EVALUATION OF MAIZE BREEDING POPULATIONS FOR RESISTANCE TO *FUSARIUM VERTICILLIOIDES* AND FUMONISIN CONTAMINATION

by

NAKISANI ELELWANI INNOCENTIA NETSHIFHEFHE

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Supervisor: Dr L.J. Rose

Co-supervisor: Prof. A. Viljoen

Prof. B.C. Flett

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SUMMARY

Maize is an important crop cultivated all around the world. It is the main source of carbohydrates for over 200 million people in Sub-Saharan Africa. Daily consumption rates can reach up to 500 g per person in certain regions of Africa. Maize production is threatened by several abiotic and biotic factors which include fungi that cause maize ear rots. *Fusarium verticillioides*, which causes Fusarium ear rot (FER), and *Aspergillus flavus*, which causes Aspergillus ear rot (AER), are the most common fungal species associated with maize produced in southern and eastern Africa, respectively. Moreover, *F. verticillioides* produces fumonisins and *A. flavus* produces aflatoxins which are toxic secondary metabolites associated with harmful effects on humans and animals. Although several management strategies can be used to reduce mycotoxin accumulation in grain, host resistance has been documented to be the most efficient, cost-effective and environmentally sound strategy to minimize contamination.

This study focused on evaluating F_1 hybrids for improved resistance to FER and fumonisin contamination under South African and Kenyan conditions. A number of hybrids exhibited improved resistance to FER, fungal and fumonisin contamination. In South Africa, hybrids R119W x CKL05015, CML495 x CKL05015 and CKL05015 x R119W were the most resistant to FER severity, *F. verticillioides* colonisation and fumonisin contamination, respectively. Under Kenyan conditions, fungal colonisation was lowest in hybrids CKL05015 x CML495 and MIRTC5 x CML495, while fumonisin concentrations were lowest in hybrids CML444 x MIRTC5 and R119W x CKL05015. Parental inbred line performance was indicative of F_1 hybrids performance. CIMMYT inbred lines CKL05015 and CML495, previously characterised as resistant to AER, exhibited significant resistance to *F. verticillioides* and its fumonisins across both countries. These lines were also found to be good general combiners for resistance to fumonisin contamination. Furthermore, F_2 populations were also evaluated and the resistant F_2 populations identified in this study can be used to produce recombinant inbred lines to utilise in genetic fingerprinting and mapping of resistant genes.

Significant genotype x environment interactions influenced FER severity, fungal and fumonisin accumulation in maize grain. General combining ability (GCA) and specific combining ability (SCA) were significant for all three infection parameters evaluated while additive gene effects were predominant in the inheritance of resistance in this set of hybrids. This study provided fundamental information on maize lines that could be used by breeders to develop resistant cultivars. Based on the findings of this study, breeding for resistance to *F. verticillioides* and its fumonisins should be successful and expedited if the parental material involved is resistant.

OPSOMMING

Mielies is 'n belangrike gewas wat regoor die wêreld verbou word. Dit is die belangrikste bron van koolhidrate vir meer as 200 miljoen mense in Sub-Sahara Afrika. 'n Daaglikse inname van tot 500 g per persoon is al in sekere streke van Afrika waargeneem. Mielieproduksie word bedreig deur abiotiese en biotiese faktore soos swamme, wat kopvrot van mielies veroorsaak. *Fusarium verticillioides*, wat Fusarium kopvrot (FKV) veroorsaak, en *Aspergillus flavus*, wat Aspergillus kopvrot (AKV) veroorsaak, is die mees algemene swamspesies wat met mielies geassosieer word wat onderskeidelik in suider- en oos-Afrika, geproduseer word. Verder, produseer *F. verticillioides* fumonisiens en *A. flavus* aflatoksiene wat giftig sekondêre metaboliete is, wat verband hou met skadelike effekte op mens en vee. Hoewel verskeie strategieë gebruik kan word om mikotoksien opeenhoping in graan te verminder, word gasheerweerstand beskou as die mees doeltreffende, koste-effektiewe en omgewingsvriendelike strategie om besoedeling te verminder.

Hierdie studie het gefokus op die evaluering van F_1 basters vir verbeterde weerstand teen FKV en fumonisien besoedeling in Suid-Afrika en Kenia. 'n Aantal basters het verbeterde weerstand teen FKV, swam- en fumonisien besmetting getoon. In Suid-Afrika het basters R119W x CKL05015, CML495 x CKL05015 en CKL05015 x R119W die meeste weerstand teen FKV, *F. verticillioides* kolonisasie en fumonisien besmetting, onderskeidelik, getoon. In Kenia was swamkolonisasie die laagste in basters CKL05015 x CML495 en MIRTC5 x CML495, terwyl fumonisien konsentrasies die laagste in basters CML444 x MIRTC5 en R119W x CKL05015 was. Ouerlike inteellyn prestasie was 'n aanduiding van F_1 baster prestasie. Keniaanse ingeteelde lyne CKL05015 en CML495, voorheen gekenmerk as weerstandig teen AER, het beduidende weerstand teenoor *F. Verticillioides,* en sy fumonisien, in albei lande getoon. Hierdie lyne is ook gevind om as goeie algemene kombineerders vir weerstand teen fumonisien besmetting te dien. Verder is F_2 bevolkings ook geëvalueer en die weerstandige F_2 bevolkings wat in hierdie studie gevind was, kan gebruik word om rekombinante ingeteelde lyne te produseer vir die doel van genetiese vingerafdrukke en kartering van weerstandige gene.

Beduidende genotipe x omgewingsinteraksies beïnvloed FKV, swam- en fumonisien opeenhoping in mielie graan. Algemene kombinasie vermoë en spesifieke kombinasie vermoë was betekenisvol vir al drie infeksie parameters geëvalueer; terwyl toevoeging geen effekte oorheersend in die erfenis van weerstand in hierdie stel basters was. Hierdie studie verskaf fundamentele inligting oor mielie-lyne, wat deur telers gebruik kan word om weerstandbiedende kultivars te ontwikkel. Op grond van die bevindinge van hierdie studie, kan die teling vir weerstand teen *F. verticillioides* en sy fumonisien suksesvol en spoedig wees, as die ouerlike materiaal betrokke, bestand is.

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CONTENTS

CHAPTER 1

Major mycotoxigenic fungi associated with maize in eastern and southern Africa

INTRODUCTION

Maize (*Zea mays* L.) is one of the major grains cultivated all around the world. It serves as the primary source of calories in eastern and southern Africa with a daily consumption of up to 500 g per person (Shephard *et al.*, 2007; Shephard, 2008). South Africa has an annual production of approximately 9.2 million tons of maize grain, making it the largest producer in Sub-Saharan Africa (SSA), followed by Ethiopia and Tanzania with a production of about 7.2 and 6.7 million tons, respectively (Abdolreza, 2006; FAO, 2014). Other countries such as Kenya, Malawi and Zambia produced over 3 million tons in 2014 (FAO, 2014). The continued successful production of maize in eastern and southern Africa is threatened by various abiotic and biotic factors which lead to significant yield losses.

African soils have been reported to have low fertility due to inadequate fertiliser usage (Buresh *et al.*, 1997; Drechsel and Gyiele, 1999). In addition to this, the El Niño phenomenon has resulted in long-lasting drought conditions and high temperatures causing a decline in production (Abbassian, 2007). Moreover, biotic stresses such as parasitic weeds, pre- and postharvest insect infestations and plant pathogens also contribute to yield losses. One of the most pressing issues, which not only threatens maize production but also threatens human and livestock health, is the infection of maize grain by mycotoxigenic fungi. Infection of grain by these fungi may lead to yield reduction, poor grain quality or, most importantly, result in mycotoxin contamination of grain. The most important fungal species associated with mycotoxin contamination in eastern and southern Africa are *Fusarium verticillioides* (Sacc.) Nirenberg and *Aspergillus flavus* Link ex Fries which cause Fusarium ear rot (FER) and Aspergillus ear rot (AER), respectively. These fungi produce mycotoxins which have been associated with various health implications in humans such as growth impairment, liver and oesophageal cancer, birth defects and several other diseases in livestock including immune suppression. Some African countries have had fatal incidents resulting from mycotoxicosis (Muture and Ogana, 2005; Mwanda *et al.,* 2005; Probst *et al.,* 2007).

Due to the detrimental effects of mycotoxin contamination in both humans and animals, management strategies to reduce losses and mycotoxins accumulation in maize have to be implemented. Noticeably infected kernels can be physically removed during or after harvest, however, kernels may appear asymptomatic yet contain mycotoxins

(Munkvold, 2003b; Henry *et al.*, 2009). This suggests that the removal of kernels does not result in the complete removal of mycotoxins in grain (Clements *et al.*, 2004). Efforts using chemical methods and heat treatment to reduce the mycotoxins, fumonisins, resulted in poor success (Headrick and Pataky, 1991; Munkvold, 2003a) and no standard cleaning methods to reduce mycotoxins in kernels are available (Munkvold and Desjardins, 1997). The development of resistant maize cultivars is the most environmentally sound, cost-effective and efficient way of controlling maize ear rot and mycotoxin contamination (Harrison *et al.*, 1990; Schjøth *et al.*, 2008). Numerous breeding strategies such as backcrossing, pedigree method, recombinant inbred line development and recurrent selection have been used to enhance host resistance to *F. verticillioides* and *A. flavus*. This review will focus on the predominant mycotoxigenic fungi causing maize ear rot in eastern and southern Africa as well as the efforts toward enhanced resistance in maize.

IMPORTANCE OF MAIZE IN SOUTHERN AND EASTERN AFRICA

The world maize production reached 851 million tons in 2010, making it the world's most cultivated crop (FAO, 2014). United States of America (USA) is the biggest producer of maize contributing 40% of the world production, followed by China which produces 20% (Abbassian, 2007; Awika *et al.*, 2011). Only 15% of the total world production is used for human consumption with the remaining being used as raw material in industrial processes and as animal feed (Awika *et al.*, 2011). Africa alone consumes 30% of the global maize production used for human consumption, with maize constituting the main staple food in SSA (Awika *et al.*, 2011). The highest consumption rate of maize in Africa is recorded for eastern and southern Africa (Macauley, 2015). Approximately 208 million people in SSA rely on maize as the basis of economic welfare and food security (Macauley, 2015). Most countries in eastern and southern Africa have an annual consumption rate of about 90 - 180 kg per person, this includes countries like Malawi, Lesotho, Kenya, Zambia and some parts of South Africa (Shephard *et al.*, 2007; Awika *et al.*, 2011; Ecker and Qaim, 2011).

There are 22 countries in the world where maize contributes the highest proportion of calorie intake of the general diet, of which sixteen are in Africa (Nuss and Tanumihardjo, 2011). Although Africa contains most of the countries that rely largely on maize compared to the rest of the world, the regional average yields are considerably lower than the global average yield of approximately 5 t/ha. The regional average yields are 1.7 t/ha, 1.5 t/ha, and 1.1 t/ha for west, east and southern Africa, respectively (Smale *et al.*, 2011). Due to the low yield of their main staple food, most African countries rely on imports from major maize producing countries such as USA, China and Argentina. Although, some countries in Africa such as South Africa frequently have significant maize surpluses for export to their neighbouring countries (Abbassian, 2007; Department of Agriculture, Forestry and Fisheries,

2014). The International Food Policy Research Institute (2000) predicted an increase in annual maize demand in Africa to reach 52 million tons by 2020. Unfortunately, the current increase in yield only averages about 1% of the required increase (Abbassian, 2007).

MAIZE PRODUCTION CONSTRAINS IN SOUTHERN AND EASTERN AFRICA

There are several factors affecting maize production in SSA, one of the major causes of which is the occurrence of El Niño (Rosenzweig *et al*., 2001; Abbassian, 2007). This is a weather phenomenon related to a considerable abnormal warming of the Pacific Ocean surface temperatures which has a global effect on weather patterns. Maize cultivated in the southern hemisphere, especially southern Africa, is largely affected by this, in the form of long-lasting dry conditions and high temperatures (Abbassian, 2007). This was evident by the sharp maize production decline in South Africa by as much as 40 to 60% during the 1980s and 1990s El Niño events (Abbassian, 2007). Southern Africa is currently experiencing severe drought resulting from one of the biggest El Niño events in the past 50 years (FAO, 2016). A loss of over 9.3 million tons was reported for the 2015/16 maize growing season in Southern Africa due to the recent El Niño event (OCHA, 2016). Another abiotic challenge to maize production in SSA is the depletion of soil fertility. Over the years, small-scale farmers used up large quantities of nutrients from the soil without the use of adequate amounts of fertiliser or manure to restore soil health (Buresh *et al.*, 1997). The average annual reduction rate per hectare of cultivated land over the last 30 years for nitrogen, phosphorus and potassium is 22 kg, 2.5 kg and 15 kg, respectively (Buresh *et al.*, 1997). The annual loss is equivalent to four billion U.S dollars in fertiliser (Buresh *et al.*, 1997; Drechsel and Gyiele, 1999).

Biotic stresses such as insect pests also pose a problem to maize production. In susceptible germplasm, a production loss of up to 15% can be caused by several species of stalk borers (such as *Busseola fusca* Fuller*).* Storage pests, such as the maize weevil (*Sitophilus zeamais* Motschulsky) and larger grain borer (LGB) (*Prostephanus trancutus* Horn) cause more extensive losses estimated at between 20 - 30% (Syngenta foundation, 2016). Reduced maize yields in Africa are also due to parasitic plants, the most important of which is Striga (*Striga hermonthica* (Delile) Benth). This obligate root parasite is dependent on the maize plant for all its water and nutrients. *Striga hermonthica* can result in yield losses as high as 50% (Parker, 1991). Plant pathogens have also presented an unremitting challenge to the production of maize in SSA. These pathogens range from viruses to fungi and bacteria. Examples include maize lethal necrosis (MLN), turcicum leaf blight (TLB), gray leaf spot (GLS), maize streak virus (MSV), southern leaf rust and maize ear rots (Macauley, 2015). Food security in eastern Africa is currently being threatened by a viral disease known as Maize Lethal Necrosis (MLN). This disease has been reported to be present in

Democratic Republic of Congo, Uganda, Rwanda, Tanzania, Ethiopia and Kenya. Since its emergence in 2011, Kenya has annual losses estimated at approximately 0.3 million tons, which is 23% of their annual production (Abbassian, 2007). Unless urgent and rigorous actions are taken, Kenya and its neighbouring countries are on the brink of severe food insecurity especially because more than 95% of their commercial maize varieties are susceptible to MLN (Prasanna, 2015; Mahuku *et al.*, 2015).

MAJOR MYCOTOXIGENIC FUNGI IN SOUTHERN AND EASTERN AFRICA

One of the major threats to food security in eastern and southern Africa is the infection of maize ears by various mycotoxigenic fungal species. There are several species associated with infection and contamination of maize grain in SSA. The prevalence and distribution of these species is highly dependent on environmental conditions (Munkvold, 2003b). *Fusarium verticillioides* is widely distributed across eastern and southern Africa while *A. flavus* is predominantly associated with maize produced in eastern Africa (Doko *et al.*, 1996; Gamanya and Sibanda, 2001; Alakonya *et al.*, 2009; Mukanga, 2009; Mukanga *et al.*, 2010; Boutigny *et al.*, 2012). Maize ear infection from these fungal species results in two different diseases namely Fusarium ear rot (FER) and Aspergillus ear rot (GER). Infection of maize grain by these mycotoxigenic fungi has been associated with yield reduction, poor grain quality, and most importantly, mycotoxin contamination of kernels (Jones *et al.*, 1980; Diener and Davis, 1987; Munkvold, 2003b; Desjardins, 2006).

Epidemiology of *Fusarium verticillioides*

The infection of maize by *F. verticillioides* is broadly affected by several factors including insect infestation, environmental conditions such as climate, temperature and relative humidity, physical damage, and pre- and postharvest practises (Fandohan *et al.*, 2003; Munkvold, 2003b). *Fusarium verticillioides* is a heterothallic fungus capable of producing perithecia and conidia, although the former are not commonly observed (Munkvold, 2003b). As opposed to perithecia, conidia are produced in abundance and these are made up of microconidia and macroconidia. These spores are the main form of inoculum for FER and asymptomatic infections on kernels (Munkvold, 2003b). Munkvold (2003b) suggested that sexual reproduction of this fungal species guarantees the increase of genetic variability rather than having an influence on the epidemiology of the disease. *Fusarium verticillioides* can infect maize during all developmental stages and may infect all parts of the plant through various pathways (Munkvold *et al.*, 1997; Bottalico, 1998; Munkvold, 2003b). Systemic kernel infection can occur by seed transmission, root, stalk or leaf infection (Munkvold and Desjardins, 1997). The most common infection pathway is through silk infection from wind-blown or water-splashed spores and insect-vectored spores (Munkvold and Desjardins,

1997). Injuries on kernels also act as a pathway for entry of the fungus (Flett and Janse van Rensburg, 1992; Munkvold, 2003b). *Fusarium verticillioides* overwinters on maize crop residue in soil and may also colonise debris of other non-host crops and weed species (Nyvall and Kommedahl, 1970; Parry *et al.*, 1995). In addition to conidia and perithecia, this endophytic pathogen produces several survival structures on maize debris in the soil or in the air around maize-producing areas including mycelia and thickened hyphae (Nyvall and Kommedahl, 1968; Gillette, 1999; Munkvold, 2003b).

The growth and germination of *F. verticillioides* is favoured by warm and dry climates with an optimum temperature of about 30°C and a minimum water activity of 0.88 a_w (Miller, 1994; Bottalico, 1998; Marín *et al.*, 1999; Reid *et al.*, 1999; Munkvold, 2003b). Furthermore, Marasas *et al.* (2000) stated that the development of FER is favoured by warm, dry weather during grain filling while drought is also associated with an increased incidence of FER and mycotoxin accumulation in grain (Miller, 2001). Another factor which influences disease development is the genetic resistance of maize hybrids, their physical traits (such as husk coverage) and the genetic variability of the pathogen population also play a significant role in disease development (Warfield and Davis, 1996; Clements *et al.*, 2003). Several scientists established that high potassium levels in the soil counteract the effect of nitrogen which in turn creates favourable conditions for *Fusarium* although this was shown for stalk rot (Otto and Everett, 1956; Younts and Musgrave, 1958). Ear rot and stalk rot, however, are strongly correlated (Mesterházy, 1983; Mesterházy *et al.*, 2000). This suggests that soil health could play a role in *F. verticillioides* development, however this area of research is not well studied (Milani, 2013).

Globally, several insect species play a major role in the development of FER. These species include corn borers (*Ostrinia nubilalis* Hübner), thrips (*Frankliniella occidentalis* Say), sap beetles (*Carpophilus* spp.), corn rootworm beetles (*Diabrotica* spp.), maize weevils (*S. zeamais*), and other grain borers such as *Prostephanus truncatus* Horn and *Rhyzopertha dominica* Fabricius (Cardwell *et al.*, 2000). Information on insect species associated with FER in Africa is limited, although the stalk borers, *B. fusca* and *Chilo partellus* Swinhoe have been the main insect pests associated with FER in South African maize fields (Flett and Janse van Rensburg, 1992; Kfir, 1997). These insects damage the plant while feeding, establishing entry wounds for the fungus. Furthermore, they act as vectors carrying spores from the plant surfaces such as leaves and transport them to maize silks where they germinate down the silk and cause kernel infection thus playing a major role in the dissemination of fungal spores (Gilbertson *et al.*, 1986; Dowd, 1998; Sobek and Munkvold, 1999). Birds also play a role by creating injuries as they feed on the grain (Munkvold *et al.*, 1997). Ako *et al.* (2003) indicated that maize ears infected with *F. verticillioides* had higher insect damage than maize not exhibiting FER symptoms. Other methods of conidia dispersal

include water and wind, although microconidia are dispersed more easily by wind when compared to macroconidia (Munkvold, 2003b). A study done by Ooka and Kommedahl (1977) revealed that spores produced by *F. verticillioides* can travel distances of up to 400 km.

Once infection is established, typical symptoms associated with *F. verticillioides* infection include white-pinkish fluffy mycelia growing between kernels and white lines radiating out from the point of silk attachment known as starburst symptom (White, 1999; Duncan and Howard, 2010). These symptoms may be scattered randomly or at the tip of the ear (Munkvold, 2003b; Bush *et al.*, 2004). However, *F. verticillioides* can also cause asymptomatic infection whereby kernels show no signs of infection (Munkvold, 2003b).

Fumonisins

Fusarium verticillioides produces fumonisins that consist of approximately 28 analogues, these are grouped into A, B, C and P series. Fumonisins A, C and P are synthesized on special media in laboratory experiments (Desjardins, 2006). On the other hand, fumonisin B (FB) is the most abundant in naturally infected maize. Furthermore, FB_1, FB_2, and FB_3 are the most common fumonisins associated with mycotoxicosis of humans and animals, with FB_1 having the most pernicious effect on humans (Rheeder *et al.*, 2002; Marasas *et al.*, 2004; Desjardins, 2006). Fumonisins have been associated with oesophageal cancer and cancer-inducing properties in humans (Marasas *et al.*, 2004; Missmer *et al.*, 2006; Rheeder *et al.*, 2009) and hinder the uptake of folic acid through the folate receptor. This is thought to be the mechanism by which birth defects in humans are caused (Stevens and Tang, 1997).

In livestock, fumonisin contamination is linked with immune suppression in chickens, porcine pulmonary oedema syndrome in pigs, hepatitis and equine leukoencephalomalacia (a lethal brain disease of horses, donkeys and rabbits) and nephrosis in sheep (Kriek *et al.*, 1981; Harrison *et al.*, 1990; Schjøth *et al.*, 2008). Fumonisin contamination of maize meal has also been linked to growth retardation of infants in Tanzania (Kimanya *et al.*, 2010). The South African Medical Research Council (MRC) and other organisations such as the International Agency for Research on Cancer (IARC) and the U.S Department of Agriculture (USDA) have evaluated fumonisins' carcinogenic potential. In 2002 the IARC classified fumonisins into group 2B, which means they are a possible carcinogen to humans (IARC, 2002). Fumonisin B_1 disrupts the metabolism of sphingolipids in cells and has the ability to change poly-unsaturated fatty acid pools which leads to degradation and death of cells (Wang *et al.*, 1991; Gelderblom *et al.*, 2001).

Currently very limited information is available in eastern and southern Africa (except South Africa) on *F. verticillioides* and fumonisin contamination of maize. This is a big concern as millions of people could unknowingly be consuming compromised grain on a

daily basis (Fandohan *et al.*, 2003). Bankole *et al.* (2006) also stated that the levels of fumonisins discovered in West African maize appear to be higher than those of east and southern Africa due to limited information available. Doko *et al.* (1996) found a 92.5% incidence of naturally occurring fumonisin contamination in 40 randomly selected samples from South Africa, Kenya, Zambia, Mozambique, Botswana, Malawi, Tanzania, Uganda and Zimbabwe. The fumonisin (FB_1 + FB_2 + FB_3) concentrations in this study ranged from 20 to 2735 ng g^{-1}, with Zimbabwe having the highest concentration. Studies done by Rheeder and colleagues (1992) in the former Transkei in South Africa have shown a relationship between fumonisin contamination in rural subsistence farming communities and a high rate of oesophageal cancer. Furthermore, a survey done by Chelule *et al.* (2001) revealed that rural households in the KwaZulu-Natal province, South Africa were at a much higher risk of consuming maize contaminated with FB_1. Maize samples collected in rural communities had 32% contamination as opposed to 6.1% in urban maize, from 50 and 49 samples, respectively (Chelule *et al.* 2001). Phoku *et al.* (2012) found that *F. verticillioides* was the most common *Fusarium* spp. associated with maize and porridge prepared from maize in a rural part of the Limpopo province in South Africa, whereas FB_1 was the most prevalent mycotoxin.

In rural communities of Tanzania, fumonisin B_1 was detected in 96% of urine samples from 148 children aged from 12 to 22 months. The toxin levels ranged from 82.8 to 327.2 ppb (Shirima *et al.*, 2013). Other studies were done in northern Tanzania where over 44% of 131 breast milk samples were contaminated with FB_1 in quantities higher than those recommended by the EU (200 ppb in infant food) (Magoha *et al.*, 2014). Several studies done in Zambia indicated that *F. verticillioides* is the most important species associated with symptomatic and asymptomatic infection and fumonisin contamination in over 114 farms across 11 districts (Doko *et al.*, 1996; Mukanga *et al.*, 2010). Surveys done in Botswana and Ethiopia revealed that *F. verticillioides* is the most dominant species causing ear rot with fumonisin concentrations reaching 1.2 µg g^{-1} and 2.4 µg g^{-1}, respectively (Siame *et al.*, 1998; Ayalew, 2010). Maize samples from Malawi had low fumonisins concentrations with a mean of 0.07 µg g^{-1} (Doko *et al.*, 1996). Atukwase *et al.* (2009) detected fumonisins ranging from 0.27 to 10.0 µg g^{-1} in maize samples from traditional storage facilities in Uganda. Alokanya *et al.* (2009) sampled maize from 24 farms across western Kenya and found FB_1 levels as high as 1348 µg kg^{-1} and approximately 5000 µg kg^{-1} in symptomless and symptomatic grain, respectively.

Epidemiology of *Aspergillus flavus*

Aspergillus flavus is mainly a saprophytic fungus that colonises and survives in various organic materials such as hay, plant rubble, cotton, compost heaps, dead insects, animal

carcasses and stored grain but it can be pathogenic to maize (Klich *et al.*, 1994; Klich, 1998; Jamie-Garcia and Cotty, 2004; Abbas *et al.*, 2008). During unfavourable conditions the hyphae thickens to form survival structures known as sclerotia which can survive in soil for several years during adverse environmental conditions (Yu, 2012). *Aspergillus flavus* overwinters as sclerotia, hyphae or asexual spores, known as conidia, in soil (Wicklow *et al.*, 1993; Payne, 1998; Horn, 2007; Abbas *et al.*, 2008). These structures serve as sources of inoculum for infection by *A. flavus*. Initial infection occurs at the beginning of the growing season when sclerotia or hyphae are exposed to the soil surface and produce conidia (Payne, 1998; Horn, 2007). Conidia are produced during the growing season and serves as secondary inoculum infecting healthy plants. The most common infection pathway is through feeding wounds created by various insects (Smart *et al.*, 1990). However, infection of the ear also occurs when *A. flavus* spores are wind-blown onto the maize silks and the fungus germinates and grows down the silk channel to the kernels (Cardwell *et al.*, 2000). According to Payne (1998), green silks are relatively resistant to infection whereas senescent silks can be colonised by *A. flavus*.

Aspergillus flavus commonly occurs in tropical and subtropical warm temperate regions (Klich *et al.*, 1994, Abbas *et al.*, 2009). It is able to grow in temperatures ranging from 12 to 48°C, but thrives in temperatures ranging from 28 to 37°C (Yu, 2012). Other factors that affect AER development include high soil and/or air temperature, drought conditions, nitrogen deficiency, high planting density and conducive environmental conditions for conidial dissemination (Diener and Davis, 1987). Furthermore, the prevalence of insect vectors, kernel injury, oxygen and carbon dioxide levels in storage, amount of initial inoculum and the presence of toxigenic strains also play a role in disease development (Horn, 2007). Although AER occurs in the field, *A. flavus* is commonly associated with post-harvest spoilage of grain in storage (Diener and Davis, 1987). This is due to the fact that it only requires about 16% moisture content in cereals to cause infection (Christensen and Meronuck, 1986). Large numbers of microconidia, produced in the field stubble, are easily wind dispersed in hot and humid weather. Another common method of dissemination of inoculum occurs through insect vectors. Insects associated with AER are *Heliothis zea* Boddie, *O. Nubilalis* and *S. zeamais* (McMillian, 1987; McMillian *et al.,* 1990). *Aspergillus flavus* is also able to infect insect vectors thus its spores are transported externally as well as internally (Fennell *et al.,* 1975). However, Lussenhop and Wicklow (1990) stated that the *Nitidulidae* (e.g. *Carpophilus lugubris* Murrey and *C. freemani* Dobson) are able to consume and transport the fungus without any negative effects.

Yellow-brown silks seem to be more susceptible to infection when compared to younger green silks (Diener and Davis, 1987). Maize ears infected by *A. flavus* exhibit an olive-green mould which normally occurs at the tip of the ear during hot and humid seasons

(Fennell *et al.*, 1975). Fungal growth usually occurs between damaged kernels. Maize ears with little husk coverage and damage (from insects, hail, high winds or early frost) exhibit more symptoms (Fennell *et al.*, 1975). Additionally, *A. flavus* can infect kernels and produce aflatoxins without causing any symptoms (Henry *et al.*, 2009).

Aflatoxins

Aflatoxins were first discovered in 1961 after an acute outbreak of Turkey "X" disease in England, which killed more than 100 000 turkeys and other livestock (Blount, 1961). Aflatoxins are the most harmful mycotoxins described in the international scientific literature. The most common aflatoxins are AFB_1, AFB_2, G_1 and G_2 which are mainly produced by *A. flavus*. These four types of aflatoxins belong to polyketides family of molecules which comprises of other structurally similar aflatoxins (Pitt *et al.*, 1993). The letters "B" and "G" represent the blue and green fluorescent colours that these mycotoxins display when placed under long wave ultraviolet light while the subscript numbers 1 and 2 indicate chromatographic mobility. Aflatoxin B_1 (AFB_1) is the most harmful and most carcinogenic toxin towards humans and livestock. It is also harmful to other animals such as primates, rodents, fish and birds (Hsieh, 1989; Eaton and Gallagher, 1994). Prolonged exposure to aflatoxins can lead to suppressed immune system, stunting in children, malnutrition, growth impairment, jaundice, proliferation of the bile duct, hepatomas, necrosis of the liver, hepatic lesions and in severe cases, death (Ngindu *et al.*, 1982; Gong *et al.*, 2002). Livestock feed that is contaminated with aflatoxins may lead to necrosis of the liver and haemorrhage in broiler chickens, cattle and pigs (Eraslan *et al.*, 2005; Osweiler, 2005). Overwhelming research resulted in aflatoxin B_1 and combinations of aflatoxins being classified as Group 1 carcinogens causing liver cancer in humans (IARC, 2002).

A. flavus is documented to reach maximum aflatoxin production at 25 to 27°C while growth in storage is favoured by >85% humidity and these conditions are prevalent in most African countries (Williams *et al.*, 2004; Abbas, 2005). According to the FAO, aflatoxins contaminate about 25% of the total global agricultural crops especially those cultivated in developing countries. Several aflatoxicosis outbreaks in Kenya, first occurrence in 1981, have rendered it the biggest hot spot for aflatoxin contamination in Africa (Ngindu *et al.*, 1982). Twelve deaths were reported in Meru North district of Kenya during 2001 due to consumption of aflatoxin contaminated maize from storage (Anonymous, 2001). In 2004, the largest occurrence of mycotoxicosis due to aflatoxins was recorded in Kenya where 125 recognised deaths were reported of 317 cases in the Eastern province of Kenya (Muture and Ogana, 2005; Mwanda *et al.,* 2005; Probst *et al.,* 2007). Most of these reports are based on incidents that occurred in subsistence farming holdings (Azziz-Baumgartner *et al.* 2005; Alakonya *et al.*, 2009; Daniel *et al.* 2011). It's uncertain whether such incidents are more

widespread in the rest of Kenya or not (Yard *et al.*, 2013). Aflatoxin contamination has also been reported in other countries. In Malawian maize, aflatoxins are predominant in hot and warm low-lying agro-ecological zones (Matumba *et al.*, 2013; Matumba *et al.*, 2015). Blood samples from umbilical cords were analysed at birth in Nigeria, Kenya and Ghana and about one third of the samples tested positive for aflatoxins which demonstrates prenatal exposure (Abbas, 2005). Furthermore, aflatoxins have also been detected in breast milk of lactating mothers in several African countries including Zimbabwe and Kenya (Abbas, 2005). Aflatoxins have not been a major issue in southern African maize. Maize sampled in Botswana had no detectable amount of aflatoxins and very small amounts were detected in sorghum (Siame *et al.*, 1998). South African Maize Board and South African Grain Laboratory (SAGL) have consistently found very little to no aflatoxins on maize samples analysed annually since 1986 (Abbas, 2005).

MANAGEMENT OF MAIZE EAR ROT AND MYCOTOXIN CONTAMINATION

Owing to the detrimental nature of mycotoxicosis, great effort has been placed into finding effective management strategies. These strategies are aimed at either obtaining resistance to the initial infection, limiting mycotoxin accumulation, or the detoxification of already contaminated maize. Cultural practices, chemical, physical, and biological control can be employed at different phases of production such as pre-harvest, postharvest, storage, transportation and processing to mitigate mycotoxin contamination (Wagacha and Muthomi, 2008).

Cultural methods

The amount inoculum present in the field, environmental conditions and the host's interaction with the pathogen all play an important role towards disease severity and mycotoxin contamination (Munkvold, 2003a). It is therefore important to put in place cultural practices that aim to reduce disease severity and mycotoxin contamination based on these aspects of disease development. Selecting a field to plant maize that does not have a history of maize ear rot infection and that has not previously been planted with maize can help ensure that there is no build-up of inoculum (CAC, 2003). Furthermore, minimum or no crop residue should be left in the soil after a planting season as the mycotoxigenic fungi may colonise senescing tissues forming infectious propagules for the following season (CAC, 2003; Munkvold, 2003b; Rankin and Grau, 2014). Krebs *et al*. (2000) and Blandino *et al*. (2010) advised that crop residue should be buried deep into the ground to reduce primary inoculum. Crop rotation with non-host crops can be used in combination with minimum tillage practices in fields previously planted with maize (Munkvold, 2003a; Rankin and Grau, 2014).

Ensuring optimal plant health can mitigate fungal infection and subsequent mycotoxin contamination. Farmers should ensure that the soil contains sufficient nitrogen and other essential nutrients (Blandino *et al.*, 2008). Soil moisture should also be adequate for optimal plant growth and planting maize in appropriate areas and planting dates can help reduce the disease incidence (Mukanga *et al.,* 2011). Since drought and high temperatures are associated with elevated levels of fumonisins and aflatoxins, it is essential to avoid these conditions by irrigating crops during critical conditions (Marín *et al*., 1998; Miller, 2001). Maize plants should also be planted at recommended row widths and plant densities to reduce water stress (Mukanga *et al.,* 2011). Maize ears should be harvested from the field as soon as possible because favourable conditions for ear rot and/or mycotoxin accumulation may occur if harvest is delayed thus leading to elevated mycotoxin levels (Chulze *et al.*, 1996, Bush *et al.*, 2004). The removal of injured and noticeably infected kernels after harvest may be practised in small farming systems, before the grain is stored in clean bins containing ventilation systems to allow for a cool environment and dry conditions (Afolabi *et al.*, 2006). However, the removal of infected kernels does not offer a full-proof control measure as *F. verticillioides*, *A. flavus* and their associated toxins can be present asymptomatically; and high concentrations of mycotoxins may be found in kernels exhibiting no visual symptoms (Munkvold *et al.*, 1997; Clements *et al.*, 2004; Afolabi *et al.*, 2007).

Certain measures can be implemented in commercial storage of maize grain to reduce fungal growth and further mycotoxin contamination. These include storing grain in low temperatures ranging from 1 to 4°C with a moisture content less than 15% and having good sanitary practices in the storage houses and milling facilities (CAC, 2003). In Zambia a statutory requirement demands that grain intended for storage should not have a moisture content greater than 13% whereas maize intended for human consumption should have less than 2% kernels exhibiting disease symptoms (FAO/WHO/UNEP, 1987). Both large and small scale farmers should ensure that grain is protected from pests, birds and damaging weather such as rain, hail and wind. This minimises further infection of grain during storage (Wagacha and Muthomi, 2008; Mukanga *et al.,* 2011). Due to limited financial resources and technical infrastructure, developing countries hardly ever have revenue to carry out conventional management practises for mycotoxin control (Small *et al.*, 2012).

Physical and chemical methods

Strategies such as heating, polishing, UV radiation, mechanical sorting and washing grain to control maize ear rots and mycotoxin contamination have been investigated (Fandohan *et al.*, 2005). Heating grain is believed to only hydrolyse the primary amino group of fumonisins which does not detoxify them as it leaves the sphingolipid backbone unbroken (Munkvold *et al.*, 1997). As a result, efforts using heat treatment to reduce fumonisin concentrations

resulted in poor success (Headrick and Pataky, 1991; Munkvold, 2003a) and no standard cleaning methods to reduce mycotoxins in kernels are present (Munkvold and Desjardins, 1997). In small scale farming systems, washing and crushing in combination with de-hulling maize grain, have been indicated to successfully prevent further accumulation of fumonisins and aflatoxins after harvest (Siwela *et al.*, 2005; Fandohan *et al.*, 2008). Physical strategies have not been very successful because they are onerous and time consuming and mycotoxins are not easily broken down by these processes as they are chemically stable (IARC, 1993; Howard *et al.*, 1998).

Several studies have been conducted to attempt detoxification of mycotoxins using chemical methods. Ammoniation and oxidising agents have been used in maize production worldwide. These agents are able to effectively detoxify aflatoxins in milled maize (Park *et al.*, 1992). On the contrary, their effectiveness in reducing FB_1 levels significantly is not consistent (Moustafa *et al.*, 2001). Pre-harvest herbicides can be applied to reduce plant stress caused by weed populations that may compete with the crop for nutrients, water and space (Jones *et al.*, 1980; Cole *et al.*, 1985). Furthermore, insecticides can also be used to reduce insect damage of kernels thus minimising entry points for the pathogens especially in the case of *F. verticillioides*, where insects play an important role in the dispersal of inoculum (Munkvold, 2003a). To date, there are no fungicides registered in South Africa for the control of the main ear rot pathogens (Janse van Rensburg, 2012). This may be due to the fact that uniform spray deposition on maize ears would be difficult to attain. Moreover, the application of fungicides may not be economically feasible for subsistence farmers (Wagacha and Muthomi, 2008).

Biological methods

Contrary to physical control, biological control strategies have shown potential in reduction of mycotoxin accumulation especially because it is environmentally sound and pathogen-specific (Meissle *et al.*, 2009). Control of fumonisin accumulation includes the use of endophytic strains of bacteria that could inhibit fungal growth which results in reduced fumonisin levels, however this has only been demonstrated in the lab (Bacon *et al.*, 2001). Bacteria strains of *Bacillus*, *Pseudomonas*, *Ralstonia* and *Burkholderia* have been observed to completely inhibit *A. flavus* and aflatoxin production (Palumbo *et al.,* 2006). *Streptomyces* was found to have antagonistic effects on *S. maydis* in maize seeds and seedlings (Bressen and Figuieredo, 2005). The use of bacteria in the field has proven to be a challenge (Dorner, 2004). Another method employed in biological control is the use of atoxigenic isolates on maize, which out-compete the toxigenic isolates within the same niche (Desjardins and Plattner, 2000). This method has shown promising results in the control of fumonisin and aflatoxin contamination in the field and is ideal as it is environmentally friendly (Meissle *et al.*,

2009). Commercial biopesticides for the control of aflatoxin contamination have been developed and includes products like Alfaguard™, used in the USA and Aflasafe™, used in some African countries (Abbas *et al.*, 2006).

LEGISLATION AGAINST MYCOTOXINS

Another way to ensure minimum levels of mycotoxins is the use of regulatory limits. International authorities have instituted maximum tolerable limits allowed for human and animal consumption as a result of the possible health risks associated with the exposure to mycotoxins. More than a hundred countries have legislation pertaining to the limits of mycotoxins in food and feed (Haumann, 1995; van Egmond *et al.*, 2007). However, in developing countries issues associated with food security have led people to choose food sufficiency over food safety (van Egmond *et al*., 2007). Consequently, most African countries only have regulations for aflatoxins especially in produce intended for export. Given that most eastern and southern African countries do not export maize, mycotoxins in this commodity are seldom regulated (Sibanda *et al.*, 1997). Countries such as Malawi, Kenya, Zimbabwe and South Africa have regulations for aflatoxins in certain foods (van Egmond, 2002). Furthermore, South Africa has amended regulations regarding the tolerances for fungus-produced toxins in foodstuffs. Raw maize grain, intended for further processing, may not contain more than 4000 pg /kg fumonisins (B1 + B2) while maize flour and maize meal, ready for human consumption may not contain more than 2000 pg /kg of fumonisins (B_1 + B_2) (Government Gazette of South Africa, 2016).

BREEDING FOR RESISTANCE

Mycotoxigenic fungi may also be controlled by the development of resistant host cultivars through breeding. This strategy is considered to be the most successful, cost-effective and environmentally sound way of controlling maize ear rots and mycotoxin contamination in maize (Schjøth *et al.*, 2008; Harrison *et al.*, 1990). Plant breeding is often defined as “the art and science of changing heredity of plants to improve their economic utility to man” (Chahal and Gosal, 2002). Through the selection of better crops from generation to generation, early farmers were applying plant breeding methods without even understanding the fundamental scientific basis (Chahal and Gosal, 2002). The use of plant breeding for resistance to ear rot in maize started in the 20th century (Afolabi *et al.*, 2007). The pursuit of enhancing natural host resistance through breeding has received renewed interest since the discovery of mycotoxins and natural resistance in maize (King and Scott, 1982; Gardner *et al*., 1987; Widstrom *et al.*, 1987; Campbell and White, 1995; Scott and Zummo, 1998).

Screening for resistance to maize ear rots and mycotoxin contamination can be laborious and time consuming. Additionally, it is important to have a well-characterised line(s) and rapid, economical methods of analysis for infection and toxin quantification (Brown *et al.*, 2013). Traditional screening methods involve planting different genotypes in different locations across different years and using various techniques to inoculate the plants to ensure disease pressure (Mesterházy *et al.*, 2012). Furthermore, the selected inoculation technique should allow discernible differences between genotypes. For evaluation of genotypes' resistance to *F. verticillioides*, Eller *et al.* (2008a) used silk channel inoculation, by injecting a spore suspension down the silk channel, as this has been proven to be the most important point of entry for this fungus. Once a suitable inoculation technique has been selected, plants are inoculated and genotypes are then selected based on several factors such as disease severity, fungal colonisation and mycotoxin concentrations.

Host resistance

Several studies employing breeding have unearthed a lot of information regarding resistance to ear rot in maize. Management of mycotoxin contamination in grain could be achieved through the inheritance of the ability to reduce fungal growth, resist entry of the fungus into the kernel and inhibit mycotoxin production (Gorman and Kang, 1991). There are numerous physical traits that have been found to play a role in resistance to infection and toxin contamination. These include kernel pericarp wax, husk tightness, kernel moisture, wounded kernel resistance and silk traits (Eller *et al.*, 2008b; Brown *et al.*, 2013). Some studies have suggested that kernel pericarp characteristics have a correlation with resistance to both *A. flavus* and *F. verticillioides.* Sampietro *et al.* (2009) found that removal of wax from the pericarp increased fumonisin accumulation and kernels that had high wax content had low fumonisin concentrations. On the other hand, Blandino and Reyneri (2007) reported that hybrids with high wax content had a higher mean of fumonisin concentration when compared to normal hybrids. Hoenisch and Davis (1994) established a correlation between resistance to *F. verticillioides* and pericarp thickness. Their hypothesis was that a thick kernel pericarp hinders insects from feeding on the grain and fungal growth. This could be the reason why popcorn is susceptible to ear rots (Mesterházy *et al.*, 2012). In contrast, Ivic *et al.* (2008) demonstrated no relationship between pericarp thickness and *F. verticillioides* resistance. Indicating that breeding for this trait would be futile.

A number of studies have demonstrated that certain natural phenolic compounds have antifungal or anti-mycotoxin activity. These are secondary metabolites produced by the plant with antioxidant properties (Atanasova-Penichon *et al*., 2016). Guiraud *et al*. (1995) and Picot *et al*. (2013) found ferulic acid to be one of the phenolic compounds aiding in plant resistance to *F. verticillioides* colonisation in maize. Atanasova-Penichon *et al*. (2014)

indicate in their study that *F. verticillioides* biotransforms chlorogenic acid and caffeic acid into products that inhibit *F. verticillioides* growth. Additionally, carvacrol, thymol, isoeugenol and eugenol have been found to have anti-fumonisin activity as a result of their molar refractivity, lipopholicity and saturated area properties (Dambolena *et al*., 2011). In agreement with these findings, Zabka and Pavela (2013) also found carvacrol and thymol to have antifungal activity against *F. verticillioides* and *A. flavus*. Gembeh *et al.* (2001) observed a significantly higher percentage of a phenol-like compound (alkylresorcinol) in the pericarp wax of a maize breeding population (GT-MAS:gk), which has been associated with resistance to *A. flavus* compared to susceptible genotypes. Alkylresorcinol has been shown to inhibit *A. flavus in vitro* (Gembeh *et al.*, 2001). Several proteins have also been associated with resistance. Harris *et al.* (2005) reported that haptoglobin-related protein (HRP) genes play a potential role in resistance to *F. verticillioides* whereas ribosome inactivating protein (RIP), zeamatin, and 14 kDa trypsin inhibitor protein (TI) have been associated with resistance to *A. flavus* resistance (Guo *et al.*, 1997; Chen *et al.*, 1998).

Phenotypic versus genotypic selection for resistance

The efficiency of selecting resistant plants based only on the expression of FER has been a contentious issue. Therefore, numerous studies have attempted to establish the relationship between these traits. Clements *et al.* (2003) identified a moderate, positive correlation between fumonisin concentration and FER yet concluded that breeding programmes should look at the two traits separately because the enhancement of resistance to ear rot may not result in adequate resistance to fumonisin accumulation. Since phenotypic correlation estimates take into account genetic and non-genetic effects, they cannot be utilised to predict the correlated response in ear rot severity, for selection on mycotoxin accumulation (Eller *et al.*, 2008a). For this reason, Robertson *et al.* (2006) carried out a study in two populations to estimate the genotypic correlation coefficients between fumonisin concentration and FER. High genotypic correlations between the two traits, r_g = 0.96 and 0.87 were established, although the phenotypic correlations were moderate, r_p = 0.40 and 0.64 across two populations. This suggests that genetic components of resistance are mainly similar for these traits, even though their phenotypic correlations are not high. Furthermore, high genotypic correlations imply that genotypes with high resistance to FER are likely to have high resistance to fumonisin accumulation (Robertson *et al.*, 2006). According to this discovery, maize variety selection based on phenotypic characteristics should be effective at improving resistance to fumonisin contamination and FER (Robertson *et al.*, 2006).

Though the genetic components of resistance may be similar for the two traits, environmental conditions which endorse ear rot do not seem to promote fumonisin

production to the same degree (Eller *et al*., 2008a). Indirect selection for fumonisin contamination by selecting against ear rot was thought to be less successful than direct selection against fumonisin contamination since fumonisin concentration had a higher heritability than FER resistance in both populations. Lower genotypic correlation (r_g = 0.56) between fumonisin concentrations and FER was determined by Eller and colleagues (2008a). Furthermore, studies screening inbred lines and cultivars for response to *F. verticillioides* infection and fumonisin accumulation have concluded that the quantification of the toxin is crucial to determine resistance (Afolabi *et al*., 2007; Small *et al*., 2012; Janse van Rensburg *et al*., 2015; Rose *et al*., 2016).

Some research indicates that aflatoxin contamination resistance is partly controlled by genetic effects (Zuber *et al.*, 1978; Darrah *et al.*, 1987; Widstrom *et al.*, 1987; Kang *et al.*, 1990; Gorman *et al.*, 1992). Moreover, additive effects were proven to be more significant that non-additive effects in determining resistance to aflatoxin contamination (Zuber *et al.,* 1978; Widstrom *et al.,* 1984; Darrah *et al.*, 1987; Gorman *et al.,* 1992). Although conversely, two studies reported the opposite could be true and resulted from different inoculation methods (Gardner *et al.,* 1987) or diverse environments (Widstrom *et al.,* 1984). Furthermore, Campbell and White (1995) found that resistance to *A. flavus* is controlled by that additive and dominance gene actions, with additive gene action being more important.

Quantitative trait loci (QTL) mapping

Several studies have mapped quantitative trait loci (QTLs) that are associated with resistance to FER and/or fumonisins (Pérez-Brito *et al*., 2001; Robertson-Hoyt *et al*., 2006; Ding *et al*., 2008; Li *et al*., 2011; Chen *et al*., 2012; Zila *et al*., 2013). Furthermore, Robertson-Hoyt *et al*. (2007) mapped QTLs to FER, AER and/or their associated mycotoxins and concluded that the genes that confer common resistances are the same or genetically linked. In the study one QTL affected FER and AER, one affected AER, FER and fumonisin accumulation and two QTLs both affected aflatoxin and fumonisin contamination (Robertson-Hoyt *et al*., 2007). Furthermore, a strong, significant genotypic correlation (r = 0.99) between FER and AER while Henry *et al.* (2009) also determined strong correlations between AER and FER severity (r = 0.72) and aflatoxin and fumonisin concentrations (r = 0.61), respectively. This implies that resistance to both ear rots and associated mycotoxin contamination may be mediated by the same genes. Furthermore, Brown *et al.* (2001) discovered that a maize breeding population (GT-MAS:gk), previously associated with *A. flavus* resistance, also inhibited *F. verticillioides* growth. They further suggested that resistance mechanisms are nonspecific for ear rot and mycotoxigenic fungi while Wisser *et al.* (2006) hypothesised that QTLs responsible for various disease resistance are grouped together in the maize genome.

Inheritance of resistance

The inheritance of resistance to FER and fumonisins has been studied on several maize breeding populations. Various aspects such as selection theory, breeding strategies, types of gene action, predicted genetic gain and mating designs have been used by breeders and researchers in population improvement. The use of molecular marker technologies to assist classical plant breeding could accelerate the development of crops resistant to fungal and mycotoxin contamination. Over the decades maize breeders and researchers have developed different methods and mating designs to use for breeding.

Pedigree breeding: Individual plants are selected from the F_2 population and the following generation and their offspring are evaluated. This method keeps record of each progeny's parent and each individual can be traced back to its parents. This method is suitable for the improvement of specific traits such as disease resistance. Brooks *et al.* (2005) used this method ($F_{2:3}$ family) to develop a genetic map for the analysis of QTLs associated with aflatoxin resistance. They identified two QTLs on chromosome 2 and 4, which mainly had additive effects, and each explained up to 18% of the variation in aflatoxin concentrations. Another study by Willcox *et al.* (2013) also used the pedigree method ($F_{2:3}$ family) to successfully identify 20 different QTLs responsible for aflatoxin resistance.

Recombinant inbred lines (RILs): The development of RILs can be used as a tool to produce genetic mapping populations. Recombinant inbred lines refers to a group of lines that incorporate an essentially permanent set of recombination events between chromosomes inherited from two or more parental lines with different traits. The selection of parental strains is dependent on the phenotype, compatibility and marker availability. Once the parental strains are identified, construction design is chosen which includes the population size, whether intercrossing will be incorporated and the number of inbreeding generations required to reach isogenecity. Then parental crosses and F_1 crosses are made resulting in F_2 population. After this, intercrossing may be implemented to increase mapping resolution by means of accumulation of additional meiotic crossover events. Then inbreeding is performed until the required generation of genetically stable recombinant lines is reached.

Recombinant inbred lines are very useful for genetic mapping of any trait that varies between the parental lines and the identification of QTLs of interest (Pollard, 2012). Another advantage of RILs is that the same mapping population can be used numerously to map a large diversity of traits, if properly stored and well maintained (Pollard, 2012). Zhang *et al.* (2016) mapped a QTL (*qaa8*) associated with aflatoxin resistance by utilising the genome-wide association analysis (GWAS) and traditional linkage mapping analysis using 228 RILs. They discovered 25 single nucleotide polymorphisms (SNPs) that significantly associated

with aflatoxin resistance and explained 6.7 to 26.8% of the phenotypic variation observed. Robertson-Hoyt *et al.* (2007) used 143 RILs to discover the relationships among *F. verticillioides* and *A. flavus* and their associated mycotoxins. They also used the same population to determine heritabilities of, and phenotypic and genotypic correlations between flowering time, ear rot and fumonisin concentration. Li *et al.* (2011) detected four QTLs for resistance to Fusarium ear rot in a population of 250 RILs on chromosome 3, 4, 5 and 6 which explained 2.5 - 10.2% of the phenotypic variation.

Backcrossing: Backcrossing is another important method of breeding for disease resistance especially because its ability to improve elite cultivars lacking a particular trait (Allard, 1960). This method is particularly beneficial when the desired trait is discovered in tropical material that is not adapted to the target environment. Backcrossing reduces the introduction of unwanted alleles from the tropical material while maintaining the desired allele(s) (Eller *et al.*, 2010). This method has been used more frequently in incorporating traits governed by a small number of loci with minimal impact from the environment as opposed to quantitatively inherited traits (Fehr, 1987). Consequently, Bliss (1981) introduced a modified backcross method suitable for quantitative inheritance namely the inbred backcross line method. This method is comprised of backcrossing and selfing at least two generation before selection of advanced lines can occur. Bliss's method has been used to successfully improve quantitative traits without loss of desirable recurrent parent phenotype in cucumber (*Cucumis sativus* L.) (Owens *et al.*, 1985) and bean (*Phaseolus vulgaris* L.) (Sullivan and Bliss, 1983a, 1983b; Schettini *et al.*, 1987), using an exotic donor parent. A similar method, called advanced backcross QTL analysis, was recommended by Tanksley and Nelson (1996) and requires that plants are advanced to the BC2 or BC3 generations with minimal selection prior to QTL mapping and employing marker assisted selection.

Sullivan and Bliss (1983b) and Tanksley and Nelson (1996) concluded that the backcross method can be beneficial in recuperating elite cultivars for quantitative traits in breeding programmes using unadapted donor cultivars because of the rapid reduction of the donor parent germplasm. Eller *et al.* (2010) also demonstrated this when using an unadapted maize inbred line with poor agronomic potential, GE440, as a donor for resistance to Fusarium ear rot and fumonisin contamination and backcrossed it with a commercial inbred, FR1064, for four generations ($BC_4F_{1:3}$). They successfully improved the commercial inbred line, FR1064, for fumonisin contamination and Fusarium ear rot resistance without significantly reducing its yield output. Robertson-Hoyt *et al.* (2007) used backcrossing to determine if breeding or selecting for resistance to *F. verticillioides* results in the loss of desirable agronomic traits. They found low correlations between agronomic traits and disease resistance, which led them to conclude that selection for increased disease

resistance should not excessively affect agronomic performance. Chen *et al*. (2012) identified three QTLs for resistance to *F. verticillioides* in a BC_2F_1 generation on chromosomes 4, 5 and 10.

Recurrent selection: The use of recurrent selection could assist to incorporate long-term improvements in quantitative resistance to FER and fumonisin accumulation in genotypes with desirable agronomic traits (Horne *et al.*, 2016). Recurrent selection is a process made up of recurrent cycles of selection for exceptional genotypes with a particular purpose in a heterozygous population and the successive recombination of the selected individuals (Lonnquist, 1952). The objective of this approach is to enhance the occurrence of favourable alleles for target traits, endorse recombination, and sustain genetic variability for continued genetic enhancement within a population from generation to generation (Hallauer *et al.*, 2010). This can be done using phenotypic evaluation and/or genotypic evaluation. Horne *et al.* (2016) evaluated three generations of lines through recurrent selection for FER and other agronomic traits. They achieved an 18% decrease in FER but the heritability decreased over the three cycles. The genotypic and phenotypic correlations were comparable to that of Robertson *et al.* (2006) and ranged from r = 0.74 to 0.87. This approach has mainly been used for yield rather than disease resistance with limited information available on the expected responses to selection for disease resistance within a population (Lambert and White, 1997; Abedon and Tracy, 1998).

Diallel cross: This mating scheme is used to investigate the genetic underpinnings of quantitative traits. Inbred lines are crossed to produce hybrids in all possible combinations and these hybrids together with the parental lines are evaluated for their response to a specific trait. This mating system is used in genetic studies for the estimation of factors such as the general and specific combining ability of genes, genetic variances, heritability, epistasis, dominance and identification of best combinations (Sneep and Hendriksen, 1979; Chahal and Gosal, 2002; Acquaah, 2007). A diallel cross can be generated using four methods. In the first method consists of making crosses form all the possible combinations (including reciprocals) and including parents in the trial. This is a complete diallel which is expressed as n^2. The second method only comprises of one set of the crosses (no reciprocals) and parents. This is the most commonly used method and is expressed by n (n + 1)/2. The third method is expressed by n (n - 1) contains two sets of crosses with no parents. The fourth involves making only one set of crosses and no parents. This diallel cross is expressed as n (n - 1)/2. The number of parents used in a diallel cross increases the number of possible crosses, it is therefore important to choose a manageable amount of parents (Hallauer *et al.*, 2010).

The most important benefit of the diallel mating designs is their capability to carry out a multifaceted approach with the purpose of testing and analysing the progenies and to acquire information that could not be found by other means (Christie and Shattuck, 1992). This method has been used extensively in maize breeding programmes (Hallauer *et al.*, 2010). Several researchers have used it to estimate heritability and combining ability of lines and hybrids with regards to Aspergillus ear rot and aflatoxin resistance (Gardner *et al.*, 1987; Zhang *et al.*, 1997; Naidoo *et al.*, 2002; Williams *et al.*, 2011) and Fusarium ear rot and fumonisin (Mukanga, 2009; Williams and Windham, 2009; Hefny *et al.*, 2012; Hung and Holland, 2012; Pádua *et al.*, 2016). The general combining ability (GCA) and specific combining ability (SCA) estimates were highly variable across these studies. Gardner *et al.* (1987) found SCA to be more important than GCA in determining the inheritance of resistance to aflatoxins while Zhang *et al.* (1997) found that they were both equally important for AER resistance. Naidoo *et al.* (2002) reported that SCA was not significant for AER and aflatoxin resistance rendering additive gene effects to be predominant for this trait in their study. Williams *et al.* (2011) also found GCA was more important in AER, *A. flavus* biomass and aflatoxin resistance, although dominant or epistatic gene actions were also observed. Mukanga (2009) observed a significant SCA and a non-significant GCA for ear rot in crosses inoculated with *F. verticillioides, A. flavus* and *Stenocarpella maydis* (Berck) Sutton in one of his experiments whereas in another experiment, both GCA and SCA were important in the inheritance of ear rot resistance. In another study of resistance to fumonisins, Williams and Windham (2009) found that SCA was not significant while GCA was significant. Hefny *et al.* (2012) and Hung and Holland (2012) found significant GCA and SCA in their studies. Both studies indicate that GCA was more important than SCA in the inheritance of resistance to *F. verticillioides* and fumonisin contamination. On the contrary, Pádua *et al.* (2016) reported that SCA was more important than GCA in resistance to fumonisin contamination in their study.

Genetic engineering

Genetic engineering of plants describes the process whereby the DNA has been altered through the introduction of a foreign gene to express a trait not inherent to the modified plant. Duvick (2001) proposed three transgene-mediated strategies for the management of FER and fumonisin accumulation in maize. These include (i) the reduction of *F. verticillioides* infection, (ii) the degrading of fumonisins, and (iii) interfering with the fumonisin biosynthetic pathway. The incorporation of antifungal and/or resistance genes, as well as the over-expression of defence-related genes could limit the fungus while catabolic enzymes from microbes have been used to detoxify fumonisins, both *in vitro* and *in situ*, before they accumulate in the plant (Yoneyama and Anzai, 1991; Zhang *et al*., 1999). Additionally,

fumonisin esterase and amine oxidase genes encoding fumonisin-degrading enzymes have been identified in *Exophiala spinifera* de Hoog and Hasse (Duvick, 2001) yet none of these genes have been successfully introduced into maize. Maize plants have been genetically engineered to interfere with the biosynthesis of aflatoxins and trichothecenes (Brown *et al*., 1999; Okubara *et al*., 2002), but not with that of fumonisins.

The most promising example of the use of genetic engineering to reduce FER and fumonisins is Bt maize (Munkvold *et al*., 1999; Abbas *et al*., 2013). This is due to the close association between kernel damage by insects and infection by *F. verticillioides* (Munkvold, 2003a). Bt maize contains a gene from *Bacillus thurigiensis* known as Bt delta endotoxin. This gene produces Cry proteins that are fatal to insects, especially the European corn borer. Bt maize limit the production of feeding wounds created by insects (Magg *et al.*, 2001). This dramatically reduces FER and fumonisins as entry through feeding wounds is an important entry for *F. verticillioides* (Munkvold *et al.*, 1999; Papst *et al.*, 2005). Munkvold *et al*. (1999) showed that Bt maize consistently had fumonisin levels below 2 ppm regardless of the weather conditions. Rheeder *et al*. (2005) observed a 39-83% difference in fumonisin levels in Bt maize compared to nontransgenic maize. The use of Bt maize to control AER and aflatoxin accumulation, however, has not been as successful (Buntin *et al*., 2001). Wu (2006) argues that this may be because AER and aflatoxin contamination are less correlated to insect damage compared to FER/fumonisin contamination. Genetically modified maize is not authorized in all countries and, consequently, conventional breeding efforts are still commonly used.

CONCLUSION

Maize ear rot infections can result in yield reduction, lessening of grain quality and infection by certain fungal species such as *F. verticillioides* and *A. flavus*. This may lead to contamination of kernels with mycotoxins that have detrimental health impacts on humans and livestock. The infection and mycotoxin contamination of maize presents great challenges for the maize producers in subsistence and commercial farming systems. Agronomic practices that help reduce disease development and lessen mycotoxin contamination have been recommended by the Codex Alimentarius Commission. Strategies that employ biological control agents and chemical methods have also been used to detoxify mycotoxins and reduce maize ear rot severity. While on the other hand, physical methods have been met with limited success. Enhanced host resistance, as part of an integrated disease management strategy, could provide a viable solution to the mycotoxin problem but requires the identification of sources of resistance combined with desirable agronomic traits. Breeding for improved ear rot and mycotoxin resistance has been slow due to its genetic complexity likely to be influenced by external factors. However, the numerous QTL mapping

studies for disease resistance in maize have provided an abundance of DNA marker trait associations (Mesterházy *et al.*, 2012). Resistance to both *A. flavus* and *F. verticillioides* is achievable which is important for African countries with both pathogens (Robertson-Hoyt *et al.*, 2007). The production of maize inbred lines that are resistant to fumonisin contamination and FER is showing great potential in managing this disease. Currently no known cultivars are resistant to *F. verticillioides* in South Africa and Kenya.

Knowledge of the inheritance of resistance to *F. verticillioides* and fumonisins is imperative to breeding resistant cultivars. The heritability and genetic effects involved in the inheritance of resistance reviewed in this chapter were highly variable in each breeding population. Therefore, the aim of this study is to gain knowledge on the inheritance of resistance to FER and fumonisin accumulation in a set of maize inbred lines and evaluate breeding populations for enhanced plant resistance to *F. verticillioides* and fumonisins under South African and Kenyan conditions. A five by five diallel study was conducted in **Chapter 2** under South African conditions. The parental inbred lines and resultant F_1 hybrids were evaluated for resistance to FER severity, *F. verticillioides* colonisation and fumonisin accumulation in the 2014/15 maize growing season at three localities in South Africa. The heritability and genetic effects of these three traits were also determined in this set of maize inbred lines. In **Chapter 3**, a six by six diallel study was performed under Kenyan conditions. The genotypes in this study were also evaluated for resistance to FER severity, *F. verticillioides* target DNA and fumonisin accumulation in the 2014 maize growing season in Kiboko, Kenya. The general and specific combining ability of inbred lines was determined together with the heritability of the evaluated traits. Furthermore, F_2 populations derived from these inbred lines were evaluated at two localities in Kenya for resistance to *F. verticillioides* colonisation and fumonisin accumulation in the 2015 maize growing season.

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CHAPTER 2

Evaluating maize hybrids for resistance to *Fusarium verticillioides* and its fumonisins under South African field conditions

ABSTRACT

Fusarium ear rot (FER) and fumonisin accumulation in maize, caused by *Fusarium verticillioides*, can lead to lower grain quality, crop yield reduction and mycotoxicosis of humans and livestock. The best approach to control this fungus is through the development of resistant maize varieties. Yet, no resistant cultivars are available in South Africa. The aim of the study, therefore, was to evaluate F_1 maize hybrids for improved resistance to *F. verticillioides* and fumonisin contamination, as well as to understand the inheritance of resistance by means of diallel analysis. Consequently, three maize inbred lines characterised for resistance to *F. verticillioides*/fumonisins under South African conditions and two inbred lines characterised for resistance to *Aspergillus flavus* under Kenyan conditions, were selected and cross-pollinated; resulting in 18 hybrids. The hybrids, together with the parental lines, were planted in replicated field trials at three localities; Makhatini, Potchefstroom and Vaalharts. All trials were artificially inoculated with *F. verticillioides*. Disease severity, *F. verticillioides* target DNA and total fumonisin concentrations were determined by visual assessment, qPCR and LCMS-MS, respectively. The resistance levels of hybrids did not differ significantly from their parental inbred lines, except for specific hybrids in certain test environments. Hybrids R119W x CKL05015, CML495 x CKL05015 and CKL05015 x R119W were the most resistant to FER severity, *F. verticillioides* colonisation and fumonisin contamination, respectively. General combining ability (GCA) and specific combining ability (SCA) were significant ($P \leq 0.05$) for all three infection parameters evaluated. The Kenyan inbred lines CKL05015 and CML495 were good general combiners for resistance to fumonisin contamination. According to SCA estimate, CKL05015 x R119W was the best hybrid combination for resistance to FER severity, *F. verticillioides* colonisation and total fumonisin contamination. Additive gene effects were predominant in the inheritance of resistance in this set of hybrids. Parental inbred line performance was indicative of F_1 hybrids performance. This study provided fundamental information on maize lines that could be used by breeders to develop resistant cultivars.

INTRODUCTION

South Africa is the biggest maize producer in the Southern African Development Community (SADC), with an annual production average of approximately 11.8 million tons p.a. between 2008 and 2013 (FAO, 2016). Over 9 000 commercial producers country-wide contribute primarily to this production, although small-scale farmers also contribute (Grain SA, 2014). The main production areas include the Free State (41%), Mpumalanga (26%) and North West (14%) provinces (DAFF, 2013). South Africa frequently has significant maize surpluses for export to neighbouring countries (DAFF, 2013). However, the successful cultivation of maize in South Africa is plummeting due to adverse growing conditions and destructive pests. NAMC (2016) estimated a total maize import of approximately 2.7 million tons for the 2016/17 season as opposed to a low 750 000 tons for the 2015/16 season (NAMC, 2015).

One of the destructive pests associated with the decrease in South African maize production is the fungus *Fusarium verticillioides* (Sacc.) Nirenberg. This pathogen is associated with the crop in all maize-growing regions of the world. It is best known for causing Fusarium ear rot (FER), but disease symptoms can range from asymptomatic infection to severe rotting of all plant parts (Munkvold, 2003). FER occurs under warm and dry environmental conditions and affects commercial, emerging and subsistence farmers, resulting in reduced grain quality and yield losses (Marín *et al*., 1999; White, 1999; Munkvold, 2003). Of greater concern is the production of mycotoxins (secondary metabolites) by *F. verticillioides*, which have potential toxicity to humans and domesticated animals (Marasas *et al*., 2000). These toxins consist primarily of fumonisins that possess cancer-promoting properties (IARC, 2002).

Fumonisins have been related to oesophageal cancer in rural areas in South Africa (Rheeder *et al*., 1992; IARC, 2002) and have been implicated in human birth defects (Marasas *et al*., 2004). Fumonisins further cause leukoencephalomalacia in equine animals (Kellerman *et al*., 1990), pulmonary oedema in pigs (Harrison *et al*., 1990) and immuno-suppression in chickens (Marijanovic *et al*., 1991; Qureshi and Hagler, 1992). Since fumonisins can be found in both symptomatic and asymptomatic kernels, minimising fumonisin accumulation in maize has become a priority in food safety research. The widespread incidence of fumonisins in maize and maize-based products intended for human and animal consumption have resulted in a number of countries introducing maximum tolerable levels for the fumonisins (Bolger *et al*., 2001; NGFA, 2011). South Africa recently created a new law for maximum tolerable limits of fumonisins. The new legislation decrees a limit of 4 mg kg^{-1} and 2 mg kg^{-1} on maize intended for processing and maize flour or maize meal intended for human consumption, respectively (Government Gazette of South Africa, 2016). Commercially produced grain, as well as grain in certain rural areas in South Africa, has been observed with fumonisin levels that are in excess of these limits (Shepard *et al*.,

2007; Shephard, 2008; Ncube *et al*., 2011; Boutigny *et al*., 2012; Janse van Rensburg *et al*., 2015). However, crop quality surveillance data indicates that commercially-produced maize is generally of good quality (SAGL, 2013). High levels of fumonisins can be found when weather or other conditions are favourable for fungal infection (Munkvold and Desjardins, 1997; Munkvold, 2003). Due to the high fumonisin levels detected in South African grain, management methods are concentrated on improving host resistance by assessing locally-adapted varieties for resistance to FER and fumonisin contamination (Small *et al*., 2012; Rose *et al*., 2016). Resistant varieties can then be used in a breeding programme for improved host resistance.

The planting of resistant cultivars, as part of an integrated disease management strategy, is regarded as an economical and effective approach to reduce FER and minimise the risk of fumonisin accumulation in maize (Harrison *et al*., 1990; Munkvold and Desjardins, 1997; Schjøth *et al.*, 2008). Sources with good resistance are needed for the development of cultivars resistant to *F. verticillioides* and fumonisin contamination (King and Scott, 1981). Moreover, knowledge on the inheritance of disease resistance would be invaluable in the development of resistant breeding material and cultivars. Historically, the diallel mating system has been extensively used in plant breeding to understand the inheritance of traits (Hallauer *et al.*, 2010). Sprague and Tatum (1942) designed a method to estimate the performance of an inbred line in hybrid combinations; known as combining ability; and classified it as general combining ability (GCA) and specific combining ability (SCA). The GCA is the average performance of a line in a series of hybrid combinations, while SCA refers to the deviation in mean performance of a hybrid relative to the average performance of the parental lines (Sprague and Tatum, 1942). This information is valuable for breeding programmes as the GCA and SCA estimates can be used to determine which parental lines can result in superior offspring (Baker, 1978). The predominant gene action in the inheritance of a trait and the relative importance of GCA and SCA were determined by the calculation of the GCA/SCA ratio of the mean squares proposed by Baker (1978): $2MS_{GCA}/(2MS_{GCA}+MS_{SCA})$, where MS_{GCA} is the GCA mean square and MS_{SCA} is the SCA mean square. The heritability of the traits was estimated according to Griffing (1956): broad sense heritability $(H^2)=2MS_{GCA}+MS_{SCA}\ /(2MS_{GCA}+MS_{SCA}+MS_e)$ and narrow sense heritability $(h^2)=2MS_{GCA}+MS_{SCA}\ /(2MS_{GCA}+MS_{SCA}+MS_e)$, where MS_{GCA} is the GCA mean square, MS_{SCA} is the SCA mean square and MS_e is the mean square error value.

The environment has played a major role in the performance of genotypes with regards to FER severity and fumonisin accumulation (Robertson-Hoyt *et al.*, 2007; Picot *et al.*, 2010). The variations in genotype performance across environments are known as genotype x environment interaction (GEI). It is, therefore, crucial to develop maize cultivars

with broad adaptability that are stable in their expression of disease resistance. Alternatively, breeding material and hybrids should be evaluated in multi-environment, multi-year trials to determine adaptability for specific environments. Data collected from the multi-environment trials can be interpreted using genotype and genotype x environment (GGE) biplot analysis, which allows graphical assessment of the relationships between genotypes, test environments and GEI (Yan *et al*., 2001; Yan and Kang, 2003). Furthermore, the Additive Main Effects and Multiplicative Interaction (AMMI) analysis of variance is often used to explain a large proportion of GEI effects and uniquely takes into account the genotype and environment effects (Gauch, 2006). This analysis is also helpful in identifying stable, adaptable genotypes and determining the magnitude of GEI (Crossa, 1990).

The development of resistant maize cultivars in mitigating fumonisin accumulation in maize grain is important. This involves the identification of resistant inbred lines for breeding purposes by evaluating such lines in multiple environments and by understanding inheritance of resistance through their combining abilities. The aim of this study, therefore, was to evaluate F_1 hybrids derived from FER/fumonisin-resistant inbred lines previously identified in South Africa and *Aspergillus flavus*-resistant inbred lines identified in Kenya, for improved resistance to FER and fumonisin accumulation across three environments in South Africa. The *A. flavus*-resistant lines were included in this study because resistance in maize to *F. verticillioides* and *A. flavus* was reported to be connected (Roberston-Hoyt *et al*., 2007)The performance of each genotype was measured according to three traits namely i) FER severity (disease expression), ii) *F. verticillioides* target DNA (fungal colonisation) and iii) fumonisin contamination (total fumonisins accumulated). Furthermore, diallel analysis was employed to understand the inheritance of resistance to *F. verticillioides* and fumonisin contamination in maize hybrids for breeding purposes.

MATERIALS AND METHODS

Field trials

Three maize inbred lines previously characterised for their response to FER and fumonisin accumulation (Small *et al*., 2012; Rose *et al*., 2016) and two lines characterised for resistance to Aspergillus ear rot (AER) (Dr Makumbi, CIMMYT, personal communication) were crossed using method 1 of Griffing's diallel mating system during the 2013/14 maize season that resulted in 18 hybrids (Table 1). Two sets of hybrids were missing due to crop failure and seed shortage. The parental inbred lines and F_1 hybrids were planted in the 2014/15 seasons at three locations with different environments in South Africa; namely Potchefstroom (grid reference: 26˚73'S, 27˚07'E; altitude, 1 349 m.a.s.l.), Makhatini (grid reference: 22˚39'S, 32˚17'E; altitude, 77 m.a.s.l.) and Vaalharts (grid reference: 27˚95'S, 24˚83'E; altitude, 1 180 m.a.s.l.). Potchefstroom and Vaalharts are located in the dry and

warm regions, while Makhatini is in the high humidity region of the South African maize-production area. Three maize seeds were planted per hole (by hand), and the weakest seedlings were removed 3 weeks after emergence, leaving only one seedling per hole. Each plot size was 10 m long and contained approximately 33 plants, with an intra-row spacing of 0.3 m and an inter-row spacing of 1 m. The trials were replicated three times in each environment. The parental inbred lines and hybrids were planted in a randomised complete block design.

The field trials were sprayed with pre- and post-emergence herbicide for the control of weeds. The pre-emergence herbicide used was flumetsum/S-metolachlor at 630 g L^{-1} (Bateleur Gold EC), whereas the post-emergence herbicide used was halosulfuron-methyl was applied at 750 g kg^{-1} (Servian 75 WG/Cyprex WP). The trials were fertilised before planting using 2:3:4 (30) + 0.5 Zn in Makhatini and 150 kg ha^{-1} + 0.5 Zn in both Potchefstroom and Vaalharts. Additionally, a top dressing of Limestone Ammonium Nitrate (28) was applied during the sixth leaf stage at the Makhatini trial at a rate of 250 kg ha^{-1}. The same top dressing was applied at the Potchefstroom and Vaalharts trials, but during the eighth leaf stage and at a rate of 100 kg ha^{-1}. Insecticides were manually administered (40 g per 50 m) into the whorl of the plants during the 10^{th} to 12^{th} leaf stage for the control of stalk borer infestation across all three trials. Beta-cyfluthrin was used for the Potchefstroom and Vaalharts trials at 0.5 g kg^{-1} (Bulldock 0.05 GR), whereas Carbaryl was used at the Makhatini trial at 25 g kg^{-1} (Kombat GR). The Vaalharts trial was flood-irrigated weekly, while the Potchefstroom trial was conducted under dryland conditions where moisture stress was monitored and overhead irrigation performed when necessary. The Makhatini trial was conducted under overhead irrigation and watered twice a week.

Inoculum preparation and inoculation of maize ears

Maize ears were artificially inoculated with *F. verticillioides* isolate MRC826, which was originally collected from the Eastern Cape (former Transkei) region of South Africa (Rheeder *et al.,* 1992). Five-day-old hyphae were used to inoculate 100 mL of Armstrong liquid medium in Erlenmeyer flasks (Booth, 1971). The flasks were incubated for 5 days in a rotary shaker at 25°C and 100 revolutions per minute (rpm). Following incubation, the liquid cultures were filtered through two layers of sterile cheesecloth into 50-mL centrifuge tubes. The suspension was centrifuged at 3 500 relative centrifugal force (rcf) for 10 min, where after the supernatant was discarded. The conidia were washed twice with de-ionized, autoclaved water (with centrifugation between washes). The spores were then suspended in water and the spore concentration adjusted to 1×10^{6} conidia mL^{-1}. Tween 20 (polyoxyethylene 20-sorbitan monolaurate) (Fisher Biotech, Fairlawn, NJ) was added to the spore suspension at a rate of three drops per litre. The primary maize ears were inoculated

by injecting 2 mL of the spore suspension down their silk channels using disposable syringes fitted with 21 G x 38 mm needles.

Disease assessment and grain processing

Maize plants were allowed to dry in the field, followed by hand-harvesting of each experimental plot, before ears were visually assessed for FER disease severity. A visual rating for each ear was conducted by estimating the percentage of the area that was visibly covered by mycelial growth or any other *F. verticillioides* symptoms (Fig. 1). These symptoms included white-pinkish fluffy mycelia growing between kernels and white lines radiating from the point of silk attachment (Clements *et al.*, 2004). After disease assessment the kernels were removed from the ears and bulked according to plot. A sample of 250 g was taken from each plot and the moisture content of the kernels lowered to approximately 10% by drying in an oven for 2 days at 37°C. This was done to increase the milling efficiency. The maize kernels were then grounded using a Husqvarna coarse steel grain grinder (Reliance, Sweden) with the grinder being cleaned thoroughly with high pressure air between each sample to avoid cross contamination. The coarsely ground maize was further ground into a fine powder using a Philips blender (400 W, 1.75 L). Sub-samples (2 g and 5 g) from each plot was accurately weighed into 50-mL Falcon tubes (BD Biosciences, USA) and kept at -20°C until DNA and fumonisin extractions were performed, respectively.

DNA extractions

From maize: DNA was isolated from milled maize samples (2 g) using a method developed by Boutigny *et al*. (2012). This method uses the DNeasy® Plant Mini kit (QIAGEN) in combination with the CTAB/PVP lysis method. Ten mL CTAB/PVP lysis buffer [0.1 M Tris, 1.4 M NaCl, 0.02 M EDTA, 2% CTAB (w/v), 1% PVP (w/v) pH 8.0] and 40 µL proteinase K (10 mg mL^{-1}) was added to each sample. The samples were placed in an incubator shaker (Labcon, USA) at 65°C for 2 hrs shaking at 200 rpm and then centrifuged for 10 min at 4 000 rpm at 25°C. One mL of the supernatant was transferred to a sterile 1.5-mL tube and 30 µL RNAse (10 mg mL^{-1}) was added to each sample and incubated at 65°C for 15 min, after which the samples were centrifuged for 10 min at 12 000 rcf. The supernatant (400 µL) was transferred to a new 1.5-mL tube and the lysate was further treated according to the protocol provided in the DNeasy® Plant Mini kit, starting from step nine in the manufacturer's protocol. The quantity of the DNA was determined using the Nanodrop ND-1000 Spectrophotometer (Inqaba Biotechnical Industries (Pty) Ltd., South Africa). The concentration of the DNA was then adjusted to 10 ng μL^{-1} by diluting the DNA in sterile water. Furthermore, DNA extractions were also performed on water-inoculated maize grain for the quantitative PCR (qPCR) matrix (maize)-matched standards.

From mycelia: In order to accurately measure the amount of *F. verticillioides*-target DNA extracted from the milled grain, pure *F. verticillioides* (isolate MRC 826) DNA was extracted from mycelia and used as a standard. The fungus was grown in 100 mL potato dextrose broth (PDB) in 250-mL Erlenmeyer flasks placed on a shaker and rotated at 200 rpm at 25°C for 10 days. The mycelia was thereafter harvested and washed twice using sterile water and two layers of sterile cheesecloth. The harvested mycelia was placed in -20°C overnight and freeze-dried the following day. After freeze-drying the mycelia was stored at -20°C until DNA extraction was performed. DNA from mycelia was extracted with the DNeasy® Plant Mini kit (QIAGEN) using a CTAB/PVP lysis method with supplementary phenol-chloroform purification steps (Boutigny *et al*., 2012).

Quantification of *F. verticillioides* target DNA in maize grain

The amount of *F. verticillioides* target DNA in maize samples was determined by qPCR according to Boutigny *et al*. (2012). A four-time dilution series was prepared using *F. verticillioides* isolate MRC 826 DNA diluted in pathogen-free maize DNA (10 ng μL^{-1}) to produce standard curves used for the quantification of *F. verticillioides* target DNA. Three replicates of each dilution set were included in the standard curve. The correlation coefficients of the standard curves used were high (R^2 > 0.99) and the slopes were M = -3.34 and M = -3.4 with reaction efficiencies of 99 and 97%, respectively. The quantification range for the assays was 0.006 - 7.088 ng μL^{-1}. Primers Fver356 fwd/F ver412 rev were used for the quantification of *F. verticillioides* (Nicolaisen *et al.,* 2009).

Quantitative PCR assays were carried out in a total reaction volume of 25 µL comprised of 200 nM of each primer, 1xSensiMix SYR (Quantace, United Kingdom) and 2 µL DNA with a concentration of 10 ng μL^{-1}. The qPCR cycling conditions were as follows: 10 min at 95°C, followed by 35 cycles of 95°C for 15 s, 60°C for 15 s and 72°C for 15 s with a melt analysis of 72°C to 95°C, with a rise of 1°C on each step (Boutigny *et al*., 2012). For analysis, two replicates of a sample were included in an assay and three replicates of a standard were added in each essay. The assay was repeated if a difference of 0.05 in the cycle threshold (ct) value was present between replicates. A no-template control containing no DNA was added to each assay. The Rotor-gene TM 6000 (Corbett Life Science Pty (Ltd), Australia) was used for all the qPCR analysis. The repeatability (intra-run variance) and reproducibility (inter-run variance) of the assays were not investigated since they were previously validated in-house by Boutigny *et al*. (2012).

Fumonisin analysis

Fumonisin B_1, B_2 and B_3 levels in milled grain were determined by liquid chromatography tandem mass spectrometry (LCMS-MS) analysis. Five grams of finely-milled maize placed in 50-mL Falcon tubes was used for fumonisin extractions. An extraction buffer was prepared containing 70% analytical grade (AR) methanol and 30% Milli-Q water (Millipore, USA). Twenty millilitre of the extraction buffer was added to each 5-g sample and shaken vigorously. Fumonisins were extracted by placing the tubes, at an angle, on a rotary shaker for 30 min at 25°C and 200 rpm. Following shaker incubation, the tubes were centrifuged for 10 min at 4 000 rpm after which 2 mL of the clear extract was filtered through a 0.20-µm recombinant cellulose (RC) syringe filter (National Separations (Pty) Ltd., South Africa) and placed in the fridge overnight. The extracts were then centrifuged for 10 min at 14 000 rpm. Clear extract (1 800 µL) was taken from each sample and transferred to a 2-mL glass vial (PromoLab (Pty) Ltd T/A Separations, South Africa) suitable for LCMS-MS analysis. Ninety five % pure standards of fumonisin B_1 (10 mg), B_2 (10 mg), and B_3 (1 mg), were obtained from the Medical Research Council-Programme on Mycotoxins and Experimental Carcinogenesis, South Africa. Working calibration standard solutions were acquired by diluting the stock calibration standard solution with 70% methanol. The working calibration standards ranged between 0.05 and 20 parts per million (mg kg^{-1}) (FB_1 and FB_2) and between 0.005 and 2 mg kg^{-1} for FB_3. The minimum limit of detection was 0.02 mg kg^{-1}, 0.002 mg kg^{-1} and 0.02 mg kg^{-1} for FB_1, FB_2 and FB_3, respectively. Samples were submitted to the Central Analytical Facility (CAF) at Stellenbosch University for fumonisin analysis by LCMS-MS.

Statistical analysis

Data transformations were performed to determine whether untransformed and transformed data is normally distributed following the Shapiro-Wilk test for normality (Shapiro and Wilk, 1965). The percentage FER severity and total fumonisins concentration were transformed using log (ln) transformations, while *F. verticillioides* target DNA values were not subjected to any transformation because the raw data was normally distributed. The Analysis of Variance (ANOVA) employing the Generalised linear model (GLM) procedure of SAS statistical software version 9.2. (SAS Institute Inc., Cary, NC, USA) was performed for FER severity, *F. verticillioides* colonisation and fumonisin contamination. Fisher's least significant difference (LSD) test was calculated at the 5% level to compare genotypes' means with reference to the variables tested and a probability level of 5% ($P < 0.05$) was regarded as significant for all significance tests. Pearson's correlation coefficients were determined, using the normalised data, to assess the relationships between FER severity, *F. verticillioides*

colonisation and total fumonisins concentration using the CORR procedure in SAS statistical software.

The data collected across the three environments was graphically analysed using the genotype and genotype x environment (GGE) biplot derived from the principal component analysis (PCA) of environment-focused data (Yan *et al*., 2000). This was done by using GenStat 15th edition (VSN International) employing the model based on singular value decomposition (SVD) of the first two principal components (PC), PC1 and PC2 (Yan, 2002). Two GGE biplots were generated namely genotype comparison biplot and mega-environment scatter plot. Furthermore, GEI was evaluated by additive main effects and multiplicative interaction (AMMI) analysis of variance (Gauch and Zobel, 1996) done by SAS statistical software version 9.2. Additive main effects and multiplicative interaction analysis, similar to GGE biplot analysis, uses both the conventional ANOVA and IPCA (interaction principal component analysis) graphically in a biplot where genotype and environment means are plotted against interaction PCA scores. Additive main effects and multiplicative interaction stability values were determined based on IPCA 1 and IPCA 2 scores according to Purchase *et al.* (2000).

GCA and SCA were estimated according to method 1 and model 1 of Griffing (1956) where parental inbred lines, F_1 offspring and reciprocal hybrids were included. Means of hybrids and reciprocal hybrids were used for the two missing hybrids to complete the diallel by using DiallelSAS05 (Zhang *et al*., 2004). This program uses a linear model to estimate combining ability which allocates the total genotypic variance into variance components i.e. GCA and SCA as well as reciprocal, maternal and non-maternal effects (Zhang *et al*., 2004). The significance of these estimates was determined by a *t*-test. The predominant gene action in the inheritance of a trait, and the relative importance of GCA and SCA, were determined by the calculation of the GCA/SCA ratio of the mean squares proposed by Baker (1978). Baker (1978) indicated that the magnitude of variation between single cross hybrids resulting from inbred lines is the sum of SCA variance and twice the GCA variance and stated that the proportion of this total that is due to GCA variation signifies the predictability of hybrid performance based on GCA alone. Baker (1978) further stated that the closer the ratio is to one, the greater the predictability of the performance of a specific single cross offspring is, based on GCA alone. Sprague and Tatum (1942) stated that the GCA and SCA estimates may be interpreted with regards to gene action. The GCA estimate is associated with genes with predominantly additive effects, whereas SCA is associated with genes that have dominant effects. A high ratio indicates predominant additive gene effects, while a low ratio indicates dominant gene effect (Griffing, 1956; Baker, 1978; Bhullar, *et al*., 1979). The heritability of the traits was estimated according to Griffing (1956).

RESULTS

Varying degrees of *F. verticillioides* infection and fumonisin accumulation were observed across test environments. GEI had a significant effect on FER symptoms expressed, *F. verticillioides* colonisation and fumonisins accumulated ($P < 0.05$) (Table 2). For this reason, the performance of all the genotypes for each trait evaluated, at each locality, is presented separately (Table 3). The environment and genotype were highly significant for FER symptoms, *F. verticillioides* colonisation and fumonisin accumulation across environments ($P < 0.0001$) (Table 2). Replicates within an environment did not differ significantly from each other for the evaluated traits (Table 2).

FER severity assessment

The mean FER severity observed in Makhatini (12.7%) and Vaalharts (11.6%) did not differ significantly from each other, but differed significantly from the mean FER in Potchefstroom (4.4%) (Table 3). Disease severity ranged from 4.0% to 31.6% in Makhatini, from 0.3% to 12.2% in Potchefstroom and 2.4% to 30.3% in Vaalharts (Table 3). The hybrid R119W x CKL05015 exhibited the lowest FER symptoms (4.0%) in Makhatini and differed significantly from the worst performing inbred lines (CML444; 31.6%, CML390; 24.6% and R119W, 22.6%) but not from the best performing inbred lines (CML495; 9.7% and CKL05015; 11.6%) (Table 3). Several hybrids did not differ significantly from R119W x CKL05015, including CML444 x CML495 (4.6%), CML495 x CML390 (5.1%), CKL05015 x CML495 (6.4%), CML495 x CKL05015 (8.2%) and CKL05015 x R119W (11.6%) (Table 3). A hybrid resulting from the crossing of two Kenyan inbred lines (CKL05015 x CML495) had the lowest signs of infection (0.3%) in Potchefstroom, which differed significantly from FER severity in all parental lines but not in hybrids CML444 x CKL05015 (1.2%) and R119W x CKL05015 (1.2%) (Table 3). In Vaalharts, the hybrid CKL05015 x CML444 developed significantly less FER symptoms (2.4%) than the parental lines (Table 3), but not significantly less than hybrids CKL05015 x CML390 (3.7%), R119W x CKL05015 (3.8%) and CKL05015 x R119W (5.2%) (Table 3). Inbred line CML390 developed most FER symptoms in Potchefstroom (12.2%) and Vaalharts (30.3%), which differed significantly from the susceptible check R119W (1.4% and 9.8%, respectively). CML390 did not have the most FER symptoms in Makhatini (24.6%) but it did not differ from the genotype with the most symptoms (CML444; 31.6%) (Table 3).

Quantification of *F. verticillioides* DNA in maize grain

The mean *F. verticillioides* DNA concentrations in maize grain differed significantly between the three locations. Makhatini had the highest mean fungal DNA content of 0.038 ng μL^{-1} and differed significantly from Vaalharts (0.021 ng μL^{-1}) and Potchefstroom (0.012 ng μL^{-1})

(Table 3). The latter localities also differed significantly from each other. The hybrid, CML495 x CML390 was least colonised by *F. verticillioides* in Makhatini (0.011 ng μL^{-1}), which was significantly less than the other inbred lines apart from parental inbred line CML495 (0.025 ng μL^{-1}) (Table 3). The hybrids that were significantly more contaminated with *F. verticillioides* than CML495 x CML390 included CML444 x CKL05015 (0.055 ng μL^{-1}), R119W x CML495 (0.055 ng μL^{-1}) and CML390 x R119W (0.076 ng μL^{-1}) (Table 3). The fungal content in CML390 x R119W (0.076 ng μL^{-1}) did not differ significantly to that measured in parental inbred lines CKL05015 (0.064 ng μL^{-1}), CML390 (0.063 ng μL^{-1}) and R119W (0.074 ng μL^{-1}) (Table 3). Potchefstroom had very low levels of *F. verticillioides* colonisation. The hybrids CKL05015 x CML444, CKL05015 x R119W, CML495 x CKL05015, CML495 x CML444, CML495 x R119W and R119W x CML495 all had fungal DNA below the limit of detection in Potchefstroom (0.006 ng μL^{-1}) (Table 3). In Potchefstroom, the lowest detectable fungal DNA was observed in the cross R119W x CML444 (0.006 ng μL^{-1}) (Table 3). The fungal content measured in this hybrid only differed significantly from hybrids CML390 x CML444 (0.038 ng μL^{-1}) and R119W x CML390 (0.035 ng μL^{-1}) (Table 3). In Vaalharts, *F. verticillioides* target DNA could not be detected in hybrids CKL05015 x CML444, CML444 x CKL05015, CML495 x CKL05015, R119W x CKL05015 and R119W x CML444 (Table 3). The hybrids CML444 x CML495 and CML495 x CML390 had the lowest detectable fungal DNA in Vaalharts (0.007 ng μL^{-1}) (Table 3), which only differed significantly from hybrid CML495 x R119W (0.045 ng uL^{-1}) and inbred lines CKL05015 (0.087 ng μL^{-1}) and CML390 (0.082 ng μL^{-1}) (Table 3). All the hybrids differed significantly from inbred line CKL05015 (0.087 ng μL^{-1}) that contained the most fungal DNA in Vaalharts (Table 3). Hybrids CKL05015 x CML390 (0.009 ng μL^{-1}) and CML390 x CKL05015 (0.012 ng μL^{-1}) had significantly less fungal DNA compared to their parental lines (CKL05015; 0.087 ng μL^{-1} and CML390; 0.082 ng μL^{-1}) in Vaalharts.

Fumonisin analysis

The means of total fumonisin concentration in maize grain differed significantly between locations, with the highest mean concentration measured in Makhatini (3.936 mg kg^{-1}) followed by Vaalharts (1.198 mg kg^{-1}) and Potchefstroom (0.322 mg kg^{-1}) (Table 3). Fumonisin concentrations ranged from 2.433 mg kg^{-1} to 6.199 mg kg^{-1} in Makhatini, from 0.027 mg kg^{-1} to 1.352 mg kg^{-1} in Potchefstroom and from 0.071 mg kg^{-1} to 6.470 mg kg^{-1} in Vaalharts. The hybrid CML495 x CML390 had the lowest fumonisin concentration (2.433 mg kg^{-1}) in Makhatini, which differed significantly from that of the worst performing parental line (CKL05015; 6.199 mg kg^{-1}) as well as parental lines CML390 (5.786 mg kg^{-1}), CML444 (3.759 mg kg^{-1}), CML495 (5.725 mg kg^{-1}) and R119W (5.627 mg kg^{-1}). The fumonisin content of most hybrids; including CML495 x R119W (2.568 mg kg^{-1}), CML444 x CML495

(2.587 mg kg^{-1}), CKL05015 x R119W (2.757 mg kg^{-1}) and CKL05015 x CML495 (2.751 mg kg^{-1}); did not differ significantly from that of hybrid CML495 x CML390 (2.433 mg kg^{-1}) (Table 3). R119W x CML495 accumulated the highest fumonisin concentration (6.281 mg kg^{-1}) among the hybrids in Makhatini, which did not differ significantly from the parental lines (Table 3). Hybrids CML495 x CML390 (2.433 mg kg^{-1}), CML495 x R119W (2.568 mg kg^{-1}) and R119W x CML390 (2.831 mg kg^{-1}) contained significantly less fumonisin levels than their parental inbred lines (CML390; 5.786 mg kg^{-1}, CML495; 5.725 mg kg^{-1} and R119W; 5.627 mg kg^{-1}) (Table 3).

Hybrid CML495 x CML390 (0.027 mg kg^{-1}) had the lowest detectable fumonisin content in Potchefstroom which it did not differ significantly from the fumonisin content in line CML495 (0.029 mg kg^{-1}) (Table 3). Fumonisin concentrations in hybrids CKL05015 x CML444, CKL05015 x R119W, CML444 x CML495, CML495 x CKL05015, CML495 x R119W and R119W x CML495 were lower than the detectable limit of 0.02 mg kg^{-1} (Table 3). In Potchefstroom, the fumonisin content of parental lines CML444 (0.573 mg kg^{-1}), CML495 (0.029 mg kg^{-1}) and CKL05015 (0.104 mg kg^{-1}) did not differ significantly from the best performing hybrid (CML495 x CML390; 0.027 mg kg^{-1}) (Table 3). In Vaalharts, lines CML 390 and CKL05015 accumulated a high fumonisin content of 6.470 mg kg^{-1} and 4.536 mg kg^{-1}, respectively (Table 3). Hybrid CML444 x CKL05015 had the lowest fumonisins (0.071 mg kg^{-1}), which did not differ significantly from that in hybrids such as CKL05015 x CML444 (0.083 mg kg^{-1}), R119W x CKL05015 (0.137 mg kg^{-1}) and CKL05015 x CML495 (0.661 mg kg^{-1}) (Table 3). In Vaalharts, hybrids CKL05015 x CML390 (0.581 mg kg^{-1}) and CML390 x CKL05015 (0.706 mg kg^{-1}) accumulated less fumonisins compared to their parental lines CML390 (6.470 mg kg^{-1}) and CKL05015 (4.536 mg kg^{-1}).

AMMI analysis

Genotypes, environments and GEI were significant sources of variation for all three traits evaluated ($P < 0.05$) (Table 4). The genotype effect (22.29%) was the most important in explaining variation in the amount of fungal target DNA retrieved. The majority of the variation in FER severity (26.45%) and fumonisin concentrations (64.25%) was attributed to the environment. The first IPCA was important in explaining the GEI for all three traits ($P < 0.05$), especially fumonisin concentration ($P < 0.001$), while the second IPCA was not significant for all three traits ($P = 0.0783$) (Table 4). The AMMI analysis for FER severity revealed that 24.67% of the total variation was explained by the genotype effects, 26.45% by the environment and 17.28% by GEI (Table 4). The genotype effects, environmental effects and GEI were accounted for 22.29%, 15.18% and 20.83% of the total variation observed in *F. verticillioides* colonisation, respectively (Table 4). The total variation observed in accumulated fumonisins was largely attributed to the environment (64.25%) while the

genotype effects and GEI explained 12.26% and 8.49% of the total variation, respectively (Table 4).

The three most stable genotypes for FER severity, *F. verticillioides* colonisation and fumonisin contamination were all hybrids (Table 5). Hybrid R119W x CKL05015 was most stable for FER symptom expression (0.0164), while CKL05015 x R119W (0.0068) and CKL444 x CML495 (0.0523) were most stable for *F. verticillioides* colonisation and fumonisin contamination, respectively. The parental lines CKL05015 (0.2018) and CML390 (1.615) had the least stability for *F. verticillioides* colonisation and fumonisin concentration, respectively (Table 5). Hybrid CKL05015 x CML444 (1.1318) was the least stable for FER severity and was the only hybrid to be the least stable across all three parameters (Table 5).

GGE biplot analysis

The first and second PC explained 59.0 and 23.4%, respectively, of the variation in FER severity, accounting for 82.4% of the total variation observed (Fig. 2). Hybrid R119W x CKL05015 (C16) had the overall highest level of resistance for FER severity, followed by CML390 x CKL05015 (C5) (Fig. 2A). These hybrids (R119W x CKL05015 and CML390 x CKL05015) also had low PC2 scores, indicating good stability (Fig. 2A and Table 5). The parental line CML390 (L2) had the most FER symptoms, followed by CML390 x R119W (C7) (Fig. 2A).

The three test environments fell into different sectors of the polygon view, indicating crossover interactions (Fig. 2B). The hybrid CKL05015 x CML495 (C3) was the vertex genotype in the Potchefstroom sector, R119W x CKL05015 (C16) in Makhatini, and CKL05015 x CML444 (C2) in the Vaalharts sector (Fig. 2B). Hybrids CKL05015 x CML495 (C3), CML444 x CKL050105 (C8), CML444 x CML495 (C9), CML495 x CML390 (C12) and CML495 x R119W (C14) developed the least FER symptoms in Potchefstroom (Fig. 2B). The hybrids CML390 x CKL05015 (C5) and R119W x CKL05015 (C16) expressed the least FER symptoms in Makhatini, while CKL05015 (L1), CKL05015 x CML390 (C1), CKL05015 x CML444 (C2), CKL05015 x R119W (C4) and CML495 x CKL05015 (C11) had the least FER symptoms in Vaalharts (Fig. 2B). The hybrids CKL05015 x CML444 (C2) and CKL05015 x CML495 (C3) were the least stable as they were the farthest from the biplot origin (Fig. 2B). The inbred line R119W (L5) was located on the origin of the biplot (Fig. 2B) indicating average performance and its lack of response to the environment.

A GGE biplot on *F. verticillioides* colonisation of maize inbred lines and hybrids explained 91.6% of the total variation found under three different environments in South Africa (PC1 = 65.1.2% and PC2 = 26.5%) (Fig. 3). The hybrid CML495 x CKL05015 (C11) contained the least fungal DNA concentration followed by CML495 x CML390 (C12) and CML444 x CML495 (C9) (Fig. 3A). Hybrid CML495 x CKL05015 (C11) and CKL05015 x

R119W (C4) were also close to the biplot origin indicating good stability (Fig. 3A). The worst performing genotypes in terms of *F. verticillioides* colonisation were inbred lines CKL05015 (L1) and CML390 (L2) (Fig. 3A). The test environments also fell into three different sectors for the *F. verticillioides* colonisation (Fig. 3B). In Makhatini, genotypes CKL05015 x CML495 (C3), CML444 x CML495 (C9), CML495 x CKL05015 (C11) and CML495 x CML390 (C12) had the highest level of resistance to *F. verticillioides* colonisation (Fig. 3A). The majority of the genotypes performed relatively similar and had the lowest *F. verticillioides* target DNA concentrations in Potchefstroom and Vaalharts (Fig. 3B). The genotypes CML495 x R119W (C14), R119W (L5) and CML390 x R119W (C7) were the least stable genotypes with regards to fungal colonisation (Fig. 3B). All the inbred lines (L1 - L5) did not fall into the same segment as any test environment (Fig. 3B).

The PC1 accounted for 76.2% and PC2 for 12.9% of the fumonisin content variation, explaining 89.1% of the total variation (Fig. 4). The genotype CML495 x CML390 (C12), accumulated the lowest fumonisin concentration followed by CKL05015 x R119W (C4) although this genotype had a lower PC2 value (0.023) (Fig. 4A and Table 5). The inbred line CML390 (L2) accumulated the highest concentration of fumonisins followed by CKL05015 (L1) (Fig. 4A). The least stable genotypes were CML495 x R119W (C14) and R119W x CML495 (C15) (Fig. 4B). Potchefstroom and Vaalharts fell into the same sector of the polygon whereas Makhatini fell into a different sector. Several hybrids performed well in Makhatini including CKL05015 x CML495 (C3), CKL05015 x R119W (C4), CML444 x CML495 (C9) and CML495 x CML390 (C12) (Fig. 4B). The inbred lines (L1 - L5) did not fall into the same segment as any test environment (Fig. 4B).

Correlations

Significant correlations between FER severity, fungal target DNA and total fumonisins were determined ($P < 0.05$). Correlations between FER severity and *F. verticillioides* colonisation within environments were low to moderate, ranging from $r = 0.33$ - 0.49, while they ranged from $r = 0.36$ - 0.48 between FER severity and fumonisin contamination (Table 6). However, the correlation between fumonisin contamination and fungal biomass within environments was good, ranging from $r = 0.73$ - 0.79 (Table 6). A moderate but significant overall correlation of $r = 0.47$ was observed between FER severity and *F. verticillioides* colonisation (Table 6). FER severity had a moderate correlation with fumonisin contamination, with an overall Pearson correlation of $r = 0.53$ (Table 6). A high overall correlation between fumonisin contamination and fungal biomass was determined ($r = 0.71$) (Table 6).

Diallel analysis

General and specific combining ability were significant for all three traits ($P < 0.05$) (Table 7). The GCA x environment and SCA x environment interactions were not significant except for the GCA x environment effect on fumonisin concentration ($P < 0.05$) (Table 7). The reciprocal, maternal and non-maternal effects were not significant for all three traits ($P < 0.05$) (Table 7). Baker's ratios of 0.83, 0.69 and 0.68 were observed for FER severity, *F. verticillioides* colonisation and fumonisin concentration, respectively (Table 7). High broad sense heritability was observed for the evaluated traits and ranged from 0.95 to 0.97 (Table 7). Whereas the narrow sense heritability ranged from 0.65 to 0.81 for FER severity, *F. verticillioides* colonisation and fumonisin content, respectively (Table 7).

Inbred lines CKL05015 had the largest negative GCA estimate (-0.44) for FER severity ($P < 0.05$) (Table 8). The inbred line CML390 (0.24) and CML444 (0.15) had the largest positive and significant GCA estimates for FER severity ($P < 0.05$) (Table 8). The Kenyan inbred line CML495 had a negative GCA estimate (-0.01) for FER severity, which was not significant ($P < 0.05$) (Table 8). The significant GCA estimates for *F. verticillioides* colonisation observed in this study were very low, 0.009 (CML495) and -0.006 (CML390) ($P < 0.05$) (Table 8). Negative significant GCA estimates for resistance to fumonisin contamination were observed in CKL05015 (-0.06) and CML495 (-0.10), with the latter having the largest value ($P < 0.05$) (Table 8). The inbred line CML390 had a significant positive GCA estimate (0.16) for fumonisin contamination ($P < 0.05$) (Table 8). The susceptible check, R119W, had no significant GCA for any of the traits evaluated ($P < 0.05$) (Table 8).

Hybrids CKL05015 x CML390 (-0.30), CKL05015 x R119W (-0.30) and CML390 x CML444 (-0.23) had the largest negative SCA estimates for FER severity, while CML390 x R119W (0.33), R119W x CKL05015 (0.32) and R119W x CML390 (0.21) had the largest positive estimates for the same trait (Table 9). The largest significant SCA estimates for *F. verticillioides* colonisation were obtained for hybrids CKL05015 x R119W (-0.01) and CKL05015 x CML390 (0.01) (Table 9) ($P < 0.05$). The hybrids CKL05015 x CML390 (-0.14) and CKL05015 x R119W (-0.18) exhibited the largest negative and significant SCA estimate for fumonisin content, while the highest positive and significant SCA estimate was observed on R119W x CML444 (0.18) (Table 9).

Weather data

Vaalharts was significantly warmer than Makhatini and Potchefstroom during the first four months of plant development (Table 10). For months 5 and 6, mean maximum temperatures in Vaalharts were significantly higher compared to Potchefstroom but not to Makhatini ($P < 0.05$). Makhatini had an average monthly temperature of 30.84°C recorded during grain

filling (month 3), which did not differ significantly from the average temperature at Potchefstroom (31.06°C), but which differed significantly to that measured at Vaalharts (35.23°C) ($P < 0.05$). Makhatini maintained a relative humidity above 89% for the entire season, while Potchefstroom and Vaalharts ranged from 78.19 - 89.81% and 73.63 - 85.04%, respectively (Table 10). Makhatini (90.66%) had significantly higher relative humidity than Potchefstroom (85.85%) and Vaalharts (75.92%) during grain filling ($P < 0.05$). The relative humidity recorded at Makhatini was significantly higher for the duration of the trial when compared to Vaalharts and Potchefstroom; with the exception of month 4 and 5 at Potchefstroom. Potchefstroom and Vaalharts experienced significantly higher levels of rainfall during the first month of the trials when compared to Makhatini (Table 10). Vaalharts had significantly more rain during the first four months of the trial when compared to Makhatini, except for month 3 when the rainfall recorded at the two localities did not differ significantly from each other ($P < 0.05$). Potchefstroom had significantly more rain compared to Makhatini and Vaalharts for the first four months of the trial ($P < 0.05$). After that Makhatini recorded significantly higher rainfall compared to Potchefstroom and Vaalharts.

DISCUSSION

Significant differences were found in FER incidence, *F. verticillioides* target DNA and fumonisins contamination of maize genotypes evaluated in this study. The genotypes most resistant to FER, fungal colonisation and fumonisin accumulation were hybrids R119W x CKL05015, CML495 x CKL05015 and CML495 x CML390, respectively. The level of resistance observed in these hybrids did not differ significantly from that of their respective parental inbred lines. The response of most other hybrids evaluated in this study to *F. verticillioides* and fumonisin accumulation was also comparable to that of their parental inbred lines. This phenomenon was previously observed in diallel studies by King and Scott (1981) and Hung and Holland (2012), and emphasises the importance of using sources of resistance with desirable agronomic traits when initiating a resistance breeding programme.

Although parental lines did not significantly different from their F_1 hybrids, certain hybrids exhibited improved resistance compared to their parental lines. This indicates that hybrids with better resistance than parental lines can be obtained by performing single crosses. Such hybrids, however, need to be further studied for resistance across locations and over several seasons. They should also be evaluated for resistance to other important diseases such as Gibberella ear rot, Diplodia ear rot and AER as well as their corresponding mycotoxins.

The inbred line CML390 was the most susceptible to FER, *F. verticillioides* colonisation and fumonisin contamination in this study. This result is in conflict with that of Small *et al.* (2012) and Rose *et al.* (2016), where the same inbred line was characterised as

resistant. Line CML390 had a high ASV, which indicated its unstable resistance across environments. Small *et al.* (2012) conducted evaluations over one season and in two localities only. It is further worth noting that the other inbred lines and hybrids in this study developed less FER symptoms, and had lower *F. verticillioides* colonisation and fumonisin levels compared to those reported by Small *et al*. (2012) and Rose *et al*. (2016). Prevailing environmental conditions in the current study may also explain why CML390 had a higher level of susceptibility compared to earlier studies. Interactions between the environment and genotypes are well known to significantly affect FER and fumonisin contamination (Munkvold, 2003; Clements *et al*., 2004; Afolabi *et al*., 2007).

In this study, GEI analysis revealed that the environment primarily contributed to FER severity and fumonisin contamination, whereas the genotype more prominently contributed to *F. verticillioides* colonisation. The environment and the genotype as the drivers of GEI variation observed in FER severity and *F. verticillioides* colonisation, respectively, is in contrast to a study by Rose *et al*. (2016) who found GEI to be the main source of variation for these traits. However, studies by de la Campa *et al*. (2005), Cao *et al*. (2014) and Rose *et al*. (2016) support the finding in this study that the environmental effect is the main source of variation for fumonisin accumulation. This indicates the importance of breeding for genotypes that are stable across environments and diverse climatic conditions, or ones that have been adapted to specific environments.

Test environments were divided into different mega-environments for FER severity and fungal colonisation, but Potchefstroom and Vaalharts grouped as a single mega-environment for fumonisin accumulation. Similar results were previously reported by Rose *et al.* (2016), and indicate that the response of genotypes to fumonisin accumulation is similar in Potchefstroom and Vaalharts. Either locality would therefore be suitable to evaluate maize genotypes for resistance to fumonisin contamination in future. This would reduce both the cost and time associated with these labour intensive field evaluations. Makhatini was the most suitable test environment for evaluating maize genotypes for resistance to fumonisin contamination. This location had a high relative humidity throughout the growing season and was relatively dry; both conditions that are favourable for *F. verticillioides* growth and fumonisin production during grain filling (month 3), which is the plant's most susceptible developmental stage (Miller, 1994; Bottalico, 1998; Marín *et al.*, 1999; Reid *et al.*, 1999). The low infection and fumonisin levels observed in Potchefstroom may be due to the high rainfall as *F. verticillioides* is favoured by dry conditions (Miller, 1994; Bottalico, 1998; Marín *et al.*, 1999; Reid *et al.*, 1999; Munkvold, 2003).

The moderate correlations found between FER severity and fumonisin content suggest that selection of superior maize genotypes based on FER resistance may not substantiate resistance to fumonisin accumulation. Clements *et al.* (2003) also found

moderate phenotypic correlations between FER and fumonisin concentrations, which made them conclude that the improvement of genotypes for FER resistance may not always produce fumonisin-resistant genotypes. On the contrary, Hung and Holland (2012) and Robertson *et al*. (2006) found good genotypic correlations above $r = 0.85$ between these two traits. The high genotypic correlation suggests that selection can be done based on either FER development or fumonisin levels, which would save time and money during resistance screening.

In this study, hybrids CKL05015 X CML495, CKL05015 X R119W, CML444 x CML495, CML495 x CKL05015 and CML495 x CML390 were resistant to both *F. verticillioides* colonisation and fumonisin contamination. This supports studies done by Janse van Rensburg *et al.* (2015) and Rose *et al*. (2016) who reported a good correlation between *F. verticillioides* target DNA and fumonisin contamination. Therefore, evaluating maize genotypes for resistance to fumonisin contamination can reflect their resistance to colonisation by *F. verticillioides*, and *vice versa*. Still, fumonisin analysis is recommended for identifying breeding materials or hybrids resistant to the mycotoxin.

Significant GCA and SCA effects observed in this study signified that additive and non-additive gene effects were both important in the inheritance of FER severity, *F. verticillioides* colonisation and fumonisin accumulation in this population. The GCA/SCA ratio, however, suggests that GCA estimates were more important in predicting resistance of hybrids than SCA estimates. This finding is in agreement with results found by Hung and Holland (2012) who also reported that GCA was more important when determining the performance of a hybrid. Still, resistance of parental inbred lines in hybrid development should be considered, as both the GCA and SCA estimates were significant.

Negative GCA and SCA effects are desirable for disease resistance based on a scale where the highest estimate corresponds to high infection (Bookmyer, *et al*., 2009; Santiago *et al*., 2009). Therefore genotypes with high positive GCA or SCA estimates should be avoided. Hybrids CKL05015 x CML390, CKL05015 x R119W and CML390 x CML495 were the best hybrid combinations for resistance to *F. verticillioides* colonisation and fumonisin accumulation based on SCA estimates. These F_1 hybrids can be used for further development of resistant maize cultivars. Selection of hybrids with good resistance to *F. verticillioides* and fumonisin contamination and high SCA could potentially result in the development of a hybrid with *F. verticillioides* and fumonisin contamination resistance for commercial release. Inbred lines that exhibited negative and significant GCA effects, such as CKL05015 (for FER severity and fumonisin contamination) and CML495 (for fumonisin), should confer resistance to their offspring. These lines can be used to start a recurrent selection programme or they can be used as a tester in a hybrid combination. Furthermore, these lines were characterised as resistant to AER/aflatoxin-resistant (Dr Makumbi,

CIMMYT, personal communication). This implies that they either have common genes responsible for resistance to both AER/aflatoxins and FER/fumonisins, or that they contain different sets of genes for resistance to both diseases. Robertson-Hoyt *et al*. (2007) previously found quantitative trait loci (QTL) that affected both ear rots and fumonisin contamination, while another QTL affected both ear rots and two additional QTLs affected aflatoxin and fumonisin contamination.

The non-significant reciprocal, maternal and non-maternal effects in the current study indicate that these effects played an insignificant role in the resistance observed in hybrids. This contradicts results by previous studies which indicated the presence of maternal effect in a genotype's resistance (Scott and King, 1984; Headrick and Pataky, 1991; Nankam and Pataky, 1996). However, the absence of reciprocal, maternal and non-maternal effects is not a novel result in breeding for ear rots. Williams *et al.* (2008) also found non-significant reciprocal, maternal and non-maternal effects in a diallel analysis study for resistance to aflatoxins. The absence of significant reciprocal, maternal and non-maternal effects indicates the lack of extra-nuclear factors in the inheritance of resistance in these set of hybrids (Headrick and Pataky, 1991; Nankam and Pataky, 1996).

Heritability estimates vary according to the inbred lines or varieties and traits evaluated (Robertson *et al*., 2006). The traits evaluated in this study (FER severity, *F. verticillioides* colonisation and fumonisin accumulation) had high broad sense heritabilities. The high heritability estimates signify that over 95% of the phenotypic variance observed on the hybrids was conferred by the parents for all three traits and explains the similarities between inbred lines and hybrids. This is especially important in marker-assisted selection, where parental resistance QTLs can be identified in the hybrids. The high narrow-sense heritability estimates observed in this study suggests that resistance can be obtained through the crossing of two parental inbred lines with good GCA effects, especially for FER severity.

The principal gene action observed in this study was additive in nature for FER severity, fungal colonisation and fumonisin production in maize. Resistance to *F. verticillioides* and fumonisin contamination was highly heritable, with resistance in the hybrids highly similar to that of their parental inbred lines. It is therefore recommended to do resistance breeding with material that contains a certain level of resistance in inbred lines. This study has provided information regarding the inheritance of resistance to FER and fumonisin contamination. This is of utmost importance in conducting a successful resistance breeding programme for the development of commercial cultivars suited to both small and large scale farmers.

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Table 1. Maize inbred lines and hybrids evaluated in Makhatini, Potchefstroom and Vaalharts during the 2014/15 maize-growing season.

Code	Genotype	Description	Origin[1]	Status[2]
L1	CKL05015	Inbred	CIMMYT-Kenya	Resistant to AER
L2	CML390	Inbred	CIMMYT-Zimbabwe	Resistant to FER/fumonisins
L3	CML444	Inbred	CIMMYT-Zimbabwe	Resistant to FER/fumonisins
L4	CML495	Inbred	CIMMYT-Kenya	Resistant to AER
L5	R119W	Inbred	ARC-GCI-South Africa	Susceptible to FER/fumonisins
C1	CKL05015 x CML390	F_1 hybrid		Unknown
C2	CKL05015 x CML444	F_1 hybrid		Unknown
C3	CKL05015 x CML495	F_1 hybrid		Unknown
C4	CKL05015 x R119W	F_1 hybrid		Unknown
C5	CML390 x CKL05015	F_1 hybrid		Unknown
C6	CML390 x CML444	F_1 hybrid		Unknown
C7	CML390 x R119W	F_1 hybrid		Unknown
C8	CML444 x CKL05015	F_1 hybrid		Unknown
C9	CML444 x CML495	F_1 hybrid		Unknown
C10	CML444 x R119W	F_1 hybrid		Unknown
C11	CML495 x CKL05015	F_1 hybrid		Unknown
C12	CML495 x CML390	F_1 hybrid		Unknown
C13	CML495 x CML444	F_1 hybrid		Unknown
C14	CML495 x R119W	F_1 hybrid		Unknown
C15	R119W x CML495	F_1 hybrid		Unknown
C16	R119W x CKL05015	F_1 hybrid		Unknown
C17	R119W x CML390	F_1 hybrid		Unknown
C18	R119W x CML444	F_1 hybrid		Unknown

[1] ARC-GCI = Agricultural Research Council – Grain Crops Institute; CIMMYT = International Maize and Wheat Improvement Centre.

[2] According to Dr Makumbi (CIMMYT, personal communication), Small *et al*. (2012) and Rose *et al*. (2016).

AER = Aspergillus ear rot.

FER = Fusarium ear rot.

Table 2. Analysis of Variance (ANOVA) for resistance to Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation and fumonisin contamination in 23 maize genotypes tested in Makhatini, Potchefstroom and Vaalharts during the 2014/15 maize-growing season.

		FER severity (%)[1]				***F. verticillioides* colonisation (ng µL^{-1})[2]**				**Fumonisins concentration (mg kg^{-1})[3]**			
Source	DF	SS	MS	F-value	Pr>F	SS	MS	F-value	Pr>F	SS	MS	F-value	Pr>F
Environment (E)	2	68.553	34.276	51.02	<.0001	0.0233	0.012	25.46	<.0001	240.311	120.156	177.16	<.0001
Rep (environment)	6	5.069	0.845	1.26	0.2814	0.004	0.001	1.32	0.2509	4.756	0.793	1.17	0.3269
Genotype (G)	22	62.546	2.843	4.23	<.0001	0.034	0.0016	3.40	<.0001	49.650	2.257	3.33	<.0001
GEI	44	47.701	1.084	1.61	0.0201	0.032	0.0007	1.59	0.0237	60.067	1.365	2.01	0.0013
Error	133	88.684	0.672	-	-	0.060	0.0005	-	-	89.527	0.678	-	-

[1] Percentage of maize ear exhibiting visual symptoms of Fusarium ear rot (FER); mean of disease severity (%) for three experimental replicates

[2] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replicates.

[3] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replicates.

GEI = genotype x environment interaction

Table 3. Genotype means for Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation and fumonisin contamination in maize evaluated in Makhatini, Potchefstroom and Vaalharts during the 2014/15 maize-growing season.

		Makhatini			Potchefstroom			Vaalharts		
Code	Genotype	FER severity (%)[123]	*F. verticillioides* colonisation (ng µL^{-1})[24]	Fumonisins concentration (mg kg^{-1}) [125]	FER severity (%)[123]	*F. verticillioides* colonisation (ng µL^{-1})[24]	Fumonisins concentration (mg kg^{-1}) [125]	FER severity (%)[123]	*F. verticillioides* colonisation (ng µL^{-1})[24]	Fumonisins concentration (mg kg^{-1})[125]
L1	CKL05015	11.6 b-g	0.064 a-c	6.199 a	4.0 a-e	0.011 c	0.104 cd	7.8 d-g	0.087 a	4.536 ab
L2	CML390	24.6 a-c	0.063 a-c	5.786 a-c	12.2 a	0.025 a-c	1.352 a	30.3 a	0.082 a	6.470 a
L3	CML444	31.6 a	0.057 a-d	3.759 a-e	4.9 a-d	0.009 c	0.573 b-d	17.8 ab	0.026 bc	0.618 c-g
L4	CML495	9.7 b-g	0.025 c-f	5.725 a-c	8.4 ab	0.009 c	0.029 d	19.0 ab	0.024 bc	1.554 b-e
L5	R119W	22.6 a-d	0.074 ab	5.627 a-d	1.4 c-e	0.012 c	1.010 a-c	9.8 b-g	0.022 bc	0.924 c-g
C1	CKL05015 x CML390	8.0 e-g	0.027 c-f	3.174 de	4.3 a-e	0.013 c	0.330 b-d	3.7 gh	0.009 bc	0.581 c-g
C2	CKL05015x CML444	9.4 c-g	0.039 a-f	3.271 c-e	5.0 a-e	ND	ND	2.4 h	ND	0.083 fg
C3	CKL05015 x CML495	6.4 e-g	0.020 d-f	2.751 e	0.3 f	0.007 c	0.087 cd	11.4 b-f	0.009 bc	0.661 c-g
C4	CKL05015 x R119W	11.6 b-g	0.026 c-f	2.757 e	2.5 b-e	ND	ND	5.2 f-h	0.012 bc	0.284 d-g
C5	CML390 x CKL05015	6.9 e-g	0.036 a-f	3.365 b-e	1.7 c-e	0.014 bc	0.483 a-d	4.9 e-g	0.012 bc	0.706 c-g
C6	CML390 x CML444	16.0 a-g	0.032 b-f	3.754 a-e	6.6 a-c	0.038 a	1.071 ab	9.0 b-g	0.032 bc	1.362 b-e
C7	CML390 x R119W	27.9 ab	0.076 a	6.103 ab	8.5 a	0.021 a-c	0.327 b-d	15.4 a-d	0.018 bc	1.159 b-f
C8	CML444 x CKL05015	14.2 a-f	0.055 a-e	4.132 a-e	1.2 d-f	0.008 c	0.322 b-d	8.5 b-g	ND	0.071 g
C9	CML444 x CML495	4.6 g	0.013 ef	2.587 e	4.2 a-e	0.013 bc	ND	16.4 a-c	0.007 c	0.443 c-g
C10	CML444 x R119W	8.9 b-g	0.032 b-f	3.658 a-e	3.9 a-e	0.019 a-c	0.649 a-d	12.4 b-e	0.022 bc	1.740 b-d
C11	CML495 x CKL05015	8.2 fg	0.018 d-f	3.566 b-e	4.7 a-d	ND	ND	7.2 c-g	ND	0.196 e-g
C12	CML495 x CML390	5.1 fg	0.011 f	2.433 e	2.0 b-e	0.007 c	0.027 d	15.3 b-e	0.007 c	0.325 d-g
C13	CML495 x CML444	9.1 b-g	0.024 c-f	2.884 e	4.3 a-d	ND	0.114 cd	13.2 b-f	0.012 bc	1.533 d-d
C14	CML495 x R119W	7.9 d-g	0.014 ef	2.568 e	1.5 c-e	ND	ND	18.0 a-c	0.045 b	2.052 b-d
C15	R119W x CML495	17.8 a-e	0.055 a-e	6.281 a-d	5.0 a-d	ND	ND	9.2 b-g	0.022 bc	0.548 c-g
C16	R119W x CKL05015	4.0 g	0.036 a-f	3.642 a-e	1.2 ef	0.008 c	0.097 cd	3.8 gh	ND	0.137 fg
C17	R119W x CML390	14.0 a-f	0.0214 c-f	2.831 e	8.4 a	0.035 ab	0.671 a-d	9.2 b-g	0.018 bc	1.377 b-e
C18	R119W x CML444	12. 6 a-g	0.043 a-f	3.665 a-e	5.5 a-d	0.006 c	0.105 cd	16.1 a-c	ND	0.195 e-g
	Mean	**12.7a**	**0.038a**	**3.936a**	**4.4b**	**0.012c**	**0.322c**	**11.6a**	**0.021b**	**1.198b**

[1] Significant letters from balanced log transformed data and for each variable. ND = Not detected.

[2] Means followed by the same letter do not differ significantly according to Fisher's least significant difference test ($P \leq 0.05$).

[3] Percentage of maize ear exhibiting visual symptoms of Fusarium ear rot; mean of disease severity (%) for three experimental replicates.

[4] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replicates.

[5] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replicates

Table 4. Analysis of Variance for Additive Main Effects and Multiplicative Interaction analysis of resistance to Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation and fumonisin contamination in 23 genotypes evaluated in Makhatini, Potchefstroom and Vaalharts during the 2014/15 maize-growing season.

		FER severity (%)[1]				***F. verticillioides* colonisation (ng µL^{-1})[2]**				**Fumonisins concentration (mg kg^{-1})[3]**			
Source	DF	SS	MS	Variation explained (%)	Pr>F	SS	MS	Variation explained (%)	Pr>F	SS	MS	Variation explained (%)	Pr>F
Total	206	217.25	1.055	-	-	0.15346	0.000745	-	-	99.79	0.484	-	-
Treatments	68	148.56	2.185	-	<0.001	0.08945	0.001316	-	<0.001	84.83	1.247	-	<0.001
Genotypes	22	53.57	2.435	24.67	<0.001	0.03420	0.001554	22.29	<0.001	12.23	0.556	12.26	<0.001
Environments	2	57.47	28.733	26.45	<0.001	0.02329	0.011647	15.18	<0.001	64.12	32.062	64.25	<0.001
Block	6	2.29	0.382	-	0.6025	0.00363	0.000605	-	0.2509	0.48	0.079	-	0.6327
Interactions	44	37.53	0.853	17.28	0.0117	0.03196	0.000726	20.83	0.0237	8.47	0.193	8.49	0.0079
IPCA 1	23	21.39	0.930	9.85	0.0167	0.01809	0.000787	11.79	0.0306	6.10	0.265	6.11	<0.001
IPCA 2	21	16.14	0.769	7.43	0.0783	0.01387	0.000660	9.04	0.1093	2.38	0.113	2.39	0.4321
Residuals	0	0.00	-	-	-	0.00000	-	-	-	0.00	-	-	-
Error	132	66.39	0.503	-	-	0.06037	0.000457	-	-	14.49	0.110	-	-

[1] Percentage of maize ear exhibiting visual symptoms of FER; mean of disease severity (%) for three experimental replicates.

[2] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replicates.

[3] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replicates.

Treatments = sum of genotype, environment effects and their interaction as sources of variation.

Interactions = genotype x environment interaction.

IPCA: interaction principal component analysis.

Table 5. Additive Main Effects and Multiplicative Interaction stability values (ASV) and interaction principal component analysis (IPCA) values of Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation and fumonisin contamination in maize evaluated in Makhatini, Potchefstroom and Vaalharts during the 2014/15 maize-growing season.

		FER severity (%)[1]				***F. verticillioides* colonisation (ng µL^{-1})**[2]				**Fumonisins concentration (mg kg^{-1})**[3]			
Code	Line/cross	Rank	ASV	IPCA1	IPCA2	Rank	ASV	IPCA1	IPCA2	Rank	ASV	IPCA1	IPCA2
L1	CKL05015	8	0.3015	-0.22574	0.0374	23	0.20178	0.11773	-0.13085	21	1.1236	-0.40085	-0.45183
L2	CML390	5	0.2709	0.20026	-0.05444	21	0.15462	0.10078	-0.08134	23	1.615	-0.62797	0.10635
L3	CML444	11	0.4329	-0.01092	0.43264	11	0.06357	-0.02848	-0.05159	9	0.382	0.14183	0.11579
L4	CML495	13	0.5249	0.19169	-0.45929	8	0.05295	0.03961	0.01154	11	0.4704	-0.0847	-0.41722
L5	R119W	20	0.7398	-0.01411	0.73957	19	0.12942	-0.08203	-0.07276	13	0.5427	0.20972	0.06956
C1	CKL05015 x CML390	18	0.5948	-0.42617	-0.18666	6	0.03733	-0.00842	0.03568	6	0.258	0.0831	0.14513
C2	CKL05015 x CML444	23	1.1318	-0.85384	-0.02549	12	0.06573	-0.04963	-0.01132	17	0.6494	0.25229	-0.04974
C3	CKL05015 x CML495	22	0.9351	0.64369	0.38302	4	0.03433	0.0093	0.03212	2	0.0902	-0.0245	0.06471
C4	CKL05015 x R119W	12	0.4381	-0.29498	0.19771	1	0.0068	0.00495	0.00211	5	0.2485	0.09641	0.02335
C5	CML390 x CKL05015	2	0.1423	0.0126	0.14131	7	0.03746	-0.02415	0.02026	7	0.2668	0.0693	0.1989
C6C	CML390 x CML444	10	0.3855	-0.27214	-0.1362	16	0.08784	0.03301	0.07656	8	0.295	-0.05113	0.26418
C7	CML390 x R119W	6	0.2757	-0.20308	0.05981	20	0.13748	-0.1003	-0.04214	4	0.248	0.04931	-0.21333
C8	CML444 x CKL05015	15	0.5583	0.08108	0.54784	18	0.1141	-0.08532	-0.02505	20	1.0221	0.39792	0.04289
C9	CML444 x CML495	21	0.8773	0.4993	-0.57605	14	0.07317	0.02027	0.06822	1	0.0523	-0.00562	0.05031
C10	CML444 x R119W	4	0.2328	0.13921	-0.14204	5	0.03679	0.01244	0.03301	10	0.4251	-0.15337	0.16052
C11	CML495 x CKL05015	14	0.555	-0.12327	-0.53041	3	0.03238	-0.00222	0.03225	12	0.5023	0.19416	-0.06377
C12	CML495 x CML390	17	0.5855	0.41442	-0.20306	10	0.06137	0.02493	0.05204	3	0.1541	0.04699	0.09586
C13	CML495 x CML444	3	0.2121	0.08453	-0.18014	2	0.01868	0.00862	0.01492	19	0.7643	-0.29772	0.01854
C14	CML495 x R119W	19	0.734	0.5492	0.09552	22	0.16023	0.12266	-0.00804	22	1.1306	-0.44054	-0.01348
C15	R119W x CML495	9	0.3226	-0.21301	0.15618	15	0.0769	-0.0364	-0.06049	16	0.6275	0.19505	-0.37836
C16	R119W x CKL05015	1	0.0164	0.00404	0.01554	9	0.05772	-0.04361	0.00976	18	0.7249	0.2817	-0.05405
C17	R119W x CML390	16	0.5766	-0.3927	-0.24841	17	0.10995	0.02034	0.1067	14	0.5489	-0.17163	0.32761
C18	R119W x CML444	7	0.2855	0.20992	-0.06435	13	0.07152	-0.05409	-0.0116	15	0.618	0.24027	-0.0419

[1] Percentage of maize ear exhibiting visual symptoms of FER; mean of disease severity (%) for three experimental replicates.

[2] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replicates.

[3] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replicates.

Table 6. Pearson's correlation coefficients between Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation and fumonisin contamination.

Variables		**Makhatini (*r*=)**	**Potchefstroom (*r*=)**	**Vaalharts (*r*=)**	**Overall (*r*=)**
FER severity	*F. verticillioides* colonisation	0.49	0.45	0.33	0.47
FER severity	Fumonisin contamination	0.48	0.36	0.46	0.53
F. verticillioides colonisation	Fumonisin contamination	0.73	0.77	0.79	0.71

All values are significant at $P < 0.05$.

Table 7. Analysis of Variance for combining ability of Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation and fumonisin concentration in maize genotypes across three environments.

		FER severity (%)[1]		***F. verticillioides* colonisation (ng µL^{-1})[2]**		**Fumonisin concentration (mg kg^{-1})[3]**	
Contrast	DF	Mean Square	Pr > F	Mean Square	Pr > F	Mean Square	Pr > F
GCA	4	6.17896207	<.0001	0.00271609	0.0002	0.92157280	<.0001
SCA	10	2.44786055	<.0001	0.00248326	<.0001	0.86360933	<.0001
GCA x ENV	4	1.10060167	0.0797	0.00107121	0.0541	0.28645039	0.0347
SCA x ENV	10	0.57177382	0.3601	0.00063384	0.1812	0.06287781	0.8236
REC	10	0.51477062	0.4485	0.00016846	0.9558	0.09992513	0.5068
REC*ENV	10	0.64095665	0.2693	0.00057350	0.2492	0.08453209	0.6407
MAT	4	0.07345459	0.9661	0.00017255	0.8199	0.07779191	0.5764
NONM	6	0.80898130	0.1607	0.00016574	0.8978	0.11468062	0.3843
MAT x ENV	4	0.19490673	0.8243	0.00029903	0.6170	0.03528270	0.8583
NONM x ENV	6	0.93832327	0.0994	0.00075648	0.1291	0.11736502	0.3692
Error	144	0.5160445	-	0.00044942	-	0.1073355	-
GCA:SCA	-	0.83	-	0.69	-	0.68	-
H^2	-	0.97	-	0.95	-	0.96	-
h^2	-	0.81	-	0.65	-	0.65	-

[1] Percentage of maize ear exhibiting visual symptoms of FER; mean of disease severity (%) for three experimental replicates.

[2] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replicates.

[3] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replicates.

GCA = general combining ability; SCA = specific combining ability; REC = reciprocal effects; MAT = maternal effects.
NONM = non-maternal effects; H^2 = broad sense heritability; h^2 = narrow sense heritability.

Table 8. General combining ability estimates for Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation and fumonisin concentration in five maize inbred lines across three environments.

	FER severity (%)[1]		***F. verticillioides* colonisation (ng µL^{-1})[2]**		**Fumonisin concentration (mg kg^{-1})[3]**	
Genotype	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|
CKL05015	-0.43825717	<.0001	0.00005556	0.9779	-0.06218293	0.0460
CML390	0.23569671	0.0007	-0.00648889	0.0015	0.16475353	<.0001
CML444	0.14931375	0.0291	-0.00001111	0.9956	-0.02461513	0.4268
CML495	-0.01391088	0.8376	0.00860000	<.0001	-0.09512004	0.0025
R119W	0.06715758	0.3231	-0.00215556	0.2826	0.01716457	0.5793

[1] Percentage of maize ear exhibiting visual symptoms of FER; mean of disease severity (%) for three experimental replicates.
[2] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replicates.
[3] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replicates.

Table 9. Specific combining ability estimates for Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation and fumonisin concentration in 18 F_1 maize hybrids across three environments.

		FER severity (%)[1]		***F. verticillioides* colonisation (ng µL^{-1})[2]**		**Fumonisin concentration (mg kg^{-1})[3]**	
Code	Genotype	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|
	SCA estimates						
C1	CKL05015 x CML390	-0.30153415	0.0325	0.01112222	0.0078	-0.13658619	0.0336
C2	CKL05015 x CML444	-0.13842556	0.3231	0.00475556	0.2504	-0.11684240	0.0686
C3	CKL05015 x CML495	0.02785956	0.8421	0.00464444	0.2615	-0.04509124	0.4800
C4	CKL05015 x R119W	-0.30307953	0.0316	-0.01045556	0.0122	-0.18308341	0.0047
C6	CML390 x CML444	-0.23725119	0.0914	-0.00414444	0.3162	0.07494402	0.2412
C7	CML390 x R119W	0.32919865	0.0197	0.00022222	0.9571	-0.03872775	0.5440
C9	CML444 x CML495	-0.12003781	0.3914	0.00254444	0.5379	-0.00088434	0.9889
C10	CML444 x R119W	0.00761048	0.9566	0.00446667	0.2802	0.00293091	0.9634
C14	CML495 x R119W	0.03655492	0.7938	-0.00703333	0.0900	0.05752116	0.3679
	Reciprocal estimates						
C5	CML390 x CKL05015	0.15856706	0.3506	0.00222222	0.6572	-0.03847546	0.6191
C8	CML444 x CKL05015	-0.19519696	0.2509	0.00377778	0.4509	-0.06780719	0.3814
C11	CML495 x CKL05015	-0.15786915	0.3527	-0.00205556	0.6814	0.03169923	0.6820
C16	R119W x CKL05015	0.31379407	0.0659	0.00122222	0.8071	-0.02702260	0.7269
C12	CML495 x CML390	0.00000000	1.0000	0.00000000	1.0000	0.00000000	1.0000
C17	R119W x CML390	0.20713375	0.2232	-0.00677778	0.1771	0.05202445	0.5016
C13	CML495 x CML444	-0.11424343	0.5009	0.00111111	0.8243	-0.10895679	0.1604
C18	R119W x CML444	-0.11912613	0.4828	-0.00327778	0.5129	0.18199157	0.0198
C15	R119W x CML495	-0.17088576	0.3145	0.00327778	0.5129	-0.00128999	0.9867

[1] Percentage of maize ear exhibiting visual symptoms of FER; mean of disease severity (%) for three experimental replicates.

[2] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replicates.

[3] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replicates.

Table 10. Maximum temperature, relative humidity and total rainfall across three environments during the 2014/15 maize-growing season.

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Temperature (°C)						
Makhatini[1,2]	26.79 b	28.74 b	30.84 b	27.60 b	28.67 a	32.02 a
Potchefstroom[1,2]	29.24 b	30.17 b	31.06 b	27.71 b	25.89 b	26.21 b
Vaalharts[1,2]	33.86 a	36.16 a	35.23 a	32.27 a	28.46 a	29.37 a
Relative humidity (%)						
Makhatini	93.41 a	92.85 a	90.66 a	89.30 a	89.98 a	90.38 a
Potchefstroom	85.58 b	87.68 b	85.85 b	89.81 a	87.75 a	78.19 b
Vaalharts	85.04 b	78.19 c	75.92 c	82.18 b	83.10 b	73.63 c
Rain (mm)						
Makhatini	0.20 b	8.50 c	9.10 b	22.20 c	65.60 a	65.80 a
Potchefstroom	114.55 a	139.19 a	55.63 a	104.65 a	28.96 b	0.76 b
Vaalharts	109.47 a	35.05 b	19.05 b	50.29 b	14.73 c	2.03 b

[1] Planting period: Makhatini: July 2014-January 2015; Potchefstroom and Vaalharts: December 2014-June 2015.
[2] Means followed by the same letter do not differ significantly according to Fisher's least significant difference test ($P < 0.05$).

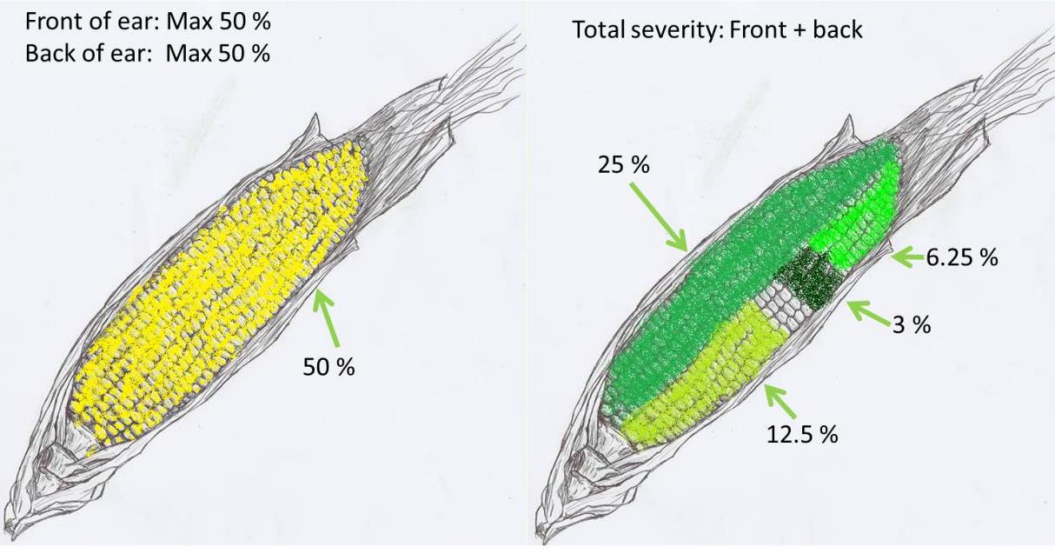

Figure 1. Visual rating scale used for Fusarium ear rot disease assessment as described by Small *et al.* (2012) (Courtesy of M. Vermeulen).

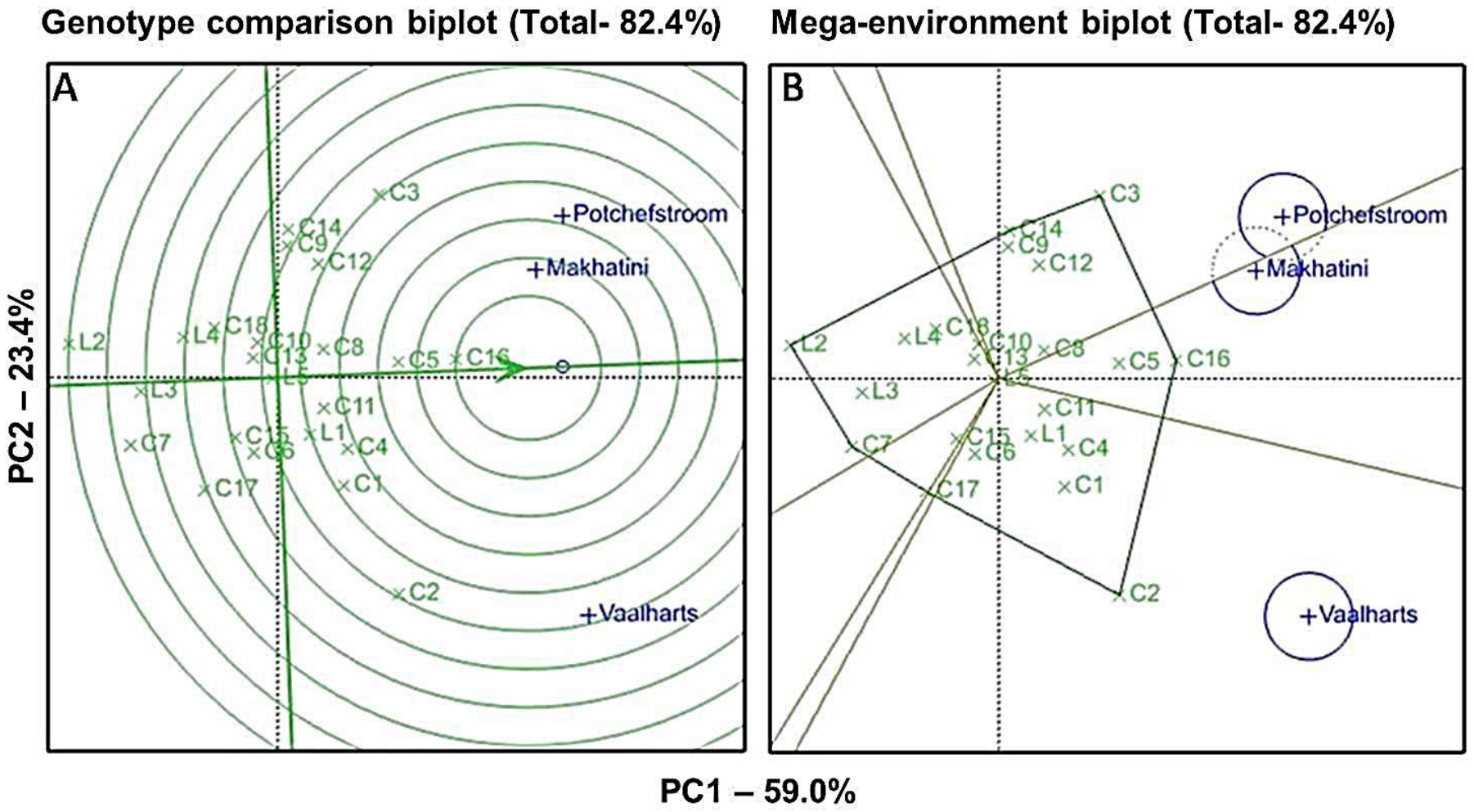

Figure 2. Genotype and genotype x environment (GGE) biplot showing Fusarium ear rot severity of five parental maize inbred lines and 18 F_1 hybrids evaluated in three environments in South Africa during the 2014/15 maize growing seasons. L1: CKL05015; L2: CML390; L3: CML444; L4: CML495; L5: R119W; C1: CKL05015 x CML390; C2: CKL05015 x CML444; C3: CKL05015 x CML495; C4: CKL05015 x R119W; C5: CML390 x CKL05015; C6: CML390 x CML444; C7: CML390 x R119W; C8: CML444 x CKL05015; C9: CML444 x CML495; C10: CML444 x R119W; C11: CML495 x CKL05015; C12: CML495 x CML390; C13: CML495 x CML444; C14: CML495 x R119W; C15: R119W x CML495; C16: R119W x CKL05015; C17: R119W x CML390; C18: R119W x CML444.

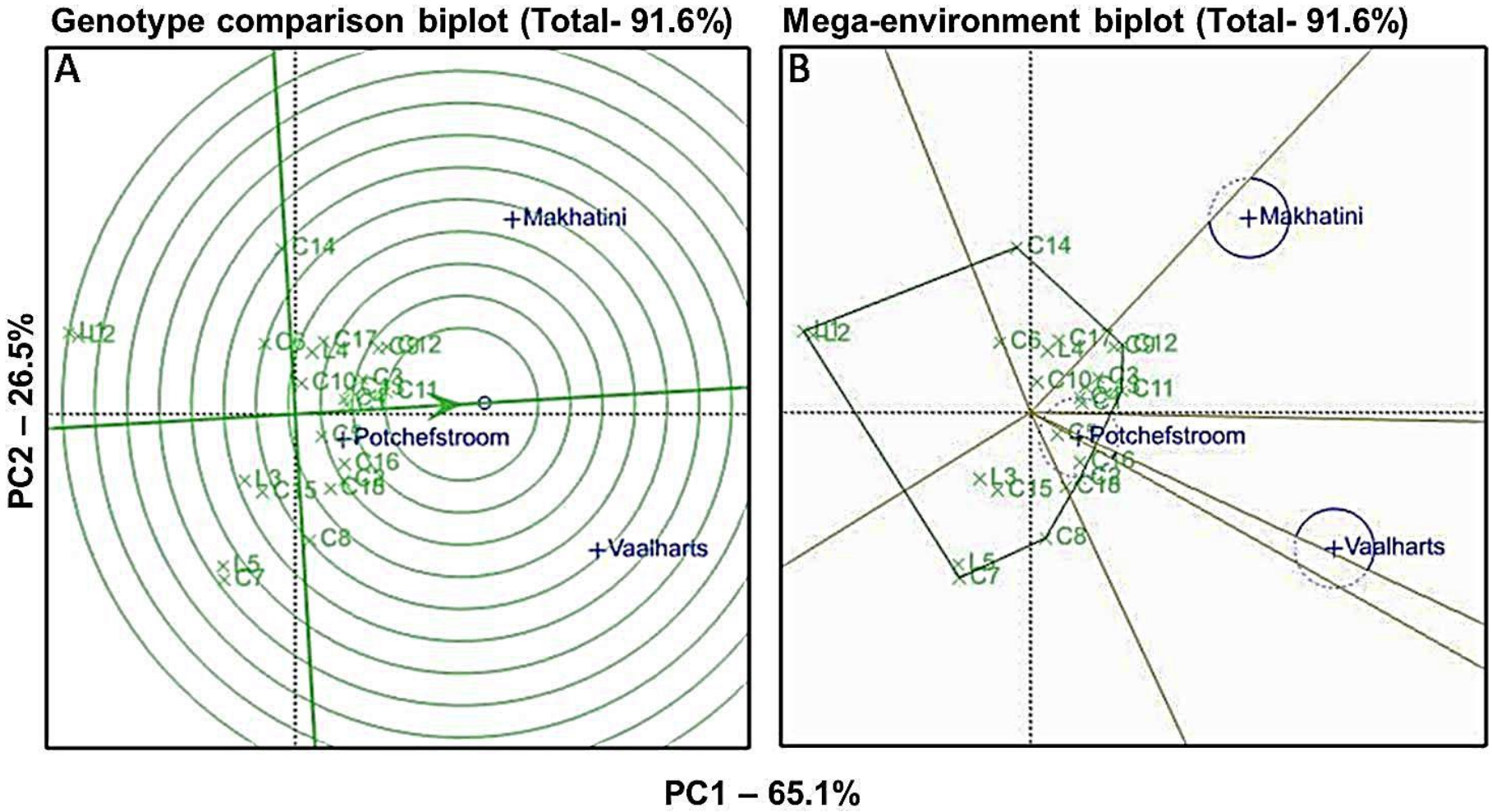

Figure 3. Genotype and genotype x environment (GGE) biplot showing *Fusarium verticillioides* colonisation of five parental maize inbred lines and 18 F_1 hybrids evaluated in three environments in South Africa during the 2014/15 maize growing seasons. L1: CKL05015; L2: CML390; L3: CML444; L4: CML495; L5: R119W; C1: CKL05015 x CML390; C2: CKL05015 x CML444; C3: CKL05015 x CML495; C4: CKL05015 x R119W; C5: CML390 x CKL05015; C6: CML390 x CML444; C7: CML390 x R119W; C8: CML444 x CKL05015; C9: CML444 x CML495; C10: CML444 x R119W; C11: CML495 x CKL05015; C12: CML495 x CML390; C13: CML495 x CML444; C14: CML495 x R119W; C15: R119W x CML495; C16: R119W x CKL05015; C17: R119W x CML390; C18: R119W x CML444.

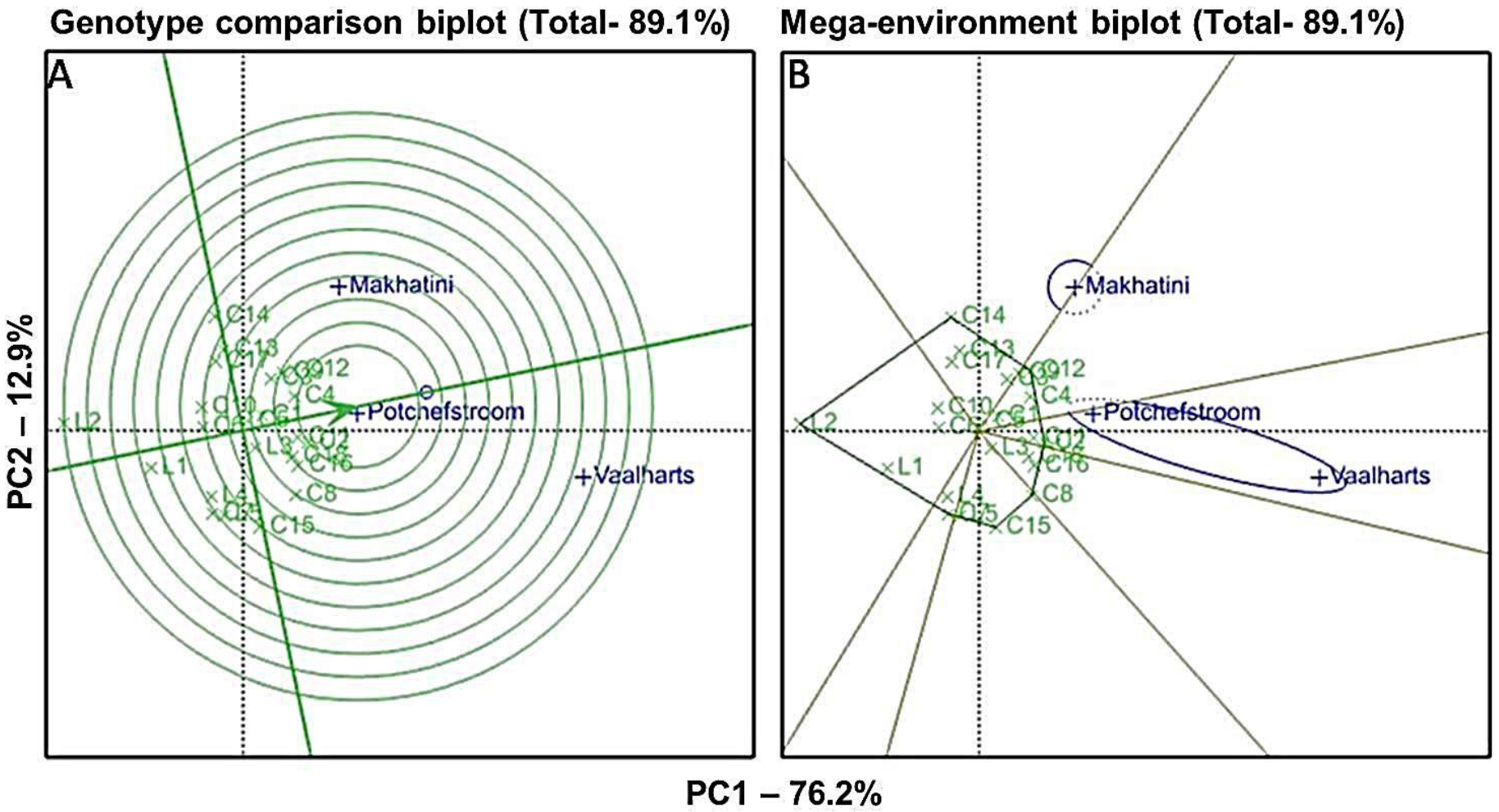

Figure 4. Genotype and genotype x environment (GGE) biplot showing fumonisin concentrations of five parental maize inbred lines and 18 F_1 hybrids evaluated in three environments in South Africa during the 2014/15 maize growing seasons. L1: CKL05015; L2: CML390; L3: CML444; L4: CML495; L5: R119W; C1: CKL05015 x CML390; C2: CKL05015 x CML444; C3: CKL05015 x CML495; C4: CKL05015 x R119W; C5: CML390 x CKL05015; C6: CML390 x CML444; C7: CML390 x R119W; C8: CML444 x CKL05015; C9: CML444 x CML495; C10: CML444 x R119W; C11: CML495 x CKL05015; C12: CML495 x CML390; C13: CML495 x CML444; C14: CML495 x R119W; C15: R119W x CML495; C16: R119W x CKL05015; C17: R119W x CML390; C18: R119W x CML444.

CHAPTER 3

Resistance to Fusarium ear rot and fumonisin contamination of maize F_1 hybrids and F_2 populations in Kenya

ABSTRACT

Maize is an important food crop and the main source of carbohydrates in eastern and southern Africa. Several fungal species, including *Fusarium verticillioides*, threaten production of the crop in these regions. *Fusarium verticillioides* causes Fusarium ear rot (FER) of maize that may result in low grain quality, yield reductions and contamination of grain with fumonisins. Breeding for resistance to FER and fumonisin contamination is the most affordable, effective and environmentally sound strategy to manage *F. verticillioides*. The aim of this study was to evaluate F_1 hybrids and F_2 breeding populations at two localities in Kenya for improved resistance to FER and fumonisin contamination. Trial plants were inoculated artificially and FER severity, *F. verticillioides* accumulation and fumonisin contamination determined. Inheritance of resistance was also determined in the F_1 hybrids. Hybrids P502C2 x CKL05015 and R119W x MIRTC5 were most affected by FER, with a disease incidence of 2.52% and 2.94%, respectively. Fungal colonisation was lowest in hybrids CKL05015 x CML495 (0.007 ng μL^{-1}) and MIRTC5 x CML495 (0.008 ng μL^{-1}), while fumonisin concentrations were lowest in hybrids CML444 x MIRTC5 (0.083 mg kg^{-1}) and R119W x CKL05015 (0.092 mg kg^{-1}). General and specific combining abilities were significant for inheritance of resistance to *F. verticillioides* colonisation, fumonisin contamination and 1000-kernel mass. Inbred lines CML495, CKL05015 and P502C2 had negative and significant general combining ability (GCA) estimates for *F. verticillioides* colonisation and fumonisin contamination, but positive and significant GCA estimates for 1000-kernel weight, respectively. The hybrid most resistant to *F. verticillioides* colonisation and fumonisin contamination was CML444 x MIRTC5. Genotype by environment interaction was the main source of variation observed in the F_2 populations. Location means for *F. verticillioides* colonisation and fumonisin contamination differed significantly, with maize grown at Katumani more severely contaminated than that grown at Kiboko. Resistant F_2 populations identified in this study can be used to produce recombinant inbred lines to utilise in genetic fingerprinting and mapping of resistant genes.

INTRODUCTION

Africa is the biggest consumer of maize in the world, consuming up to 30% of annual global production (Awika *et al*., 2011). The cereal serves as the main source of carbohydrates in eastern and southern Africa (Macauley, 2015), with annual consumption ranging from 90 - 180 kg per person (Shephard *et al*., 2007; Awika *et al*., 2011; Ecker and Qaim, 2011). Average yield in eastern and southern Africa is 1.5 t/ha and 1.1 t/ha, respectively, which is much lower than the average global yield of 5 t/ha (Smale *et al*., 2011). Maize production is affected by several biotic stresses, such as mycotoxigenic fungi, which threaten food security and safety in the region. Mycotoxigenic fungi are associated with maize ear rots and include *Aspergillus flavus* Link ex Fries, which causes Aspergillus ear rot (AER), and *Fusarium verticillioides* (Sacc.) Nirenberg, which causes Fusarium ear rot (FER) (Doko *et al*., 1996; Afolabi *et al*., 2007; Barros *et al*., 2008; Janse van Rensburg and Flett, 2010; Snyman *et al*., 2011). *Aspergillus flavus* further deposits toxic aflatoxins in maize grain, while *F. verticillioides* contaminates grain with fumonisins. Of the two, aflatoxin is considered the more important mycotoxin in eastern Africa, where it has resulted in the mortality of people due to acute poisoning (Muture and Ogana, 2005; Mwanda *et al.,* 2005; Probst *et al.,* 2007).

The importance of *F. verticillioides* as a mycotoxigenic fungus in eastern Africa is often underestimated. Infection by *F. verticillioides* can result in yield losses, poor grain quality and decreased nutritive value in addition to the production of fumonisins. The mycotoxin has been associated with oesophageal cancer and birth defects in humans, and with lethal animal diseases such as porcine pulmonary oedema syndrome and equine leukoencephalomalacia (Harrison *et al*., 1990; Rheeder *et al*., 2002; Marasas *et al*., 2004). Alarming levels of fumonisins have been detected in eastern and southern African maize. In a random sampling study across eastern and southern African countries, Doko *et al*. (1996) detected fumonisins in 93% of maize samples. Prolonged exposure to fumonisins has also been linked to growth retardation of infants in Tanzania (Kimanya *et al*., 2010). In western Kenya, fumonisin levels in over 50% of the 985 maize samples collected were above the allowed by the European Union (1 mg kg^{-1}) (Mutiga *et al*., 2015). In an assessment of locally brewed beer made from maize and millet, Kurui *et al*. (2014) found fumonisin concentrations ranging from 0.2 – 4 mg kg^{-1}. In another study, Alakonya *et al*. (2009) detected fumonisin concentrations of up to 4.4 mg kg^{-1} in symptomless grain and over 5 mg kg^{-1} in rotten grain sampled from the Western province of Kenya. Rotten grain in this region is often used in brewing, as feed for livestock, and is milled with symptomless grain into flour during maize paucity (Alakonya *et al*., 2009). The high levels of fumonisins detected in Kenya are concerning, the country has become the epitome of aflatoxicosis due to recurring incidents over the years.

Reducing mycotoxin accumulation in grain can prevent mycotoxicosis of both humans and livestock. Common agricultural practices; such as the application of herbicides to eliminate competition with weeds for nutrients, irrigation to lessen drought stress, insecticides to minimise insect damage and fertilizers to ensure adequate nutrients are supplied; can decrease fumonisin contamination in grain (Jones *et al*., 1980; Cole *et al*., 1985; Miller, 2001; Munkvold, 2003; Blandino *et al*., 2008; Mukanga *et al*., 2011). Alakonya (2011) and Olubandwa *et al*. (2011), however, indicated that small-scale farmers are not aware of the dangers associated with consuming mouldy grain and do not have sufficient knowledge of enhanced agricultural practices and the technical experience to reduce mycotoxin production in maize. Furthermore, subsistence farmers in developing countries barely have enough income to implement management strategies to minimize mycotoxin contamination (Olubandwa *et al*., 2011; Small *et al*., 2012). This is especially worrisome in a country such as Kenya, where small-scale farmers produce more than 70% of all the maize in the country (Government of Kenya, 2008; Tegemeo Institute and East African Grain Council, 2009; Oluoch-Kosura, 2011). The most affordable and practical means to reduce FER and fumonisins contamination of maize in Kenya, therefore, is the planting of *F. verticillioides*-resistant genotypes (Harrison *et al*., 1990; Munkvold and Desjardins, 1997; Schjøth *et al.*, 2008).

The development of hybrids resistant to *Aspergillus flavus*, *F. verticillioides* and their mycotoxins is required to mitigate mycotoxin contamination of maize grain in eastern and southern Africa. Robertson-Hoyt *et al.* (2007a) have identified several overlapping quantitative trait loci (QTLs) in the maize genome that affect AER, FER, aflatoxin and fumonisin contamination, and Wisser *et al.* (2006) found that QTLs that account for various disease resistances in the maize genome occur in clusters. *Fusarium* spp. can also reduce *A. flavus* growth, which in turn results in reduced aflatoxins (Zummo and Scott, 1992) because both fungi interact with the host in similar ways (Robertson-Hoyt *et al.,* 2007a).

A number of inbred lines were evaluated for resistance to AER/aflatoxin and FER/fumonisin contamination in South Africa and Kenya before (Dr Makumbi, CIMMYT, personal communication; Rose *et al*., 2016; Rose *et al*., 2017). In this study, F_1 hybrids and F_2 populations derived from FER/fumonisin-characterised and AER-resistant inbred lines will be evaluated for resistance to *F. verticillioides* and fumonisin accumulation under Kenyan climatic conditions. The inheritance of kernel weight, resistance to *F. verticillioides* and fumonisin accumulation was also investigated using Griffing's diallel mating system.

MATERIALS AND METHODS

Planting material and field sites

Two maize inbred lines characterised, as resistant (CML444) and susceptible (R119W) to FER/fumonisin contamination (Small *et al*., 2012; Rose *et al*., 2016), and four inbred lines (CKL05015, CML495, MIRTC5, and P502) characterised as resistant to FER/fumonisin contamination and AER (Rose *et al*., 2017; Dr Makumbi, CIMMYT, personal communication), were used in this study (Table 1). These inbred lines were cross-pollinated including reciprocal crosses, using the diallel mating system, during the 2013 maize-growing season. Subsequently, 30 F_1 hybrids were planted in Kiboko, Kenya (grid reference: 2˚15' S, 37˚75' E; altitude 975 m.a.s.l.) together with their parental inbred lines (Table 1) in 2014. The F_1 hybrids were then self-pollinated to create 27 F_2 populations that were planted with three inbred lines (CKL05015, MIRTC5 and R119W) in Katumani (grid reference: 1˚35'S, 37˚14'E; altitude 1600 m.a.s.l.) and Kiboko during the 2014/15 season (Table 1). Kiboko and Katumani are in the semi-arid counties of Makueni and Machakos, respectively, in eastern Kenya. Katumani has a warm and dry climate, while Kiboko is hot and wet.

Standard agricultural practices were followed for both trials. Maize seeds were manually planted with two seeds per hole, and seedlings were thinned to one, 3 weeks after emergence. Thirty-three plants of each genotype were planted in a 10-m row, which represented a plot. Each plot had three replicates. Therefore, each F_1 and F_2 population consisted of approximately 100 individuals. The plots were 1 m apart, with an intra-row spacing of 0.3 m. The trials were planted in a randomised complete block design with the inbred lines and hybrids randomised separately. Drip irrigation was applied at all trials.

Inoculum preparation

Three *F. verticillioides* isolates (UONK005, UONK038 and UONK048); obtained from the University of Nairobi, School of Biological Sciences' culture collection; were used as inoculum. These isolates were initially isolated from severely infected grain in the Nandi district. *Fusarium verticillioides* hyphae, grown on potato dextrose agar (PDA) for 5 days, were used for spore production in 100 mL of Armstrong liquid medium (Booth, 1971). The inoculated medium was placed on an incubator shaker at 25°C shaking at 100 revolutions per minute (rpm). Isolates were kept separate during incubation period. After 5 days the suspension was filtered through two layers of autoclaved cheesecloth and centrifuged for 10 min at 3 500 relative centrifugal force (rcf). The supernatant was discarded and the conidia washed with autoclaved, de-ionized water. This was repeated twice, with a centrifuge step between washes. Conidia were then dissolved in 500 mL sterile distilled water and the spore suspension was adjusted to 1×10^6 conidia mL^{-1}. Three drops of Tween 20 (polyoxyethylene

20-sorbitan monolaurate) (Fisher Biotech, Fairlawn, NJ) were added per litre spore suspension.

Inoculation of maize ears and disease assessment

The primary ears of individual maize plants were artificially inoculated to ensure adequate disease pressure to discriminate between inbred lines and hybrids. This was done by injecting 2 mL of the 1×10^6 *F. verticillioides* spores down the silk channel using disposable syringes fitted with 21 G x 38 mm needles. Once the plants reached physiological maturity, the ears were hand harvested and their husks removed. FER severity was then determined according to the rating scale described by Small *et al*. (2012). The ears collected in each plot were thereafter shelled and bulked. F_2 populations were also bulked according to plot. A 250-g grain sample from each plot was then milled using a Husqvarna coarse steel grain grinder (Reliance, Sweden). Coarsely milled grain was further milled to flour using a Philips blender (400 W, 1.75 L). Two sub-samples were taken for DNA and fumonisin extractions.

DNA extractions and Quantification of *F. verticillioides* target DNA in maize grain

DNA was isolated from milled maize grain (2 g) and *F. verticillioides* mycelia as described by Boutigny *et al*. (2012) and the amount of *F. verticillioides* target DNA determined by qPCR. The slope of the standard curve used in this study was M = -3.34 while the correlation coefficient was R^2 = 0.99905. The efficiency of the reaction was 99% and the limit of detection was varied from 0.006 to 7.088 ng μL^{-1}. The reproducibility (inter-run variance) and repeatability (intra-run variance) of the qPCR was not investigated, as this was previously performed by Boutigny *et al*. (2012) under the same laboratory conditions using the same analytical equipment. The amount of *F. verticillioides* target DNA in a sample represented fungal colonisation (Adejumo *et al*., 2009; Ncube, *et al*., 2011; Janse van Rensburg *et al*., 2015; Rose *et al*., 2016).

Fumonisin analysis

Fumonisin B_1, B_2 and B_3 levels in 5-g samples of milled grain were determined by liquid chromatography tandem mass spectrometry (LCMS-MS) according to Rose *et al*. (2016) at the Central Analytical Facility, Stellenbosch University. The limits of detection were 0.02, 0.002, and 0.02 mg kg^{-1} for FB_1, FB_2 and FB_3, respectively.

Statistical analysis

Data from the three trials was statistically analysed using the Shapiro-Wilk test for normality (Shapiro and Wilk, 1965). The 1 000-kernel weight and *F. verticillioides* target DNA data was normally distributed, therefore no transformations were performed. Total fumonisins

concentration data, however, was log-transformed to achieve normality. The percentage FER severity was subjected to arcsin and log transformations. The Analysis of Variance (ANOVA) was performed for 1 000-kernel weight, FER severity, *F. verticillioides* colonisation and fumonisin contamination employing the generalized linear model (GLM) procedure of SAS statistical software version 9.2. (SAS Institute Inc., Cary, NC, USA). The Fisher's least significant difference (LSD) test was used at the 5% level to compare inbred lines and hybrids means for all the parameters evaluated. Significance was regarded to be true at a probability level of 5% ($P < 0.05$) for all tests. Pearson's correlation coefficients were determined to assess the relationships between FER severity, *F. verticillioides* colonisation and total fumonisins concentration using the CORR procedure in SAS.

Diallel analysis was performed using DiallelSAS05 (Zhang *et al*., 2004). This program uses a linear model to estimate combining abilities which allocates the total genotypic variance into variance components, i.e. general combining ability (GCA) and specific combining ability (SCA) as well as reciprocal, maternal and non-maternal effects (Zhang *et al*., 2004). Diallel analysis was performed according to method 1 and model 1 of Griffing (1956), which included parental inbred lines, their F_1 offspring and reciprocal hybrids. T-tests were used to determine the significance of these estimates. The heritability of the different traits was evaluated using Griffing's (1956) equations: broad sense heritability (H^2) = 2MSGCA+MSSCA/(2MSGCA+MSSCA+MSe) and narrow sense heritability (h^2) = 2MSGCA/(2MSGCA+MSSCA+MSe), where MSGCA is the GCA mean square, MSSCA is the SCA mean square and MSe is the mean square error value. Furthermore, the predominant gene action in the inheritance of a trait was determined by Baker's (1978) calculation of the GCA/SCA ratio of the mean squares: 2MSGCA/(2MSGCA+MSSCA), where MSGCA is the GCA mean square and MSSCA is the SCA mean square.

RESULTS

F_1 hybrids trial

A significant difference ($P < 0.05$) in *F. verticillioides* colonisation, fumonisin contamination and 1 000-kernel weight was found between genotypes in the F_1 hybrid trial, but not in FER severity ($P < 0.5258$) (Table 2). The replications had a significant effect on FER severity ($P < 0.05$), but not on *F. verticillioides* colonisation ($P = 0.2272$), fumonisin contamination ($P = 0.1624$) and 1000-kernel weight ($P = 0.1003$) (Table 2). Despite transformations, FER severity data could not reach a normal distribution. Of the genotypes evaluated, 22 out of 36 exhibited no FER symptoms (Table 3). The mean FER severity was 0.43%, and the highest percentage FER symptoms was 2.94% (Table 3). Only six genotypes exhibited FER symptoms above 1% namely; CML495, CKL05015 x R119W, CML495 x MIRTC5, CML495 x R119W, P502C2 x CKL05015 and R119W x MIRTC5 (Table 3).

Fusarium verticillioides target DNA in the different genotypes evaluated ranged from 0.007 to 0.093 ng μL^{-1}, with a mean of 0.02 ng μL^{-1} (Table 3). Inbred lines CML444 (0.093 ng μL^{-1}) and P502C2 (0.069 ng μL^{-1}) were most colonised by *F. verticillioides*, with significantly more target DNA than the other genotypes ($P < 0.05$) (Table 3). The hybrid CKL05015 x CML495 (0.007 ng μL^{-1}) and CML444 x CKL05015 (0.007 ng μL^{-1}) had the lowest fungal DNA content, which did not differ significantly from parental lines CKL05015 (0.024 ng μL^{-1}) and CML495 (0.017 ng μL^{-1}) (Table 3). None of the other hybrids differed significantly ($P < 0.05$) from the best performing hybrids CKL05015 x CML495 (0.007 ng μL^{-1}) and CML444 x CKL05015 (0.007 ng μL^{-1}) (Table 3).

The fumonisin concentrations quantified ranged from 0.083 to 2.25 mg kg^{-1} with a mean of 0.054 mg kg^{-1} (Table 3). The hybrid CML444 x MIRTC5 (0.083 mg kg^{-1}) accumulated the lowest concentration of fumonisins and it differed significantly from the worst performing genotypes which included CML444 (2.370 mg kg^{-1}), P502C2 (2.546 mg kg^{-1}) and R119W (1.522 mg kg^{-1}) (Table 3). The parental lines CKL05015, CML495 and MIRTC5 did not differ significantly from hybrid CML444 x MIRTC5 (Table 3). The inbred line P502C2 (2.546 mg kg^{-1}) had the highest levels of fumonisins but did not differ significantly from CML444 (2.370 mg kg^{-1}) and R119W (1.522 mg kg^{-1}) (Table 3). The hybrid P502C2 x CML444 (1.312 mg kg^{-1}) was the only hybrid that contained significantly less fumonisins than the worst performing genotypes ($P < 0.05$) (Table 3).

The overall mean of the 1 000-kernel weight for all genotypes evaluated at Kiboko was 221.39 g, and the individual genotype means ranged from 151.10 to 306.70 g/1 000 kernels (Table 3). Hybrid P502C2 x CML444 (306.70 g/1 000 kernels) had the highest 1 000-kernel weight, which differed significantly from all the other inbred lines. It did not, however, differ significantly from hybrids CKL05015 x P502C2, CML444 x P502C2, CML444 x R119W and P502C2 x R119W (Table 3). The inbred line P502C2 had the highest 1 000-kernel weight (234.53 g) among the inbred lines, but this did not differ significantly from CKL05015, CML444, CML495 and MIRTC5 ($P \geq 0.05$) (Table 3). Line R119W had the lowest 1 000-kernel weight (151.10 g) when compared to other inbred lines. The hybrid MIRTC5 x CML444 (157.73 g) had the lowest 1 000-kernel weight and did not differ significantly from R119W (Table 3).

FER severity had a low and non-significant correlation with *F. verticillioides* colonisation and fumonisin contamination ($P \geq 0.05$) (Table 4). Fumonisin contamination and *F. verticillioides* colonisation had a highly significant correlation of $r = 0.85$ ($P < 0.05$) (Table 4). The 1 000-kernel weight was negatively and non-significantly correlated with FER severity and *F. verticillioides* colonisation ($P \geq 0.05$) (Table 4). Pearson's correlation coefficient between 1 000-kernel weight and fumonisin contamination was not significant ($P \geq 0.05$) (Table 4).

Diallel analysis

GCA and SCA were significant for fungal colonisation, fumonisin contamination and 1 000-kernel weight, but not for FER severity ($P \geq 0.05$) (Table 5). The reciprocal, maternal and non-maternal effects were not significant for any of the variables ($P \geq 0.05$) (Table 5). Baker's ratio was relatively high for the 1 000-kernel weight (0.87) and FER severity (0.78), and moderate for the *F. verticillioides* target DNA (0.47) and fumonisin contamination (0.65) (Table 5). The broad sense heritability estimates ranged from 0.79 to 0.94, where 1 000-kernel weight was the highest and FER severity the lowest (Table 5). Narrow sense heritability estimates ranged from 0.44 (*F. verticillioides* target DNA) to 0.82 (1 000-kernel weight) (Table 5).

All the parental lines had non-significant GCA estimates for FER severity except for R119W (0.232), which had a positive GCA estimate (Table 6). The GCA estimates of CKL05015, MIRTC5 and R119W were not significant for *F. verticillioides* target DNA ($P < 0.05$) (Table 6). The inbred line CML444 (0.0060) has a significant but positive GCA for *F. verticillioides* target DNA, while CML495 (-0.0065) had a significant but negative GCA ($P < 0.05$) (Table 6). For fumonisin contamination CKL05015 (0.105) was a general good combiner, and its GCA estimate was significant. The inbred lines CML444 (0.091) and P502C2 (0.118) had positive and significant GCA estimates for fumonisin contamination ($P < 0.05$) (Table 6). The inbred lines CML495, MIRTC5 and R119W had non-significant GCA estimates for fumonisin ($P < 0.05$) (Table 6). Inbred line P502C2 had a highly significant and positive GCA estimate (21.275) for 1 000-kernel weight ($P < 0.05$) (Table 6). Inbred lines CKL05015, CML444 and CML495 had non-significant GCA estimates ($P < 0.05$) (Table 6). The inbred lines MIRTC5 (-15.670) and R119W (-16.199) had significant GCA estimates, but were not good general combiners for 1 000-kernel weight ($P < 0.05$) (Table 6).

The hybrids CML495 x P502C2 (-0.227), MIRTC5 x P502C2 (-0.243), MIRTC5 x CML495 (-0.363), R119W x CKL05015 (-0.367) and R119W x CML495 (-.467) had good SCA estimates for FER severity, but these were not significant ($P < 0.05$) (Table 7). The worst significant SCA estimates for FER severity were observed on the hybrid CKL05015 x P502C2 (0.531) and its reciprocal hybrid (0.660) had ($P < 0.05$) (Table 7). Genotypes CML444 x MIRTC5 (-0.0153), CML444 x P502C2 (-0.0164) and P502C2 x R119W (-0.0111) had significant SCA estimates and were the best combinations for *F. verticillioides* target DNA in this population ($P < 0.05$) (Table 7). The highest positive SCA estimates for *F. verticillioides* target DNA were observed on hybrids CML495 x R119W (0.0057) and MIRTC5 x CKL05015 (0.0073), but they were not significant ($P < 0.05$) (Table 7). The best combinations for fumonisin accumulation with significant SCA estimates were CML444 x MIRTC5 (-0.254) and P502C2 x R119W (-0.272) ($P < 0.05$) (Table 7). The hybrid MIRTC5 x CKL05015 (0.252) had the highest positive and significant SCA estimate for fumonisin

contamination ($P < 0.05$) (Table 7). Hybrids such as P502C2 x CKL05015 (0.226) and P502C2 x CML444 (0.233) had high positive SCA estimates, but were not significant for fumonisin contamination ($P < 0.05$) (Table 7). The most desirable combinations for 1 000-kernel mass were hybrids CML444 x P502C2 (32.502) and CML444 x MIRTC5 (30.832) (Table 7). The hybrid CML444 x MIRTC5 (-27.153) and its reciprocal hybrid (30.05) had the largest significant and negative SCA estimates for 1 000-kernel weight ($P < 0.05$) (Table 7). Good SCA estimates for 1 000-kernel mass were determined for hybrids CKL05015 x MIRTC5 (20.075), R119W x CML495 (25.733) and P502C2 x MIRTC5 (22.400), but these were not statistically significant ($P < 0.05$) (Table 7).

F_2 populations trials

FER expression was minimal in both trials and did not provide any valuable information concerning F_2 populations that were resistant to ear rot (data not shown). Significant genotype x environment interactions affected the accumulation of *F. verticillioides* and fumonisins ($P < 0.05$) (Table 8). The variation between replications at each locality was insignificant ($P \geq 0.05$) (Table 8). The overall genotype mean of the *F. verticillioides* target DNA in Katumani was 0.116 ng μL^{-1} and the individual genotype means ranged from 0.010 to 0.524 ng μL^{-1} (Table 9). In Katumani, the F_2 family CML495 x MIRTC5 and its reciprocal had the lowest fungal DNA of 0.010 ng μL^{-1} and 0.014 ng μL^{-1}, respectively (Table 9). These F_2 populations differed significantly from the genotype containing the highest *F. verticillioides* target DNA in Katumani, namely R119W x CKL05015 (0.524 ng μL^{-1}) (Table 9). All the other F_2 families differed significantly from this genotype ($P < 0.05$) (Table 9). The parental lines CKL05015 (0.021 ng μL^{-1}) and R119W (0.075 ng μL^{-1}) did not differ significantly from the best performing genotype in fungal colonisation in Katumani ($P < 0.05$) (Table 8). The mean *F. verticillioides* target DNA in Kiboko was 0.050 ng μL^{-1} and the concentrations ranged from 0.006 to 0.287 ng μL^{-1} (Table 9). The lowest fungal DNA was obtained from populations R119W x CKL05015 (0.006 ng μL^{-1}) and MIRTC5 x CML495 (0.008 ng μL^{-1}) (Table 9). No F_2 populations differed significantly from these genotypes except for R119W x CML495 (0.104 ng μL^{-1}) ($P < 0.05$). The inbred lines MIRTC5 (0.287 ng μL^{-1}) and R119W (0.252 ng μL^{-1}) had the highest fungal DNA quantified, and they differed significantly from all the genotypes evaluated ($P < 0.05$) (Table 9).

Fumonisin levels in Katumani ranged from 0.184 to 9.272 mg kg^{-1} with an average of 2.093 mg kg^{-1} (Table 9). The F_2 population MIRTC5 x CML495 had the lowest fumonisin concentration of 0.184 mg kg^{-1} but did not differ significantly from inbred line CKL05015 (0.296 mg kg^{-1}) and several other F_2 populations such as CML495 x MIRTC5 and P502C2 x CML444 ($P < 0.05$) (Table 9). The highest levels of fumonisins detected in Katumani were from the R119W x CKL05015 population (9.272 mg kg^{-1}) (Table 9). This population did not

differ significantly from genotypes such as MIRTC5, R119W x MIRTC5 and MIRTC5 x CML444 ($P < 0.05$) (Table 9). The fumonisin contamination mean in Kiboko was 0.741 mg kg^{-1} and the genotype means ranged from 0.034 to 5.107 mg kg^{-1} (Table 9). The F_2 populations CML444 x CKL05015 (0.054 mg kg^{-1}), CML495 x CML444 (0.066 mg kg^{-1}) and R119W x CKL05015 (0.034 mg kg^{-1}) had the lowest fumonisin levels in Kiboko, which differed significantly from the worst performing genotype ($P < 0.05$) (Table 9). The inbred lines MIRTC5 (5.107 mg kg^{-1}) and R119W (3.088 mg kg^{-1}) had the highest fumonisin levels, which differed significantly from each other and that was significantly higher than that produced by other genotypes ($P < 0.05$) (Table 9). The overall correlation between *F. verticillioides* colonisation and fumonisin contamination was $r = 0.78$ ($P < 0.0001$). The correlation between these variables was $r = 0.77$ in Katumani and $r = 0.86$ in Kiboko ($P < 0.0001$).

DISCUSSION

Developing maize cultivars with resistance to FER/fumonisins is complex and requires an integrated understanding of cultivar performance across environments as well as the inheritance of resistance in maize plants. In this study F_1 hybrid CKL05015 x CML495 and CML444 x CKL05015 were most resistant to *F. verticillioides* colonisation, while CKL05015 x R119W accumulated the least fumonisins. The improved resistance in these hybrids did not incur a yield penalty when the 1 000-kernel weight of the hybrids was compared to their parental inbred lines. The F_2 family derived from hybrid MIRTC5 x CML495 was the most resistant to *F. verticillioides* colonisation in both localities and fumonisin accumulation in Katumani. These results indicate that improved resistance to FER and fumonisins is possible using a single cross. However, F_1 hybrid performance was not indicative of F_2 family performance in response to *F. verticillioides* infection. This highlights the need to evaluate F_2 and later generations for resistance to FER and fumonisins, and to determine the impact of resistance on yield by means of testcross evaluations with elite maize lines.

Hybrids CML444 x P502C2, MIRTC5 x CML495, R119W x CKL05015, MIRTC5 x R119W and CML495 x MIRTC5 were least colonised by *F. verticillioides* and contaminated with fumonisins. Still, their response was not significantly better than that of their parental lines. The same result was also observed by King and Scott (1981) and Hung and Holland (2012), and suggests that inbred line evaluations can provide breeders with a good prediction of the response of hybrids when breeding for resistance to FER/fumonisins (Hung and Holland, 2012). Mesterházy *et al*. (2000) also stated that it is easier to predict the resistance of a hybrid if both parental lines are resistant. Inbred lines with resistance to FER/fumonisins thus provide a good basis for a resistance-breeding programme.

Genotypes in the F_1 and F_2 generations exhibited varying degrees of resistance to FER, fungal colonisation and fumonisin production. Genotypes performances were comparable between the F_1 generation and the F_2 generations. The environment, however, played a significant role on the amount of variation observed in fungal colonisation and fumonisin contamination of the F_2 study. This was evident in the differences in the genotypes' responses between the two locations. For example, R119W and R119W x CKL05015 had an opposite responses in the two test locations. In Katumani, for instance, R119W compared well to the most resistant genotypes in FER development and fumonisin contamination. However, this line performed poorly in Kiboko, where it was one of the worst performing genotypes. Also, R119W x CKL05015 was the best performing inbred line in the Kiboko F_2 hybrid trial, but it was the worst performing genotype in Katumani.

The Kenyan inbred line CKL05015 consistently showed resistance to FER symptoms, *F. verticillioides* colonisation and fumonisin contamination. This line has been previously characterised as resistant to AER under Kenyan climatic conditions (Dr Makumbi, CIMMYT, personal communication). This result indicates that this inbred line could have resistance to both AER and FER and their associated mycotoxins. Common resistance mechanisms to AER and FER have been documented before (Robertson-Hoyt *et al*., 2007a; Williams and Windham, 2009). However, not all AER-resistant genotypes are resistant to FER/fumonisins. The AER-resistant Kenyan inbred lines MIRTC5 and P502C2, for instance, were susceptible to *F. verticillioides* colonisation and fumonisin accumulation. Inbred line CML495 was a good general combiner for resistance to fumonisin accumulation, and can be invaluable in countries where both fumonisins and aflatoxins co-contaminate maize grain. The inbred line CML444 (previously characterised as resistant) did not differ significantly from R119W (previously characterised as susceptible) (Rose *et al*., 2016). Although previously described as resistant to FER, fungal colonisation and fumonisin contamination, the inbred line CML444 has shown to perform inconsistently across environments.

FER severity had low and non-significant correlations with *F. verticillioides* colonisation and fumonisin contamination in the F_1 population. Colonisation of grain with *F. verticillioides* and fumonisin contamination of such grain, however, were significantly correlated. This suggests that fungal colonisation of grain is a better indication of fumonisin accumulation than FER development Similar correlations between FER, *F. verticillioides* colonisation and fumonisin contamination were described by Janse van Rensburg *et al*. (2015) and Rose *et al*. (2016) before. This may be due to the measurement of *F. verticillioides* target DNA being an absolute measure compared to the subjective nature of FER severity. However, FER severity is much cheaper and requires less expertise to execute when compared to the quantification of fungal DNA and fumonisin analysis. While it

is useful to evaluate maize for FER, it is important to determine the content of fumonisins when evaluating maize genotypes against *F. verticillioides*.

The relative importance of additive and non-additive genes in *F. verticillioides* resistance has been studied in different maize populations before (Williams and Windham, 2009; Hefny *et al*., 2012; Hung and Holland, 2012; Pádua *et al*., 2016). The results of these studies portrayed either GCA or SCA effects to be more important for resistance to *F. verticillioides* colonisation and fumonisin contamination. The predominant gene effects involved in resistance appear to be dependent on the population evaluated. The additive and non-additive gene effects were almost equally important for inheritance of resistance to *F. verticillioides* colonisation and fumonisin contamination, whereas additive gene effects were important for the 1 000-kernel weight in this study. This in agreement with studies done by Srdić *et al*. (2007) and Rashmi *et al*. (2013) who also found additive gene effects to be the principal gene effect for 1 000-kernel weight. The maternal and non-maternal effects of the genotypes evaluated in this study were not significant. Williams *et al*. (2008) observed the same result in their diallel study of resistance to *A. flavus* and aflatoxins and proposed this was due to the lack of cytoplasmic factors in the inheritance of resistance to FER and fumonisin contamination in this set of genotypes.

Significant negative GCA estimates are desirable for the inheritance of resistance traits. In this study the Kenyan lines CML495 and CKL05015, previously characterised for resistance to AER, exhibited significantly positive GCA estimates for *F. verticillioides* colonisation and fumonisin contamination, respectively. These lines can thus be used in a breeding programme to improve resistance to both *A. flavus* and *F. verticillioides*. Conversely, the lines with significant positive GCA estimates for fungal colonisation (CML444) and fumonisin contamination (CML444 and P502C2) should be avoided. The hybrids CML444 x MIRTC5 and P502C2 x R119W were the best combinations for both fungal colonisation and reduced fumonisins with significant SCA estimates. The hybrid CML444 x P502C2 was also a good combination for fungal colonisation, but its SCA estimate for fumonisin contamination (though negative) was not significant. These hybrids can be studied further to investigate the potential nuclear factors involved in the inheritance of resistance. No hybrid had a significant SCA estimate for FER resistance and this may be due to the low expression of FER symptoms.

Understanding the inheritance of resistance to *F. verticillioides* infection and fumonisin contamination in maize is vital in breeding resistant maize cultivars. This study determined that both GCA and SCA were important for hybrid resistance. Furthermore, hybrids with improved resistance to *F. verticillioides* infection and fumonisins were generated and parental lines served as a good indicator of a hybrid's performance to infection. The resistance of Kenyan maize inbred lines to multiple ear rot pathogens and their associated

mycotoxins was demonstrated in this study. Developing hybrids from these lines could be cost-effective and time-saving when compared to developing hybrids for each ear rot pathogen individually. This is essential for countries like Kenya where aflatoxins and fumonisins co-occur.

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Table 1. Maize inbred lines and hybrids evaluated in Katumani and Kiboko, Kenya, during the 2014/15 maize-growing seasons.

Code	Inbred line	Description	Origin[a]	Status[b]
P1	CKL05015	Inbred	CIMMYT-Kenya	AER/aflatoxin-resistant
P2	CML444	Inbred	CIMMYT-Zimbabwe	FER/fumonisin-resistant
P3	CML495	Inbred	CIMMYT-Kenya	AER/aflatoxin-resistant
P4	MIRTC5	Inbred	CIMMYT-Kenya	AER/aflatoxin-resistant
P5	P502	Inbred	CIMMYT-Kenya	AER/aflatoxin-resistant
P6	R119W	Inbred	ARC-GCI-South Africa	FER/fumonisin-susceptible

Code	Hybrid	Trial sites		
		F_1 - Kiboko	F_2 - Katumani	F_2 - Kiboko
H1	CKL05015 x CML444	✓	✓	✓
H2	CKL05015 x CML495	✓	✓	✓
H3	CKL05015 x MIRTC5	✓	✓	X
H4	CKL05015 x P502	✓	✓	✓
H5	CKL05015 x R119W	✓	X	✓
H6	CML444 x CKL05015	✓	✓	✓
H7	CML444 x CML495	✓	✓	✓
H8	CML444 x MIRTC5	✓	✓	✓
H9	CML444 x P502	✓	✓	✓
H10	CML444 x R119W	✓	X	X
H11	CML495 x CKL05015	✓	✓	✓
H12	CML495 x CML444	✓	✓	✓
H13	CML495 x MIRTC5	✓	✓	✓
H14	CML495 x P502	✓	✓	✓
H15	CML495 x R119W	✓	✓	✓
H16	MIRTC5 x CKL05015	✓	✓	✓
H17	MIRTC5 x CML444	✓	✓	✓
H18	MIRTC5 x CML495	✓	✓	✓
H19	MIRTC5 x P502	✓	✓	✓
H20	MIRTC5 x R119W	✓	✓	✓
H21	P502 x CKL05015	✓	✓	✓
H22	P502 x CML444	✓	✓	✓
H23	P502 x CML495	✓	✓	✓
H24	P502 x MIRTC5	✓	✓	✓
H25	P502 x R119W	✓	✓	✓
H26	R119W x CKL05015	✓	✓	✓
H27	R119W x CML495	✓	✓	✓
H28	R119W x MIRTC5	✓	✓	✓
H29	R119W x P502	✓	✓	✓
H30	R119W x CML444	✓	X	X

[a] ARC-GCI = Agricultural Research Council - Grain Crops Institute; CIMMYT = International Maize and Wheat Improvement Centre.
[b] According to Small *et al*. (2012), Rose *et al*. (2016) and Dr Makumbi (CIMMYT, personal communication).
✓ = Genotype present in the trial.
X = Genotype missing in the trial.
AER = Aspergillus ear rot.
FER = Fusarium ear rot.

Table 2. Analysis of Variance for Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation, fumonisin concentration and 1000-kernel weight of maize genotypes evaluated in Kiboko in the 2014 maize-growing season.

FER severity (%)[x]

Source of variation	DF	MS	F-value	Pr > F
Replications	2	8.0329	4.21	0.0188
Genotype	35	1.8545278	0.97	0.5258

***F. verticillioides* colonisation (ng µL^{-1})[y]**

Source of variation	DF	MS	F-value	Pr > F
Replications	2	0.0004	1.51	0.2272
Genotype	35	0.0009	3.38	<0.0001

Fumonisins concentration (mg kg^{-1})[z]

Source of variation	DF	MS	F-value	Pr > F
Replications	2	0.8115	1.87	0.1624
Genotype	35	1.1047	2.54	0.0005

1 000-kernel weight (g/1 000 kernels)[a]

Source of variation	DF	MS	F-value	Pr > F
Replications	2	2725.8669	2.38	0.1003
Genotype	35	2880.7163	2.51	0.0005

[x] Percentage of maize ears exhibiting symptoms of FER; mean of disease severity (%) for three experimental replications.
[y] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replications.
[z] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replications.
[a] Weight of 1 000 maize kernels.

Table 3. Means of Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation, fumonisin concentration and 1 000-kernel weight of 36 maize genotypes evaluated in Kiboko in the 2014 maize-growing season.

Code	Genotype	FER severity (%)[x]	*F. verticillioides* colonisation (ng μL^{-1})[y]	Fumonisins Concentration (mg kg^{-1})[z]	1 000-kernel weight (g/1 000 kernels)[a]
P1	CKL05015	0 b	0.024 b-d	0.255 e-h	200.70 d-i
P2	CML444	0 b	0.093 a	2.370 a	224.77 b-f
P3	CML495	1.52 ab	0.017 b-d	0.365 d-h	208.83 c-h
P4	MIRTC5	0.65 ab	0.036 b	0.553 d-h	192.87 e-i
P5	P502C2	0 b	0.069 a	2.546 ab	234.53 b-f
P6	R119W	0.63 ab	0.034 bc	1.522 a-c	151.10 i
H1	CKL05015 x CML444	0 b	0.016 b-d	0.426 d-h	234.50 b-f
H2	CKL05015 x CML495	0 b	0.007 d	0.148 gh	222.83 b-f
H3	CKL05015 x MIRTC5	0 b	0.011 b-d	0.101 h	218.27 b-g
H4	CKL05015 x P502C2	0 b	0.015 b-d	0.182 f-h	269.50 ab
H5	CKL05015 x R119W	1.29 ab	0.022 b-d	0.106 h	205.50 d-i
H6	CML444 x CKL05015	0 b	0.007 d	0.097 h	221.70 b-f
H7	CML444 x CML495	0 b	0.013 b-d	0.225 e-h	213.37 c-g
H8	CML444 x MIRTC5	0.07 b	0.008 cd	0.083 h	217.83 b-g
H9	CML444 x P502C2	0.18 b	0.012 b-d	0.366 d-h	262.07 a-c
H10	CML444 x R119W	0 b	0.011 b-d	0.216 e-h	269.97 ab
H11	CML495 x CKL05015	0 b	0.010 b-d	0.321 e-h	244.67 b-e
H12	CML495 x CML444	0 b	0.008 cd	0.207 e-h	213.37 c-g
H13	CML495 x MIRTC5	1.25 ab	0.016 b-d	0.687 c-h	222.20 b-f
H14	CML495 x P502C2	0 b	0.013 b-d	0.285 e-h	239.77 b-f
H15	CML495 x R119W	2.38 ab	0.026 b-d	1.172 b-e	163.43 g-i
H16	MIRTC5 x CKL05015	0 b	0.026 b-d	1.146 c-d	242.03 b-f
H17	MIRTC5 x CML444	0 b	0.013 b-d	0.173 f-h	157.73 hi
H18	MIRTC5 x CML495	0 b	0.008 cd	0.128 gh	213.10 c-g
H19	MIRTC5 x P502C2	0 b	0.014 b-d	0.221 e-h	187.47 f-i
H20	MIRTC5 x R119W	0.36 ab	0.020 b-d	0.213 e-h	195.07 e-i
H21	P502C2 x CKL05015	2.52 a	0.019 b-d	1.163 c-f	222.13 b-f
H22	P502C2 x CML444	0.32 ab	0.015 b-d	1.312 a-d	306.70 a
H23	P502C2 x CML495	0 b	0.011 b-d	0.347 d-h	255.63 a-d
H24	P502C2 x MIRTC5	0 b	0.020 b-d	0.440 d-h	232.27 b-f
H25	P502C2 x R119W	0 b	0.012 b-d	0.249 e-h	231.43 b-f
H26	R119W x CKL05015	0 b	0.009 cd	0.092 h	226.40 b-f
H27	R119W x CML495	0 b	0.013 b-d	0.191 e-h	214.90 b-g
H28	R119W x MIRTC5	2.94 a	0.027 b-d	0.515 e-h	196.93 e-i
H29	R119W x P502C2	0.88 ab	0.014 b-d	0.287 e-h	235.93 b-f
H30	R119W x CML444	0.62 ab	0.022 b-d	0.846 c-g	220.51 b-f
Mean		**0.43**	**0.020**	**0.543**	**221.39**

[x] Percentage of maize ears exhibiting symptoms of Fusarium ear rot; mean of disease severity (%) for three experimental replications.
[y] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replications.
[z] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replications.
[a] Weight of 1 000 maize kernels.

Table 4. Pearson correlation coefficients between Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation, fumonisin concentration and 1 000-kernel weight of maize genotypes evaluated in Kiboko in the 2014 maize growing season.

	FER Severity (%)[x]	***F. verticillioides* colonisation (ng µL^{-1})[y]**	**Fumonisins concentration (mg kg^{-1})[z]**
FER severity (%)[x]	-	0.08778 (*P* = 0.6107)	0.17719 (*P* = 0.3012)
***F. verticillioides* colonisation (ng µL^{-1})[y]**	0.08778 (*P* = 0.6107)	-	0.85095 (*P* = 0.0001)
1 000-kernel weight (g/1 000 kernels)[a]	-0.31946 (*P* = 0.0575)	-0.1296 (*P* = 0.4513)	0.01364 (*P* = 0.9371)

[x] Percentage of maize ears exhibiting symptoms of FER; mean of disease severity (%) for three experimental replications.
[y] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replications.
[z] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replications
[a] Weight of 1 000 maize kernels.

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Table 5. Analysis of Variance for combining ability of Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation, fumonisin concentration and 1 000-kernel weight of maize genotypes evaluated in Kiboko in the 2014 maize-growing season.

		FER severity (%)[x]		***F. verticillioides* colonisation (ng µL^{-1})[y]**		**Fumonisin concentration (mg kg^{-1})[z]**		**1 000-kernel weight (g/1 000 kernels)[a]**	
Contrast	DF	Mean Square	Pr > F	Mean Square	Pr > F	Mean Square	Pr > F	Mean Square	Pr > F
GCA	5	0.589	0.202	7728.262	<0.0001	0.001	0.015	0.316	0.006
SCA	15	0.340	0.598	2340.185	0.027	0.002	<0.0001	0.345	<0.0001
REC	15	0.492	0.258	1694.999	0.138	0.0001	0.973	0.135	0.113
MAT	5	0.322	0.541	813.827	0.618	0.0001	0.955	0.203	0.051
NONM	15	0.577	0.171	2135.586	0.066	0.0001	0.885	0.100	0.339
Error	70	0.393	-	1146.696	-	0.0003	-	0.087	-
GCA:SCA		0.78	-	0.87	-	0.47	-	0.65	-
H^2		0.79	-	0.94	-	0.93	-	0.92	-
h^2		0.61	-	0.82	-	0.44	-	0.59	-

[x] Percentage of maize ears exhibiting symptoms of FER; mean of disease severity (%) for three experimental replications.
[y] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replications.
[z] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replications.
[a] Weight of 1 000 maize kernels.
GCA = general combining ability; SCA = specific combining ability; REC = reciprocal effects.
MAT = maternal effects; NONM = non-maternal effects.
H^2 = broad sense heritability; h^2 = narrow sense heritability.

Table 6. General combining ability estimates for Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation, fumonisin concentration and 1 000-kernel weight of six maize inbred lines evaluated in Kiboko in the 2014 maize-growing season.

	FER severity (%)[x]		***F. verticillioides* colonisation (ng µL^{-1})[y]**		**Fumonisin concentration (ppm)[z]**		**1 000-kernel weight (g/1 000 kernels)[a]**	
Genotype	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|
CKL05015	-0.090	0.351	-0.0038	0.119	-0.105	0.022	4.355	0.401
CML444	-0.133	0.169	0.0060	0.015	0.091	0.046	9.217	0.078
CML495	0.009	0.929	-0.0065	0.010	-0.070	0.123	-2.978	0.565
MIRTC5	0.024	0.799	-0.0001	0.958	-0.067	0.140	-15.670	0.003
P502	-0.042	0.659	0.0038	0.120	0.118	0.011	21.275	<0.0001
R119W	0.232	0.018	0.0006	0.816	0.033	0.464	-16.199	0.002

[x] Percentage of maize ears exhibiting symptoms of FER; mean of disease severity (%) for three experimental replications.
[y] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replications.
[z] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replications.
[a] Weight of 1 000 maize kernels.

Table 7. Specific combining ability estimates for Fusarium ear rot (FER) severity, *Fusarium verticillioides* colonisation, fumonisin concentration and 1 000-kernel weight of F_1 maize hybrids evaluated in Kiboko in the 2014 maize-growing season.

		FER Severity (%)[x]		***F. verticillioides* colonisation (ng µL^{-1})[y]**		**Fumonisin concentration (mg kg^{-1})[z]**		**1 000-kernel weight (g/1 000 kernels)[a]**	
Code	Genotype	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|
SCA estimates									
H1	CKL05015 x CML444	-0.038	0.860	-6.862	0.561	-0.0109	0.054	-0.120	0.246
H2	CKL05015 x CML495	-0.180	0.412	10.984	0.353	-0.0007	0.903	0.019	0.856
H3	CKL05015 x MIRTC5	-0.196	0.372	20.075	0.092	0.0030	0.593	0.169	0.104
H4	CKL05015 x P502C2	0.531	0.017	-1.202	0.919	-0.0028	0.613	0.028	0.782
H5	CKL05015 x R119W	-0.036	0.870	6.405	0.587	-0.0010	0.862	-0.182	0.080
H7	CML444 x CML495	-0.137	0.532	-14.262	0.229	-0.0086	0.127	-0.179	0.085
H8	CML444 x MIRTC5	-0.091	0.677	-27.153	0.024	-0.0153	0.007	-0.254	0.015
H9	CML444 x P502C2	0.255	0.246	32.502	0.007	-0.0164	0.004	-0.044	0.666
H10	CML444 x R119W	0.007	0.975	30.832	0.011	-0.0096	0.087	-0.084	0.413
H13	CML495 x MIRTC5	0.069	0.751	14.909	0.209	-0.0011	0.850	0.096	0.351
H14	CML495 x P502C2	-0.227	0.300	8.014	0.497	-0.0052	0.354	-0.129	0.212
H15	CML495 x R119W	-0.034	0.875	-13.045	0.271	0.0057	0.310	0.102	0.324
H19	MIRTC5 x P502C2	-0.243	0.268	-17.127	0.149	-0.0065	0.243	-0.127	0.219
H20	MIRTC5 x R119W	0.233	0.287	6.480	0.583	0.0033	0.551	-0.053	0.605
H25	P502C2 x R119W	-0.140	0.522	7.219	0.541	-0.0111	0.048	-0.272	0.010
Reciprocal estimates									
H6	CML444 x CKL05015	0.000	1.000	-6.400	0.645	-0.0045	0.492	-0.127	0.296
H11	CML495 x CKL05015	0.000	1.000	10.917	0.432	0.0015	0.819	0.058	0.631
H16	MIRTC5 x CKL05015	0.000	1.000	11.883	0.393	0.0073	0.264	0.252	0.041
H21	P502C2 x CKL05015	0.660	0.012	-23.683	0.091	0.0020	0.760	0.226	0.065
H26	R119W x CKL05015	-0.367	0.156	10.450	0.452	-0.0069	0.292	-0.006	0.959
H12	CML495 x CML444	0.000	1.000	0.000	1.000	-0.0027	0.684	-0.011	0.927
H17	MIRTC5 x CML444	-0.061	0.812	-30.050	0.033	0.0023	0.731	0.038	0.752
H22	P502C2 x CML444	0.098	0.704	22.317	0.111	0.0016	0.804	0.233	0.057
H18	MIRTC5 x CML495	-0.363	0.161	-4.550	0.743	-0.0038	0.558	-0.189	0.122
H30	R119W x CML444	0.367	0.157	-24.728	0.078	0.0053	0.416	0.205	0.093
H23	P502C2 x CML495	0.000	1.000	7.933	0.568	-0.0013	0.839	0.017	0.891
H27	R119W x CML495	-0.467	0.073	25.733	0.067	-0.0066	0.316	-0.239	0.051
H24	P502C2 x MIRTC5	0.000	1.000	22.400	0.110	0.0033	0.611	0.076	0.530
H28	R119W x MIRTC5	0.256	0.322	0.933	0.946	0.0039	0.550	0.075	0.537
H29	R119W x P502C2	0.310	0.230	2.250	0.871	0.0006	0.929	0.015	0.905

[x] Percentage of maize ears exhibiting symptoms of FER; mean of disease severity (%) for three experimental replications.
[y] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replications.
[z] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replications.
[a] Weight of 1 000 maize kernels.

Table 8. Analysis of Variance *Fusarium verticillioides* colonisation and fumonisin contamination in maize genotypes tested in Katumani and Kiboko during the 2014/15 maize-growing season.

***F. verticillioides* colonisation (ng μL^{-1})[y]**

Source of variation	DF	MS	F-value	Pr>F
Location	1	0.192152	27.4736	<0.0001
Replications	1	0.001032	0.1475	0.7016
Genotype	31	0.02245	3.2099	<0.0001
Location x Genotype	28	0.019905	2.846	<0.0001
Error	121	0.006994		

Fumonisins concentration (mg kg^{-1})[z]

Source of variation	DF	MS	F-value	Pr>F
Location	1	115.827	18.168	<0.0001
Replications	1	5.57	0.8737	0.35179
Genotype	31	10.347	1.623	0.0337
Location x Genotype	28	12.331	1.9341	0.0077
Error	121	6.375		

[y] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replications.
[z] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replications.

Table 9. Genotype means of *Fusarium verticillioides* colonisation and fumonisin concentration in maize evaluated in Katumani and Kiboko in the 2014/15 maize-growing season.

		Katumani		Kiboko	
Code	**Genotype**	***F. verticillioides* colonisation (ng µL^{-1})^{y}**	**Fumonisins concentration (mg kg^{-1})^{z}**	***F. verticillioides* colonisation (ng µL^{-1})^{y}**	**Fumonisins concentration (mg kg^{-1})^{z}**
P1	CKL05015	0.021 de	0.296 f	0.039 b-d	0.577c-i
P4	MIRTC5	0.219 bc	2.840 a-d	0.287 a	5.107 a
P6	R119W	0.075 c-e	1.038 c-f	0.252 a	3.088 b
H1	CKL05015 x CML444	0.101 b-e	0.870 c-f	0.043 b-d	0.399 c-i
H2	CKL05015 x CML495	0.095 b-e	1.392 c-f	0.030 cd	0.253 f-i
H3	CKL05015 x MIRTC5	0.081 c-e	1.459 c-f	x	x
H4	CKL05015 x P502C2	0.179 b-d	2.972 a-f	0.077 bc	1.218 c-e
H5	CKL05015 x R119W	x	x	0.035 cd	0.990 c-e
H6	CML444 x CKL05015	0.096 b-e	2.532 a-f	0.034 cd	0.054 i
H7	CML444 x CML495	0.067 c-e	0.748 c-f	0.015 cd	0.535 c-i
H8	CML444 x MIRTC5	0.071 c-e	1.665 a-f	0.045 b-d	1.109 c-e
H9	CML444 x P502C2	0.108 b-e	2.799 a-e	0.039 b-d	0.576 c-i
H11	CML495 x CKL05015	0.106 b-e	1.083 c-f	0.015 cd	0.187 f-i
H12	CML495 x CML444	0.039 de	0.600 c-f	0.028 cd	0.066 i
H13	CML495 x MIRTC5	0.010 e	0.372d-f	0.015 cd	0.125 hi
H14	CML495 x P502C2	0.072 c-e	0.886 c-f	0.062 b-d	1.248 c
H15	CML495 x R119W	0.080 c-e	1.923 a-f	0.024 cd	0.331 d-i
H16	MIRTC5 x CKL05015	0.057 de	0.733 c-f	0.011 cd	0.104 hi
H17	MIRTC5 x CML444	0.270 b	3.988 a-c	0.016 cd	0.138 hi
H18	MIRTC5 x CML495	0.014 e	0.184 f	0.008 d	0.308 e-i
H19	MIRTC5 x P502C2	0.045 de	0.657 c-f	0.037 b-d	0.382 e-i
H20	MIRTC5 x R119W	0.107 b-e	1.283 c-f	0.017 cd	0.168 hi
H21	P502C2 x CKL05015	0.249 b	5.723 ab	0.075 bc	0.940 c-f
H22	P502C2 x CML444	0.037 de	0.453 c-f	0.036 b-d	0.792 c-h
H23	P502C2 x CML495	0.073 c-e	1.633 c-f	0.012 cd	0.217 f-i
H24	P502C2 x MIRTC5	0.143 b-e	1.260 c-f	0.067 b-d	1.107 cd
H25	P502C2 x R119W	0.140 b-e	1.907 c-f	0.015 cd	0.543 c-i
H26	R119W x CKL05015	0.524 a	9.272 a	0.006 d	0.034 i
H27	R119W x CML495	0.180 b-d	3.124 a-f	0.104 b	0.981 c-g
H28	R119W x MIRTC5	0.072 c-e	5.337 a-c	0.039 b-d	0.495 c-i
H29	R119W x P502C2	0.137 b-e	3.748 a-e	0.013 cd	0.172 g-i
Mean		**0.116 a**	**2.093 a**	**0.050 b**	**0.741 b**

[y] Mean of *F. verticillioides* target DNA present in maize grain from three experimental replications.
[z] Fumonisin concentration = total of FB_1 + FB_2 + FB_3; mean of fumonisin concentration from three experimental replications.
x = Missing population due to crop failure.

GENERAL CONCLUSION

Maize populations evaluated in this study demonstrated different levels of resistance to Fusarium ear rot severity (FER), *F. verticillioides* colonisation and fumonisin contamination. The level of resistance in the genotypes was highly affected by environmental conditions and genotype by environment interactions. Hybrids R119W x CKL05015, CML495 x CKL05015 and CKL05015 x R119W were the most resistant to FER severity, *F. verticillioides* colonisation and fumonisin contamination under South African conditions, respectively. Hybrids CKL05015 x CML495 and R119W x CKL05015 had the least *F. verticillioides* colonisation and fumonisin content under Kenyan conditions, respectively. These genotypes should be evaluated across different seasons in South Africa and Kenya to determine the stability of their resistance response.

CIMMYT inbred lines CKL05015 and CML495, previously characterised as resistant to AER, were found to be good general combiners for resistance to *F. verticillioides* colonisation and fumonisin contamination. The potential for combined resistance to *F. verticillioides* and *A. flavus*, therefore, exists and should be further investigated. The availability of maize varieties with resistance to several maize ear rots would be of great value, especially in Africa, where mycotoxigenic fungi often co-occur.

Due to the high narrow sense heritability observed for resistance to FER, *F. verticillioides* colonisation and fumonisin contamination, inbred line performance was indicative of F_1 hybrids performance. This signifies that phenotypic selection performed in earlier generations can be effective in developing material resistant to *F. verticillioides* and fumonisins. Therefore, the populations identified as resistant to parameters evaluated in this study, can be further developed into recombinant inbred lines with stable, homogenous resistance. Furthermore, the developed RILs can be used to detect quantitative trait loci associated with resistance to FER and fumonisins through the use of genetic linkage analysis. Future research should also investigate the possibility of employing marker assisted selection as a means of improving the efficiency of resistance breeding.

The correlation between FER and *F. verticillioides* colonisation as well as FER and fumonisin contamination were significant and moderate under South African conditions yet non-significant under Kenyan conditions. Strong, significant correlations were consistently observed between *F. verticillioides* target DNA and fumonisin contamination across both countries. This indicates that selection for resistance based on either method would result in genotypes that are resistant to fumonisins. The method used, however, will vary according to the institution, availability of resources, technical skills and cost of analysis.

The 1000-kernel weight measurements revealed that resistance to FER/fumonisins does not potentially result in reduced yield. However, this was only tested in one season in this study and has to be repeated. Furthermore, later generations should be test crossed

with high-yielding elite lines to properly determine the affect breeding for resistance has on yield.

Both general and specific combining ability were important in the inheritance of resistance to *F. verticillioides*. This signifies the importance of utilising selection methods that exploit both the additive and non-additive gene effects involved in the inheritance of resistance. Therefore, breeding strategies such as cross breeding and marker assisted selection that would enable pyramiding of resistance QTLs or genes would greatly enhance the efficiency of developing genotypes resistant to *F. verticillioides* and fumonisin accumulation. This study provided important information on maize inbred lines and hybrids that can be utilised by breeders to develop resistant varieties in the respective countries.