Chapter 1

Northern corn leaf blight as a destructive foliar disease of maize

1.1. Introduction

The three most widely produced cereals worldwide are maize, wheat and rice (Anonymous, 2005). Maize serves as an important source of food in developing countries such as Sub-Saharan Africa and Latin America, and as a source of fodder in industrialized countries such as the United States of America (Hoohafkan & Stewart, 2008). The current world population of seven billion is estimated to increase up to nine billion by 2050, and will require a 70% increase in food supply (Anonymous, 2003). Production of maize is severely affected by yield-limiting diseases, such as Northern corn leaf blight (NCLB), a devastating foliar disease which can result in yield losses of up to 50% (Raymundo & Hooker, 1981). NCLB occurs worldwide and results from infection of maize leaves with the fungal pathogen, *Exserohilum turcicum* (Pass.) (Leonard & Suggs, 1974). Resistant maize cultivars are the most widespread method to control NCLB, and maize resistance to *E. turcicum* can be either quantitative (polygenic − mediated by several genes) or qualitative (monogenic − inherited by single major genes) (Balint-Kurti & Johal, 2009, Welz & Geiger, 2000). Maize disease resistance is frequently combined with chemical control and cultural practices to reduce yield losses (Kloppers & Tweer, 2009). Ensuring food security for the future will rely on effective disease management strategies in order for maximum crop yields to be obtained from current agricultural land.

The aim of this review is to provide a synopsis of our current understanding of the fungal biology, plant-pathogen interactions and plant defence responses with regards to the *E. turcicum*-maize pathosystem. Furthermore, a summary is given of some of the key research that has been conducted on *E. turcicum* to-date. Maize yield losses due to NCLB can effectively be decreased and prevented when an in-depth understanding of the pathogen, the interaction with its host (*Zea mays*), and its population genetics are reached.

1.2. The *Zea mays* – *Exserohilum turcicum* pathosystem

1.2.1. Pathogen biology and life cycle

Exserohilum turcicum is a hemibiotrophic fungus that lives biotrophically on living host tissue, before switching to a necrotrophic lifestyle which results in death of the infected host cells

(Munch et al., 2008, Perfect & Green, 2001). Optimal environmental conditions, such as long dew periods, relatively high humidity (90 – 100%), cool to moderate temperatures (20 – 25°C), short photoperiods and low luminosity, seem to have a great impact on disease incidence (Bentolila et al., 1991, Levy, 1991). Conidia serve as the primary inoculum by which NCLB is spread from plant-to-plant and from field-to-field.

Maize, sorghum (*Sorghum bicolor*) and related grass species, such as Johnson grass (*Sorghum halopense*) or Sudan grass (*Sorghum sudanense*), serve as hosts for *E. turcicum* (Agrios, 2005). Reports have indicated that isolates of *E. turcicum* may be specific to the crop from which it was isolated (Bunker et al., 2006). Further studies are therefore needed to clarify host specificity.

Proliferation of *E. turcicum* can occur through sexual or asexual propagation. The sexual stage of *E. turcicum* has not been observed in the field although it can be induced under laboratory conditions (Borchardt et al., 1998c, Ramathani et al., 2011). Crossings are performed by placing a 0.5 mm mycelial plug from a 10 day old culture to be tested, with a 0.5 mm mycelial plug from a tester strain onto the appropriate substrate. Two sterile pieces of barley or Johnson grass stalks embedded in Sach's agar are often used (Ferguson & Carson, 2007, Luttrell, 1958, Pedersen & Brandenburg, 1986). Stalks are placed 1 cm apart, mycelial plugs are placed onto the stalks and incubated at 25°C for 3 – 4 weeks (Pedersen & Brandenburg, 1986).

The sexual stage is characterized by black, ellipsoid to globose ascocarps containing ascospores (Luttrell, 1964). The conidia of *E. turcicum* are olive grey to brown, measure 73 – 137 µm in length and 18 – 23 µm in width, and have 2 – 7 septae (Luttrell, 1964). Conidia may be fusiform, cylindrical or obclavate with a straight to curved shape (Fig. 1) (Sivanesan, 1987). The conidiophores are cylindrical in shape and olive to brown in colour with 3 – 7 septae (Leonard & Suggs, 1974, Luttrell, 1964). The mycelium is either brown, or grey to black (Sivanesan, 1987).

The *E. turcicum* lifestyle closely follows that of maize. Maize is planted in early summer and is harvested from mid- to late summer (Anonymous, 2014). At the end of the maize growing season, *E. turcicum* overwinters in and on infected crop debris as mycelia and conidia (Kloppers & Tweer, 2009). When temperatures increase and moist conditions prevail, new conidia formed from mycelia and conidia that have overwintered are spread to the lower leaves of young maize plants (Robert & Findley, 1952). Wind or rain spreads conidia onto the middle and upper leaves

of infected plants as well as to surrounding plants (Elliott & Jenkins, 1946). Maize plants susceptible to *E. turcicum* subsequently develop disease. The fungus once again overwinters on crop debris after maize has been harvested, ready to sporulate at the start of the next growing season.
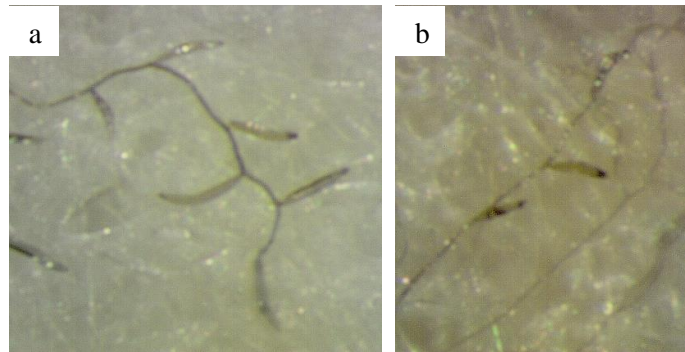

Fig. 1 Conidial shape of *Exserohilum turcicum* isolates – conidia have a characteristic oval shape and protruding hilum (a – b). Multiple conidia are formed on single conidiophores (a). (Photos: MP Haasbroek).

1.2.2.   Infection strategy and disease aetiology

Moisture availability and photoperiod play important roles in successful infection of maize once *E. turcicum* conidia land on the leaf surface (Braun & Howard, 1994). Free moisture on the leaf surface is required for infection by the NCLB pathogen to occur (Anonymous, 2013). Field observations indicated that shorter photoperiods resulted in an increase in the rate of disease progression, while photoperiods exceeding 12 hours effectively prevented repeated infection cycles requiring conidial production (Benedict, 1979).

The infection strategy of *E. turcicum* is summarized in Fig. 2. Infection of the host takes place via a fine infection hypha (the penetration peg) that develops from the lower surface of the appressorium (Knox-Davies, 1974). It has been noted that more than one infection hypha can develop from the appressorium (Knox-Davies, 1974). In the absence of conidia, appressoria can also develop from hyphae (Hilu & Hooker, 1965). Penetration of the host epidermal cell layer by the penetration peg leads to thickening at the site of infection and invagination of the plasma

membrane. An intracellular vesicle is formed giving rise to stout colonization hyphae which extend into adjacent cells and results in intracellular growth (Knox-Davies, 1974). In rare cases, the infection hypha was observed to form hand-like structures between the cuticle and epidermis (Knox-Davies, 1974). Penetration of the epidermis occurred by one or more hyphae and growth took place as described earlier. At later stages of infection, hyphae spread and branch out into the mesophyll tissue and subsequently invade the xylem tissue (Wisser et al., 2006).
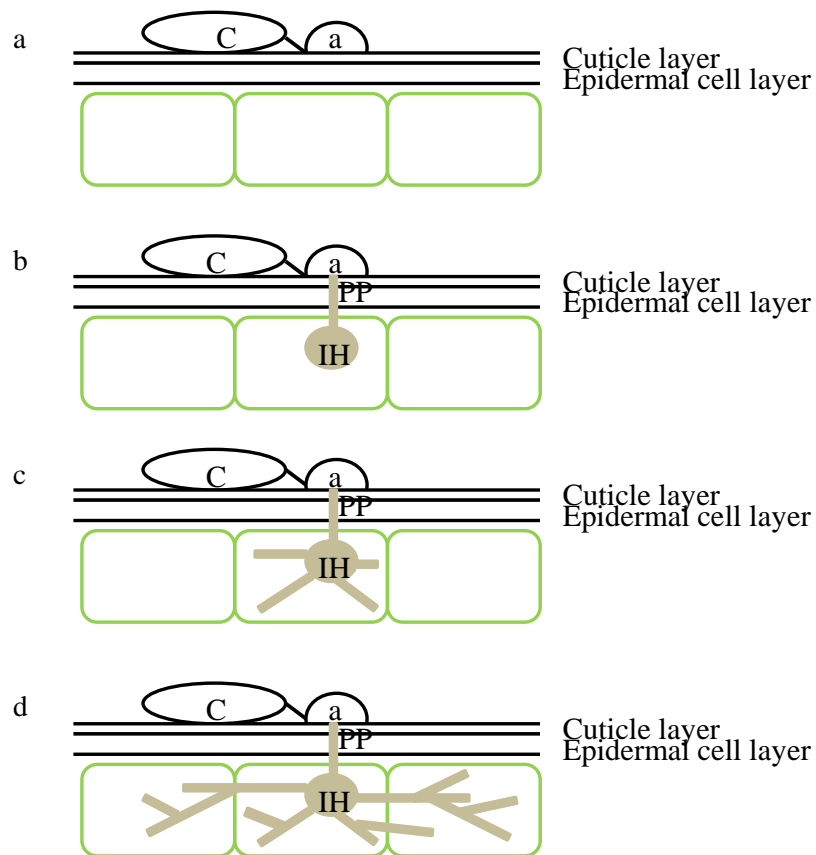

Fig. 2 The infection process of *Exserohilum turcicum* – Germination of the conidium (C) results in formation of an appressorium (a) (a). Penetration of the cuticle layer and epidermal cell layer occurs via a penetration peg (PP) (b). The PP gives rise to an invasive hypha (IH) that branches into numerous smaller hyphae that enables intracellular growth to take place (1c – d).

Growth, symptom development and spread of *E. turcicum in planta* differ between susceptible and resistant maize plants. In susceptible interactions, the fungus thrives in the xylem and later grows from vessels and tracheids into adjacent living bundle sheaths and chlorenchyma (Hilu &

Hooker, 1965). Death of these cells follows rapidly and results in enlarged necrotic lesions. Cell death may result from the presence of toxic compounds or cell wall degrading enzymes (CWDEs) (Cuq et al., 1993, Perfect et al., 1999). The necrotic lesions characteristic of NCLB have been attributed to a phytotoxic compound called monocerin (Cuq et al., 1993). Monocerin was first isolated from *E. monoceras,* but has also been isolated from the entomogenous fungus *Fusarium larvarum* (Robeson & Strobel, 1982), *Microdochium bolleyi* (Sappapan et al., 2008) as well as from an *E. turcicum* isolate collected from *Sorghum halopense* (Johnson grass) (Cuq et al., 1993). Monocerin is non-specific; i.e. it is toxic towards various plants such as maize, Johnson grass, radish, cucumber and tomato. It has been reported that *E. turcicum* produces not only monocerin, but other non-specific toxins in culture as well as water-soluble compounds (Bashan et al., 1995). CDWEs in hemibiotrophs are recognized to be important during the onset of disease symptoms by aiding host colonization and increased access to nutrients (Degefu et al., 2004, Perfect et al., 1999).

In maize plants with resistance to *E. turcicum*, hyphal growth of the fungus within the plant is sparse and lesion enlargement is restricted by the slowly advancing hyphae in the mesophyll (Hilu & Hooker, 1965). When moderate temperatures and long dew periods prevail, hyphae can grow rapidly in lesions of plants with quantitative (polygenic) resistance (Bentolila et al., 1991, Hilu & Hooker, 1965, Kloppers & Tweer, 2009, Levy, 1991). The subsequent sporulation is then of the same magnitude as on lesions of susceptible plants. In qualitative (monogenic) resistant plants, hyphal growth in leaves remains limited, conidial production is either absent or very sparse and growth in the xylem is greatly restricted (Hilu & Hooker, 1965).

1.2.3.   Northern corn leaf blight symptoms

Northern corn leaf blight lesions are characterized by small, water-soaked spots which elongate to become elliptical (or cigar-shaped) and grey-green in colour (Agrios, 2005, Smith & Kinsey, 1980, White, 1999) (Fig. 3). Mature lesions are tan with distinct dark zones of sporulation and are often surrounded by pale-green, water-soaked borders. In the final stage of disease, lesions are straw-coloured to grey and coalesce to kill large parts of the leaves and, in severe cases, the entire plant (Kloppers & Tweer, 2009).

Expression of NCLB symptoms differ between resistant maize lines and susceptible lines. Maize plants that are resistant to *E. turcicum* typically produce chlorotic lesions that are characterized by a yellow border, which is absent in susceptible interactions (Fig. 4a – b). In resistant interactions, pathogen sporulation is minimal on maize leaf surfaces and hyphal growth within the plant is severely limited, resulting in restricted lesion development (Elliott & Jenkins, 1946). Prolific fungal growth results in enlarged necrotic lesions in susceptible interactions.
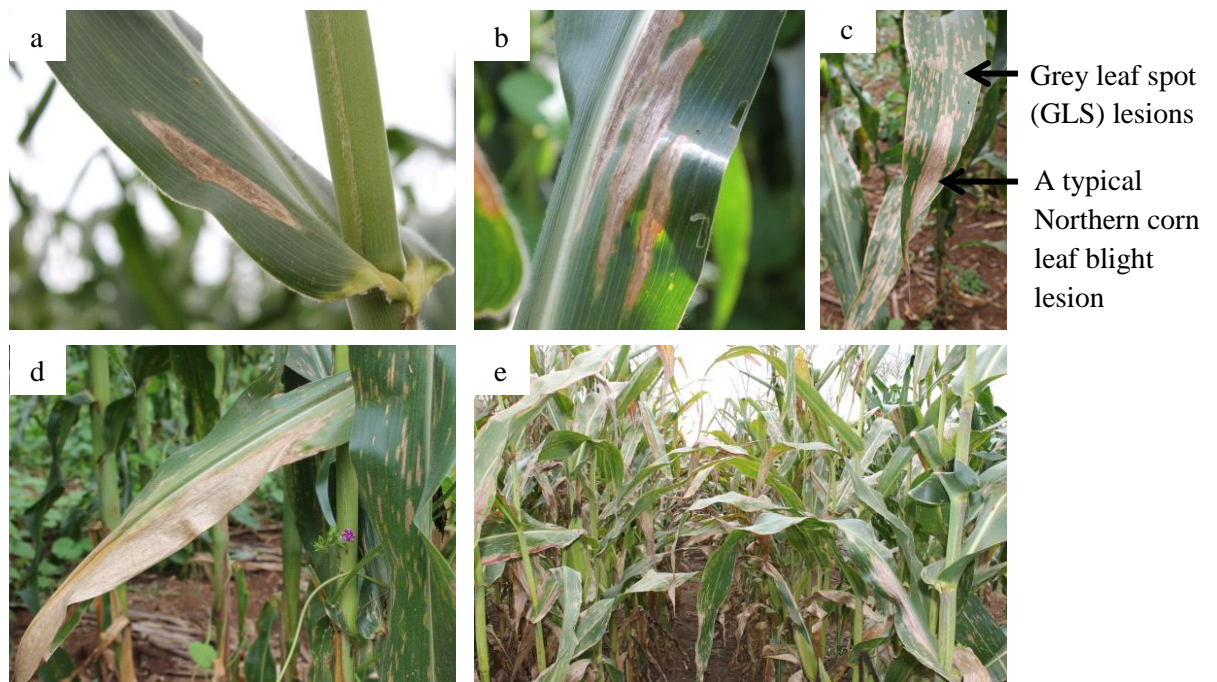

Fig. 3 Characteristic symptoms of Northern corn leaf blight – Typical NCLB lesions are cigar-shaped and grey to straw in colour (a). Multiple NCLB lesions may occur on the same plant (b). NCLB is often found in conjunction with other diseases such as grey leaf spot (c). Coalescing lesions can cause severe damage to leaves (d – e). (Photos: MP Haasbroek).

1.2.4.   Disease severity and economic importance

The developmental stage of the plant and environmental conditions are important when infection occurs. Disease phenotype is predicted to be more severe when infection occurs before tasseling due to extensive defoliation which affects the grain filling process (Carson, 1995a). Tropical and sub-tropical regions with long dew periods and moderate temperatures are particularly favourable for disease development and explain the high disease incidence observed in these

climates (Borchardt et al., 1998c). However, epidemics have occurred under suboptimal conditions and have been absent under ideal conditions (Levy, 1989). Although the reason for this is unclear, it may relate to the resistance of cultivars planted during different environmental conditions or high inoculum load within specific years.
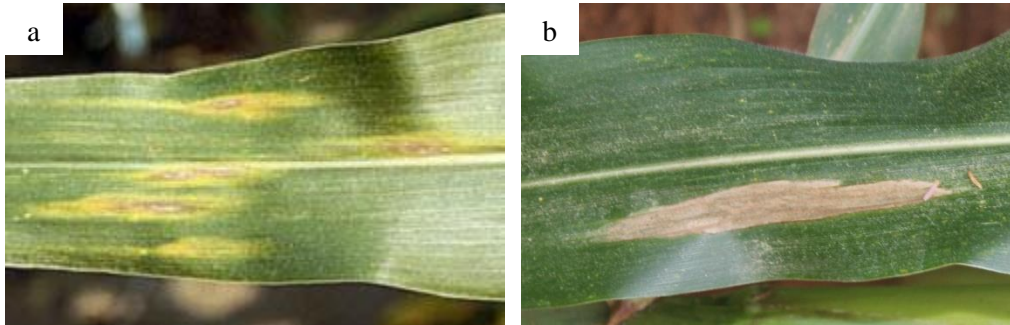

Fig. 4 Lesion phenotypes of plants resistant and susceptible to *Exserohilum turcicum* – Plants resistant to *E. turcicum* will often show yellowing of the leaf around the area of infection (a), in plants susceptible to *E. turcicum,* tan and cigar-shaped lesions develop that decrease the photosynthetic potential of the leaf (b). (Photos: a - Svec and Dolezal (2014), b – MP Haasbroek).

Severe yield losses due to NCLB may exceed 50% due extensive leaf damage during the grain-filling period (Raymundo & Hooker, 1981). In South Africa, typical yield losses range between 15 – 30% and occur from loss in photosynthetic leaf material (Fig. 4b) (Kloppers & Tweer, 2009). Infection with *E. turcicum* predisposes maize plants to infections with other pathogens, thereby aggravating yield losses (Fajemisin & Hooker, 1974, Raymundo & Hooker, 1981).

1.2.5.   Distribution of NCLB

The first report of NCLB was in Parma, Italy in 1876 (Drechsler, 1923) and since then the disease has spread worldwide and is now found in most of the maize-producing countries (Table 1). The maize producing areas that are most affected by NCLB are the North Eastern United States, Sub-Saharan Africa, and areas of China, Latin America and India (Adipala et al., 1995, Dingerdissen et al., 1996). In South Africa, NCLB is estimated to be the most widespread foliar

disease of maize and is most severe in the Eastern parts of the country, such as KwaZulu-Natal and Mpumalanga where environmental conditions are conducive to disease development (Kloppers & Tweer, 2009). Other areas where NCLB is prevalent include the Orange-, Modder- and Vaal river system, as well as the Groblersdal-Loskopdam area (Craven & Morey, 2011).

Table 1 First observations of Northern corn leaf blight – Since it was first observed in Parma, Italy in 1876, Northern corn leaf blight has spread worldwide. Below are the known dates of when NCLB was first observed in various countries.

Location	Year	Reference
Parma, Italy	1876	Drechsler (1923)
New Jersey, United States of America	1878	Jordan et al. (1983)
China	1899	Dong et al. (2008)
Southwest France	Early 1900's	Drechsler (1923)
New England	Early 1900's	Drechsler (1923)
Mid-Atlantic States	Early 1900's	Drechsler (1923)
India	1907	Harlapur (2005)
Uganda	1924	Emechebe (1975)
North western Yugoslavia	1947	Spehar and Rojc (1971)
Ethiopia	1952	Abebe and Singburaudom (2006)
South Africa	Pre-1956*	Bogyo (1956)
Austria	Late 1980's	Borchardt et al. (1998a)
Switzerland	Early 1990's	Borchardt et al. (1998a)
Upper Rhine valley, Germany	1995	Borchardt et al. (1998a)

* In South Africa, maize cultivars with resistance to *Exserohilum turcicum* was first reported in 1956 (Bogyo, 1956).

1.2.6.   Control of Northern corn leaf blight

Effective control of NCLB can be achieved through the use of fungicides, resistant cultivars, crop rotation or biological control. The use of fungicides is not sustainable as fungi may become resistant to fungicides over time (Brent & Hollomon, 2007). In South Africa, it has already been

noted that a particular fungicide may work extremely well in one field, but not in an adjacent field (Rikus Kloppers, personal communication).

Resistant maize cultivars are widely used to control NCLB and are very effective (Borchardt et al., 1998b). Tillage practices and crop rotation with a non-host of *E. turcicum* planted in between maize growing years, reduces mycelia from the previous season thereby decreasing the inoculum load and leading to lower disease incidence in subsequent years (Kloppers & Tweer, 2009).

A biocontrol agent, *Chaetomium globosum,* has recently been identified that secretes anti-fungal compounds effective against *E. turcicum* (Zhang et al., 2013). *C. globosum* displays biological activity against other plant-pathogenic fungi, such as *Pythium ultimum,* the causal agent of damping off in many agricultural crops (Hendrix & Campbell, 1973, Martin & Loper, 1999).

1.3. Taxonomy, morphology and phylogenetics of the *Exserohilum* genus

The genus *Helminthosporium* originally consisted of species that naturally occurred on diverse grass species (Luttrell, 1958). *Exserohilum turcicum* was initially assigned to the *Helminthosporium* genus in 1876 due to its' occurrence on maize, sorghum, Sudan grass and Johnson grass, and was designated as *H. turcicum* (Pass). In 1959, species from the genus *Helminthosporium* were subdivided into three genera (*Drechlera, Bipolaris* and *Helminthosporium*) based on the method of conidium germination (Shoemaker, 1959). Graminicolous species with cylindric conidia that conidiate from any cell were assigned to *Dreschlera,* while other graminicolous species that formed fusoid conidia and showed bipolar germination were assigned to the genus *Bipolaris*. The genus *Helminthosporium* was retained for lignicolous, or wood-decaying, species. In 1959, *E. turcicum* was therefore renamed as *Bipolaris turcica*, although the species name *H. turcicum* was still used until the 1970's. A new genus, *Exserohilum,* was created by Leonard and Suggs (1974) to include species with a distinct protruding hilum previously considered to belong to the genera *Helminthosporium* or *Bipolaris*. The asexual stage was renamed as *Exserohilum turcicum* and has been classified as a filamentous Ascomycete of the class Dothideomycetes and falls in the Pleosporaceae family (Fig. 5) (Kodsueb et al., 2006), along with other pathogens of maize, such as *Cochliobolus*

heterostrophus. The protuberant hilum of species in the *Exserohilum* genus sets them apart from other pathogens of maize such as *C. heterostrophus* (Fig. 6a – b).
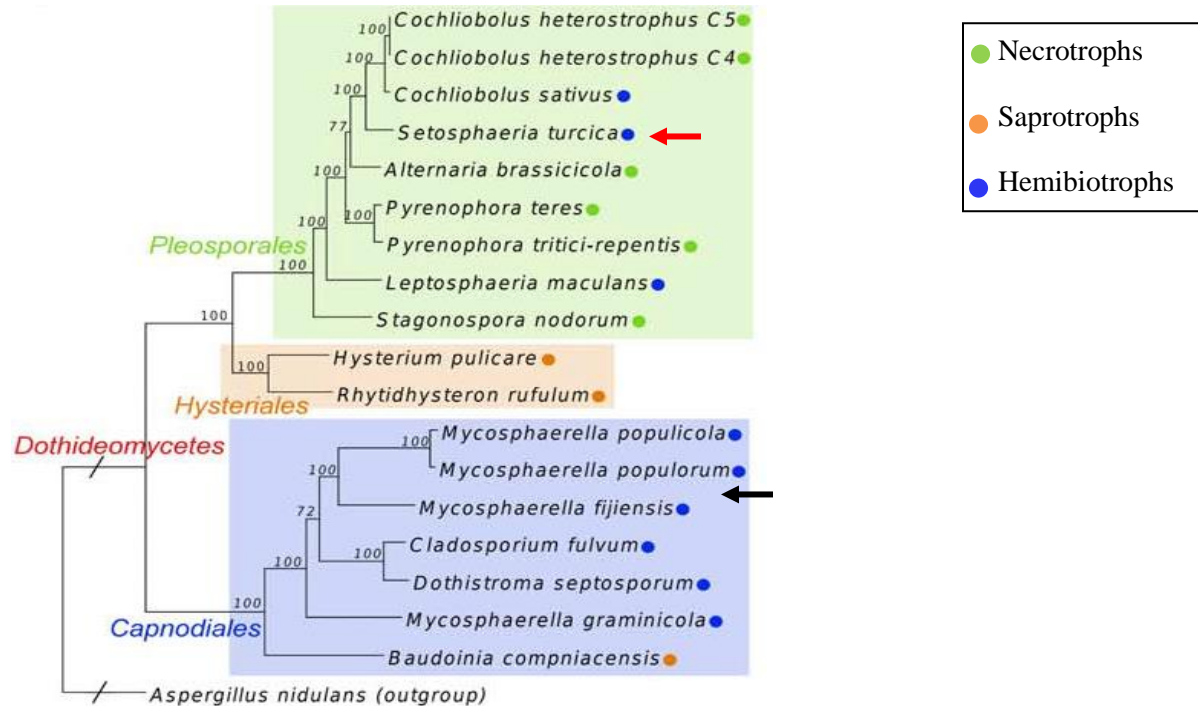

Fig. 5 Phylogenetic tree based on the genomes of 18 Dothideomycetes – The tree was constructed using 51 conserved protein families. Bootstrap values are indicated on branches. The phylogenetic placement of *Exserohilum turcicum* (presented here in the sexual state, *Setosphaeria turcica*) is indicated by the red arrow. *Cercospora zeina* is not indicated on this tree, but its' sister species *C. zeae-maydis* groups between *Mycosphaerella populorum* and *M. fijiensis* (position indicated by the black arrow) (Ohm et al., 2012). [Tree constructed by: Ohm RA, Feau N, Henrissat B, Schoch CL, et al. (2012) Diverse Lifestyles and Strategies of Plant Pathogenesis Encoded in the Genomes of Eighteen Dothideomycetes Fungi. PLoS Pathog 8(12): e1003037. doi:10.1371/journal.ppat.1003037, Figure 2 (http://www.plospathogens.org)].

The sexual stage was first described from crosses made on Barley culm between isolates of the NCLB pathogen from Georgia in the United States of America (Luttrell, 1957). The experiment was repeated using more isolates collected from maize, sorghum, Sudan grass and Johnson grass from larger regions of Georgia (Luttrell, 1958). The sexual stage was described as a new species of *Trichometasphaeria* due to the size of the ascospores and conidial morphology (Luttrell, 1958). The sexual state was separated from the *Trichometasphaeria* species based on

morphological characteristics of the sexual structures (such as absence of a shield-like structure that covers perithecia, called the clypeus) and was designated as *Setosphaeria turcica* (Luttrel) (Leonard & Suggs, 1974).
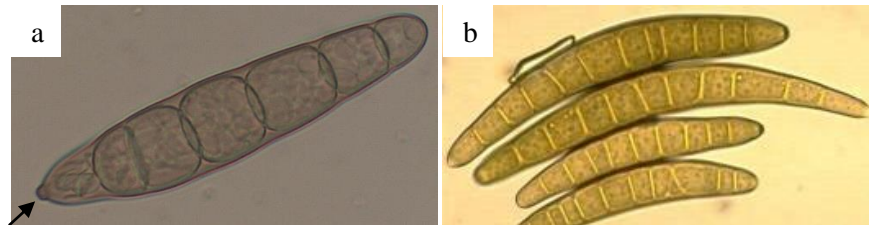

Fig. 6 Conidia of maize pathogens *Exserohilum turcicum* and *Cochliobolus heterostrophus* – The hilum of *Exserohilum turcicum* (a) is strongly protuberant, as indicated by the black arrow. The conidium of *Cochliobolus heterostrophus* has a smooth apex (b) (Photos: a – Dr. Maryke Craven, Agricultural Research Council – Grain Crops Institute; b – Copyright held by T. Tukiboshi, image available at http://www.niaes.affrc.go.jp).

To date, 36 species are accommodated in the genus *Exserohilum.* Species are differentiated based mainly on conidial size and shape (Luttrell, 1958, Shoemaker, 1959). For example, the conidia of *E. turcicum* are characteristically fusiforme, with rounded cells at the end, while *E. rostratum* conidia are rostrate to ellipsoid (Leonard & Suggs, 1974). Within this genus, *E. rostratum, E. longirostratum* and *E. macginnissi* are recognized pathogens of humans and animals causing phaeohyphomycosis. Infection with *E. pedicellatum* and *E. rostratum* results in Exserohilum root rot and rostratum leaf spot of maize, respectively (Lin et al., 2011).

The internal transcribed spacer (ITS) region is the barcode gene used to delineate fungal species (Schoch et al., 2012). The ITS region separates the 18S and 28S nuclear ribosomal DNA (rDNA) (Fig. 7). It further consists of two spacer regions, ITS1 and ITS2, as well as the non-variable 5.8S coding sequence. The ITS1 and ITS2 regions are the variable regions and can be amplified using the universal primers, ITS1 and ITS4 (White et al., 1990). ITS sequencing analysis revealed potential synonymy of *E. rostratum, E. longirostratum* and *E. mcginnisii* (da Cunha et al., 2012). Used in conjunction with other genes, such as elongation factor 1-α, β-tubulin, small and large subunit RNA and cytochrome oxidase I, evolutionary relationships among *Exserohilum* species will become clearer (Collado-Romero et al., 2008, Schoch et al., 2006).
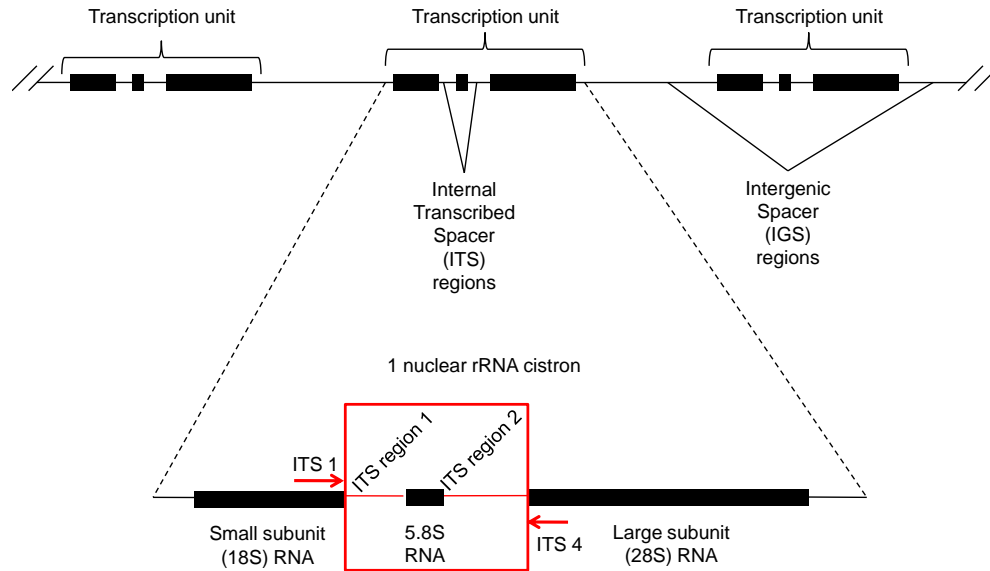
Fig. 7 Schematic representation of the internal transcribed spacer (ITS) region – The nuclear ribosomal RNA cistron consists of the 18S, 5.8S and 28S genes which are transcribed as a unit by RNA polymerase I. Multiple copies of the cistron occurs in tandem. The 18S, 5.8S and 28S genes are separated by two Internal Transcribed Spacer (ITS) regions, which are spliced out during posttranscriptional processes. ITS regions mutate more rapidly than other parts of the cistron, making it the fungal barcode marker of choice to differentiate between species. The ITS 1 and ITS 2 regions can be amplified using the universal primers ITS1 and ITS4 (as indicated by red arrows). Adapted from: White et al. (1990).

The dual nomenclature for naming both the sexual and asexual states of fungi has been abolished by the Amsterdam convention (Hawksworth et al., 2011). The nomenclature *anamorph* and *teleomorph* has been replaced by sexual and asexual morphs. The *one fungus = one name* movement aims to reduce confusion when referring to fungal species with sexual and asexual states (Norvell, 2011, Wingfield et al., 2011). The Committee for Fungal Nomenclature, General Committee and subcommittees will review current nomenclature and provide lists with accepted and rejected names of fungi, from which it will be clear whether *S. turcica* or *E. turcicum* will be the accepted name for this fungus (Norvell, 2011). Up until now, the nomenclature of both the sexual state *Setosphaeria turcica* (Shen et al., 2013, Xue et al., 2013, Zhang et al., 2013), as well as the asexual state *Exserohilum turcicum* (Kim et al., 2012, Ram et al., 2012, Wang et al., 2012), have been used. The current nomenclature for the asexual state, *Exserohilum turcicum*, will be used for the purpose of this review since the sexual stage has not been observed under field conditions.

1.4. Genetics of resistance to *E. turcicum*

1.4.1.   Qualitative resistance of maize

Qualitative resistance is based on single resistance genes (*R*-genes) and provides race-specific, high-level resistance (Balint-Kurti & Johal, 2009). *R*-gene mediated resistance in plants typically results in the hypersensitive response (HR), or programmed cell death, at the site of pathogen infection (McDowell & Simon, 2006). Qualitative resistance is based on gene-for-gene interactions, which occur between products secreted by corresponding gene pairs that control a) the inherent immunity of the plant (*R*-genes), and b) the ability of the pathogen to cause disease (effectors) (Flor, 1942). The genetic factor in plants that conveys resistance is the *R*-gene product. The genetic factor in the pathogen that results in the ability to cause disease was previously known as the avirulence (*Avr*) factor, but is now referred to as an effector molecule (Bent & Mackey, 2007). Effectors are usually species, race or strain specific and contribute to pathogen virulence (Thomma et al., 2011). Plants with the appropriate *R*-gene can recognize effectors secreted by a pathogen and will be resistant to attack, whereas a plant incapable of recognizing the effector will be susceptible.

Monogenic resistance to *E. turcicum* is mediated by specific resistance genes called the *Ht* genes, the name of which was derived from the previous species name, *Helminthosporium turcicum.* The four major dominant resistance genes found in maize are *Ht1, Ht2, Ht3* and *HtN* (Gevers, 1975, Hooker, 1963a, Hooker, 1963b, Hooker, 1977, Hooker, 1978, Hooker, 1981). Other dominant *R*-genes that have been identified, are *HtM* (Robbins & Warren, 1993), *HtP* (Ogliari et al., 2005) and *HtNB* (Xu et al., 1987). Although it was initially assumed that *HtNB* may be the same as or a homologue of *HtN*, a recent fine mapping study by Wang et al. (2012) revealed that *HtNB* is a new gene conveying resistance to NCLB. Recessive resistance genes *ht4* and *rt* have also been reported in maize (Carson, 1995b, Ogliari et al., 2005). Most of the *R*-genes are race-specific, but genes *rt* and *HtP* are potentially capable of conveying wide resistance to *E. turcicum* races (Ogliari et al., 2005). Chromosomal locations have been determined for the majority of the *R*-genes (Table 2), although none of these genes have yet been isolated or characterized. A potential inhibitor of *Ht2, Ht3* and *HtN,* called *Sht1*, has been identified which suppresses the expression of these genes (Ceballos & Gracen, 1989).

Table 2 Summary of fine-mapping studies conducted to-date that identified chromosomal locations of the known *R*-genes in maize conveying resistance to *Exserohilum turcicum* – Chromosomal location is given as the position on the chromosome (e.g. 2L refers to the long arm of chromosome 2) and bin number. Known resistance phenotypes induced by the major *R*-genes are indicated. Unknown chromosomal locations or resistance phenotypes are indicated by (-).

R-gene	Chromosomal location		Resistance phenotypes	Reference
	Position	**Bin**		
Ht1	2L	2.06	Chlorosis with minimal pathogen sporulation	Bentolila et al. (1991); Leonard (1989)
Ht2	8L	8.05	Chlorosis with minimal pathogen sporulation	Simcox & Bennetzen (1993); Leonard et al. (1989)
Ht3	-	-	Chlorosis with minimal pathogen sporulation	Leonard (1989)
HtN	8L	8.06	Prolonged latent period	Simcox & Bennetzen (1993); Leonard (1989)
HtP	2L	2.08	-	Ogliari et al. (2005)
HtNB	8L	8.07	-	Wang et al. (2012)
rt	3L	3.06	-	Ogliari et al. (2005)
ht4	1S	-	Chlorotic halo	Carson (1995b)
HtM	-	-	Complete resistance	(Robbins & Warren, 1993)

A study was undertaken by Martin et al. (2011) to identify genes controlling the resistance of maize and sorghum to NCLB by using amplified fragment length polymorphism (AFLP) based transcript profiling (cDNA-AFLP) of susceptible and resistant maize and sorghum lines infected with *E. turcicum*. Genes that were up-regulated in response to pathogen infection were silenced in sorghum using virus induced gene silencing (VIGS) to confirm their function in host defence responses. A putative *R*-gene was identified on chromosome two of maize that conveys resistance against *E. turcicum*. The gene encodes a coiled-coil, nucleotide binding, Leucine-rich repeat (CC-NB-LRR). This gene was found to be uniquely expressed in resistant maize genotypes and further studies revealed six orthologous genes in a cluster of three pairs in sorghum. Genome searches of related grass species revealed that orthologs of the gene identified in maize are highly conserved amongst maize, sorghum, rice, foxtail millet (*Setaria italica*) and purple false brome (*Brachypodium distachyon*). Clusters of *R*-genes may be the result of factors marking *R*-gene evolution, e.g. gene duplication, unequal crossing over, recombination and

diversification (McDowell & Simon, 2006, Meyers et al., 2005). The putative *R*-gene found by Martin et al. (2011) may be the *Ht1* gene. Further molecular studies are needed to confirm the identity of this putative *R*-gene.

1.4.2.   Quantitative resistance of maize

Quantitative (or polygenic) resistance of plants to pathogens has a multigenic basis and often confers intermediate levels of resistance that is more durable than qualitative resistance (Balint-Kurti & Johal, 2009). Resistance is mediated by genomic regions, or loci, known as quantitative trait loci (QTLs). Chromosomal locations of QTLs are expressed as *bins*, which indicate the fixed interval between two core loci and are numbered by a code designating the linkage group and the location within the linkage group (Gardiner et al., 1993). Quantitative resistance to NCLB is common and has been identified in various maize inbred lines and cultivars, as summarized by Welz & Geiger (2000). QTLs conveying resistance to NCLB have been identified in the US corn Germplasm set (Dingerdissen et al., 1996, Freymark et al., 1994, Freymark et al., 1993), in tropical African maize lines (Schechert et al., 1999) and in European maize populations (Welz et al., 1999). Some QTLs were found to be common between cultivars. One such shared QTL is located on chromosome 8 and is of special importance due to the presence of qualitative resistance genes *Ht2, HtN* and *HtNB* (Table 1) in the same chromosomal region (Wang et al., 2012). However, further fine mapping of this particular region is necessary to elucidate the exact location of major (qualitative) and minor (quantitative) *R*-genes. QTL analysis was conducted on three foliar diseases (NCLB, southern corn leaf blight and grey leaf spot) to find loci conveying multiple disease resistance (Zwonitzer et al., 2010). Unfortunately, although evidence suggests the presence of multiple disease resistance loci, the authors did not find a locus conveying resistance to all three diseases. Quantitative resistance is more often used by maize breeders since it is non-race specific and more difficult to overcome than a single *R*-gene (Balint-Kurti & Johal, 2009). In resistance to NCLB, quantitative resistance appears to be insensitive to light and temperature variations (Carson & Vandyke, 1994), making it a valuable tool for maize breeders. The quantitative resistance phenotype (regardless of the QTL involved) displays fewer and smaller lesions and an extended latent period. The amount of sporulation of the fungus, however, does not decrease (Carson, 1995b, Ullstrup, 1970).

1.5. Races of *E. turcicum*

Races of fungal pathogens refer to isolates that respond differentially to varieties of a single host plant (Van Der Plank, 1969). Prior to 1972, maize lines with the *Ht1* gene were considered resistant to NCLB. Bergquist and Masias (1974), however, observed isolates of *E. turcicum* virulent on maize lines carrying the *Ht1* gene. Isolates that were unable to cause disease on maize lines with *Ht1* were designated as race 1, and isolates virulent to maize lines with *Ht1* were designated as race 2. Observation of isolates with differential responses to the other *Ht* genes followed swiftly and race designations became confusing. In 1989, it was proposed that race nomenclature should follow the resistance formula, i.e. genes to which isolates are susceptible/resistant (Leonard et al., 1989). Races of *E. turcicum* are characterized based on pathogenicity, and depend on the pathogen's ability to overcome the major *Ht* resistance genes *(Ht1, Ht2, Ht3* and *HtN)*. In the case of race 12, the fungal isolate is able to overcome the *Ht1* and *Ht2* genes and cause disease in plants carrying these *R*-genes, but not in plants carrying any of the other major *R*-genes (Fig. 8). The resistance formula of this isolate would then be *Ht3, HtN/Ht1, Ht2*. Similarly, an isolate only capable of causing disease on plants encoding the *H2, Ht3* and *HtN* genes is classified as race 23N. Race 0 describes an isolate that is unable to cause disease on plants carrying any one of the *Ht* genes. Race typing is conducted by observing the phenotypic effect when isolates are inoculated onto a set of maize differential lines under controlled greenhouse conditions (Craven & Fourie, 2011). Ideally, a maize differential set consists of maize lines with only one type of resistance gene per set of plants (e.g. one maize line will have only the *Ht1* gene, while another line will have resistance gene *HtN, Ht2* or *Ht3*). The maize plants are then inoculated with the pathogen and carefully monitored for the development of disease symptoms.
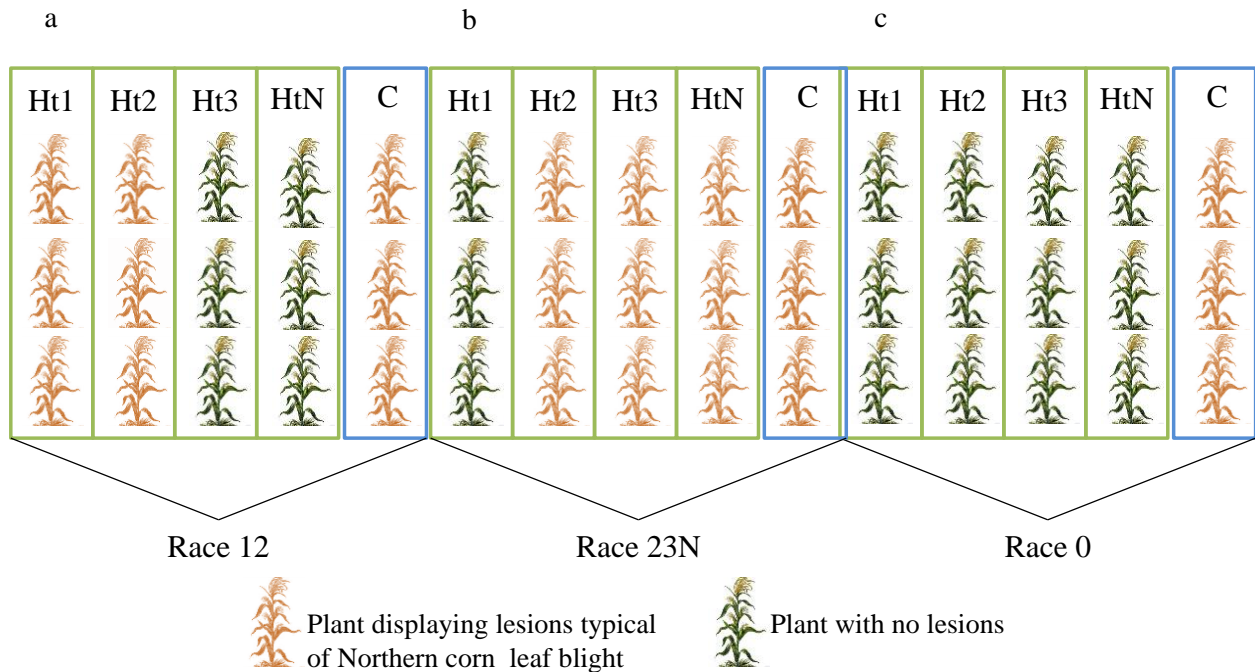
Fig. 8 Race typing of *Exserohilum turcicum* isolates on maize differential sets – Maize differential lines contain one type of resistance gene per maize set, with one control (C) plant that does not carry an *R*-gene. Maize lines are inoculated with an isolate of *E. turcicum* and carefully monitored for lesion development. Races are classified based on their ability to overcome the *R*-genes and result in lesions characteristic of Northern corn leaf blight (NCLB). The above isolates represent a) Race 12; b) Race 23N; and c) Race 0.

In South Africa, seven out of the possible 16 races have been characterized, and these are races 0, 3, 23, 13N, 23N, 3N and 123N (M. Craven, personal communication). Globally, race 0 seems to be the most prevalent followed by races 1, 2, 3 and 12 (Bigirwa et al., 1993, Fallah Moghaddam & Pataky, 1994, Muiru et al., 2010a, Muiru et al., 2010b, Ogliari et al., 2005, Pratt, 2003). Race identification is an extremely tedious process and has shown some inconsistencies. Thakur et al. (1989) found two isolates that were virulent on *B73Ht3* at 22°C day/18°C night temperatures, but not at 26°C day/22°C night temperatures, thereby resulting in different race designations between experiments. In South Africa, repeated infection trials to determine race types of field isolates indicated differences in race characterization between experiments (M. Craven, personal communication). This may be due to the breakdown of virulence when the pathogen is exposed to non-optimal environmental conditions, e.g. low light intensities or high temperatures (Leath et al., 1990). The breakdown of *R*-gene mediated disease resistance at high temperatures has been well documented (Hua, 2013), such as in the *Pseudomonas syringae –*

Arabidopsis thaliana pathosystem (Wang et al., 2009). The mechanism underlying loss of plant disease resistance at elevated temperatures remains poorly understood.

Due to the increasing reports of inconsistencies using maize differential lines, other, more robust, methods are needed to characterize races of *E. turcicum.* One possibility is to use microsatellite markers, as was used to differentiate isolates of *Fusarium oxysporum* forma speciales (f. sp.) *cicero* into four pre-defined race groups (Barve et al., 2001). Although Barve et al. (2001) concluded that further studies were needed to corroborate the results, the study provided evidence to suggest that it is possible to develop molecular markers that can differentiate the different races of a pathogen.

1.6. Studies on genetic variability of fungi

1.6.1. Tools to assess fungal population structure

There are a number of different methods available to study the population genetic structure of fungi. These include markers such as simple sequence repeat (SSRs), random amplification of polymorphic DNA (RAPDs), AFLP, restriction fragment length polymorphism (RFLPs) and single nucleotide polymorphisms (SNPs) (Table 3). Due to their ease of use and variability, SSRs are popular markers to study the genetic structure of fungal populations (Buschiazzo & Gemmell, 2006). SSRs are composed of $1 - 6$ nucleotides tandemly repeated throughout the genomes of most organisms (Chambers & MacAvoy, 2000). The variability of SSRs is derived mainly from length polymorphisms (Ellegren, 2004). Three mechanisms have been proposed that result in polymorphism of markers; 1) unequal crossing over during meiosis, 2) retrotransposition, and 3) strand slippage during replication, although the latter is generally assumed to be the main mechanism of mutation (Fan & Chu, 2007). The majority of simple repeats occur in non-coding DNA (introns or intergenic regions) since the expansion of these repeats (excluding trinucleotide repeats) will be hindered by selection against frameshift mutations (Metzgar et al., 2000, Metzgar & Wills, 2000). Microsatellites are assumed to evolve neutrally and the degree of polymorphism is expected to be proportional to the underlying rate of mutation (Ellegren, 2004). Their high variability makes them valuable markers in population genetic studies to identify the biological history of a species (Chambers & MacAvoy, 2000).

Table 3 Descriptions, advantages and disadvantages of various molecular methods often used to study fungal population structure

Tool	Description	Advantages	Disadvantages	Reference(s)
Random amplification of polymorphic DNA (RAPD)	Degenerate primers (8-10 nucleotides long) bind randomly to genomic DNA. Resultant PCR products are visualized on an agarose gel and analysed by scoring bands as absent or present.	-No prior sequence knowledge required -Inexpensive	-Often inconsistent -Hard to reproduce -Dominant marker -Difficult to interpret	McDonald (1997)
Restriction fragment length polymorphism (RFLP)	Enzymatic digestion of DNA, followed by separation of fragments using gel electrophoresis. Polymorphism results from differences in restriction enzyme cleavage sites.	-Inexpensive -Co-dominant marker -Selectively neutral	-Incomplete gel separation may lead to merging of two bands (collision)	McDonald & McDermott (1993); McDonald (1997)
Amplified fragment length polymorphism (AFLP)	Restriction digestion of genomic DNA. Ligation of specific adaptor sequences onto DNA fragments and selective amplification of adaptor-ligated DNA fragments. Banding patterns are visualized on polyacrylamide gels.	-Co-dominant marker -Selectively neutral	-Homoplasy -Collision	McDonald & McDermott (1993)
Simple sequence repeat (SSR)	Sequence-specific primers are designed to amplify SSR regions. Polymorphic markers are labelled with fluorescent dyes, combined in PCR reaction and visualized with capillary electrophoresis.	-Easy to use -Easy to reproduce -Highly variable -High throughput in multiplex reactions -Co-dominant marker -High mutation rate -Highly informative	-Sequence knowledge required -Heterozygous individuals appear homozygous due to null alleles -Loci may be linked -Often species specific	Buschiazzo and Gemmell (2006); Capote et al. (2012); Ellegren (2004); Pompanon et al. (2005)
Single nucleotide polymorphism (SNP)	Single nucleotide differences between samples within genomic DNA. Occurs frequently.	-High frequency in genome -Easy to analyse (only two alleles per locus)	-Requires sequence data	

1.6.2.   Assessing the population genetic structure of *E. turcicum*

Genetic structure refers to the amount and distribution of genetic variation within and between populations (McDonald & McDermott, 1993). The factors affecting genetic structure are mutation, genetic drift, gene flow, method of reproduction and selection (McDonald & McDermott, 1993). Larger population sizes are assumed to evolve more rapidly, due to more individuals potentially undergoing mutations. In smaller populations, alleles are often lost due to random recombination events, a phenomenon known as genetic drift (Linde et al., 2009). Fungi can spread over large distances and the resultant increased gene flow leads to decreased genetic diversity between populations. A high rate of gene flow in fungal populations poses a serious threat to farmers as it enables fungicide resistance genes or new effector genes to spread more quickly (Goodwin et al., 1994). Sexual reproduction is advantageous to fungal populations since it results in increased genetic diversity during random recombination events (Ali et al., 2014, Kim et al., 2013). Strong directional selection favours pathogens to overcome a single major resistance gene, but the effect of selection can be minimized when crops with different resistance genes are grown in a single field (Zhu et al., 2000).

Our current understanding of the genetic structure within *E. turcicum* populations is based on studies that used RAPD haplotypes, isozymes or AFLP profiles to infer gene and genotypic diversity, and to determine if migration was taking place (Boora et al., 1999, Borchardt et al., 1998a, Borchardt et al., 1998b, Borchardt et al., 1998c, Dong et al., 2008, Ferguson & Carson, 2004, Ferguson & Carson, 2007, Muiru et al., 2010a, Simcox et al., 1993). RAPD markers have been used to study the population structure between *E. turcicum* isolates from tropical (Mexico, Kenya and Southern China) and temperate (Europe and Northern China) climates, amongst race groups and between sampling years (Borchardt et al., 1998a, Borchardt et al., 1998b, Borchardt et al., 1998c, Dong et al., 2008, Ferguson & Carson, 2004, Ferguson & Carson, 2007). Genotypic diversity was higher amongst isolates from tropical climates than temperate climates, potentially due to the increased likelihood of sexual reproduction occurring in tropical climates (Borchardt et al., 1998c). In addition to comparing isolates from different regions, Dong et al. (2008) conducted the only study thus far to compare genetic differentiation between race groups. The results indicated high genetic diversity in *E. turcicum* populations, and that genetic similarity was high within race groups and low between race groups. The high genotypic diversity

observed between isolates from the Eastern United States did not differ between sampling years (1974 – 1978, 1980 – 1986, and 1990 – 1994) (Ferguson & Carson, 2004, Ferguson & Carson, 2007) and although new haplotypes were observed, specific haplotypes persisted over time, indicating the high fitness of these haplotypes. Factors proposed to be responsible for the high level of genetic diversity among populations of *E. turcicum*, were migration, mutation and/or sexual reproduction.

Migration of *E. turcicum* isolates seems to be a common phenomenon between geographical regions not divided by physical barriers, such as large mountain ranges or sea masses (Borchardt et al., 1998c, Dong et al., 2008). A comparison of isolates from Africa, South America, Asia and Europe revealed that certain haplotypes were unique to each continent (Borchardt et al., 1998c). A high number of private alleles specific to each continent further indicated that movement of isolates between continents was either very low or absent. Migration is often observed between nearby regions where migration is not impeded by physical barriers. A high number of shared RAPD haplotypes indicated that migration was frequent in some states of the North Eastern United States, but not all. Shared haplotypes were often detected between locations within Central Kenya or within Western Kenya, but differed quite extensively between Central and West Kenya (Borchardt et al., 1998b). This indicates that migration is most likely occurring within each region, but not between regions, which may be attributed to the large distance between Central and West Kenya (320 km). Furthermore, these regions are separated by the Rift valley, a great trench running through Kenya from North to South that breaks the continuous zone of arable land for maize production. The rate of migration within regions varied, with an estimated 20.6 migrants per year between locations in Western Kenya, and an estimated 5.8 migrants per year between locations in Central Kenya. *Exserohilum turcicum* conidia are spread via wind or rain splash and is not seed-transmitted, which may explain why migration is high amongst regions not separated by physical barriers but limited between continents (Robert & Findley, 1952). Restricted gene flow due to physical barriers further indicates that *E. turcicum* conidia are not spread by anthropogenic movement.

Studies that compared the RAPD profiles of *E. turcicum* from alternative hosts did not find shared haplotypes between maize-infecting isolates, and those from sorghum or Johnson grass (Borchardt et al., 1998a, Ferguson & Carson, 2004). Borchardt et al. (1998a) compared the

RAPD profiles of seven isolates from Johnson grass to 12 isolates from maize from the same location in Switzerland, and to a further 61 isolates from various other locations in Switzerland (9), Germany (24), France (9), Austria (12) and Hungary (7). The RAPD profiles of the Johnson grass isolates differed significantly from the profiles obtained from maize isolates. Furthermore, a comparison of the RAPD profiles of three *E. turcicum* isolates from sorghum, three isolates from Johnson grass and 251 isolates from maize collected from different locations in the North Eastern United States over a period of 20 years, did not reveal any shared haplotypes between isolates from maize and those from sorghum species (Ferguson & Carson, 2004). The variability may be attributed to differences in hosts, i.e. isolates from maize and sorghum or Johnson grass may not interbreed readily. However, due to the small number of isolates sampled from different locations and different years, shared haplotypes might have been missed.

Other techniques have been used to study the population diversity of *E. turcicum* isolates (Muiru et al., 2010a, Simcox et al., 1993). Simcox et al. (1993) determined isozyme diversity in 40 *E. turcicum* isolates from maize collected from the United States Corn belt during 1979 – 1987. Electrophoretic phenograms obtained from isozyme analysis could clearly distinguish between isolates from races 0, 1 and 23. Isolates of race 0 clustered into a single phenogram, irrespective of the year collected or location from which it was collected. Four phenograms were obtained for isolates of race 1, although the majority of isolates clustered into a single phenotype. A diagnostic isozyme polymorphism was obtained that could differentiate race 23 from the other races and a polymorphism unique to race 1 was also observed. Race 1 was found to be more similar to race 0 than to race 23. The use of AFLP markers to determine variability within *E. turcicum* isolates was explored by Muiru et al. (2010a). Using three primer combinations, the authors generated 9 AFLP groups from Kenyan (56 isolates), German (26 isolates) and Austrian (7 isolates) isolates. The groups were not specific to any region, and isolates from different locations grouped together. Genotypic differences between isolates were observed and the clustering analysis revealed that isolates from Austria were more similar to those from Kenya than those from Germany. These results were similar to a further study by Muiru et al. (2010c), which used short intergenic repeat sequences to study strain differences in *E. turcicum*. These sequences were first identified in bacteria and consist of two classes, repetitive extragenic palindromic (REP) sequences and enterobacterial repetitive intergenic consensus (ERIC) sequences (de Bruijn, 1992). Primers designed to conserved regions within these repeat

sequences are available and successful amplification results in a fingerprint, or banding pattern, on an agarose gel. Although these primers amplified in *E. turcicum*, amplification success was low; especially with REP sequences. Only nine out of 17 samples amplified successfully with primers specific to REP sequences. The finding that Kenyan and Austrian isolates were more similar than Austrian and German isolates, is in contrast to previous studies that found strong continental isolation and incomplete differentiation between samples from Eastern Europe (Austria and Hungary) and Western Europe (Germany, Switzerland and France) (Borchardt et al., 1998a, Borchardt et al., 1998c). To elucidate the global population structure of *E. turcicum*, large sample sizes representative of the genetic variability of all maize producing countries will be needed for genetic analyses.

SSRs have been successfully used in population studies of species closely related to *E. turcicum,* such as *Cochliobolus sativus* (Ceballos & Gracen, 1989), *Alternaria alternata* (Mace & Veech, 1973) and *Pyrenophora teres* (Braun & Howard, 1994) (Fig. 6). Gurung et al. (2011) used SSRs to show significant subpopulation structuring in the wheat pathogen, *Mycosphaerella graminicola*, from the United States. In addition, SSRs have also been used to study differences in the distantly related anther smut fungus, *Microbotryum violaceum,* on two closely related host species (*Silene latifolia* and *S. dioica*, flowering plants commonly known as White- and Red Campion, respectively) (Bucheli et al., 2001). A high number of private alleles and no overlap in allele sizes for the majority of SSR loci indicated high genetic differentiation between isolates from these two hosts and suggest that little or no sexual recombination took place over a long period of time (Hendrix & Campbell, 1973). Population studies utilizing SSR markers can be used to elucidate a species' genetic structure, migratory patterns, mating mechanisms within populations and between populations from different hosts, evolutionary relationships among populations from different countries and the geographical origin of a species (Barnes et al., 2001, Chambers & MacAvoy, 2000).

It is hypothesized that an organism's genetic diversity will be highest at its centre of origin, and decrease with increasing distance from the centre (Vavilov, 1923 cited from (Borchardt et al., 1998c)). Two hypotheses dictate where *E. turcicum* might originate from; 1) it co-evolved with maize in Mexico, or 2) it co-evolved with sorghum in East Africa (Borchardt et al., 1998c). Assuming that *E. turcicum* co-evolved with maize, Ferguson & Carson (2004) tested the

hypothesis that genotypic diversity will be lower in the areas further from the possible centre of origin, Mexico (Ferguson & Carson, 2004). The authors considered isolates collected from 20 states in the North Eastern United States. Unfortunately, no isolates from Mexico were collected during the study. No statistical support was found for their hypothesis that genetic diversity will be lower in the states further away from the possible centre of origin (Mexico), which may indicate that migration is frequent within this region.

Studies outlined above on the genetic variability of *E. turcicum* suggest that 1) the genetic variability within this pathogen is high, 2) sexual recombination may be frequent in tropical climates, and 3) migration occurs between populations. However, no population studies have been conducted on *E. turcicum* in South Africa thus far. Understanding the population structure of *E. turcicum* is important for disease management, and will provide a foundation for future studies of this pathogen in South Africa.

1.7. Mating types of *Exserohilum turcicum*

The nomenclature for naming mating (MAT) types in filamentous ascomycetes was proposed by Turgeon and Yoder (2000) and will be applied for the purpose of this review. Sexual reproduction in filamentous ascomycetes is determined by the MAT locus. The alleles (variants) of the genes at the MAT locus share very little sequence homology and are therefore termed *idiomorphs* (Metzenberg & Glass, 1990). Since the MAT genes occur between similar flanking regions, suppression of recombination between these genes preserves their dissimilarity (Coppin et al., 1997, Kronstad & Staben, 1997). The MAT1-1 idiomorph consists of an open reading frame (ORF) encoding the alpha (α) box motif, but can be associated with other genes, e.g. metallothionein in *Pyrenopeziza brassicae*, designated as MAT1-1-4 (Collado-Romero et al., 2008, Singh & Ashby, 1998). MAT1-2 is a regulatory protein encoded by a single ORF and contains a high mobility group (HMG) motif and is the only gene associated with this phenotype (Martin et al., 2013, Souza et al., 2003). Heterothallic individuals are self-sterile, meaning they carry only one MAT gene and require another individual of the compatible mating type to undergo sexual recombination (Souza et al., 2003, Turgeon, 1998). Homothallic individuals carry both MAT genes, are capable of selfing and are therefore referred to as self-fertile. MAT

genes are believed to encode transcription factors and to be responsible for a cell's nuclear identity (whether a cell/nucleus is of mating type 1 or 2) by directing expression of mating-type-specific genes such as pheromones and receptors (Lee et al., 2010, Souza et al., 2003).

The presence or frequency of the different mating type genes in a population is an indication of the frequency of sexual recombination (Duong et al., 2013, Groenewald et al., 2008). The closer the frequencies of the MAT-genes are to each other, the higher the likelihood that sexual recombination is taking place in the population (Bihon et al., 2014). Previous studies to determine the distributions of MAT1-1 and MAT1-2 idiomorphs among field collected isolates of *E. turcicum* were based on crosses with isolates of known MAT type, followed by careful observation for the development of sexual structures (Borchardt et al., 1998c, Fan et al., 2007, Ferguson & Carson, 2004). Polymerase chain reaction (PCR) primer pairs have been developed from the mating type idiomorph sequences of *E. turcicum* which amplify different sized fragments from genomic DNA (Ramathani et al., 2011). The size difference between fragments amplified from MAT1-1 and MAT1-2, however, is only 43 base pairs (bp) which is difficult to visualize using 2% agarose gel electrophoresis. Studies on the sexual stage of *E. turcicum* determined that this fungus is heterothallic with two mating types (MAT1-1 and MAT1-2) (Luttrell, 1957). Isolates from maize that could mate with both MAT1-1 and MAT1-2 tester isolates have been identified from Northern China as well as Uganda (Fan et al., 2007, Martin, 2011). It is possible that these isolates were not obtained from single spores, or that the MAT locus is far more complex than we initially thought and requires in-depth research to elucidate its true structure. Obtaining sequences for the genes located at the MAT locus (from isolates that test positive for both mating types) may reveal whether these isolates are true hybrids (i.e. carrying both MAT1-1 and MAT1-2 genes).

The sexual stage of *E. turcicum* can be induced under laboratory conditions, but has not been observed in the field (Luttrell, 1957). The observed absence of the sexual stage does not exclude sexual recombination occurring in the field, and studies into the distributions of mating types have been undertaken to determine whether sexual reproduction is taking place. Unequal frequencies of the MAT idiomorphs in *E. turcicum* populations were found in temperate regions, but near-equal frequencies were found in tropical regions (Adipala et al., 1993, Borchardt et al., 1998c, Fan et al., 2007, Pedersen & Brandenburg, 1986). This may suggest that sexual

recombination occurs frequently in tropical climates (e.g. Mexico and Kenya), but most likely only occurs in temperate climates (e.g. China and Europe) when environmental conditions are conducive to disease. It is possible that due to low disease incidence in temperate regions, different mating types aren't in close enough proximity for mating to take place. Borchardt et al. (1998c) suggested that natural populations of *E. turcicum* consist of both clonal and sexually reproducing populations, which was corroborated by Ferguson & Carson (2004). High genotypic diversity and near equal mating type frequencies in the Eastern United States indicated that sexual reproduction is occurring, however, focus on particular regions revealed that in some states, populations seemed clonal (Ferguson & Carson, 2004). It is also possible that individuals are migrating from the tropics (e.g. Mexico) where sexual recombination is suggested to be high (Ferguson & Carson, 2004) to the Eastern United States, thus creating the impression that sexual recombination is occurring.

Sexual reproduction can increase host range and pathogenicity (Nelson et al., 1965, Rodriguez & Ullstrup, 1962). Fan et al. (2007) found that the virulence of progeny isolates differ from that of parental isolates. It is therefore possible that the mating of *E. turcicum* isolates of different races could result in the production of new races with varying levels of pathogenicity. Sexual recombination is expected to be a major source of variation in *E. turcicum*.

1.8. Conclusion

NCLB was of minor importance in Uganda until it resulted in significant yield losses in 1988 (Adipala et al., 1993). Sporadic outbreaks of NCLB have occurred in Kenya, and NCLB is currently estimated to be the most widespread foliar disease in South Africa (Kloppers & Tweer, 2009, Martin, 2011). The increased disease incidence may be attributed to a change in the pathogen population (Martin et al., 2011). Epidemics may arise if new races of the pathogen evolve, or if previously scarce races that are able to overcome current monogenic resistance control measures dramatically increase in frequency. The interaction between host and pathogen is a dynamic system in which the pathogen and host are continually adapting between host immunity and pathogen strategies to evade host detection. Since quantitative resistance is not

dependent on light and temperature, it is more durable, and a combination of quantitative and qualitative resistances is combined to obtain the best results (Carson & Vandyke, 1994).

The availability of the genome sequence (Ohm et al., 2012) of *E. turcicum* enables rapid mining for genes of interest (e.g. putative effector genes) as well as potential markers (e.g. repeat sequences). SSRs have a high mutation rate and the pattern of variation at these loci are therefore indicative of a species' recent evolutionary history (Ellegren, 2004). Results from population studies can also reveal the level of genetic diversity within a group of individuals. The amount of genetic diversity maintained can be an indicator of how fast the pathogen is adapting, and thus how long it will take the pathogen to overcome fungicides and resistant hosts (McDonald & McDermott, 1993). We propose that SSRs will be a valuable tool to assess the level of diversity within South African populations of *E. turcicum*. Using SSRs to study population structure of *E. turcicum* will be novel and highly informative. SSRs are highly reproducible and can be useful to study samples collected from worldwide populations to gain a global understanding of *E. turcicum* population structure.

There is a lack of knowledge regarding the reproductive biology of *E. turcicum* and more studies are needed. Despite the absence of an observed sexual stage in the field, the possibility that sexual reproduction is taking place cannot be excluded. Presumably, sexual reproduction will occur at the end of the season on senescing plants. It is possible that sexual recombination is absent in the field due to non-optimal environmental conditions (Fan et al., 2007), which would imply that any variation is attributable to mutation or migration. It has been noted in populations from Europe, however, that the high genotypic diversity cannot be explained by mutation alone (Borchardt et al., 1998a). A molecular tool, such as PCR primers specific to each mating type idiomorph, allows rapid screening of large sample sets to determine mating types of isolates in a field. Equal ratios of MAT1-1 and MAT1-2 could be indicative of sexual reproducing populations, while non-equal ratios are indicative of clonal reproduction (Bihon et al., 2014, Duong et al., 2013, Groenewald et al., 2008).

The environmental conditions in South Africa are conducive to disease development and sexual reproduction. The hypothesis for this study was therefore that isolates from different regions within South Africa will be non-clonal due to sexual recombination. The research aim of this project was to determine the genetic variability of *E. turcicum* isolates throughout South African

maize production areas. To achieve this aim, the study focussed on two research questions; 1) what is the level of genetic differentiation between *E. turcicum* isolates from different regions?, and 2) is there evidence for sexual recombination taking place in the field? We propose to use SSR markers with mating type-diagnostic PCR primers to answer these questions. SSRs were used to elucidate the population structure of South African *E. turcicum* isolates. The presence of sexual reproduction in a field will be determined from mating type frequencies as well as the level of SSR diversity. A clear understating of the population structure, in addition to the knowledge on the biology of this pathogen, will aid in designing effective control strategies for NCLB.

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