*
2016.ZA.UMT.056	South Africa	Mthatha	158	465	242	223	209	196	175	240	329	251	134	*MAT1-2*
2016.ZA.UMT.057	South Africa	Mthatha	155	459	242	223	209	196	175	236	329	251	134	*MAT1-2*
2016.ZA.UMT.060	South Africa	Mthatha	155	465	242	220	209	196	175	240	329	251	134	*MAT1-2*
2016.ZA.UMT.063	South Africa	Mthatha	158	462	248	223	230	196	191	240	329	251	137	*MAT1-2*
2016.UG.FTP.001	Uganda	Fortportal	155	462	242	223	197	196	172	244	329	245	137	*MAT1-2*
2016.UG.FTP.003	Uganda	Fortportal	155	465	245	223	203	202	169	236	324	242	134	*MAT1-1*
2016.UG.FTP.005	Uganda	Fortportal	155	462	245	223	200	199	172	244	329	248	134	*MAT1-2*
2016.UG.FTP.007	Uganda	Fortportal	155	462	245	220	209	193	175	244	329	245	137	*MAT1-1*
2016.UG.FTP.008	Uganda	Fortportal	155	462	242	223	209	193	172	260	329	245	134	*MAT1-1*
2016.UG.FTP.009	Uganda	Fortportal	158	462	245	223	209	196	175	260	349	245	134	*MAT1-1*
2016.UG.FTP.010	Uganda	Fortportal	155	462	242	220	209	193	172	244	329	245	134	*MAT1-1*
2016.UG.FTP.013	Uganda	Fortportal	158	456	245	223	230	196	175	244	329	245	137	*MAT1-2*
2016.UG.FTP.015	Uganda	Fortportal	161	462	242	223	209	196	172	244	329	245	134	*MAT1-1*
2016.UG.FTP.016	Uganda	Fortportal	155	462	245	220	233	193	175	244	329	245	137	*MAT1-1*
2016.UG.FTP.018	Uganda	Fortportal	158	462	239	223	209	193	169	240	329	242	137	*MAT1-2*
2016.UG.FTP.019	Uganda	Fortportal	158	468	242	223	209	193	175	240	329	245	137	*MAT1-2*
2016.UG.FTP.020	Uganda	Fortportal	158	456	242	223	230	193	172	244	329	245	140	*MAT1-1*
2016.UG.FTP.024	Uganda	Fortportal	155	462	245	223	212	196	172	244	329	245	140	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2016.UG.FTP.029	Uganda	Fortportal	158	462	248	220	230	193	172	248	329	248	140	*MAT1-1*
2016.UG.FTP.028	Uganda	Fortportal	155	465	248	220	209	196	172	244	329	245	137	*MAT1-1*
2016.UG.FTP.030	Uganda	Fortportal	155	465	245	220	227	193	175	244	324	245	137	*MAT1-2*
2016.UG.FTP.031	Uganda	Fortportal	158	465	245	223	209	199	178	244	329	245	134	*MAT1-2*
2016.UG.FTP.032	Uganda	Fortportal	161	465	245	223	209	199	172	244	329	245	134	*MAT1-1*
2016.UG.FTP.033	Uganda	Fortportal	161	462	239	220	209	196	172	244	329	242	134	*MAT1-1*
2016.UG.FTP.036	Uganda	Fortportal	155	465	242	220	206	196	172	244	329	245	134	*MAT1-2*
2016.UG.FTP.039	Uganda	Fortportal	155	468	242	226	209	202	172	244	329	245	137	*MAT1-1*
2016.UG.FTP.041	Uganda	Fortportal	155	468	245	220	197	196	241	260	329	245	137	*MAT1-2*
2016.UG.FTP.042	Uganda	Fortportal	155	462	242	217	209	196	172	244	329	245	137	*MAT1-1*
2016.UG.FTP.044	Uganda	Fortportal	158	462	245	220	230	196	241	260	329	245	134	*MAT1-1*
2016.UG.FTP.046	Uganda	Fortportal	158	462	248	220	209	196	175	244	329	245	134	*MAT1-1*
2016.UG.FTP.051	Uganda	Fortportal	158	462	242	220	230	196	172	244	329	245	140	*MAT1-2*
2016.UG.FTP.053	Uganda	Fortportal	158	462	242	220	209	196	172	244	329	245	134	*MAT1-2*
2016.UG.FTP.058	Uganda	Fortportal	158	462	248	223	209	196	175	244	329	242	134	*MAT1-2*
2016.UG.FTP.063	Uganda	Fortportal	158	468	242	223	197	196	175	244	329	245	134	*MAT1-2*
2016.UG.FTP.065	Uganda	Fortportal	158	462	248	223	209	196	187	244	329	245	134	*MAT1-2*
2016.UG.FTP.067	Uganda	Fortportal	155	465	242	223	209	196	172	244	329	242	134	*MAT1-2*
2016.UG.FTP.068	Uganda	Fortportal	158	462	242	220	209	193	172	212	329	245	140	*MAT1-2*
2016.UG.FTP.069	Uganda	Fortportal	158	462	248	223	212	196	172	244	329	245	137	*MAT1-2*
2016.UG.FTP.070	Uganda	Fortportal	158	465	245	220	209	199	172	236	329	248	137	*MAT1-1*
2016.UG.FTP.071	Uganda	Fortportal	161	447	245	220	209	193	175	244	329	245	140	*MAT1-2*
2016.UG.FTP.072	Uganda	Fortportal	158	462	245	220	209	193	169	240	324	242	134	*MAT1-1*
2016.UG.FTP.073	Uganda	Fortportal	158	462	248	223	209	175	169	244	329	245	134	*MAT1-1*
2016.UG.FTP.074	Uganda	Fortportal	161	459	245	220	230	193	169	244	329	245	140	*MAT1-2*
2016.UG.FTP.075	Uganda	Fortportal	158	462	248	220	209	193	172	244	329	248	140	*MAT1-2*
2016.UG.FTP.076	Uganda	Fortportal	161	465	245	223	209	199	172	244	329	245	140	*MAT1-2*
2016.UG.FTP.080	Uganda	Fortportal	158	462	248	223	230	196	172	244	329	245	140	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2016.UG.FTP.011	Uganda	Fortportal	158	462	245	223	197	196	244	236	329	245	137	*MAT1-1*
2016.UG.FTP.025	Uganda	Fortportal	158	462	242	223	200	202	172	244	329	245	137	*MAT1-1*
2016.UG.FTP.034	Uganda	Fortportal	158	462	242	220	209	196	172	244	329	245	140	*MAT1-2*
2016.UG.FTP.040	Uganda	Fortportal	161	462	242	223	248	196	172	244	329	245	140	*MAT1-2*
2016.UG.FTP.043	Uganda	Fortportal	158	465	245	223	200	199	178	244	329	245	134	*MAT1-1*
2016.UG.FTP.045	Uganda	Fortportal	158	465	245	220	209	196	175	244	329	245	134	*MAT1-1*
2016.UG.FTP.064	Uganda	Fortportal	158	465	242	223	209	193	175	244	329	242	137	*MAT1-2*
2016.UG.KPC.004	Uganda	Kapchorwa	158	462	242	223	209	199	172	244	329	245	134	*MAT1-1*
2016.UG.KPC.006	Uganda	Kapchorwa	155	462	245	220	200	196	175	260	329	245	137	*MAT1-2*
2016.UG.KPC.014	Uganda	Kapchorwa	155	465	245	220	230	193	175	244	329	245	137	*MAT1-1*
2016.UG.KPC.017	Uganda	Kapchorwa	158	462	245	223	209	196	172	244	329	245	134	*MAT1-1*
2016.UG.KPC.027	Uganda	Kapchorwa	158	465	242	223	230	196	172	244	329	245	134	*MAT1-2*
2016.UG.KPC.038	Uganda	Kapchorwa	155	462	245	226	209	208	175	244	329	245	134	*MAT1-1*
2016.UG.KPC.047	Uganda	Kapchorwa	155	465	245	223	209	196	175	236	329	239	134	*MAT1-2*
2016.UG.KPC.048	Uganda	Kapchorwa	155	462	242	220	209	193	172	244	329	245	137	*MAT1-2*
2016.UG.KPC.049	Uganda	Kapchorwa	158	462	242	223	209	196	172	244	329	245	134	*MAT1-1*
2016.UG.KPC.050	Uganda	Kapchorwa	161	462	248	226	209	193	187	244	324	245	137	*MAT1-2*
2016.UG.KPC.052	Uganda	Kapchorwa	161	462	245	223	230	199	175	244	329	245	134	*MAT1-2*
2016.UG.KPC.054	Uganda	Kapchorwa	155	465	248	220	209	196	172	244	329	248	134	*MAT1-2*
2016.UG.KPC.055	Uganda	Kapchorwa	158	462	242	220	209	196	172	244	329	245	140	*MAT1-1*
2016.UG.KPC.060	Uganda	Kapchorwa	158	462	245	220	206	193	172	244	329	248	140	*MAT1-1*
2016.UG.KPC.066	Uganda	Kapchorwa	155	465	245	223	230	196	172	244	329	248	134	*MAT1-1*
2016.UG.KPC.037	Uganda	Kapchorwa	158	465	245	223	230	199	244	244	329	245	137	*MAT1-2*
2016.UG.KPC.062	Uganda	Kapchorwa	158	465	248	220	209	193	172	248	329	248	137	*MAT1-2*
2016.UG.LIR.086	Uganda	Lira	158	462	248	223	209	196	172	248	329	248	134	*MAT1-1*
2016.UG.LIR.089	Uganda	Lira	158	465	248	223	209	196	172	244	329	248	134	*MAT1-1*
2016.UG.LIR.095	Uganda	Lira	158	465	242	220	230	196	175	244	329	245	134	*MAT1-2*
2016.UG.LIR.097	Uganda	Lira	161	462	248	220	209	193	169	244	329	245	137	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2016.UG.LIR.098	Uganda	Lira	158	465	248	220	230	193	190	244	329	245	134	*MAT1-2*
2016.UG.LIR.101	Uganda	Lira	158	462	248	220	209	193	169	248	329	245	140	*MAT1-2*
2016.UG.LIR.102	Uganda	Lira	158	462	242	220	209	196	190	244	329	245	134	*MAT1-1*
2016.UG.LIR.108	Uganda	Lira	158	462	245	220	209	193	175	244	329	245	134	*MAT1-1*
2016.UG.LIR.110	Uganda	Lira	158	465	248	220	200	199	175	244	329	248	137	*MAT1-2*
2016.UG.LIR.115	Uganda	Lira	158	465	245	223	209	196	169	244	329	245	140	*MAT1-1*
2016.UG.LIR.117	Uganda	Lira	158	462	248	220	209	196	175	248	329	248	134	*MAT1-2*
2016.UG.LIR.120	Uganda	Lira	158	465	245	220	230	193	175	244	329	245	137	*MAT1-2*
2016.UG.LIR.121	Uganda	Lira	158	462	245	220	209	193	175	240	329	245	137	*MAT1-2*
2016.UG.LIR.127	Uganda	Lira	158	462	239	223	209	196	172	240	329	242	137	*MAT1-2*
2016.UG.LIR.134	Uganda	Lira	158	462	242	220	209	193	172	244	329	245	137	*MAT1-2*
2016.UG.LIR.135	Uganda	Lira	158	462	242	220	209	193	172	244	329	245	134	*MAT1-1*
2016.UG.LIR.136	Uganda	Lira	158	462	239	223	209	196	172	240	329	242	137	*MAT1-2*
2016.UG.LIR.138	Uganda	Lira	158	462	245	223	209	196	175	240	329	245	134	*MAT1-1*
2017.UG.MSK.001	Uganda	Masaka	155	468	242	229	236	196	169	244	329	245	134	*MAT1-1*
2017.UG.MSK.002	Uganda	Masaka	155	465	248	223	209	199	175	244	329	245	137	*MAT1-2*
2017.UG.MSK.003	Uganda	Masaka	158	465	251	223	209	223	175	244	329	248	134	*MAT1-2*
2017.UG.MSK.005	Uganda	Masaka	158	459	245	226	206	196	169	244	329	248	137	*MAT1-2*
2017.UG.MSK.006	Uganda	Masaka	158	462	239	223	209	196	175	244	329	242	134	*MAT1-2*
2017.UG.MSK.007	Uganda	Masaka	158	462	245	223	212	196	175	244	329	245	134	*MAT1-1*
2017.UG.MSK.008	Uganda	Masaka	158	462	245	220	212	199	175	248	329	245	134	*MAT1-2*
2017.UG.MSK.010	Uganda	Masaka	158	462	245	220	209	193	190	244	329	245	134	*MAT1-2*
2017.UG.MSK.012	Uganda	Masaka	158	462	248	223	209	196	175	240	329	248	134	*MAT1-1*
2017.UG.MSK.013	Uganda	Masaka	158	462	245	223	209	196	172	244	329	245	134	*MAT1-1*
2017.UG.MSK.015	Uganda	Masaka	158	462	245	223	209	202	190	244	329	245	134	*MAT1-2*
2017.UG.MSK.016	Uganda	Masaka	158	462	245	223	209	193	175	260	329	245	134	*MAT1-1*
2017.UG.MSK.017	Uganda	Masaka	158	465	245	220	230	196	175	244	329	245	134	*MAT1-2*
2017.UG.MSK.068	Uganda	Masaka	158	462	245	235	209	193	175	244	329	245	137	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.UG.MSK.069	Uganda	Masaka	158	459	242	223	209	196	175	256	329	245	137	*MAT1-2*
2017.UG.MSK.077	Uganda	Masaka	158	462	245	220	230	196	175	244	329	245	137	*MAT1-2*
2017.UG.MSK.079	Uganda	Masaka	158	462	245	220	230	193	175	244	329	245	134	*MAT1-1*
2017.UG.MSK.080	Uganda	Masaka	158	462	245	220	209	193	175	240	329	245	134	*MAT1-2*
2017.UG.MSK.081	Uganda	Masaka	158	462	245	220	209	193	175	244	329	245	134	*MAT1-2*
2017.UG.MSK.082	Uganda	Masaka	158	465	248	223	209	196	175	244	329	248	137	*MAT1-2*
2017.UG.MSK.084	Uganda	Masaka	155	462	245	220	230	196	175	244	329	245	137	*MAT1-2*
2017.UG.MSK.090	Uganda	Masaka	158	459	245	220	209	193	175	240	329	245	134	*MAT1-2*
2017.UG.MSK.092	Uganda	Masaka	158	462	245	220	209	193	175	244	329	245	137	*MAT1-2*
2017.UG.MSK.095	Uganda	Masaka	158	465	245	220	209	193	175	236	329	245	134	*MAT1-2*
2017.UG.MSK.096	Uganda	Masaka	155	465	245	220	209	193	175	240	329	245	137	*MAT1-2*
2017.UG.MSK.100	Uganda	Masaka	155	465	242	220	209	193	175	236	329	242	134	*MAT1-1*
2017.UG.MSK.113	Uganda	Masaka	155	465	245	223	230	196	175	240	329	245	134	*MAT1-2*
2017.UG.MSK.114	Uganda	Masaka	155	462	242	223	227	193	241	244	329	245	134	*MAT1-1*
2017.UG.MSK.115	Uganda	Masaka	158	468	245	220	206	193	175	244	329	245	137	*MAT1-1*
2017.UG.MSK.116	Uganda	Masaka	158	462	242	232	224	193	175	244	329	242	137	*MAT1-2*
2017.UG.MSK.122	Uganda	Masaka	158	468	245	220	209	193	175	244	329	245	140	*MAT1-1*
2017.UG.MSK.123	Uganda	Masaka	158	462	242	220	209	202	175	244	329	242	134	*MAT1-2*
2017.UG.MSK.009	Uganda	Masaka	155	462	245	223	209	196	175	244	329	245	134	*MAT1-2*
2017.UG.MSK.070	Uganda	Masaka	158	462	245	220	209	193	175	236	329	245	134	*MAT1-1*
2017.UG.MSK.073	Uganda	Masaka	158	462	242	220	209	199	172	256	329	245	137	*MAT1-2*
2017.UG.MSK.085	Uganda	Masaka	158	462	245	220	209	193	175	240	329	245	137	*MAT1-1*
2017.UG.MSK.093	Uganda	Masaka	158	462	239	223	209	199	175	240	329	242	134	*MAT1-2*
2017.UG.MSK.101	Uganda	Masaka	158	462	245	220	209	193	175	260	329	245	134	*MAT1-1*
2017.UG.MSK.110	Uganda	Masaka	158	465	245	220	209	196	175	240	329	245	134	*MAT1-2*
2017.UG.MSK.112	Uganda	Masaka	158	462	239	220	209	193	190	244	329	239	137	*MAT1-2*
2017.UG.MSK.117	Uganda	Masaka	158	462	245	220	209	196	175	244	329	245	134	*MAT1-2*
2017.UG.MSK.120	Uganda	Masaka	161	462	245	223	209	193	175	244	329	245	134	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.UG.MSK.125	Uganda	Masaka	158	462	248	220	209	193	175	244	329	248	137	*MAT1-2*
2017.UG.MSK.105	Uganda	Masaka	158	462	245	223	209	199	175	236	329	176	137	*MAT1-2*
2017.UG.GYZ.019	Uganda	Wakiso	158	462	245	220	209	193	175	244	329	245	134	*MAT1-1*
2017.UG.GYZ.020	Uganda	Wakiso	158	462	242	220	230	193	175	240	329	245	134	*MAT1-1*
2017.UG.GYZ.021	Uganda	Wakiso	164	462	242	220	230	196	175	244	329	242	134	*MAT1-2*
2017.UG.GYZ.023	Uganda	Wakiso	158	462	242	223	209	196	175	244	329	242	134	*MAT1-2*
2017.UG.GYZ.024	Uganda	Wakiso	161	465	245	223	209	193	175	244	329	245	137	*MAT1-2*
2017.UG.GYZ.025	Uganda	Wakiso	155	462	245	220	224	196	175	244	329	245	134	*MAT1-1*
2017.UG.GYZ.026	Uganda	Wakiso	158	462	245	220	209	196	175	244	329	245	134	*MAT1-1*
2017.UG.GYZ.028	Uganda	Wakiso	155	465	245	220	209	193	175	244	329	242	134	*MAT1-2*
2017.UG.GYZ.031	Uganda	Wakiso	161	462	245	223	197	196	175	244	329	176	137	*MAT1-1*
2017.UG.GYZ.032	Uganda	Wakiso	155	465	245	220	233	193	190	260	329	245	137	*MAT1-2*
2017.UG.GYZ.033	Uganda	Wakiso	155	462	245	223	209	199	190	240	329	245	137	*MAT1-1*
2017.UG.GYZ.034	Uganda	Wakiso	158	462	245	220	209	196	190	236	329	245	137	*MAT1-1*
2017.UG.GYZ.035	Uganda	Wakiso	158	462	245	220	209	193	190	244	329	245	137	*MAT1-2*
2017.UG.GYZ.036	Uganda	Wakiso	158	465	245	220	209	193	175	244	329	245	134	*MAT1-1*
2017.UG.GYZ.037	Uganda	Wakiso	155	459	245	235	209	196	190	244	329	245	134	*MAT1-2*
2017.UG.GYZ.043	Uganda	Wakiso	158	465	245	223	209	193	175	240	329	245	137	*MAT1-2*
2017.UG.GYZ.044	Uganda	Wakiso	158	462	245	220	209	193	175	244	329	245	134	*MAT1-2*
2017.UG.GYZ.053	Uganda	Wakiso	161	462	245	223	209	193	175	260	329	245	134	*MAT1-2*
2017.UG.GYZ.060	Uganda	Wakiso	158	462	248	220	209	193	175	244	329	248	134	*MAT1-2*
2017.UG.GYZ.061	Uganda	Wakiso	158	462	245	223	209	193	175	240	329	245	137	*MAT1-2*
2017.UG.GYZ.022	Uganda	Wakiso	158	462	242	223	209	199	175	212	329	242	134	*MAT1-1*
2017.UG.GYZ.027	Uganda	Wakiso	158	465	245	223	209	199	175	244	329	245	134	*MAT1-1*
2017.UG.GYZ.029	Uganda	Wakiso	149	465	242	220	230	196	175	244	329	242	134	*MAT1-1*
2017.UG.GYZ.038	Uganda	Wakiso	155	462	245	220	209	196	175	244	329	245	134	*MAT1-1*
2017.UG.GYZ.052	Uganda	Wakiso	161	462	245	220	209	193	175	232	329	245	134	*MAT1-2*
2017.UG.GYZ.054	Uganda	Wakiso	161	465	245	220	233	193	175	260	329	245	134	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.UG.GYZ.062	Uganda	Wakiso	158	462	245	220	209	193	175	240	329	245	137	*MAT1-2*
2017.UG.GYZ.040	Uganda	Wakiso	161	465	242	220	233	193	175	228	329	245	137	*MAT1-2*
2017.UG.GYZ.047	Uganda	Wakiso	158	462	245	220	230	196	175	260	329	245	134	*MAT1-1*
2016.UG.NML.092	Uganda	Wakiso	161	432	245	220	209	202	172	244	324	245	137	*MAT1-1*
2016.UG.NML.103	Uganda	Wakiso	158	462	245	223	230	196	172	244	329	248	134	*MAT1-2*
2016.UG.NML.104	Uganda	Wakiso	158	462	245	223	209	196	175	260	329	245	134	*MAT1-2*
2016.UG.NML.107	Uganda	Wakiso	158	468	245	223	209	196	175	244	329	245	134	*MAT1-2*
2016.UG.NML.109	Uganda	Wakiso	158	465	245	223	212	199	172	244	329	176	134	*MAT1-1*
2016.UG.NML.111	Uganda	Wakiso	158	462	248	220	209	193	193	240	329	248	134	*MAT1-2*
2016.UG.NML.113	Uganda	Wakiso	158	459	242	223	209	199	190	244	329	245	140	*MAT1-2*
2016.UG.NML.114	Uganda	Wakiso	158	462	245	223	230	196	175	260	329	245	134	*MAT1-1*
2016.UG.NML.118	Uganda	Wakiso	161	462	248	220	209	196	175	244	329	248	134	*MAT1-1*
2016.UG.NML.122	Uganda	Wakiso	158	459	242	220	209	199	193	240	329	242	137	*MAT1-2*
2016.UG.NML.124	Uganda	Wakiso	158	459	245	223	209	199	175	240	329	245	137	*MAT1-2*
2016.UG.NML.128	Uganda	Wakiso	161	459	242	223	197	196	175	240	329	245	134	*MAT1-2*
2015.ZM.CHL.055	Zambia	Chilanga	158	465	223	209	199	245	191	244	329	251	134	*MAT1-2*
2015.ZM.CHL.056	Zambia	Chilanga	155	462	223	230	199	242	191	240	329	245	134	*MAT1-2*
2015.ZM.CHL.057	Zambia	Chilanga	155	462	236	230	199	245	175	244	329	245	137	*MAT1-2*
2015.ZM.CHL.064	Zambia	Chilanga	155	471	223	230	202	242	191	240	329	245	137	*MAT1-2*
2015.ZM.CHL.065	Zambia	Chilanga	158	462	236	230	196	245	187	236	329	248	134	*MAT1-1*
2015.ZM.CHL.066	Zambia	Chilanga	155	465	223	230	196	248	194	240	329	245	137	*MAT1-2*
2015.ZM.CHL.067	Zambia	Chilanga	158	462	236	209	196	245	175	244	329	245	137	*MAT1-2*
2015.ZM.CHL.068	Zambia	Chilanga	158	462	236	209	196	245	175	244	329	245	137	*MAT1-2*
2015.ZM.CHL.070	Zambia	Chilanga	158	465	223	209	199	242	191	240	329	251	137	*MAT1-2*
2015.ZM.CHL.071	Zambia	Chilanga	158	462	236	230	196	245	191	240	329	245	137	*MAT1-1*
2015.ZM.CHL.072	Zambia	Chilanga	158	462	223	230	196	245	175	248	329	245	134	*MAT1-2*
2015.ZM.CHL.073	Zambia	Chilanga	155	462	236	214	196	242	191	240	329	245	137	*MAT1-1*
2015.ZM.CHL.074	Zambia	Chilanga	158	462	223	230	196	245	191	240	329	245	137	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZM.CHL.075	Zambia	Chilanga	158	462	223	230	196	245	191	240	329	245	137	*MAT1-2*
2015.ZM.CHL.076	Zambia	Chilanga	158	465	223	209	199	242	191	240	329	245	134	*MAT1-2*
2015.ZM.CHL.077	Zambia	Chilanga	158	465	223	209	199	242	191	240	329	245	134	*MAT1-2*
2015.ZM.CHL.078	Zambia	Chilanga	158	465	223	230	196	245	175	240	329	245	137	*MAT1-1*
2015.ZM.CHL.079	Zambia	Chilanga	155	462	223	209	196	242	175	240	329	251	137	*MAT1-1*
2015.ZM.CHL.080	Zambia	Chilanga	158	462	223	230	196	245	191	240	329	245	137	*MAT1-2*
2015.ZM.CHL.081	Zambia	Chilanga	155	462	223	230	196	245	191	240	329	245	137	*MAT1-1*
2015.ZM.CHL.082	Zambia	Chilanga	155	462	223	230	199	242	191	240	329	245	137	*MAT1-1*
2015.ZM.CHL.083	Zambia	Chilanga	158	462	236	242	199	242	175	240	329	251	134	*MAT1-1*
2015.ZM.CHL.084	Zambia	Chilanga	158	462	236	242	199	242	175	240	329	251	134	*MAT1-1*
2015.ZM.CHL.087	Zambia	Chilanga	155	462	236	230	196	242	191	240	329	251	137	*MAT1-2*
2015.ZM.CHL.088	Zambia	Chilanga	155	465	223	230	199	245	191	240	329	251	137	*MAT1-1*
2015.ZM.CHL.089	Zambia	Chilanga	155	465	236	245	199	245	172	240	329	245	134	*MAT1-1*
2015.ZM.CHL.102	Zambia	Chilanga	155	465	236	245	199	245	175	240	329	245	134	*MAT1-1*
2015.ZM.CHL.103	Zambia	Chilanga	155	462	236	214	214	242	191	240	329	251	137	*MAT1-2*
2015.ZM.CHL.104	Zambia	Chilanga	155	462	236	212	214	242	191	240	329	251	137	*MAT1-2*
2015.ZM.CHS.001	Zambia	Chisamba	155	462	223	230	199	242	191	240	329	251	134	*MAT1-1*
2015.ZM.CHS.002	Zambia	Chisamba	158	462	223	230	199	242	191	240	329	251	134	*MAT1-2*
2015.ZM.CHS.003	Zambia	Chisamba	158	462	236	230	196	242	191	240	329	251	137	*MAT1-1*
2015.ZM.CHS.004	Zambia	Chisamba	155	465	236	230	199	242	172	236	329	251	134	*MAT1-1*
2015.ZM.CHS.005	Zambia	Chisamba	158	462	223	230	199	242	191	240	329	251	134	*MAT1-2*
2015.ZM.CHS.006	Zambia	Chisamba	155	459	223	230	199	242	187	240	329	245	137	*MAT1-1*
2015.ZM.CHS.007	Zambia	Chisamba	155	459	223	230	199	242	191	240	329	245	137	*MAT1-1*
2015.ZM.CHS.008	Zambia	Chisamba	155	459	223	230	199	242	191	240	329	245	137	*MAT1-1*
2015.ZM.CHS.009	Zambia	Chisamba	158	462	223	233	199	242	191	240	329	245	134	*MAT1-2*
2015.ZM.CHS.011	Zambia	Chisamba	155	459	223	230	199	242	187	240	329	245	137	*MAT1-1*
2015.ZM.CHS.012	Zambia	Chisamba	155	462	236	230	199	242	194	240	329	248	137	*MAT1-2*
2015.ZM.CHS.013	Zambia	Chisamba	155	456	223	209	199	242	187	240	329	251	134	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZM.CHS.014	Zambia	Chisamba	155	465	236	230	199	242	191	240	329	248	137	*MAT1-2*
2015.ZM.CHS.015	Zambia	Chisamba	155	465	223	233	199	242	187	240	329	245	134	*MAT1-2*
2015.ZM.CHS.016	Zambia	Chisamba	155	465	236	230	199	242	187	236	329	251	137	*MAT1-2*
2015.ZM.CHS.017	Zambia	Chisamba	155	471	236	209	196	242	175	240	329	245	134	*MAT1-2*
2015.ZM.CHS.018	Zambia	Chisamba	155	465	223	233	199	242	187	240	329	245	134	*MAT1-2*
2015.ZM.CHS.019	Zambia	Chisamba	155	471	236	209	196	242	172	240	329	245	134	*MAT1-2*
2015.ZM.CHS.020	Zambia	Chisamba	155	471	236	209	196	242	175	240	329	245	134	*MAT1-2*
2015.ZM.CHS.021	Zambia	Chisamba	158	462	236	230	199	245	191	240	329	251	137	*MAT1-2*
2015.ZM.CHS.022	Zambia	Chisamba	158	462	236	230	199	242	191	240	329	251	137	*MAT1-2*
2015.ZM.CHS.023	Zambia	Chisamba	158	462	236	230	199	245	191	240	329	251	137	*MAT1-2*
2015.ZM.CHS.024	Zambia	Chisamba	158	462	236	230	199	242	187	240	329	248	137	*MAT1-2*
2015.ZM.CHS.025	Zambia	Chisamba	158	462	236	230	199	245	191	240	329	251	137	*MAT1-2*
2015.ZM.CHS.026	Zambia	Chisamba	158	462	236	230	199	242	187	240	329	251	137	*MAT1-2*
2015.ZM.CHS.027	Zambia	Chisamba	155	465	223	233	199	245	187	240	329	245	134	*MAT1-1*
2015.ZM.CHS.028	Zambia	Chisamba	155	465	223	230	196	245	191	240	329	245	137	*MAT1-1*
2015.ZM.CHS.029	Zambia	Chisamba	155	465	223	230	196	245	187	240	329	245	137	*MAT1-1*
2015.ZM.CHS.030	Zambia	Chisamba	158	462	236	230	199	242	187	240	329	245	134	*MAT1-1*
2015.ZM.CHS.031	Zambia	Chisamba	158	465	236	230	199	245	187	244	329	251	134	*MAT1-1*
2015.ZM.CHS.032	Zambia	Chisamba	158	462	236	230	199	245	172	240	329	245	137	*MAT1-1*
2015.ZM.CHS.035	Zambia	Chisamba	155	462	236	230	199	245	175	240	329	251	134	*MAT1-2*
2015.ZM.CHS.036	Zambia	Chisamba	155	462	236	230	199	245	175	240	329	251	134	*MAT1-2*
2015.ZM.CHS.037	Zambia	Chisamba	155	462	236	209	196	242	187	240	329	251	134	*MAT1-2*
2015.ZM.CHS.038	Zambia	Chisamba	155	462	236	230	196	242	172	240	329	245	137	*MAT1-2*
2015.ZM.CHS.039	Zambia	Chisamba	155	462	236	230	196	242	175	240	329	245	137	*MAT1-2*
2015.ZM.CHS.040	Zambia	Chisamba	155	462	236	230	196	242	175	240	329	245	137	*MAT1-2*
2015.ZM.CHS.041	Zambia	Chisamba	155	465	223	230	196	245	175	240	329	251	137	*MAT1-2*
2015.ZM.CHS.042	Zambia	Chisamba	155	465	223	230	196	245	172	240	329	251	137	*MAT1-2*
2015.ZM.CHS.043	Zambia	Chisamba	155	465	223	230	196	245	187	240	329	251	137	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2015.ZM.CHS.044	Zambia	Chisamba	155	465	223	230	196	245	187	240	329	251	137	*MAT1-1*
2015.ZM.CHS.045	Zambia	Chisamba	155	465	223	230	196	245	191	240	329	251	137	*MAT1-1*
2015.ZM.CHS.046	Zambia	Chisamba	155	465	236	220	196	242	191	240	329	251	134	*MAT1-1*
2015.ZM.CHS.058	Zambia	Chisamba	155	462	236	209	199	245	175	240	329	251	134	*MAT1-2*
2015.ZM.CHS.059	Zambia	Chisamba	158	465	223	230	199	242	191	240	329	251	137	*MAT1-1*
2015.ZM.CHS.060	Zambia	Chisamba	158	465	223	230	199	245	191	228	329	251	137	*MAT1-1*
2015.ZM.CHS.061	Zambia	Chisamba	155	465	236	230	199	242	191	240	329	245	137	*MAT1-2*
2015.ZM.CHS.062	Zambia	Chisamba	155	465	223	230	199	245	187	240	329	251	137	*MAT1-2*
2015.ZM.CHS.063	Zambia	Chisamba	155	465	223	230	199	245	187	240	329	251	137	*MAT1-2*
2015.ZM.CHS.069	Zambia	Chisamba	155	465	223	230	196	242	175	244	329	251	134	*MAT1-1*
2015.ZM.CHS.085	Zambia	Chisamba	155	462	223	230	196	245	191	240	329	251	134	*MAT1-2*
2015.ZM.CHS.086	Zambia	Chisamba	155	462	223	230	196	245	187	244	329	251	134	*MAT1-2*
2015.ZM.CHS.090	Zambia	Chisamba	155	462	223	230	196	245	191	240	329	251	134	*MAT1-2*
2015.ZM.CHS.091	Zambia	Chisamba	155	465	223	230	199	245	175	244	329	245	137	*MAT1-2*
2015.ZM.CHS.092	Zambia	Chisamba	155	462	236	214	214	242	191	240	329	251	137	*MAT1-2*
2015.ZM.CHS.093	Zambia	Chisamba	155	462	236	214	214	242	191	240	329	251	137	*MAT1-2*
2015.ZM.CHS.094	Zambia	Chisamba	155	462	233	230	199	242	172	240	329	245	134	*MAT1-2*
2015.ZM.CHS.095	Zambia	Chisamba	155	465	236	226	199	242	187	240	329	245	134	*MAT1-2*
2015.ZM.CHS.096	Zambia	Chisamba	155	465	236	230	199	242	191	240	329	245	134	*MAT1-2*
2015.ZM.CHS.097	Zambia	Chisamba	155	462	236	209	196	242	191	240	329	245	134	*MAT1-1*
2015.ZM.CHS.098	Zambia	Chisamba	155	462	223	230	199	242	191	240	329	251	134	*MAT1-1*
2015.ZM.CHS.099	Zambia	Chisamba	155	465	236	209	199	248	191	240	329	251	134	*MAT1-2*
2015.ZM.CHS.100	Zambia	Chisamba	158	462	236	230	199	242	175	240	329	251	137	*MAT1-1*
2015.ZM.CHS.101	Zambia	Chisamba	155	465	223	230	199	242	175	244	329	251	137	*MAT1-1*
2017.ZW.ACT.056	Zimbabwe	KweKwe	155	462	242	223	212	196	190	240	329	245	137	*MAT1-2*
2017.ZW.ACT.060	Zimbabwe	KweKwe	155	462	242	223	229	199	175	240	329	245	137	*MAT1-2*
2017.ZW.ACT.075	Zimbabwe	KweKwe	158	462	242	236	229	199	175	244	329	245	137	*MAT1-2*
2017.ZW.ACT.101	Zimbabwe	KweKwe	155	462	242	223	229	199	175	240	329	245	137	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.ZW.ACT.112	Zimbabwe	KweKwe	155	462	245	223	229	199	175	236	329	245	137	*MAT1-2*
2017.ZW.ACT.113	Zimbabwe	KweKwe	155	462	245	223	229	199	185	242	329	245	134	*MAT1-1*
2017.ZW.ACT.116	Zimbabwe	KweKwe	155	465	242	236	226	196	242	245	329	245	139	*MAT1-2*
2017.ZW.ACT.135	Zimbabwe	KweKwe	158	462	239	236	229	199	175	244	329	245	137	*MAT1-2*
2017.ZW.ACT.136	Zimbabwe	KweKwe	158	462	242	236	229	199	175	244	329	245	137	*MAT1-2*
2017.ZW.ACT.142	Zimbabwe	KweKwe	158	462	242	236	229	199	175	244	329	245	137	*MAT1-2*
2017.ZW.ACT.146	Zimbabwe	KweKwe	158	462	242	236	229	199	175	244	329	245	137	*MAT1-2*
2017.ZW.RRS.262	Zimbabwe	KweKwe	158	462	245	236	229	199	175	240	329	245	137	*MAT1-1*
2017.ZW.RRS.263	Zimbabwe	KweKwe	158	462	248	236	229	199	172	236	329	245	137	*MAT1-1*
2017.ZW.RRS.274	Zimbabwe	KweKwe	158	462	245	236	229	199	161	242	329	245	137	*MAT1-2*
2017.ZW.RRS.279	Zimbabwe	KweKwe	158	462	242	236	229	199	175	236	329	251	137	*MAT1-1*
2017.ZW.RRS.286	Zimbabwe	KweKwe	158	462	245	236	229	199	242	242	329	248	137	*MAT1-1*
2017.ZW.RRS.287	Zimbabwe	KweKwe	161	462	245	236	229	199	175	244	329	245	137	*MAT1-1*
2017.ZW.RRS.309	Zimbabwe	KweKwe	155	465	245	223	233	199	196	245	329	251	134	*MAT1-2*
2017.ZW.RRS.317	Zimbabwe	KweKwe	158	462	242	223	229	203	196	242	329	251	137	*MAT1-1*
2017.ZW.RRS.320	Zimbabwe	KweKwe	155	462	248	236	209	196	196	242	329	245	134	*MAT1-2*
2017.ZW.RRS.327	Zimbabwe	KweKwe	158	462	242	236	229	203	242	263	329	251	137	*MAT1-1*
2017.ZW.AFR.238	Zimbabwe	Mutare	158	462	242	236	235	193	242	242	329	245	137	*MAT1-1*
2017.ZW.AFR.243	Zimbabwe	Mutare	155	462	242	236	209	196	172	240	329	245	137	*MAT1-1*
2017.ZW.AFR.247	Zimbabwe	Mutare	155	467	245	236	229	196	190	240	329	251	137	*MAT1-2*
2017.ZW.AFR.255	Zimbabwe	Mutare	155	465	245	236	229	196	190	240	329	245	137	*MAT1-1*
2017.ZW.AFR.258	Zimbabwe	Mutare	158	462	242	236	229	193	242	240	329	245	137	*MAT1-2*
2017.ZW.AFR.259	Zimbabwe	Mutare	158	462	242	236	229	196	175	240	329	245	137	*MAT1-2*
2017.ZW.AFR.260	Zimbabwe	Mutare	155	465	246	236	229	199	190	240	329	251	137	*MAT1-2*
2017.ZW.AFR.265	Zimbabwe	Mutare	158	465	242	223	229	196	190	236	329	245	137	*MAT1-1*
2017.ZW.AFR.266	Zimbabwe	Mutare	155	462	245	223	229	196	190	240	329	251	137	*MAT1-1*
2017.ZW.AFR.268	Zimbabwe	Mutare	158	465	242	223	229	199	190	240	329	245	137	*MAT1-2*
2017.ZW.AFR.269	Zimbabwe	Mutare	158	462	245	223	238	196	175	240	329	245	137	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.ZW.AFR.270	Zimbabwe	Mutare	158	462	242	223	229	196	190	244	329	251	134	*MAT1-1*
2017.ZW.AFR.271	Zimbabwe	Mutare	155	462	242	236	209	196	242	242	349	245	137	*MAT1-1*
2017.ZW.AFR.316	Zimbabwe	Mutare	155	465	248	236	229	199	242	242	329	251	134	*MAT1-1*
2017.ZW.AFR.319	Zimbabwe	Mutare	155	462	242	223	229	199	242	240	329	245	134	*MAT1-1*
2017.ZW.MUT.039	Zimbabwe	Mutare	155	465	242	236	229	199	242	242	329	245	134	*MAT1-1*
2017.ZW.MUT.068	Zimbabwe	Mutare	158	462	245	236	229	196	190	240	329	251	134	*MAT1-1*
2017.ZW.MUT.072	Zimbabwe	Mutare	155	465	245	236	229	199	190	236	329	245	134	*MAT1-2*
2017.ZW.MUT.093	Zimbabwe	Mutare	158	462	242	236	229	199	190	240	329	251	134	*MAT1-2*
2017.ZW.MUT.099	Zimbabwe	Mutare	158	462	242	236	229	196	190	240	329	251	134	*MAT1-1*
2017.ZW.MUT.109	Zimbabwe	Mutare	158	465	242	223	209	196	175	240	329	245	134	*MAT1-1*
2017.ZW.MUT.120	Zimbabwe	Mutare	155	465	242	236	229	199	175	236	329	245	137	*MAT1-2*
2017.ZW.MUT.121	Zimbabwe	Mutare	155	465	242	236	229	199	190	244	329	251	137	*MAT1-1*
2017.ZW.MUT.122	Zimbabwe	Mutare	158	462	242	223	235	199	190	240	329	245	137	*MAT1-2*
2017.ZW.MUT.143	Zimbabwe	Mutare	155	462	242	236	229	196	175	240	329	251	137	*MAT1-2*
2017.ZW.MUT.149	Zimbabwe	Mutare	155	465	242	236	226	196	242	242	329	245	137	*MAT1-2*
2017.ZW.MUT.153	Zimbabwe	Mutare	155	465	245	236	229	203	242	242	329	251	137	*MAT1-2*
2017.ZW.CHN.308	Zimbabwe	Chinhoyi	155	462	242	236	229	199	242	245	329	245	137	*MAT1-1*
2017.ZW.CHN.314	Zimbabwe	Chinhoyi	155	462	242	236	229	199	251	242	329	245	137	*MAT1-1*
2017.ZW.CHN.315	Zimbabwe	Chinhoyi	158	467	245	236	235	199	190	248	329	251	137	*MAT1-2*
2017.ZW.CHN.321	Zimbabwe	Chinhoyi	155	462	245	223	209	196	242	236	329	245	137	*MAT1-1*
2017.ZW.CHN.329	Zimbabwe	Chinhoyi	155	465	245	236	229	199	242	242	329	245	134	*MAT1-1*
2017.ZW.CHN.034	Zimbabwe	Chinhoyi	155	465	242	236	229	196	242	242	329	245	134	*MAT1-1*
2017.ZW.CHN.077	Zimbabwe	Chinhoyi	158	462	242	223	229	199	175	240	329	245	137	*MAT1-2*
2017.ZW.CHN.126	Zimbabwe	Chinhoyi	158	465	245	236	229	199	190	240	329	245	137	*MAT1-1*
2017.ZW.ART.057	Zimbabwe	Harare	155	465	245	236	229	199	190	240	329	245	137	*MAT1-2*
2017.ZW.ART.065	Zimbabwe	Harare	155	465	245	236	229	199	190	240	329	245	137	*MAT1-1*
2017.ZW.ART.069	Zimbabwe	Harare	155	465	242	239	229	199	175	240	329	245	137	*MAT1-1*
2017.ZW.ART.138	Zimbabwe	Harare	158	462	245	223	229	196	190	240	329	245	137	*MAT1-2*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.ZW.ART.139	Zimbabwe	Harare	155	462	245	223	229	199	175	240	329	251	134	*MAT1-2*
2017.ZW.ART.151	Zimbabwe	Harare	158	465	245	223	209	199	242	242	329	251	137	*MAT1-2*
2017.ZW.ART.159	Zimbabwe	Harare	158	462	245	236	229	196	242	245	329	251	137	*MAT1-2*
2017.ZW.ART.162	Zimbabwe	Harare	158	462	245	223	229	196	196	245	329	251	134	*MAT1-2*
2017.ZW.ART.169	Zimbabwe	Harare	155	462	242	236	229	196	175	244	329	245	137	*MAT1-1*
2017.ZW.ART.185	Zimbabwe	Harare	155	462	245	236	229	196	242	242	329	245	137	*MAT1-1*
2017.ZW.ART.188	Zimbabwe	Harare	155	462	248	236	229	199	175	240	329	245	137	*MAT1-1*
2017.ZW.ART.310	Zimbabwe	Harare	155	465	248	236	229	199	196	242	329	245	134	*MAT1-1*
2017.ZW.ART.330	Zimbabwe	Harare	155	467	245	236	209	199	242	242	329	251	137	*MAT1-1*
2017.ZW.CMH.210	Zimbabwe	Harare	155	462	245	223	229	199	190	240	329	251	137	*MAT1-1*
2017.ZW.CMH.214	Zimbabwe	Harare	158	462	245	223	209	196	190	248	329	251	134	*MAT1-2*
2017.ZW.CMH.215	Zimbabwe	Harare	155	462	242	223	229	199	175	244	329	245	137	*MAT1-2*
2017.ZW.CMH.216	Zimbabwe	Harare	158	462	245	236	209	199	175	240	329	245	137	*MAT1-2*
2017.ZW.CMH.217	Zimbabwe	Harare	155	462	242	236	229	199	190	213	329	245	137	*MAT1-1*
2017.ZW.CMH.219	Zimbabwe	Harare	155	462	242	223	229	199	190	240	329	245	134	*MAT1-2*
2017.ZW.CMH.221	Zimbabwe	Harare	155	467	242	236	229	199	175	236	329	251	137	*MAT1-2*
2017.ZW.CMH.222	Zimbabwe	Harare	155	465	245	223	229	199	190	240	329	245	137	*MAT1-1*
2017.ZW.CMH.223	Zimbabwe	Harare	155	462	245	236	235	196	242	242	329	245	137	*MAT1-1*
2017.ZW.CMH.225	Zimbabwe	Harare	158	465	245	236	229	199	188	236	329	245	137	*MAT1-2*
2017.ZW.CMH.226	Zimbabwe	Harare	158	462	242	223	229	199	190	240	329	245	134	*MAT1-2*
2017.ZW.CMH.227	Zimbabwe	Harare	158	462	245	223	229	199	242	242	329	251	137	*MAT1-1*
2017.ZW.CMH.228	Zimbabwe	Harare	158	465	245	223	229	196	175	240	329	251	137	*MAT1-1*
2017.ZW.CMH.229	Zimbabwe	Harare	155	462	242	223	229	199	242	213	329	251	137	*MAT1-2*
2017.ZW.CMH.231	Zimbabwe	Harare	155	462	245	236	235	196	175	240	329	245	137	*MAT1-1*
2017.ZW.CMH.232	Zimbabwe	Harare	158	462	242	223	229	199	190	240	329	245	137	*MAT1-1*
2017.ZW.CMH.233	Zimbabwe	Harare	158	465	245	223	229	196	242	242	349	251	137	*MAT1-1*
2017.ZW.CMH.235	Zimbabwe	Harare	155	462	242	236	229	199	190	240	329	245	137	*MAT1-2*
2017.ZW.CMH.236	Zimbabwe	Harare	158	462	245	223	229	199	172	240	329	245	134	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.ZW.CMH.242	Zimbabwe	Harare	158	462	248	223	229	199	188	236	329	245	137	*MAT1-2*
2017.ZW.CMH.244	Zimbabwe	Harare	155	467	242	236	209	196	196	240	329	251	137	*MAT1-2*
2017.ZW.CMH.245	Zimbabwe	Harare	155	462	248	236	229	199	172	236	329	245	137	*MAT1-1*
2017.ZW.CMH.246	Zimbabwe	Harare	158	465	245	236	229	199	190	240	329	245	134	*MAT1-2*
2017.ZW.CMH.248	Zimbabwe	Harare	158	462	245	223	229	196	175	240	334	245	137	*MAT1-2*
2017.ZW.CMH.250	Zimbabwe	Harare	155	465	248	236	229	196	175	248	329	245	134	*MAT1-2*
2017.ZW.CMH.252	Zimbabwe	Harare	155	459	242	236	229	199	190	240	329	251	137	*MAT1-2*
2017.ZW.CMH.254	Zimbabwe	Harare	158	462	245	223	229	199	190	240	329	251	137	*MAT1-2*
2017.ZW.CMH.304	Zimbabwe	Harare	161	462	248	236	229	203	242	242	329	245	134	*MAT1-1*
2017.ZW.CMH.306	Zimbabwe	Harare	155	462	245	236	229	196	242	245	329	251	134	*MAT1-1*
2017.ZW.CMH.322	Zimbabwe	Harare	158	467	245	236	209	193	242	242	329	245	137	*MAT1-2*
2017.ZW.CMH.324	Zimbabwe	Harare	158	467	245	236	209	193	242	245	329	251	134	*MAT1-2*
2017.ZW.CMH.326	Zimbabwe	Harare	155	462	242	236	233	203	236	248	329	245	134	*MAT1-1*
2017.ZW.SHM.063	Zimbabwe	Harare	158	462	242	223	229	196	190	240	329	245	134	*MAT1-2*
2017.ZW.SHM.118	Zimbabwe	Harare	155	462	242	223	229	199	175	240	329	245	134	*MAT1-2*
2017.ZW.SHM.295	Zimbabwe	Harare	155	462	242	236	209	196	175	236	329	245	137	*MAT1-1*
2017.ZW.SHM.296	Zimbabwe	Harare	155	467	245	223	209	199	242	245	329	245	137	*MAT1-2*
2017.ZW.SHM.300	Zimbabwe	Harare	158	462	242	236	229	196	242	240	329	242	137	*MAT1-1*
2017.ZW.STP.038	Zimbabwe	Harare	155	465	239	236	229	199	242	242	329	245	134	*MAT1-1*
2017.ZW.STP.061	Zimbabwe	Harare	158	465	245	223	209	199	190	240	329	245	137	*MAT1-2*
2017.ZW.STP.082	Zimbabwe	Harare	158	462	242	236	229	199	175	244	329	245	137	*MAT1-2*
2017.ZW.STP.127	Zimbabwe	Harare	158	462	242	223	209	196	175	240	329	251	137	*MAT1-1*
2017.ZW.STP.130	Zimbabwe	Harare	158	462	242	236	229	199	175	244	329	245	137	*MAT1-2*
2017.ZW.STP.133	Zimbabwe	Harare	155	462	242	236	226	196	242	242	329	245	137	*MAT1-1*
2017.ZW.STP.141	Zimbabwe	Harare	158	465	242	236	229	196	175	240	329	251	134	*MAT1-2*
2017.ZW.STP.145	Zimbabwe	Harare	158	462	242	236	229	199	175	244	329	245	137	*MAT1-2*
2017.ZW.STP.158	Zimbabwe	Harare	158	465	248	236	209	196	188	240	329	245	139	*MAT1-2*
2017.ZW.STP.166	Zimbabwe	Harare	158	467	242	223	209	196	175	244	329	251	137	*MAT1-1*

Isolate	Country	District	01	04	05	06	07	08	10	12	13	15	18	Mating types
2017.ZW.STP.171	Zimbabwe	Harare	159	462	242	223	229	199	242	242	329	251	134	*MAT1-1*
2017.ZW.STP.173	Zimbabwe	Harare	155	465	242	223	209	196	190	240	329	245	137	*MAT1-2*
2017.ZW.STP.174	Zimbabwe	Harare	158	465	248	236	209	196	242	242	329	251	134	*MAT1-2*
2017.ZW.STP.178	Zimbabwe	Harare	155	462	248	236	229	199	188	236	329	251	137	*MAT1-1*
2017.ZW.STP.180	Zimbabwe	Harare	155	462	245	236	229	196	242	242	329	251	134	*MAT1-2*
2017.ZW.STP.183	Zimbabwe	Harare	155	462	242	236	229	196	175	240	329	251	137	*MAT1-2*
2017.ZW.STP.186	Zimbabwe	Harare	158	465	245	236	229	199	190	242	329	251	137	*MAT1-1*
2017.ZW.STP.192	Zimbabwe	Harare	155	462	242	223	229	196	190	240	329	251	137	*MAT1-1*
2017.ZW.STP.193	Zimbabwe	Harare	155	465	245	236	209	199	242	245	329	245	134	*MAT1-2*
2017.ZW.STP.194	Zimbabwe	Harare	155	465	245	236	209	199	175	240	329	245	134	*MAT1-2*
2017.ZW.STP.195	Zimbabwe	Harare	155	462	242	223	229	199	190	240	329	245	134	*MAT1-2*
2017.ZW.STP.198	Zimbabwe	Harare	158	462	242	236	229	184	175	240	329	245	137	*MAT1-1*
2017.ZW.STP.201	Zimbabwe	Harare	158	465	245	236	229	199	246	245	329	251	137	*MAT1-1*
2017.ZW.STP.203	Zimbabwe	Harare	155	462	245	236	229	199	196	242	329	245	134	*MAT1-1*
2017.ZW.STP.204	Zimbabwe	Harare	158	465	245	223	229	203	190	240	329	245	137	*MAT1-2*
2017.ZW.STP.206	Zimbabwe	Harare	155	465	245	236	209	199	242	245	329	245	134	*MAT1-2*
2017.ZW.STP.207	Zimbabwe	Harare	155	465	245	236	229	199	175	240	329	245	137	*MAT1-2*
2017.ZW.STP.208	Zimbabwe	Harare	155	462	242	236	229	199	190	240	329	251	137	*MAT1-1*
2017.ZW.STP.209	Zimbabwe	Harare	155	467	242	236	229	199	175	244	329	245	137	*MAT1-1*
2017.ZW.STP.211	Zimbabwe	Harare	155	462	245	236	229	199	175	240	329	245	137	*MAT1-2*
2017.ZW.STP.212	Zimbabwe	Harare	158	462	245	239	229	199	175	240	329	245	137	*MAT1-1*
2017.ZW.STP.218	Zimbabwe	Harare	155	462	242	223	238	199	190	244	329	245	134	*MAT1-2*
2017.ZW.STP.220	Zimbabwe	Harare	155	465	245	236	229	199	172	236	329	245	134	*MAT1-1*
2017.ZW.STP.230	Zimbabwe	Harare	155	462	245	236	229	203	175	240	329	251	137	*MAT1-2*