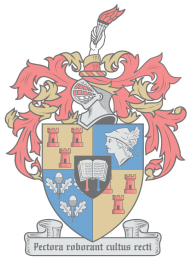
EFFECT OF PHENOLIC COMPOUNDS ON MAIZE EAR ROT PATHOGENS AND THEIR ASSOCIATED MYCOTOXINS

by

ASHEEQAH CASSIEM



UNIVERSITEIT
iYUNIVESITHI
STELLENBOSCH
UNIVERSITY

Thesis presented in partial fulfilment of the requirements for the degree Master of Science in AgriSciences at Stellenbosch University

Supervisor: Dr L.J. Rose

Co-supervisor: Prof A. Viljoen

December 2018

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2018

SUMMARY

Maize (*Zea mays* L.) is one of the most important staple food crops in South Africa. It is significantly affected by Fusarium ear rot (FER) pathogens such as *F. verticillioides, F. proliferatum* and *F. subglutinans*, as well as Gibberella ear rot (GER) pathogens such as *F. boothii* and *F. graminearum* which results in poor grain quality and yield. Grain contamination with these pathogens also poses a significant food safety concern, as they are known to produce mycotoxins. Mycotoxins have been associated with a number of humans and animal diseases. Mycotoxins persist during food processing as they are heat-stable molecules and thus not fully eliminated. Therefore, the most effective way to prevent mycotoxins contamination would be to limit their production and accumulation in the field.

Numerous cultural methods may reduce *Fusarium* inoculum, however, none of these methods are totally effective under high disease pressure and ideal circumstances for mycotoxin production. Phenolic compounds, naturally produced by plants, have been shown to reduce the growth and mycotoxin production of *Fusarium* spp. Pre-existing phenolic compounds can act as a physical barrier to pathogens, while induced phenolic compounds have been known to accumulate in response to biotic stress such as fungal infections. Phenolic compounds are associated with plant defence mechanisms and can be seen as a potential management strategy.

In this study, the phenolic compounds vanillic, ferulic, caffeic, coumaric, chlorogenic and sinapic acid were used at different concentrations (0; 0.5; 1.5 and 2.5 mM) *in vitro* to evaluate the effect of phenolic compounds on FER and GER causal pathogens. A significant isolate by compound by concentration interaction was observed. The growth study illustrated significant reduction in growth of all *Fusarium* species evaluated. The growth of all isolates was inhibited by ferulic acid (2.5 mM). Ferulic acid combined with caffeic acid did not differ significantly from ferulic acid (2.5 mM) individually, while ferulic acid combined with chlorogenic acid had a synergistic effect when compared to the compounds individually. The biomass of the FER pathogens were reduced by caffeic acid while vanillic and coumaric acid reduced the biomass of the GER pathogens. Several phenolic compounds were able to reduce the production of mycotoxins, with chlorogenic acid significantly reducing the production of all mycotoxins evaluated.

Phenolic profiles in response to infection by *F. verticillioides*, over kernels maturation stages, of a resistant (CML 390) and susceptible (R2565y) maize inbred line was evaluated. Ferulic and caffeic acid was found at higher concentrations in the susceptible line than in the resistant line. Moreover, higher concentratrions of ferulic, sinapic and caffeic acid was observed in the water-inoculated grain of both lines compared to the fungal-inoculated grain.. No clear differences in phenolic content were established in the fungal-inoculated grain of both lines. In addition, gene expression of phenylalanine ammonia lyase (PAL) 3 was determined

by reverse transcription quantitative PCR. The susceptible line displayed a delayed response to infection as *PAL3* was only upregulated at 28 days after inoculation (dai). However, the resistant line illustrated a much faster defence response as *PAL3* was up-regulated from 7 dai.

The information obtained in this study could be used to enhance integrated disease management strategies to prevent infection by mycotoxigenic fungi and subsequent contamination with mycotoxins.

OPSOMMING

Mielies (*Zea mays* L.) is een van die belangrikste stapelvoedselgewasse in Suid-Afrika. Dit word beduidend beïnvloed deur Fusarium kopvrot (FKV) patogene soos *F. verticillioides*, *F. proliferatum* en *F. subglutinans*, asook Gibberella kopvrot (GKV) patogene soos *F. boothii* en *F. graminearum* wat tot swak graankwaliteit en opbrengs lei. Graanbesmetting met hierdie patogene vorm ook 'n belangrike voedselveiligheid kwessie, aangesien hulle daarvoor bekend is om mikotoksiene te produseer. Mikotoksiene word met 'n aantal mens- en dieresiektes geassosieer. Mikotoksiene word nie tydens voedselverwerking vernietig nie, aangesien dit hitte-stabiele molekules is en dus nie heeltemal vernietig word nie. Daarom is die mees doeltreffende manier om kontaminasie van mikotoksiene te voorkom, om hul produksie en akkumulasie in die veld te beperk.

Verskeie verbouingsmetodes kan *Fusarium* inokulum verminder, maar geeneen van hierdie metodes is heeltemal effektief onder hoë siektedruk en ideale omstandighede vir mikotoksienproduksie nie. Daar is getoon dat fenoliese verbindings, wat natuurlik deur plante geproduseer word, die groei en mikotoksienproduksie van *Fusarium* spp verminder. Vooraf-bestaande fenoliese verbindings kan as 'n fisiese versperring vir patogene optree, terwyl geïnduseerde fenoliese verbindings daarvoor bekend is om in reaksie op biotiese stres soos swaminfeksies, te akkumuleer. Fenoliese verbindings word met plantverdedigingsmeganismes geassosieer en kan as 'n potensiële bestuurstrategie gesien word.

In hierdie studie is die fenoliese verbindings vanille-, ferul-, kafeïen-, kumariese-, chlorogene- en sinapiensuur in verskillende konsentrasies (0; 0.5, 1.5 en 2.5 mM) *in vitro* gebruik om die effek van fenoliese verbindings op FKV en GKV veroorsakende patogene te evalueer. 'n Betekenisvolle isolaat en verbinding en konsentrasie interaksie is waargeneem. Die groeistudie het 'n betekenisvolle vermindering in die groei van alle *Fusarium* spesies wat geëvalueer is, getoon. Die groei van alle isolate is deur feruliensuur (2.5 mM) geïnhibeer. Ferulinsuur, gekombineer met kafeïensuur, verskil nie betekenisvol van feruliensuur (2.5 mM) alleen nie, terwyl feruliensuur, gekombineer met chlorogene suur, 'n sinergistiese effek gehad het wanneer met die verbindings individueel vergelyk is. Die biomassa van die FKV patogene is deur kafeïensuur verminder, terwyl die vanilliese en kumariese suur die biomassa van die GKV patogene verminder het. Verskeie fenoliese verbindings was in staat om die produksie van mikotoksiene met chlorogene suur betekenisvol te verminder, wat die produksie van alle mikotoksiene wat geëvalueer is, aansienlik verminder het.

Fenoliese profiele in reaksie op infeksie deur *F. verticillioides*, oor die veroudering van pitte, van 'n weerstandbiedende (CML 390) en vatbare (R2565y) mielie ingebore lyn, is geëvalueer. Ferulien- en kafeïensuur is by 'n hoër konsentrasie in die vatbare lyn as in die weerstandbiedende lyn gevind. Verder is hoër konsentrasies van ferulien-, sinapic- en

kafeïensuur in die water-geïnokuleerde graan van beide lyne gevind teenoor swam-geïnokuleerde graan. Geen duidelike verskille in fenoliese inhoud is in die swam-geïnokuleerde graan van beide lyne vasgestel nie. Daarbenewens is geen-uitdrukking van fenielalanien ammoniakliase (*PAL*) 3 deur middel van omgekeerde transkripsie kwantitatiewe PKR bepaal. Die vatbare lyn vertoon 'n vertraagde reaksie op infeksie, aangesien *PAL3* slegs by 28 dae na inokulasie (dni) opgereguleer is. Die weerstandbiedende lyn het egter 'n baie vinniger verdedigingsreaksie getoon, aangesien *PAL3* vanaf 7 dni opgereguleer is.

Die inligting wat in hierdie studie verkry word, kan gebruik word om geïntegreerde siektebestuurstrategieë te verbeter ten einde infeksie deur mikotoksigeniese swamme en daaropvolgende kontaminasie met mikotoksiene, te voorkom.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to the following persons and institution:

- **Dr Lindy J. Rose** and **Prof A. Viljoen** for their insight, guidance and support as my supervisors.
- **The South African Maize Trust** for the research funds to execute my project.
- **The South African Maize Trust** and **National Research Foundation** for my Master's bursaries.
- **Karlien van Zyl** for all the technical assistance in the field and laboratory.
- **Stefan Links** and **Sahabne Ullah** for laboratory assistants and constant encouragement.
- **Privat Ndayihanzamaso** for assistance and advice in the laboratory.
- **Khanya Mahlalela** for technical assistance.
- Members of the **Fusarium Research Group** for moral support.
- My parents, **Munnawar** and **Fowzia Cassiem**, and **Maqsood Cassiem** for their compassion and motivation.
- My husband, **Ridaw Daniels**, for constant support and encouragement.
- Most importantly, the **Almighty Allah SWT**, for giving me the strength and perseverance to carry out my research project.

CONTENTS

CHAPTER 1

An overview of the role of phenolic compounds against *Fusarium* species that cause maize ear rot

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important food crops produced worldwide (Byerlee and Eicher, 1997) and the predominant source of nutrition for many South Africans. In certain areas of the country the consumption levels of maize is as high as 300 – 500 g per person daily (Shephard, 2008).

Maize production in South Africa is continuously under pressure by the fungal genus *Fusarium* (Ncube *et al.*, 2011; Boutigny *et al.*, 2012; Small *et al.*, 2012) which causes seedling diseases as well as root, stalk, and ear rot (Munkvold and Desjardins, 1997). Of these fungal diseases, Fusarium ear rot (FER) is an economically important disease which can affect the yield and quality of the maize grain. FER is predominantly caused by *Fusarium verticillioides* (Saccardo) Nirenberg (syn = *F. moniliforme* Sheldon), while *F. proliferatum* (Matsushima) Nirenberg and *F. subglutinans* (Wollenweber and Reinking) Nelson, Toussoun and Marasas, are also responsible for the disease. In South Africa, *F. verticillioides* is the most predominant *Fusarium* spp. associated with FER of maize (Ncube *et al.*, 2011; Boutigny *et al.*, 2012). *Fusarium verticillioides*, *F. proliferatum* and *F. subglutinans* produce a variety of toxic secondary metabolites, or mycotoxins, of which fumonisins are the most important (Gelderblom *et al.*, 1988), however, *F. subglutinans* are known to produce little or no fumonisins (Nelson *et al*., 1992; Reynoso *et al*., 2004). These mycotoxins have lethal effects on humans and animals.

Gibberella ear rot (GER) is caused by the *F. graminearum* species complex (FGSC). In South Africa the main species that affect maize ears are *F. boothii* (Boutigny *et al*., 2011) as well as *F. graminearum* sensu stricto under rotational systems (Gokul, 2015). These causal pathogens are also know to produce mycotoxins such as zearalenone (ZEA) and trichothecenes (TCT). TCTs are further divided into nivalenol (NIV), deoxynivalenol (DON) and its acetylated derivative.

Neither conventional nor unconventional breeding efforts have been successful in producing maize genotypes that are completely resistant to FER and/or fumonisin contamination. Currently, no fungicides are registered for the control of FER (Nel *et al*., 2003). Consequently, there is an urgent need for an alternative strategy to increase the resistance of the maize plant to *Fusarium* diseases. Plant secondary metabolites, such as phenolic compounds, are known to be produced in response to fungal infection and forms part of the induced defence response (Nicholson and Hammerschmidt, 1992; Santiago *et al*., 2007).

Studies have shown that phenolic compounds have the ability to inhibit the growth of several fungal species (Purushothaman, 1976; Baranowski *et al*., 1980; Santiago *et al*., 2007) including several *Fusarium* species (Kasenberg and Traquair, 1988; Assabgui *et al*., 1993; McKeehen *et al*., 1999; Santiago *et al*., 2007). Therefore, phenolic compounds may be a promising, environmentally-sound, alternative strategy to manage FER and the associated fumonisin contamination of the maize grain.

The aim of the literature review is to discuss the pathogens associated with FER and GER of maize and the potential use of phenolic compounds in their management.

THE IMPORTANCE OF MAIZE IN SOUTH AFRICA

South Africa's average maize production in 2017 was approximately 16.7 million tons of which 8 million tons is consumed as a staple food by approximately 200 million individuals as well as fodder for cattle and livestock (Shephard *et al*., 2007; Shephard, 2008; DAFF, 2018). Maize is produced through two types of production systems; commercial farming systems and subsistence farming systems (DAFF, 2018). Maize surplus is exported to the neighbouring countries (DAFF, 2018) thereby earning foreign currency.

Strategies are incorporated such as rapid drying, use of resistant crops and the use of upgraded storage structures to reduce the loss of commercial farming systems; however, the subsistence farmers lack the resources that are necessary to ensure quality grain is produced for consumption (Bankole and Adebanjo, 2003). Subsistence farmers produce products of poor quality due to the fungal infections, pest damage, poor soil conditions and lack management strategies such as pesticides, hybrid seeds and fertilisers (Ncube *et al*., 2011).

MAIZE PLANT MORPHOLOGY

Maize plants are monocotyledonous, whose seeds comprise of only one embryonic leaf, and forms part of the *Gramineae* (syn. *Poaceae*) family. The maize kernel usually produces grain protected with a coat, namely the pericarp with the kernel further comprised of the germ and endosperm, which is covered by the aleurone layer (Serna-Saldivar, 2012).

The average height of a typical maize plant is usually one to four meters (Paliwal, 2000; Farnham *et al.*, 2003). It has a stalk which grows vertically and is made up of nodes and internodes (Fig. 1). Each leaf is attached to the stalk at the node and develops in two opposite directions. A fully developed maize plant could consist of up to 20 leaves (Du Plessis, 2003). The maize plant is monoecious, it produces male inflorescence on the tassels and female inflorescence on the lateral ears (Bortiri and Hake, 2007). The ear (female reproductive part) arises in the axillary buds (Irish and Nelson, 1989) and consists of flowers that develop in rows and elongate to the tip of the ear, creating the silks (Fig. 1) (Irish and Nelson, 1989; Sleper and

Poehlman, 2006; Bortiri and Hake, 2007). The ear and silks are protected by layers of leaf covering, known as the husk (Fig. 1) (Bassetti and Westgate, 1993). Each flower on the tassel is made up of three anthers (male reproductive part) in which the pollen is produced (Acquaah, 2007). Pollen is released from the mature tassels under warm and dry conditions (Oldenburg *et al.,* 2011). The pollen is received by the silks once they have emerged from the husk covering (Bassetti and Westgate, 1993) and pollen grain germination follows within minutes (Nielsen, 2016b). The ovule is fertilised when the pollen tube grows and develops inside the silk. Even though many grains of pollen might land and germinate in the silk, only one pollen grain usually fertilises the ovule (Nielsen, 2016b). This is the silking stage or growth stage R1 (Nielsen, 2016a)

KERNEL DEVELOPMENT

The development of maize kernels consist of 6 stages, namely; silking stage (R1), kernel blister stage (R2), kernel milk stage (R3), kernel dough stage (R4), kernel dent stage (R5) and finally physiological maturity (R6) (Hanway, 1966; Nielsen, 2016a). Kernel developmental stages can be linked to the number of days after silking; however, this can be significantly impacted by environmental conditions.

Maize kernel development begins at the blister stage, R2, which usually occurs 10-14 days after silking (Fig. 2). The kernels contain ample amount of clear fluid and the kernel appears to look like a white blister (Nielsen, 2016b). At this stage the starch starts accumulating in the endosperm and the dry weight of the kernel increase at a rapid pace (Hanway, 1966; Nielsen, 2016a). In the embryo the radicle (embryonic root), the coleoptile (protective sheath) and the first embryonic leaf is formed. The kernel moisture content at this stage is approximately 85%. Kernel milk stage, R3, occurs about 18 - 22 days after silking (Fig. 2). During this stage the kernel contain a white fluid that is considered “milky” and the starch continues to gather and collect in the endosperm (Nielsen, 2016b). The constant growth in this stage is largely due the expansion of the cells as well as the accumulation of starch, while the moisture content of the kernel is around 80%. Kernel dough stage, R4, is approximately 24 - 28 days after silking (Fig. 2). During this stage the inner white “milky” fluid becomes more of a dough consistency this occurs due to the constant accumulation of starch in the endosperm. In the course of this stage there is the formation on the four embryonic leaves, while cell division has ceased in the epidermal layers of the endosperm (Hanway, 1966; Nielsen, 2016a). By this stage the kernel has reached 50% of its mature dry weight and the moisture content of the kernel is 70% (Hanway, 1966; Nielsen, 2016a). Dent stage, R5, usually starts 35 - 42 days after silking (Fig. 2) (Nielsen, 2016). Denting of most of the kernel near the crown occurs and embryonic leaves and the radicle are fully differentiated at this stage. The lateral seminal roots also start forming at this stage. The moisture content of the kernel at this stage is about 55% (Hanway, 1966;

Nielsen, 2016). When all the kernels are fully dented it is an indication of a morphologically mature embryo (Hanway, 1966). The final stage is physiological maturity, R6, which usually occurs 55 - 65 days after silking (Fig. 2). The accumulation of dry matter in the kernel has ceased (Hanway, 1966) and the kernel usually reached its maximum dry weight while the kernel continues to lose moisture. The moisture content of the kernel is usually around 30% but it could be between 25 - 40% (Hanway, 1966; Nielsen, 2016).

Pathogens have the ability to affect the maize plant at different developmental stages. The site of infection can vary from the infected seed, coleoptile infection, through the silk or lesions (Kurger, 1962). For optimal emergence of the maize seedling soil temperature should be above 15°C and warmer weather is preferred as cooler weather limits the uptake of nutrient from the soil. If the seedling takes a longer time to emerge during this period, it would be at higher risk of pathogenic infection as the pathogens penetrate the coleoptile (Kurger, 1962). During the R1 stage of kernel development, the maize plant is susceptible to stressors. These include climatic conditions and temperature and could cause the dry mature accumulation to cease prematurely during kernel development that leads to lower grain yield (Hanft and Jones, 1986). Exposure to extreme weather conditions such as hail, might damage the plant or create openings for fungal pathogens to enter. During stages R2- R6 active grain filling occurs, in which the plants directs carbohydrates to the kernel. Infection of maize leaves, during this grain filling period, will cause premature death of leaves which would severely impact the production of photosynthates to the kernels, thereby reducing grain quality (Ward *et al*., 1999).

EAR ROT DISEASES OF MAIZE CAUSED BY *FUSARIUM* SPECIES

Fusarium ear rot of maize

The main causal agent of FER is *F. verticillioides* along with *F*. *proliferatum* and *F*. *subglutinans*. Recently *F. temperatum* Scauflaire and Munaut has also been associated with FER in South Africa and may have possibly been misidentified as *F*. *subglutinans* (Schoeman *et al*., 2018). These fungi produce numerous toxic secondary metabolites, mycotoxins, of which fumonisins are the most significant. *Fusarium verticillioides* has been found on maize, rice and sorghum, *F. subglutinans* has only been isolated from South African maize and sorghum (Rabie and Lübben, 1984; Boutigny *et al*., 2012) while *F. proliferatum* has a wide host range including the cereal crops as well as pine trees, asparagus, wheat and barley (Conner *et al*., 1996; Desjardins, 2003; Marín *et al*., 2010).

Epidemiology

FER is more likely to develop under hot, dry weather conditions at the flowering stage and after the flowering stage this is mainly true for *F. verticillioides* (Marín *et al.*, 1999; Reid *et al.*, 1999, White, 1999). The optimal temperature for *F. verticillioides* is around 25°C and 30°C

(Marín *et al.*, 1999; Munkvold, 2003; Reid *et al.*, 1999) while *Fusarium subglutinans* and *F. proliferatum* is associated with cooler areas (Edwards, 1935; White, 1999; Leslie and Summerell, 2006). The optimal temperature for *F. subglutinans* is 25°C, while the optimal temperature *F. proliferatum* is between 16°C and 25°C (Marín *et al.*, 1999; Munkvold, 2003; Leslie and Summerell, 2006). Asexual spores (macroconidia and microconidia) of *Fusarium* spp. overwinter on the maize debris from a previous season (Fig. 3) (Smith and White, 1988). This maize residue is the primary source of inoculum which infects maize kernels (Munkvold, 2003). Microconidia are numerous and are dispersed by the wind (Gillette, 1999; Munkvold, 2003) causing systemic infection of the maize plant through the silks (Jones *et al.*, 1980; Headrick and Pataky, 1991) or following insect and animal damage (Warfield and Davis, 1996; Munkvold *et al.*, 1999). Infection can also take place through plant roots (Desjardins, 2003; Yates and Sparks, 2008) and after planting of contaminated seeds (Foley, 1962; Sumner, 1968; Yates *et al.*, 1999).

Symptoms

FER is characterised by white to light pink mould typically found on random kernels, on groups of kernels or on kernels that have been physically injured (Fig. 3) (White, 1999; Munkvold, 2003). Kernels that are infected can also display starburst symptoms; white streaks that radiate from the point of silk attachment (Koehler, 1942). The streaks are caused by fungal growth and are corroded channels found within the pericarp (Duncan and Howard, 2010). *Fusarium verticillioides* can colonise kernels without showing any symptoms and these asymptomatic infections further complicates the disease assessment (Foley, 1962). Disease development is ultimately influenced by the environment and the genetic background of the plant and the pathogen (Kedera *et al.*, 1994; Maiorano, 2009; Duncan and Howard, 2010).

Mycotoxins

The production of fumonisins by *F. verticillioides* is limited to maize but the production of fumonisins by *F. proliferatum* has a wider host range such as pine trees, asparagus, wheat and barley (Conner *et al*., 1996; Marín *et al*., 2010). *Fusarium subglutinans* are known to produce little to no fumonisins (Nelson *et al*., 1992; Reynoso *et al*., 2004) but do produce moniliformin (Farber *et al.,* 1988; Marasas *et al*., 1986; Low *et al*., 1996; Sewram *et al*., 1999). Fumonisins are classified as Group 2B by the International Agency for Research on Cancer (IARC) indicating it is possibly carcinogenic to humans (Marasas *et al*., 2004; Marín *et al*., 2010). The ingestion of fumonisin-contaminated grain has been linked to oesophageal cancer in humans in South Africa, China, Iran and the United States (Marasas *et al*., 1981; Marasas *et al*., 1988, Sydenham *et al*., 1990; Rheeder *et al*., 1992; Norred and Voss, 1994), leukoencephalomalacia in horses (Marasas *et al.*, 1988) and pulmonary oedema in pigs

(Gelderblom *et al*., 1988; Ross *et al*., 1990; Gelderblom *et al*., 2004; Atanasova-Penichon *et al.*, 2014).

The environmental and biological role of fumonisins has been under discussion. It has been suggested that fumonisins has a role in the infection process on *F. verticillioides* and *F. proliferatum* (Covarelli *et al*., 2012). Fumonisins have been shown to be phytotoxic to maize in culture (Lamprecht *et al*., 1994). However, mutated strains of *F. verticillioides* which do not produce fumonisins had the same ability to infect maize kernels and cause ear rot as a strain that produced fumonisins (Proctor *et al*., 1999; Desjardins and Plattner, 2000). For *F. verticillioides*, the production of fumonisins is also believed to provide the fungus with a competitive advantage over other fungi occupying the same ecological niche (Fox and Howlett, 2008).

Infection process

There are several infection pathways for *Fusarium* species that have been identified (Munkvold, 2003). These infection pathways include; silk infection, injury by insects as well as systemic transmission (Munkvold *et al.*, 1997; Sobek and Munkvold, 1999; Munkvold, 2003). Infection may also occur through openings in the pericarp, such as the point of silk emergence (Nelson, 1992). According to Nelson (1992), developing kernels may be infected via the silk and symptomless infection usually occurs when the maize ears are infected through the silk (Desjardins *et al*., 2002; Munkvold *et al.*, 1997; Nelson, 1992). Silk inoculation is the only method that improved the overall incidence infection in the maize kernels (Munkvold *et al.*, 1997). Insects have been associated with damaging the kernels (creating wounds or openings) and has been identified in acting as a vector for *F. verticillioides* (Munkvold *et al.*, 1997; Sobek and Munkvold, 1999). Infection process can also occur by systemic development of *F. verticillioides* on the maize plant through the seed and infection of the stalk (Munkvold *et al.*, 1997; Nelson, 1992).

Gibberella ear rot of maize

The causal organism of Gibberella ear rot (GER) is *Fusarium graminearum* (Schwabe) (Ward *et al*., 2002, Leslie and Summerell, 2006). *Fusarium graminearum* was thought to be only a single species (O'Donnell *et al*., 2004; 2008). However, recent studies has demonstrated that *F. graminearum* is part of a species complex, the *F. graminearum* species complex (FGSC) that consists of 16 distinct species (van der Lee *et al*., 2015). *Fusarium graminearum* has been found on maize, wheat as well as barely and it is known to produce secondary metabolites that are toxic (Leslie and Summerell, 2006).

FGSC associated with maize grain in SA

Of the 16 species in the FGSC only three species has been discovered on maize in South Africa, they are *F. boothii*, *F. graminearum* and *F. meridionale* (Beukes *et al*., 2017). The most dominant species that affect maize ears in South Africa is *F. boothii* (Boutigny *et al.*, 2011) while the disease can also be caused by *F. graminearum* sensu stricto under rotational systems (Gokul, 2015).

Epidemiology

GER predominates in cooler areas with higher precipitation during the growth season. In South Africa, it predominates in warm, wet conditions (Koehler, 1959; Smith and White, 1988; Logrieco *et al*., 1993; Bottalico, 1998; Munkvold, 2003). *Fusarium graminearum* produces chlamydospores that can survive between periods of different hosts (Sutton, 1982; Munkvold, 2003). It forms perithecia on residues and the fruiting structures release ascospores into the air (Fig. 4) (Munkvold, 2003). Ascospore release requires a period of dehydration which follows a diurnal pattern (recurs daily) with the release occurring mainly at night (Munkvold, 2003). *Fusarium graminearum* can be transmitted from the seed to the seedling (Cotton, 1996; Kabeere *et al*., 1997; Munkvold, 2003). The macroconidia are dispersed by water splashes and they can be dispersed over long distances once they are in the air (dispersed from the sporodochium) (Bergstrom and Shields, 2002; Munkvold, 2003).

Symptoms

Gibberella ear rot usually start with white fungal mycelia which is seen at the tip of the ear (White, 1999). The mycelia grows down the ear towards the base of the ear and eventually the fungal growth on the infected kernel will turn red or pink (Fig. 4) (White, 1999). If an infection occurs at an earlier stage, the whole ear may rot and will be enclosed by a pinkish mycelium that will cause the husk to tightly adhere to the ear (White, 1999). Previous studies has shown that when rainfall occurs at the time of silking, there is an increase in the severity of ear rot symptoms (Sutton, 1982; Vigier *et al*., 2001).

Mycotoxins

Fusarium graminearum produces the mycotoxin zearalenone (ZEA) and type B trichothecenes (TCTB), which include nivalenol (NIV), deoxynivalenol (DON) and its acetylated derivative 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON) (Atanasova *et al.*, 2012). In South Africa, *F. boothii* which produces the 15-ADON chemotype, has been exclusively associated with commercial maize produced in South Africa (Boutigny *et al*., 2011) It has been under discussion that the incidence of ZEA in maize is correlated with summer precipitation (Sutton *et al*., 1980) and a regression model has been developed by Vigier *et al*. (1997). It has also been discovered that *F. graminearum* strains that lacked TCT production

yielded different results to strains the produces TCT and strains that lacked DON production were less capable of causing diseases than their wild-type equivalents (Desjardins *et al*., 1996; Harris *et al*., 1999; Munkvold, 2003). The ingestion of these mycotoxins could induce acute or chronic effects on both humans and animals, for instance hepatotoxicity (liver damage); nephrotoxicity (poisonous effects on the kidney); teratogenesis (disrupt the development of an embryo) and it has carcinogenic effects (Bryden, 2012; Da Rocha *et al*., 2014; Smith *et al*., 2016). Mycotoxins such as zearalenone (ZEA) have been associated with reproductive disorders in animals (Kuiper-Goodman *et al*., 1987; Beukes, *et al*., 2017). Mycotoxins has been associated various effects on female reproduction resulting in oocyte impairment (Santos *et al*., 2013). An *in vitro* study by Ahamed *et al*. (2001) showed that ZEA has the ability to stimulate the growth of breast cancer cells.

Infection process

One of the most important infection pathways of *F. graminearum* which causes maize ear rot is the silk-channel (Reid *et al*., 1992; Vigier *et al*., 2001). There are other infection pathways in which *F. graminearum* may infect the maize plant including through wounds that have been made by insects, animals, birds and in certain cases even extreme weather conditions such as storms or hail (Sutton *et al*., 1980; Reid *et al*., 1999; Vigier *et al*., 2001; Oldenburg and Ellner, 2015).

HEALTH RISKS OF FUMONISINS AND TRICHOTHECENES

Mycotoxins are known to cause sever mycotoxicosis in humans and animals (Jurado, *et al*., 2009). Certain mycotoxins such as FB_1 are known to cause acute diseases and have been classified in 2B group carcinogens by the IARC (Jurado, *et al*., 2009). Due to the accumulation of mycotoxins in maize grain, it increases the importance of these pathogens in an agricultural context (Sutton, 1982; Fernando *et al*., 1997) such as decline in the agricultural economies (worldwide as well as Africa) since contaminated food products could be between 25% and 50% of the worlds food crops estimated by the Food and Agriculture Organisation (FAO) (Mannon and Johnson, 1985; Fernando *et al*., 1997; Fandohan *et al*., 2003).

In South Africa the level of mycotoxin contamination of commercial maize are generally low and routinely monitored by the South African Grain Laboratory. However, when weather conditions for fungal infections are favourable high levels of mycotoxins can be found (Beukes *et al*., 2017). Subsistence maize production, however, has been shown to contain much higher fumonisin levels ranging from 0 to 21 800 µg/kg and is not subjected to mycotoxin surveillance (Ncube *et al*., 2011). This places resource-poor farmers and their communities at much higher risk for mycotoxin exposure.

Mycotoxins may cause serious disease and illnesses on human upon consumption, in addition, it has been associated with death in humans (Marasas *et al*., 2004; Zain, 2011). Mycotoxins such as fumonisin have also been associated with oesophageal cancer in humans (Nelson *et al*., 1993). When contaminated grain is ingested by animals it may cause reproductive disorders in poultry, swine and cattle (Fernando *et al*., 1997). FB_1 has been associated with leukoencephalomalacia in horses, pulmonary oedema in swine in addition to liver damage and cancer in other animals (Ross *et al*., 1990; Nelson *et al*., 1993; Cotton and Munkvold, 1998). Based a study done by Pestka and Smolinski (2005) it was shown that trichothecenes causes diarrhoea, vomiting as well as gastrointestinal haemorrhage and at higher doses it eventually leads to death.

Due to the health risks that are associated with the consumption of contaminated maize or maize-based products, their incidence rate is presently under regulation in many countries including the European Union (EU, 2006). South Africa amended the act on fungus toxins in food to legislate a maximum allowable level of fumonisin and DON in maize and maize-based food in September 2016. The maximum allowable level for fumonisin is 4000 µg kg^{-1} for further processing and 2000 µg kg^{-1} for human consumption, while DON is 2000 µg kg^{-1} for further processing and 1000 µg kg^{-1} for human consumption (South Africa Department of Health, 2016). Mycotoxins are heat-stable and are not fully eliminated during food processing (Gelderblom *et al.*, 2004; Picot *et al.*, 2013; Atanasova-Penichon *et al.*, 2014). An optimal way to reduce contamination of maize-based foods and feeds would be to control or regulate mycotoxin biosynthesis (Atanasova-Penichon *et al.*, 2014).

MANAGEMENT AND CONTROL

Fusarium spp. could be controlled through both pre-harvest and post-harvest management strategies. When focussing on pre-harvest management the emphasis should be placed on cultural practices. For post-harvest management emphasis should be on factors such as harvesting time, moisture management, drying as well as storage. Another focal point in management of *Fusarium* spp. could be focussing on host resistance, of which conventional and unconventional breeding efforts could be looked into.

Pre-harvest management strategies

Numerous cultural practices have been shown to reduce *Fusarium* spp and mycotoxin contamination. Maize seeds should be planted when the temperature of the soil and groundwater are appropriate for germination (Du Plessis, 2003). It has previously been documented that planting the maize seeds late and harvesting occurring in wet conditions are favourable to *F. verticillioides* (Bilgrani and Choudhary, 1998; Fandohan *et al*., 2003). It has also been documented that the occurrence of *F. verticillioides* and *F. graminearum* increases

with wet weather (Sutton, 1982; Al-Heeti, 1987; Vigier *et al*., 2001; Fandohan *et al*., 2003). Crop rotation can be seen to have two benefits if maize is in rotation with a non-host crop of *Fusarium* (Fandohan *et al*., 2003; Thomison, 2013). When maize is in rotation with a non-host crop of the *Fusarium* species, the disease outbreak was considerably lower (Fandohan *et al*., 2003). Crop rotations allows for better weed control and it could aid in the removal of weeds that are hosts for *Fusarium* spp. (Fandohan *et al*., 2003; Thomison, 2013). Another aspect to take into account would be soil tillage. Soil tillage allows for optimal growth of the maize plant by changing the soil structure and hydraulic properties (Du Plessis, 2003; Desai, 2004). It also affects the ratio of the type of soil and the ability of the soil to store water (Desai, 2004; Du Plessis, 2003). The type of soil and date of planting will greatly affect the planting depth, for more sandy soil the planting depth should be deeper than that of heavier soil (Du Plessis, 2003). When focusing on the interaction between infection by *Fusarium* spp. and tillage there have been many inconsistent findings (Steinkellner and Langer, 2004). According to Damm (1998) and Weber and colleagues (2001), there was a decrease in *Fusarium* rot, while Bailey and Duczek (1996) revealed an increase in Fusarium rot and Arnold-Reimer (1994) and Swan and co-workers (2000) determined that there was no effect of tillage on *Fusarium* (Steinkellner and Langer, 2004).

Post-harvest management strategies

When taking post-harvest management strategies into consideration, harvesting time is one of the aspects that can be controlled. Maize ears can be harvested with a moisture content of 30% although it should be delayed until the moisture content is around 15% (Desai, 2004; Ncube, 2012). However, Bush and colleagues (2004) suggest that harvesting earlier (with a moisture content of 25%) may reduce the levels of fumonisin contamination. Harvesters should be regulated in such a way that it prevents any form of kernel damage during harvesting as it could allow for the entry to fungal spores (Fandohan *et al*., 2003; Ncube, 2012). Precautionary measures should be taken into account to prevent infection by *Fusarium* spp. and the mycotoxin accumulation during drying practices.

In western Nigeria, the African Rural Storage Centre has established an improved system (NAS, 1978; Desai, 2004) where local storage cribs has reached a drying rate of 1% in 10 days with the cobs having an initial moisture content of 21% (Comes and Riley, 1962; Desai, 2004). When the initial moisture content is around 30-40% forced air ventilation could be used, however, the air should not be heated. Heated air may be used in humid areas in order to reduce the relative humidity to about 70% (Desai, 2004). Mechanical driers could be used with temperature between 44-60°C (Arora *et al*., 1973; Salunkhe *et al*., 1985; Desai, 2004). When kernels are being stored for short periods of time it is suggested that the moisture content should be around 14% and when kernels are being stored for longer periods should be around 10-12%, while the moisture content of seed stock should be 8-10% (Desai, 2004). For storage

purposes, metal or concrete bins in a well aerated area with low moisture content and favourable temperatures are recommended (Ncube, 2012) as well as the storage of the grain over the cob, because the cob is more susceptible to infestation (Hindmarch and MacDonald, 1980; Desai, 2004).

Host resistance

Host resistance is considered an environmentally safe and feasible strategy to manage Fusarium ear rot, Gibberella ear rot and the mycotoxin contamination (Munkvold, 2003; Wang *et al*., 2016; Lanubile *et al*., 2017). Host resistance can be improved or enhanced by using conventional breeding strategies, unconventional breeding strategies and resistance enhancement to combat infection by *Fusarium* spp. and the mycotoxin contamination.

Conventional breeding strategies, such as the screening of inbred lines, can be looked at from a phenotypic or genotypic point of view. Breeders search for specific phenotypic quantitative traits in populations, such as the crop yield, grain quality, stalk quality and disease resistance, to develop inbred lines through controlled artificial pollination (Acquaah, 2007). Pedigree selection then takes places over a number of generations until a desired outcome in performance and a high level of uniformity is achieved (Acquaah, 2007). Many studies have shown that there is genetic variation in resistance to FER and the mycotoxin accumulation (Lanubile *et al.*, 2017). Quantitative trait loci (QTL) mapping studies can be used to determine whether a certain trait in maize is an indication of resistance. DNA markers that have been associated with QTL's may also be used in marker-assisted selection for breeding. It has been established, through QTL mapping studies, that resistance to FER and GER in maize is a quantitative trait (Lanubile *et al.*, 2017). However, the progress in QTL mapping studies has slowed due to many influential factors such as the environment and thus affected the accuracy of marker-assisted selection (Lanubile *et al.*, 2017). QTLs usually have small effects (Lanubile *et al.*, 2017) but the effects might not be well established enough to be effective in different populations (Wang *et al.*, 2016).

Unconventional breeding strategies consist of techniques such as mutation breeding and genetic modification. Mutation breeding is when mutagens are used to create new alleles which are then introduced into existing cultivars. The aim of mutation breeding is to produce plants with superior traits such as increase in quality (Acquaah, 2007). Genetic modification or genetic engineering is when biotechnology is used to manipulate the plants gene thereby changing the genetic makeup of the plant. In a study by Bakan *et al.* (2002) maize was genetically modified with the *cry*1A(b) gene from *Bacillus thuringiensis*, which would produce the Cry-1A(b) protein to protect the maize plant from insects thus limiting openings for infection by *Fusarium* spp. The study indicated that the modified plant had a lower fungal biomass when compared to the isogenic non-modified hybrids. Fumonisin levels in the modified maize ranged

from 0.05 to 0.3 ppm while the fumonisin levels in the isogenic maize were 0.4 to 9 ppm (Bakan *et al.,* 2002).

Resistance enhancement

Resistance enhancements can be focused on by two aspects. Firstly, by investigating the host-pathogen interaction for defence responses in maize and secondly the application of biochemical stimulants or metabolites to obtain systemic acquired resistance. To investigate host-pathogen interaction emphasis can place on the signalling response pathway. The plant generates a signal in response to pathogenic molecules that may then control a vast range of responses that allow genes to control many actions (Duvick 2001; Wang *et al.*, 2016). The responses that follow could be programmed cell death in the infected tissue and the initiation of the defence pathway (Ryals, 1996; Duvick 2001). Biochemical stimulants or phytochemicals are synthesised in plants to produce secondary metabolites such as anthocyanins, carotenoids and phenolic compounds (Lopez-Martinez *et al.*, 2009). Phenolic compounds are widely distributed and have been linked to mechanisms of disease resistance (Reid *et al.*, 1992a; Santiago *et al.*, 2007). Phenolic compounds can act as resistance barriers to insects as well as antimicrobial substances (Samapundo *et al.*, 2007). Phenolic compounds can also accumulate in response to host-pathogen interaction (Reid *et al.*, 1992; Samapundo *et al.*, 2007).

Mechanisms of resistance

The mechanisms of resistance in the maize plant can be sub-divided into three categories namely structural characteristics focussing on silk length, husk coverage, husk tightness, pericarp thickness and kernel hardness, the biochemical characteristics which focuses on factors such as pH, sugar starch, total nitrogen, carbon-nitrogen ratio, moisture content and phenolic acids and the genetic characteristics such as defence related genes and certain secondary metabolites to acquire systemic resistance.

Maize ears can be infected through two main pathways; by wounds on the kernel and through the silk (Mesterházy *et al*., 2012). When maize plants are infected via the silk fungal spores are deposited on the silk (Cao *et al*., 2014) and the fungus has to grow down the silk before it eventually reaches the kernel (Mesterházy *et al.*, 2012). Hence, longer silk length should hinder the infection by fungi. It has also been stated that maize ears cannot be infected via the silk once the silk has dried out (Reid *et al.*, 1992). Maize silks differ in colour, length and abundance and might also differ in biochemical factors, all these elements could play a role in disease resistance (Reid *et al*., 1992). Thicker pericarps might be an important factor when considering infection through wounds made by insects. According to a 2-year study, resistant and intermediate hybrids had thicker pericarps than the susceptible hybrids (Hoenisch and Davis, 1994). Husk coverage has been associated with susceptibility to maize

ear rot pathogens. Loose husks has been correlated with high incidence of FER while tighter husks are thought to be more resistant (Koehler, 1942; Warfiels and Davis, 1996; Demissie *et al.*, 2008). Furthermore, coverage of the entire developing ear has related to a reduced in the percentage of the infected kernels when compared to an exposed ear (Kommedahl and Windels, 1981; Warfiels and Davis, 1996).

Biochemical factors such as pH have been known to repress fumonisin production. According to Smith *et al*. (2012) acidic conditions such as a pH of 3 are advantageous or beneficial to fumonisin production while alkaline conditions such as pH of 8 might suppress the production of fumonisin. Another biochemical component that affects the biosynthesis on fumonisin is starch (Kim and Woloshuk, 2008). The amount of starch increases tremendously during the maturity of the maize kernels (Bluhm *et al*., 2008). Immature kernels lack starch and at this stage there is no production of fumonisin while a mature kernel has a high starch content and at this stage there are high levels of fumonisin production (Bluhm *et al.*, 2008) Other key regulators for fumonisin production are nitrogen source as well as the carbon to nitrogen ratio (Smith *et al*., 2012). Phenolic acids, which are biochemical compounds, have been known to reduce the colony size of *Fusarium* spp. as well as reducing the toxin production (Samapundo *et al.*, 2007).

As previously discussed, in terms of genetic host resistance, focus on the defence-signalling pathway generated by the host-pathogen interaction would be of significant interest. Recently, a number of studies have compared maize plants resistant and susceptible to FER using genomic approaches (Lanubile *et al*., 2017). Such research has identified candidate genes that potentially contribute to resistance. The genes still require functional annotation but could be utilised by genetic engineering to confer resistance to maize. The signalling response pathway could aid in the identification of defence genes that are being expressed. To provide a rapid response the defence genes could be overexpressed which could result in a disease resistance phenotype (Duvick, 2001).

PHENOLIC COMPOUNDS

Phenolic compounds are naturally occurring compounds derived from plants (Beekrum *et al*., 2003). These compounds are synthesised through the shikimate pathway. It starts with two amino acids (phenylalanine and tyrosine) that loses an ammonia group via the phenylalanine ammonia-lyase (*PAL*) enzyme to produce cinnamic and p-coumaric acid, respectively (Salinas-Moreno *et al.*, 2017). These two compounds then go through the phenylpropanoid pathway to produce the array of phenolic compounds previously identified (Salinas-Moreno *et al.*, 2017). Phenolic compounds occur in two forms either soluble (free phenolic compounds) or insoluble (bound phenolic compounds). Free phenolic compounds are found in lesser

quantities than bound phenolic compounds, but the diversity of the free phenolic compounds is much greater than that of the bound (Salinas-Moreno *et al.*, 2017).

Phenolic compounds and their role in maize resistance

Phenolic compounds are a group of structurally diverse plant secondary metabolites. Plants contain some constitutive phenolic compounds and those produced during the defence response against microbial infection. Phenolic compounds can have a direct or indirect effect on the fungus. Phenolic compounds has been shown to decrease fungal growth and mycotoxin production when Phenolics are in direct contact with the fungus (Beekrum *et al*., 2003; Samapundo *et al*., 2007; Boutigny *et al* 2009; Ponts *et al*., 2011). Phenolic compounds such as phenols can have an indirect effect by being oxidised to quinones. Quinones then produce reactive oxygen species (ROS) which is toxic to the fungus. The free radicals then act directly as antimicrobial agents by disrupting the membrane of the fungal cell wall (Low and Merida, 1996; Apel and Hirt, 2004; Obach and Kalgutkar, 2010). Another method of indirect effect would be the detoxification of mycotoxins to a less toxic metabolite (Galvano *et al*., 2001). Furthermore, phenolic compounds break down the fungal membrane permeability barrier, by causing leakage of ions and other chemicals through the lipid bilayers. Phenolic compounds can also form pores and modify the electric potential of the membrane (Atanasova-Penichon *et al.,* 2016).

Pre-existing phenolic compounds

Pre-existing, constitutive, phenolic compounds contribute to mechanical resistance against insects and are directly antimicrobial substances. In maize, the bulk of phenolic compounds are situated in the pericarp, cell wall and aleurone layers of the kernels (Salinas-Moreno *et al.*, 2017). These phenolic compounds are primarily bound to hemi-cellulose such as ferulic acids and their corresponding dehydrodimers (Kato and Nevins, 1985; Fincher and Stone, 1986) or cross-linked through the production of dimers (Eraso and Hartley, 1990). These phenolic compounds have been shown to be important resistant factors against the maize weevil, *Sitophilus zeamais*, and the grain borer, *Prostephanus truncatu* (Serratos *et al.*, 1987; Classen *et al.*, 1990; Arnason *et al.*, 1992).

Induced phenolic compounds

Several studies have investigated the role of phenolic compounds in resistance to *Fusarium* spp. and the associated mycotoxin contamination (Beekrum *et al*., 2002; Siranidou *et al*., 2002; Atanasova-Penichon *et al*., 2012; Atanasova-Penichon *et al*., 2014). Induced phenolic compounds are a frequently observed biochemical event following successful infection (Reid *et al.*, 1992a; Reid *et al.*, 1992b). In addition, elevated levels of pericarp phenylpropanoids

serve as resistance factors to *F. verticillioides* in resistant maize genotypes (Sampietro *et al.*, 2013) in the cereal cell walls.

It has been shown that secondary metabolites in grains, such as antioxidants and phenolic compounds, could lead to the inhibition of fungal development and mycotoxin deposition (Boutigny *et al.*, 2009). Lipophilic properties of phenolic compounds are known to have an effect on the fungal growth inhibition (Ponts *et al*., 2011*).* There is a strong correlation between the phenolic compounds and their lipophilic properties; a higher level of lipophilicity signifies a greater effect on the fungal growth inhibition (Ponts *et al.,* 2011). Recently, the phenolic content of developing kernels from two lines, one resistant and one susceptible, to *F. graminearum* has been studied (Atanasova-Penichon *et al.*, 2012). Atanasova-Penichon and colleagues (2012) demonstrated that *Fusarium* infection leads to an increase in chlorogenic acid. Moreover, this phenolic compound, together with ferulic acid, was more abundant in the resistant than in the susceptible line. However, such data are not yet available on maize inbred lines resistant to *F. verticillioides*.

Studies have shown that the inhibition of fungal growth is not an indication of the reduction of the mycotoxin accumulation (Chipley and Uriah, 1980; Beekrum *et al*., 2003). Phenolic compounds are anti-oxidants, and mycotoxin biosynthesis is strongly affected by plant metabolites that have pro-oxidant properties (Ponts *et al*., 2011). Phenolic compounds have the ability to inhibit the production of several mycotoxins, including fumonisins (Chipley and Uriah, 1980; Norton, 1999; Bakan *et al*., 2003; Beekrum *et al*., 2003; Samapundo *et al*., 2006; Boutigny *et al.*, 2009; Ponts *et al.*, 2011). In a study by Ferrochio *et al.* (2013) it was shown on maize-based media that high concentrations of ferulic acid can reduce fumonisin production by *F. verticillioides* and *F. proliferatum*. The study concluded that ferulic acid can aid as a post-harvest management strategy to reduce fumonisin accumulation on maize.

BIOSYNTHESIS AND REGULATION OF PHENOLIC COMPOUNDS

Research interest in the regulation of phenolic compounds in maize, should it be pre-existing or induced, has increased over the last few years. Gene expression studies on the *F. verticillioides*-maize pathosystem have shown that numerous phenolic-encoding genes i.e. 3-deoxyanthocyanidin flavonoids, a defective lipoxygenase, shikimate pathway-encoding genes, and phenylalanine ammonia-lyase (*PAL*) are transcriptionally induced upon infection (Lanubile *et al.*, 2010; 2012a; 2012b; 2014). These genes were found to be transcribed at much higher levels in resistant maize genotypes when compared to susceptible maize genotypes (Gao *et al.*, 2007; Lanubile *et al.*, 2010; Lanubile *et al.*, 2012a; Lanubile *et al.*, 2012b; Lanubile *et al.*, 2014; Van Zyl, 2015).

Phenolic compounds are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathway (Randhir *et al*., 2004; Balasundram *et al*., 2006). In a study by Starr

and colleges (2014) it states that the *PAL* gene is an important enzyme in the phenylpropanoid metabolism in plants. This study revealed six *PAL* genes in the initial evaluation of the maize genome. The up-regulation of *ZmPAL*4 was observed in the inoculated plant when compared to the non-inoculated plant (Starr *et al*., 2014); however, these maize plants were inoculated with *Meloidogyne incognita*.

CONCLUSION

The presence of fumonisins in food and feed, needs to be reduced. Several studies have been investigating the role of natural phenolic compounds and antioxidants as a method to manage toxigenic fungi associated with maize. However, more research is required on these plant secondary metabolites to ultimately use these molecules in an integrated management plan. Therefore, the research objective of **Chapter 2** is to test the efficacy of selected phenolic compounds against the growth and mycotoxin production of *Fusarium* species *in vitro*. Phenolic compounds could be developed for spray application to plants similar to fungicides and for the development of resistant GMO inbred lines through the overexpression of genes encoding for phenolic compounds. The employment of local maize cultivars with enhanced resistance to FER presents an ideal disease management strategy. The evaluation of genes that regulate the production of phenolics may therefore be useful to identify lines with increased tolerance. In **Chapter 3** the objective is to extract and characterise phenolic compounds in maize kernels, at different developmental stages, of a resistant and susceptible inbred line as well as determine the differential gene response of phenolic-encoding genes in maize kernels, at different developmental stages, of a resistant and susceptible inbred line. Mycotoxin risk management practices in the maize supply chain should be supported to ensure the delivery of safe products to the consumers as well as developing convenient, affordable and environmentally-friendly methods to manage toxigenic fungi responsible for ear rot diseases in maize.

REFERENCES

Acquaah, G. 2007. Principles of plant genetics and breeding. Blackwell publishing Ltd., Malden, USA, 569 pp.

Ahamed, S., Foster, J. S. Bukovsky, A. and Wimalasena J. 2001. Signal transduction through the Ras/Erk pathway is essential for the mycoestrogen zearalenone-induced cell-cycle progression in mcf-7 cells. Molecular Carcinogenesis 30:88-98.

Al-Heeti, A.A. 1987. Pathological, toxicological and biological evaluations of *Fusarium* species associated with ear rot of maize. PhD thesis. Wisconsin, Madison University.

Apel, K. and Hirt, H. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology 55: 373-399.

Arnold-Reimer, K. 1994. Influence of conservation tillage on plant diseases and weeds in cereals and consequences for targeted crop protection. Thesis, University Goettingen, Germany.

Arora, B.K., Bhatangar, A.P. and Bakshi, A.S. 1973. Critical temperature for drying maize seeds. Journal of Agricultural Engineering 10: 112-116.

Assabgui, R.A., Reid, L.M., Hamilton, R.I. and Arnason, J.T. 1993. Correlation of kernel (E)-ferulic acid content of maize with resistance to *Fusarium graminearum*. Phytopathology 83: 949-953.

Atanasova-Penichon, V., Barreau, C. and Richard-Forget, F. 2016. Antioxidant secondary metabolites in cereals: Potential involvement in resistance to *Fusarium* and mycotoxin accumulation. Frontiers in Microbiology 7: 566.

Atanasova-Penichon, V., Bernillon, S., Marchegay, G., Lornac, A., Ponts, N., Zehraoui, E., Barreau, C. and Richard-forget, F. 2014. Bioguided isolation, characterization, and biotransformation by *Fusarium verticillioides* of maize kernel compounds that inhibit fumonisin production. Molecular Plant-Microbe Interactions 27: 1148-1158.

Atanasova-Penichon, V., Pons, S., Pinson-Gadais, L., Picot, A., Marchegay, G., Bonnin-Verdal, M.-N., Ducos, C., Barreau, C., Roucolle, J., Sehabiague, P., Carolo, P. and Richard-Forget, F. 2012. Chlorogenic acid and maize ear rot resistance: a dynamic study investigating *Fusarium graminearum* development, deoxynivalenol production, and phenolic acid accumulation. Molecular Plant-Microbe Interactions 25: 1605-1616.

Bailey, K.L. and Duczek, L.J. 1996. Managing cereal diseases under reduced tillage. Canadian Journal of Plant Pathology 18: 159-167.

Bakan B, Melcion D, Richard-Molard D, Cahagnier B. 2002. Fungal growth and *Fusarium* mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. Journal of Agricultural and Food Chemistry 50: 728-731.

Balasundram, N., Sundram, K. and Samman, S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry 99: 191-203.

Bankole, S. A. and A. Adebanjo. 2003. Mycotoxins in food in West Africa: current situation and possibilities of controlling it. African Journal of Biotechnology 2: 254-263.

Baranowski, J.D., Davidson, P.M., Nagel, C.W. and Branen, A.L. 1980. Inhibition of *Saccharomyces cerevisiae* by naturally occurring hydroxycinnamates. Journal of Food Science 45: 592-594.

Bassetti, P. and Westgate, M.E. 1993. Emergence, elongation, and senescence of maize silks. Crop Science 33: 271-275.

Beekrum, S., Govinden, R., Padayachee, T. and Odhav, B. 2003. Naturally occurring phenols: A detoxification strategy for fumonisin B_1. Food Additives and Contaminants 20: 490-493.

Bergstrom, G.C. and Shields, E.J. 2002. Atmospheric spore dispersal and regional epidemiology of the Fusarium head blight fungus. Phytopathology 92: S93 (Abstr.)

Beukes, I., Rose, L. J., Shephard, G.S., Flett, B. C. and Viljoen, A. 2017. Mycotoxigenic *Fusarium* species associated with grain crops in South Africa – A review. South African Journal of Science. 113: 1-12.

Bilgrami, K.S. and Choudhary, A.K. 1998. Mycotoxins in preharvest contamination of agricultural crops. Page 43 in: Mycotoxins in Agriculture and Food Safety (K.K. Sinha and D. Bhatnagar, ed.). Marcel Dekker, Inc., New York, USA.

Bluhm, B.H., Kim, H., Butchko, R.A.E. and Woloshuk, C.P. 2008. Involvement of *ZFR1* of *Fusarium verticillioides* in kernel colonization and the regulation of *FST1*, a putative sugar transporter gene required for fumonisin biosynthesis on maize kernels. Molecular Plant Pathology 9: 203-211.

Bortiri, E. and Hake, S. 2007. Flowering and determinacy in maize. Journal of Experimental Botany 58: 909-916.

Bottalico, A. 1998. *Fusarium* diseases of cereals: species complex and related mycotoxin profiles, in Europe. Journal of plant Pathology 80: 85-103.

Boutigny, A.L., Barreau, C., Atanasova-Penichon, V., Verdal-Bonnin, M.N., Pinson-Gadais, L. and Richard-Forget, F. 2009. Ferulic acid, an efficient inhibitor of type B trichothecene biosynthesis and *Tri* gene expression in *Fusarium* liquid cultures. Mycological Research 113: 746-753.

Boutigny, A.L., Ward, T.J., Van Coller, G.J., Flett, B., Lamprecht, S.C., O'Donnell, K. and Viljoen, A., 2011. Analysis of the Fusarium graminearum species complex from wheat, barley and maize in South Africa provides evidence of species-specific differences in host preference. Fungal Genetics and Biology, 48: 914-920.

Boutigny, A.L., Beukes, I., Small, I., Zühlke, S., Spiteller, M., Van Rensburg, B.J., Flett, B. and Viljoen, A. 2012. Quantitative detection of *Fusarium* pathogens and their mycotoxins in South African maize. Plant Pathology 61: 522-531.

Bryden, W.L. 2012. Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security. Animal Feed Science and Technology 173: 134-158.

Bush, B.J., Carson, M.L., Cubeta, M.A., Hagler, W.M. and Payne, G.A. 2004. Infection and fumonisin production by *Fusarium verticillioides* in developing maize kernels. Phytopathology 94: 88-93.

Byerlee, D. and Eicher, C.K. 1997. Evolution of the African maize economy. In: Africa's Emerging Maize Revolution (D. Byerlee and C.K. Eicher, eds.). Lynne Rienner Publishers, London, United Kingdom, 9-22 pp.

Cao, A., Butrón, A., Ramos, A.J., Marín, S., Souto, C. and Santiago, R. 2014. Assessing white maize resistance to fumonisin contamination. European Journal of Plant Pathology 138: 283-292.

Chipley, J. R., and Uraih, N. 1980. Inhibition of *Aspergillus* growth and aflatoxin release by derivatives of benzoic-acid. Applied and Environmental Microbiology 40:352-357.

Classen, D., Amason, J.T., Serratos, J.A., Lambert, J.D.H., Nozzolillo, C. and Philoghe, B.J.R. 1990. Correlation of phenolic acid content of maize to resistance to *Sitophilus zeamais*, the maize weevil in CIMMYT's collections. Journal of Chemical Ecology 16: 301-315.

Comes, M.A. and Riley, J. 1962. An investigation of drying rates and insects control in a maize crib with improved ventilation. West African Stored Products Research Unit. Annual technical report: 12, 72 pp.

Conner, R.L., Hwang, S.F. and Stevens, R.R. 1996. *Fusarium proliferatum*: a new causal agent of black point in wheat. Canadian Journal of Plant Pathology 18: 419-423.

Cotten, T.K. and Munkvold, G.P. 1998. Survival of *Fusarium moniliforme*, *F. proliferatum*, and *F. subglutinans* in Maize Stalk Residue. Phytopathology 88: 550-555.

Cotton, T.K. 1996. Survival and seed transmission of *Fusarium moniliforme*, *Fusarium proliferatum*, and *Fusarium Subglutinans* in maize. MSc Thesis, Iowa State University, USA, 75 pp

Covarelli, L., Beccari, G., Salvi, S. 2011. Infection by mycotoxigenic fungal species and mycotoxin contamination of maize grain in Umbria, central Italy. Food and Chemical Toxicology 49: 2365-2369.

Da Rocha, M.E.B., da Freire, F.C.O., Maia, F.E.F., Guedes, M.I.F. and Rondina, D. 2014. Mycotoxins and their effects on human and animal health. Food Control 36: 159-165.

Damm, U. 1998. Soil mycoflora in two differently managed wheat stocks with special consideration of the *Fusarium* species. Institute on Microbiology 357: 90-91.

Demissie, G., Tefera, T. and Tadesse, A. 2008. Importance of husk covering on field infestation of maize by *Sitophilus zeamais* Motsch (Coleoptera: *Curculionidea*) at Bako, Western Ethiopia. African Journal of Biotechnology: 7.

Department of Agriculture, Forestry and Fisheries (DAFF). 2018. Crops estimates committee. https://www.daff.gov.za/docs/Cropsestimates/Media%20June%202018.pdf (8 October 2018).

Desai, B.B. 2004. Seeds Handbook: Biology, Production, processing and storage. Second edition Revised and expanded. Marcel Dekker, Inc., NY, USA.

Desjardins, A. E. and Plattner, R. D. 2000. Fumonisin B_1 – nonproducing strains of *Fusarium verticillioides* cause maize (*Zea mays*) ear infection and ear rot. Journal of Agricultural and Food Chemistry 48: 5773-5780.

Desjardins, A.E. 2003. *Gibberella* from *A (venacceae)* to *Z (eae)*. Annual Review Phytopathology 41: 177-198.

Du Plessis, J. 2003. Maize production. Resource Centre Directorate Agricultural Information Services Private Bag X144, Pretoria, 1-38 pp.

Duncan, K.E. and Howard, R.J. 2010. Biology of maize kernel infection by *Fusarium verticillioides*. Molecular Plant-Microbe Interactions 23: 6-16.

Duvick, J. 2001. Prospects for reducing fumonisin contamination of maize through genetic modification. Environmental Health Perspectives 109: 337-342.

Edwards, E.T. 1935. Studies on *Gibberella fujikuroi var. subglutinans* the hitherto undescribed ascigerous stage of *Fusarium monilifornie var. subglutinans* and on its pathogenicity on maize in New South Wales. PhD thesis, Department of Agriculture, New South Wales.

Eraso, F. and Hartley, R.D. 1990. Monomeric and dimeric phenolic constituents of plant cell walls. Journal of Science and Food Agriculture 51: 163-170.

European Union (EU). 2006. EU regulation on mycotoxins. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs https://ec.europa.eu/jrc/en/eurl/mycotoxins/legislation.

Fandohan, P., Hell, K., Marasas, W.F.O. and Wingfield, M.J. 2003. Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. African Journal of Biotechnology 2: 570-579.

Farber, J.M., Sanders, G.W., Lawrence, G.A. and Scott, P.M. 1988. Production of moniliformin by Canadian isolates of *Fusarium*. Mycopathologia 101: 187- 190.

Farnham, D.E., Benson, G.O. and Pearce, R.B. 2003. Corn perspective and culture. Pages 30-33 in: Corn: Chemistry and Technology (P.J. White and L.A. Johnson, eds.) American Association of Cereal Chemicals, Inc., St. Paul, Minesota, USA.

Fernando, W.G.D., Paulitz, T.C., Seaman, W.L., Dutilleul, P. and Miller, J. D. 1997. Head blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. Phytopathology 87:414-421.

Ferrochio, L., Cendoya, E., Farnochi, M.C., Massad, W and Ramirez, M.L. 2013. Evaluation of ability of ferulic acid to control growth and fumonisin production of *Fusarium verticillioides* and *Fusarium proliferatum* on maize based media. International Journal of food Microbiology 167: 215-220.

Fincher, G.B. and Stone, B.A. 1986. Cell walls and their components in cereal grain technology. Advances in Cereal Science and Technology 8: 207-295.

Foley, D.C. 1962. Systemic infection of corn by *Fusarium moniliforme*. Phytopathology 52: 870-872.

Fox, M.E. and Howlett, B.J. 2008. Secondary metabolism: regulation and role in fungal biology. Current Opinion in Microbiology 11: 481-487.

Galvano, F., Piva, A., Ritieni, A. and Galvano, G., 2001. Dietary strategies to counteract the effects of mycotoxins: A review. Journal of food protection 64: 120-131.

Gao, X., Shim, W.B., Göbel, C., Kunze, S., Feussner, I., Meeley, R., Balint-Kurti, P. and Kolomiets, M. 2007. Disruption of a maize 9-lipoxygenase results in increased resistance to fungal pathogens and reduced levels of contamination with mycotoxin fumonisin. Molecular Plant-Microbe Interactions 20: 922-933.

Gelderblom, W.C.A., Jaskiewicz, K., Marasas, W.F.O., Thiel, P.G., Horak, R.M., Vleggaar, R. and Krick, N.P.J. 1988. Fumonisins - Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Applied and Environmental Microbiology 54: 1806-1811.

Gelderblom, W.C.A., Rheeder, J.P., Leggott, N., Stockenstrom, S., Humphreys, J., Shephard, G.S. and Marasas, W.F.O. 2004. Fumonisin contamination of a corn sample associated with the induction of hepatocarcinogenesis in rats-role of dietary deficiencies. Food and Chemical Toxicology 42: 471-479.

Gillette, K.S. 1999. Biodiversity of *Fusarium* species in Iowa maize fields and kernels: Preharvest and postharvest. M.S. Thesis, Iowa State University, USA, 123 pp.

Gokul, A. 2015. *Fusarium graminearum* species complex (FGSC) composition in South African wheat and maize grown in rotation. MScAgric thesis, University of Stellenbosch, Stellenbosch, South Africa.

Hanft, J.M. and Jones, R.J. 1986. Kernel abortion in maize. Plant Physiology 81: 503-510.

Hanway, J.J. 1966. How a corn plant develops. Special report. Iowa State University.

Harris, L.J., Desjardins, A.E., Plattner, R.D., Nicholson, P., Butler, G., Young, J.C., Weston, G., Proctor, R.H. and Hohn, T.M. 1999. Possible role of trichothecene mycotoxins in virulence of *Fusarium graminearum* on maize. Plant Disease 83: 954-960.

Headrick, J.M. and Pataky, J.K. 1991. Maternal influence on the resistance of sweet corn lines to kernel infection by *Fusarium moniliforme*. Phytopathology 81: 268-274.

Hindmarsh, P.S. and MacDonald, T. A. 1980. Field trials to control insect pests of farm stored maize in Zambia. Journal of Stored Products Research 16: 9-18.

Hoenisch, R. and Davis, R. 1994. Relationship between kernel pericarp and FER. Plant Disease 78: 517-519.

Irish, E.E. and Nelson, T. 1989. Sex determination in monoecious and dioecious plants. The Plant Cell 1: 737.

Jones, R.K., Duncan, H.E., Payne, G.A. and Leonard, K.J. 1980. Factors influencing infection by *Aspergillus flavus* in silk-inoculated corn. Plant Disease 64: 859-863.

Jurado, M., Marín, P., Magan, N. and González-Jaén, M.T. 2008. Relationship between solute, matric potential stress, temperature, growth, and *FUM1* gene expression in two *Fusarium verticillioides* strains from Spain. Applied and Environmental Microbiology 74: 2032-2036.

Kabeere, F., Hampton, J.G. and Hill, M.J. 1997. Transmission of *Fusarium graminearum* (Schwabe) from maize seeds to seedlings. Seed Science Technology 25: 245-252.

Kasenberg, T.R and Traquair, J.A. 1988. Effects of phenolics on growth of *Fusarium oxysporum* f.sp. *radicis-lycopersici in vitro.* Canadian Journal of Botany 66: 1174-1177.

Kato, Y. and Nevins, D.J. 1985. Isolation and identification of 2-045'- 0-trans-feruloyl-B-L-arabinofuranosy1)-(1-3)-0-B-D xylopyranosyl-(l-4)-D-xylopyranose as a component of *Zea* shoot cell walls. Carbohydrates Research 137: 139-150.

Kedera C.J., Leslie, J.F. and Claflin, L.E. 1994. Genetic diversity of *Fusarium* section Liseola (*Gibberella fujikuroi*) in individual maize stalks. Phytopathology 84: 603-607.

Kim, H. and Woloshuk, C.P. 2008. Role of *AREA*, a regulator of nitrogen metabolism, during colonization of maize kernels and fumonisin biosynthesis in *Fusarium verticillioides*. Fungal Genetics and Biology 45: 947-953.

Koehler, B. 1942. Natural mode of entrance of fungi into corn ears and some symptoms that indicate infection. Journal of Agricultural Research 64.

Kommedahl, T. and Windels, C.E. 1981. Root-, stalk-, and ear-infecting *Fusarium* species on corn in the USA. Pages 94-103 in: Fusarium: Diseases, Biology, and Taxonomy (P.E. Nelson, T.A. Toussoun, and R.J. Cook, eds.). Pennsylvania State University, University Park.

Kruger, W. 1962. *Sphacelotheca reiliana* on maize I. Infection and control studies. South African Journal of Agricultural Science 5: 43-56.

Kuiper-Goodman, T., Scott, P. M. and Watanabe, H. 1987. Risk assessment of the mycotoxin zearalenone. Regulatory Toxicology and Pharmacology 7: 253-306.

Lamprecht, S.C., Marasas, W.F.O., Alberts, J.F., Cawood, M.E., Gelderblom, W.C.A., Shepard, G.S., Thiel, P.G. and Calitz, F.J. 1994. Phytotoxicity of fumonisins and TA-toxin to corn and tomato. Phytopathology 84: 383-391.

Lanubile, A., Bernardi, J., Battilani, P., Logrieco, A. and Marocco, A. 2012a. Resistant and susceptible maize genotypes activate different transcriptional responses against *Fusarium verticillioides*. Physiological and Molecular Plant Pathology 77: 52-59.

Lanubile, A., Bernardi, J., Marocco, A., Logrieco, A. and Paciolla, C. 2012b. Differential activation of defense genes and enzymes in maize genotypes with contrasting levels of resistance to *Fusarium verticillioides*. Environmental and Experimental Botany 78: 39-46.

Lanubile, A., Ferrarini, A., Maschietto, V., Delledonne, M., Marocco, A. and Bellin, D. 2014. Functional genomic analysis of constitutive and inducible defense responses to *Fusarium verticillioides* infection in maize genotypes with contrasting ear rot resistance. BMC Genomics 15:710.

Lanubile, A., Maschietto, V., Borrelli, V.M., Stagnati, L., Logrieco, A.F. and Marocco, A. 2017. Molecular basis of resistance to Fusarium ear rot in maize. Frontiers in Plant Science 8: 1774.

Lanubile, A., Pasini, L. and Marocco, A. 2010. Differential gene expression in kernels and silks of maize lines with contrasting levels of ear rot resistance after *Fusarium verticillioides* infection. Journal of Plant Physiology 167: 1398-1406.

Leslie, J.F. and Summerell, B.A. 2006. *Fusarium verticillioides* (Saccardo) Nirenberg. The *Fusarium* Laboratory Manual (J.F. Leslie and B.A. Summerell, eds.). Blackwell Publishing, Oxford, UK.

Logrieco, A., Moretti, A., Altomare, C., Bottalico, A. and Torres, E.C., 1993. Occurrence and toxicity of *Fusarium subglutinans* from Peruvian maize. Mycopathologia 122: 185-190.

Lopez-Martinez, L.X., Oliart-Ros, R.M., Valerio-Alfaro, G., Lee, C.H., Parkin, K.L. and Garcia, H.S. 2009. Antioxidant activity, phenolic compounds and anthocyanins content of eighteen strains of Mexican maize. Food Science and Technology 42: 1187-1192.

Low, P.S. and Merida, J.R., 1996. The oxidative burst in plant defense: Function and signal transduction. Physiologia Plantarum 96: 533-542.

Maiorano, A., Reyneri, A., Magni, A., and Ramponi, C. 2009a. A decision tool for evaluating the agronomic risk of exposure to fumonisins of different maize crop management systems in Italy. Agricultural Systems 102: 17-23.

Maiorano, A., Reyneri, A., Sacco, D., Magni, A. and Ramponi, C. 2009. A dynamic risk assessment model (FUMAgrain) of fumonisin synthesis by *Fusarium verticillioides* in maize grain in Italy. Crop Protection 28: 243-256.

Mannon, J. and Johnson, E. 1985. Fungi down on the farm. New Scientist 105: 12-16.

Marasas, W.F.O., Kellerman, T.S., Gelderblom, W.C.A., Coetzer, J.A.W., Thiel, P.G. and Van der Lugt, J.J. 1988. Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. Journal of Veterinary Research 55: 197-203.

Marasas, W.F.O., Riley, R.T., Hendricks, K.A., Stevens, V.L., Sadler, T.W., Gelineau-van Waes, J., Missmer, S.A., Cabrera, J., Torres, O., Gelderblom, W.C.A., Allegood, J., Martínez, C., Maddox, J., Miller, J.D., Starr, L. Sullards, M. C., Roman,A.V., Voss, K. A., Wang, E. and Merrill, A.H. Jr. 2004. Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and *in vivo*: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. The Journal of Nutrition 134: 711-716.

Marasas, W.F.O., Thiel, P.G., Rabie, C.J., Nelson, P.E. and Toussoun, T.A .1986. Moniliformin production in *Fusarium* section Liseola. Mycologia 78: 242-247.

Marasas, W.F.O., Wehner, F.C., Van Rensburg, S.J. and Van Schalkwyk, D.J. 1981. Mycoflora of corn produced in human esophageal cancer areas in Transkei, southern Africa. Phytopathology 71: 792-796.

Marín, S., Homedesa, V., Sanchis, A.J., Ramos, N. and Magan, N. 1999. Impact of *Fusarium moniliforme* and *F. proliferatum* colonisation of maize on calorific losses and fumonisin production under different environmental conditions. Journal of Stored Products and Research 35: 15-26.

Marín, P., Magan, N., Vazquez, C. and Gonzalez-Jaen, M.T. 2010. Differential effect of environmental conditions on the growth and regulation of the fumonisin biosynthetic gene *FUM1* in the maize pathogens and fumonisin producers Fusarium verticillioides and *Fusarium proliferatum*. Federation of European Microbiological Societies Microbiology and Ecology 73: 303-311.

McKeehen, J.D., Busch, R.H., and Fulcher, R.G. 1999. Evaluation of wheat (*Triticum aestivum* L.) phenolic acids during grain development and their contribution to *Fusarium resistance*. Journal of Agricultural and Food Chemistry 47: 1476-1482.

Mesterházy, Á., Lemmens, M. and Reid, L.M. 2012. Breeding for resistance to ear rots caused by *Fusarium* spp. in maize - A review. Plant Breeding 131: 1-19.

Munkvold, G.P. 2003. Cultural and genetic approaches to managing mycotoxins in maize. Annual Review of Phytopathology 41: 99-116.

Munkvold, G.P. and Desjardins, A.E. 1997. Fumonisins in maize: Can we reduce their occurrence? Plant Disease 81: 556-565.

Munkvold, G.P., Hellmich, R.L. and Rice, L.G. 1999. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and non-transgenic hybrids. Plant Disease 83: 130-138.

National Academy of Science (NAS). 1978. Postharvest food losses in developing countries. Washington.

Ncube, E., Flett, B.C., Waalwijk, C. and Viljoen, A. 2011. *Fusarium* spp. and levels of fumonisins in maize produced by subsistence farmers in South Africa. South African Journal of Science 107: 1-7.

Nel, A., Krause, M., and Khelawanlall, N. 2003. A guide for the control of plant diseases. National Department of Agriculture, Pretoria, South Africa.

Nelson, P.E., 1992. Taxonomy and biology of *Fusarium moniliforme*. Mycopathologia 117: 29-36.

Nicholson, R. L., and Hammerschmidt, R. 1992. Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology 30: 369-389.

Nielsen, R.L. 2016a. Grain filling stages in corn. Corny News Network, Purdue University of Agronomy. http://www.kingcorn.org/news/timeless/GrainFill.html (June 2018).

Nielsen, R.L. 2016b. Silk emergence. Corny News Network, Purdue University of Agronomy. http://www.kingcorn.org/news/timeless/Silks.html (June 2018).

Norred, W.P. and Voss, K.A. 1994. Toxicity and role of fumonisin in animal diseases and human oesophageal cancer. Journal of Food Protection. 57: 522-527.

Norton, R.A. 1999. Inhibition of aflatoxin B-1 biosynthesis in *Aspergillus flavus* by anthocyanidins and related flavonoids. Journal of Agricultural and Food Chemistry. 47: 1230-1235.

O'Donnell, K. Ward, T. J., Geiser, D. M., Kistler H. C and Aoki, T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal genetics and biology. 41: 600-623.

O'Donnell, K., Ward, T. J., Aberra, D., Kistler, H. C., Aoki, T., Orwig, N., Kimura, M., Bjørnstad, A. and Klemsdal, S. S. 2008. Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the *Fusarium graminearum* species complex from Ethiopia. Fungal Genetics and Biology 45: 1514-1522.

Obach, R. Scott, and A. S. Kalgutkar. 2010. Reactive electrophiles and metabolic activation. Comprehensive Toxicology (Second Edition) 1: 309-347.

Oldenburg, E. and Ellner, F. 2015. Distribution of disease symptoms and mycotoxins in maize ears infected by *Fusarium culmorum* and *Fusarium graminearum*. Mycotoxin Research 31: 117-126.

Oldenburg, M., Petersen, A. and Baur, X. 2011. Maize pollen is an important allergen in occupationally exposed workers. Journal of Occupational Medicine and Toxicology 6: 32.

Paliwal, R.L. 2000. Tropical maize morphology. Pages 13-20 in: Tropical Maize: Improvement and Production (R.L. Paliwal, G. Granados, H.R. Lafitte, A.D Vlollci, eds.). Food and Agriculture Organization of the United Nations, Rome.

Pestka, J.J. and Smolinski, A.T. 2005. Deoxynivalenol: Toxicology and potential effects on humans. Journal of Toxicology and Environmental Health, Part B 8: 39-69.

Picot, A., Atanasova-Pénichon, V., Pons, S., Marchegay, G., Barreau, C., Pinson-Gadais, L., Roucolle, J., Daveau, F., Caron, D. and Richard-Forget, F. 2013. Maize kernel antioxidants and their potential involvement in Fusarium ear rot resistance. Journal of Agricultural and Food Chemistry 61: 3389-3395.

Ponts, N., Pinson-Gadais, L., Boutigny, A.L., Barreau, C. and Richard-Forget, F. 2011. Cinnamic-derived acids significantly affect *Fusarium graminearum* growth and in vitro synthesis of type B trichothecenes. Phytopathology 101: 929-934.

Proctor, R.H., Desjardins, A.E., Plattner, R.D. and Hohn, T.M. 1999. A polyketide synthase gene required for biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* mating population A. Fungal Genetics and Biology 27: 100-112.

Purushothaman, D. 1976. Changes in phenolic compounds in rice cultivars as influenced by *Xanthomonas oryzae*. Riso 25: 88-91

Rabie, C.J. and Lübben, A. 1984. The mycoflora of sorghum: *Sorghum caffrorum* malt. South African Journal of Botany 3: 251-255.

Randhir, R., Lin, Y.T. and Shetty, K. 2004. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asia Pacific Journal of Clinical Nutrition 13: 295-307.

Reid, L.M., Mather, D.E., Arnason, J.T., Hamilton, R.I. and Bolton, A.T. 1992. Changes in phenolic constituents of maize silk infected with *Fusarium graminearum*. Canadian Journal of Botany 70: 1697-1702.

Reid, L.M., Nicol, R.W., Ouellet, T., Savard, M., Miller, J.D., Young, J.C., Stewart, D.W. and Schaafsma, A.W. 1999. Interaction of *Fusarium graminearum* and *F. moniliforme* in maize ears: disease progress, fungal biomass, and mycotoxin accumulation. Phytopathology 89: 1028-1037.

Reynoso, M.M., Torres, A.M. and Chulze, S.N. 2004. Fusaproliferin, beauvericin and fumonisin production by different mating population among *Gibberella fujikuroi* species complex isolated from maize. Mycological Research 108: 154-160.

Rheeder, J. P., Marasas, W. F. O., Thiel, P. G., Syndenham, E. W., Shephard, G.S. and van Schalkwyk, D. J. 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human oesophageal caner in Transkei. Phytopathology 82: 353-357.

Ross, P.F., Nelson, P.E., Richard, J.L., Osweiler, G.D., Rice, L.G., Plattner, R.D. and Wilson, T.M. 1990. Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary edema syndrome in swine. Applied and Environmental Microbiology 56: 3225-3226.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y. and Hunt, M.D. 1996. Systemic acquired resistance. Plant Cell 8: 1809-1819.

Salinas-Moreno, Y., García-Salinas, C., Ramírez-Díaz, J.L. and Alemán-de la Torre, I. 2017. Phenolic compounds in maize grains and its nixtamalized products. In: Phenolic Compounds-Natural Sources, Importance and Applications.

Salunkhe, D.K., Chavan, J.K and Kadam, S.S. 1985. Postharvest biotechnology of cereals. CRC Press. Boca Raton, Florida.

Samapundo, S., De Meulenaer, B., Osei-Nimoh, D., Lamboni, Y., Debevere, J. and Devlieghere, F. 2007. Can phenolic compounds be used for the protection of corn from fungal invasion and mycotoxin contamination during storage? Food Microbiology 24: 465-473.

Sampietro, D.A., Fauguel, C.M., Vattuone, M.A., Presello, D.A. and Catalán, C.A.N. 2013. Phenylpropanoids from maize pericarp: resistance factors to kernel infection and fumonisin accumulation by *Fusarium verticillioides*. European Journal of Plant Pathology 135: 105-113.

Santiago, R., Reid, L.M., Arnason, J.T., Zhu, X., Martinez, N. and Malvar, R.A. 2007. Phenolics in maize genotypes differing in susceptibility to Gibberella stalk rot (*Fusarium graminearum* Schwabe). Journal of Agricultural and Food Chemistry 55: 5186-5193.

Santos, R.R., Schoevers, E.J., Roelen, B.A.J. and Fink-Gremmels, J. 2013. Mycotoxins and female reproduction: *in vitro* approaches. World Mycotoxin Journal 6: 245-253.

Schoeman, A., Flett, B.C., van Rensburg, B.J., Ncube, E. and Viljoen, A. 2018. Pathogenicity and toxigenicity of *Fusarium verticillioides* isolates collected from maize roots, stems and ears in South Africa. European Journal of Plant Pathology, 152: 677-689.

Serna-Saldivar, S.O. 2012. Cereal grains: Laboratory reference and procedures manual. CRC Press.

Serratos, J.A., Amason, J.T., Nozzolillo, C., Lambert, J.D.H., Philogene, B.J.R., Fulcher, R. G., Davidson, K., Peacock, L., Atkinson, J. and Morand, P. 1987. The factors contributing to resistance of exotic maize populations to maize weevil *Sitophilus zeamais*. Journal of Chemical Ecology 13: 751-762.

Sewram, V., Nieuwoudt, T.W., Marasas, W.F.O., Shephard, G.S. and Ritieni, A., 1999. Determination of the mycotoxin moniliformin in cultures of *Fusarium subglutinans* and in naturally contaminated maize by high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. Journal of Chromatography A 848: 185-191.

Shephard, G.S. 2008. Impact of mycotoxins on human health in developing countries. Food Additives and Contaminants 25: 146-151.

Shephard, G.S., Marasas, W.F.O., Burger, H.M., Somdyala, N.I.M, Rheeder, J.P, Van der Westhuizon, L., Gatyeni, P and Van Schalkwyk, D.J. 2007. Exposure assessment for fumonisins in the former Transkei region of South Africa. Food Additives and Contaminants 24: 621-629.

Siranidou, E., Kang, Z. and Buchenauer, H. 2002. Studies on symptom development, phenolic compounds and morphological defence responses in wheat cultivars differing in resistance to Fusarium head blight. Journal of Phytopathology 150: 200-208.

Sleper, D.A. and Poehlman, J.M. 2006. Breeding corn (maize). Pages 277-296 in: Breeding Field Crops (D.A. Sleper and J.M. Poehlman, eds.). Blackwell Publishing, USA.

Small, I.M., Flett, B.C., Marasas, W.F.O., McLeod, A., Stander, M.A. and Viljoen, A. 2012. Resistance in maize inbred lines to *Fusarium verticillioides* and fumonisin accumulation in South Africa. Plant Disease 96: 881-888.

Smith D.R and White D.G. 1988. Disease on corn. Pages 687-766 in: Corn and Corn Improvements, 3rd Edition, Agronomy Series No. 18 (G.F. Sprague and J.W. Dudley, eds.). American Society of Agronomy, Madison, WI, USA.

Smith, M.C., Madec, S., Coton, E. and Hymery, N. 2016. Natural co-occurrence of mycotoxins in foods and feeds and their in vitro combined toxicological effects. Toxins 8: 94.

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

South African Department of Health. 2016. Foodstuffs, cosmetics and disinfectants Act, 1972 (Act 54 of 1972) Regulations governing tolerance for fungus-produced toxins in foodstuffs: amendment. Government Gazette.

Starr, J.L., Yang, W., Yan, Y., Crutcher, F. and Kolomiets, M., 2014. Expression of phenylalanine ammonia lyase genes in maize lines differing in susceptibility to *Meloidogyne incognita*. Journal of Nematology 46: 360.

Steinkellner, S. and Langer, I. 2004. Impact of tillage on the incidence of *Fusarium* spp. in soil. Plant and Soil 267: 13-22.

Sumner, D.R. 1968. Ecology of corn stalk rot in Nebraska. Phytopathology 58: 755-760.

Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Canadian Journal of Plant Pathology 4: 195-209.

Sutton, J.C., Baliko, W. and Funnell, H.S. 1980. Relation of weather variables to incidence of zearalenone in southern Ontario. Canadian Journal of Plant Science 60: 149-155.

Swan, L.J., Backhouse, D. and Burgess, L.W., 2000. Surface soil moisture and stubble management practice effects on the progress of infection of wheat by *Fusarium pseudograminearum*. Australian Journal of Experimental Agriculture 40: 693-698.

Sydenham, E.W., Van Der Westhuizen, L., Stockenström, S., Shephard, G.S. and Thiel, P.G. 1994. Fumonisin-contaminated maize: physical treatment for the partial decontamination of bulk shipments. Food Additives and Contaminants 11: 25-32.

Thomison, P.R. 2013. Cultural practices for optimizing maize seed yield and quality in production fields.

Van der Lee, T., Zhang, H., van Diepeningen, A. and Waalwijk, C. 2015. Biogeography of *Fusarium graminearum* species complex and chemotypes: A review. Food Additives and Contaminant 32: 453-460.

Van Zyl, K. 2015. Resistance in maize to infection and toxin production by *Fusarium verticillioides*. MSc Agric thesis, University of Stellenbosch, Stellenbosch, South Africa.

Vigier, B., Reid, L.M., Dwyer, L.M., Stewart, D.W., Sinha, R.C., Arnason, J.T. and Butler G. 2001. Maize resistance to Gibberella ear rot: symptoms, deoxynivalenol, and yield. Canadian Journal of Plant Pathology 23: 99-105.

Vigier, B., Reid, L.M., Seifert, K.A. Stewart, D.W. and Hamilton, R.I. 1997. Distribution and prediction of *Fusarium* species associated with maize ear rot in Ontario. Canadian Journal of Plant Pathology 19: 60-65.

Wang, Y., Zhou, Z., Gao, J., Wu, Y., Xia, Z., Zhang, H. and Wu, J. 2016. The mechanisms of maize resistance to *Fusarium verticillioides* by comprehensive analysis of RNA-seq data. Frontiers in Plant Science 7: 1654.

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

Ward, T.J., Bielawski, J.P., Kistler, H.C., Sullivan, E. and O'Donnell, K. 2002. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. Proceedings of the National Academy of Sciences 99: 9278-9283.

Warfield, C.Y. and Davis, R.M. 1996. Importance of the husk covering on the susceptibility of corn hybrids to Fusarium ear rot. Plant Disease 80: 208-210.

Weber, R., Hrynczuk, B., Runowska-Hrynczuk, B. and Kita, W. 2001. Influence of the mode of tillage on diseases of culm base in some winter wheat varieties, oats and spring wheat. Phytopathological journal 149: 3-4.

White, D. 1999. *Fusarium* kernel or ear rot. Pages 20-21 in: Compendium of Corn Diseases. American Phytopathological Society Press, St. Paul, USA.

Yates, I.E. and Sparks, D. 2008. *Fusarium verticillioides* dissemination among maize ears of field-grown plants. Crop Protection 27: 606-613.

Yates, I.E., Meredith, F., Smart, W., Bacon, C.W. and Jaworski, A.J. 1999. *Trichoderma viride* suppresses fumonisin B_1 production by *Fusarium moniliforme*. Journal of Food Protection 62: 1326-1332.

Zain, M.E. 2011. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society 15: 129-144.

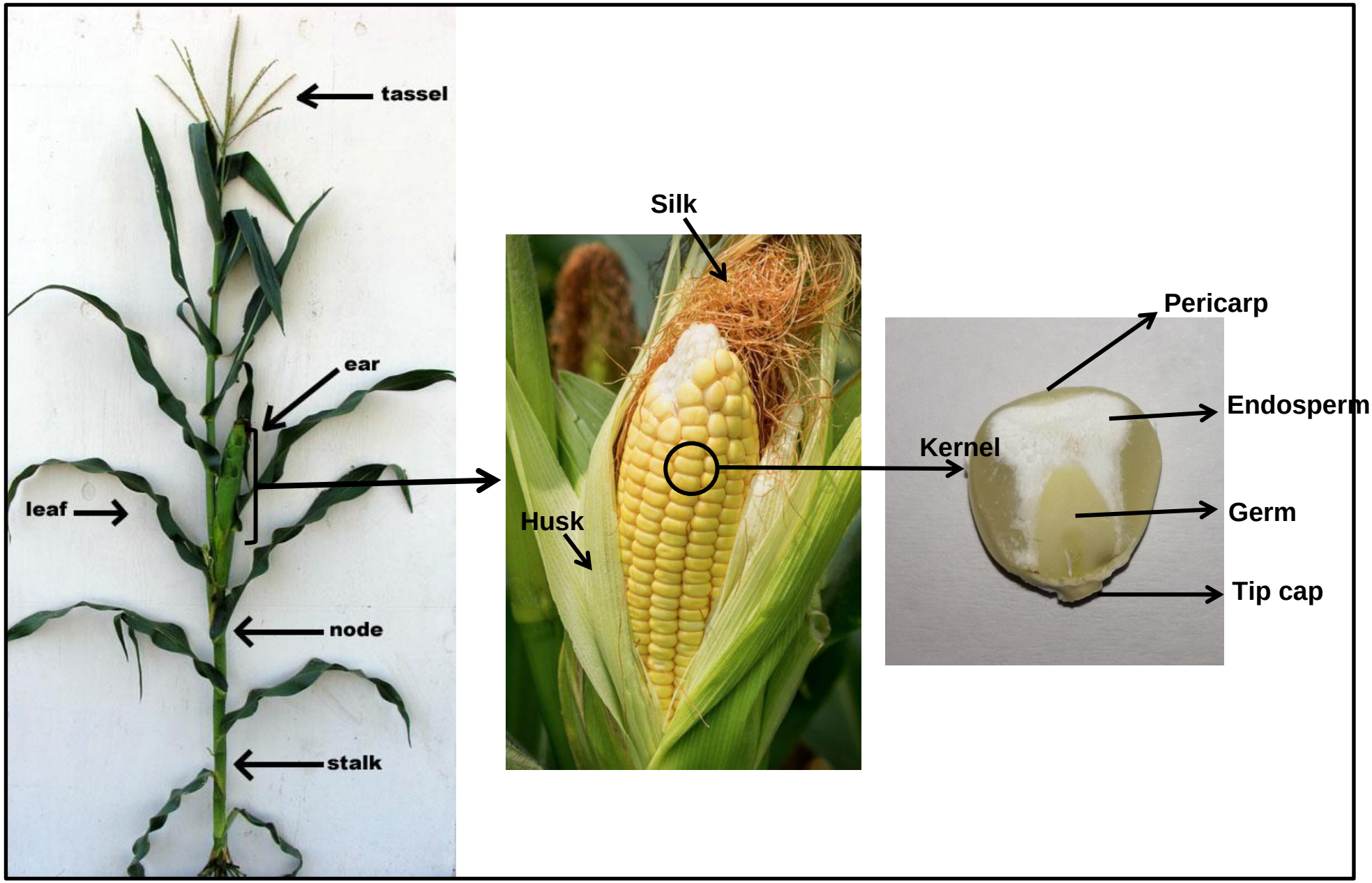


Figure 1. Maize plant and kernel morphology.

(Source: http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=1075412493&topicorder=3&maxto=12 and https://www.farmcrowdy.com/farm/maize-farm-kaduna/).

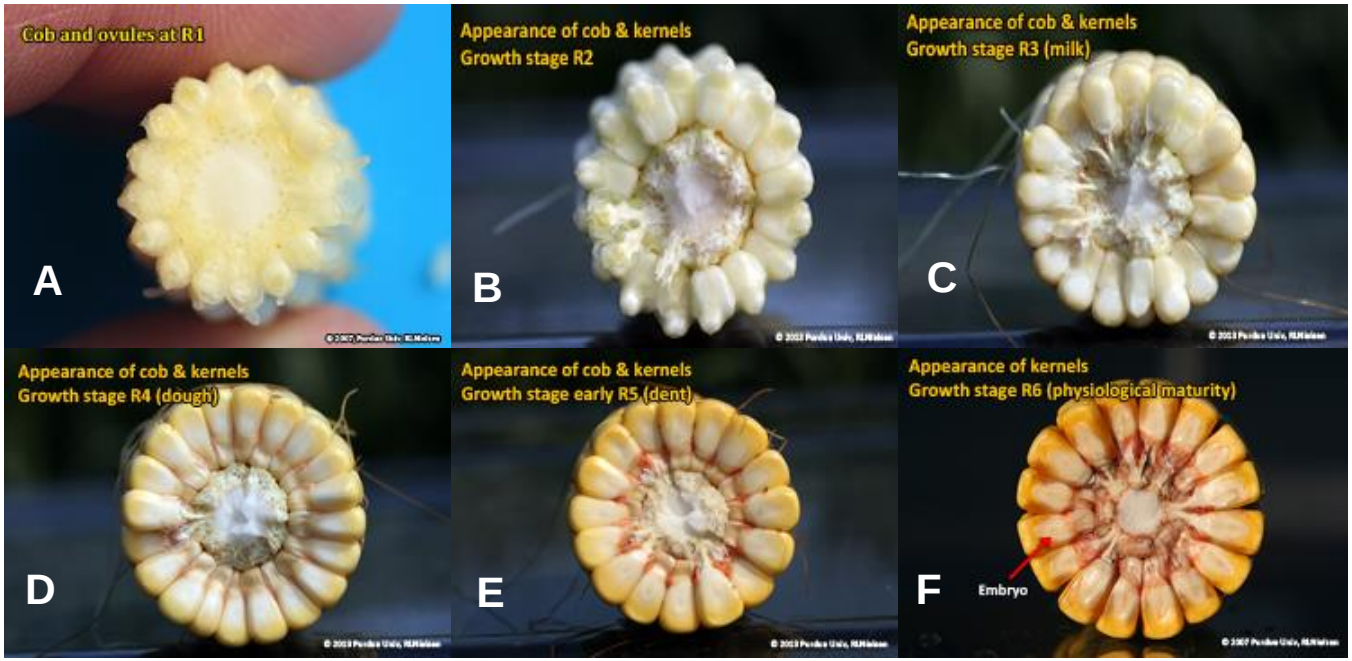


Figure 2. Cross section through the kernel at different development stages. A: silking stage, B: blister stage, C: milk stage, D: dough stage, E: dent stage, F: physiological maturity (Source: https://www.agry.purdue.edu/ext/corn/news/timeless/GrainFill.html).

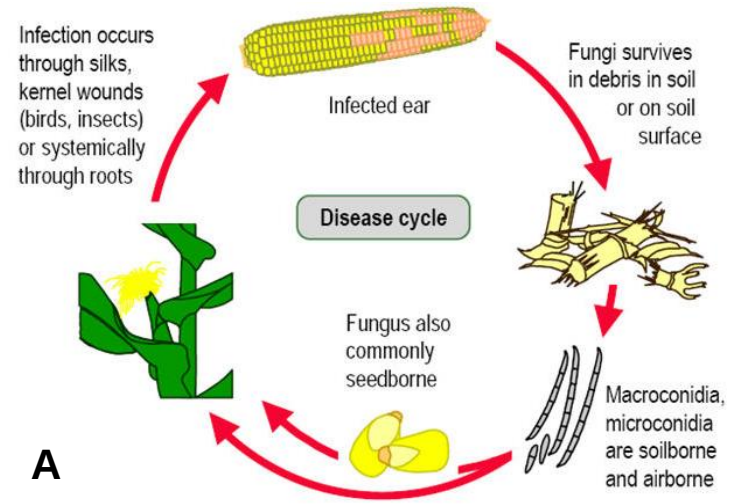


Figure 3. Fusarium ear rot caused by *F. verticillioides*. A: Disease cycle, B: Star burst symptoms (Source https://www.pioneer.com/home/site/us/agronomy/crop-management/corn-insect-disease/fusarium-ear-rot/).

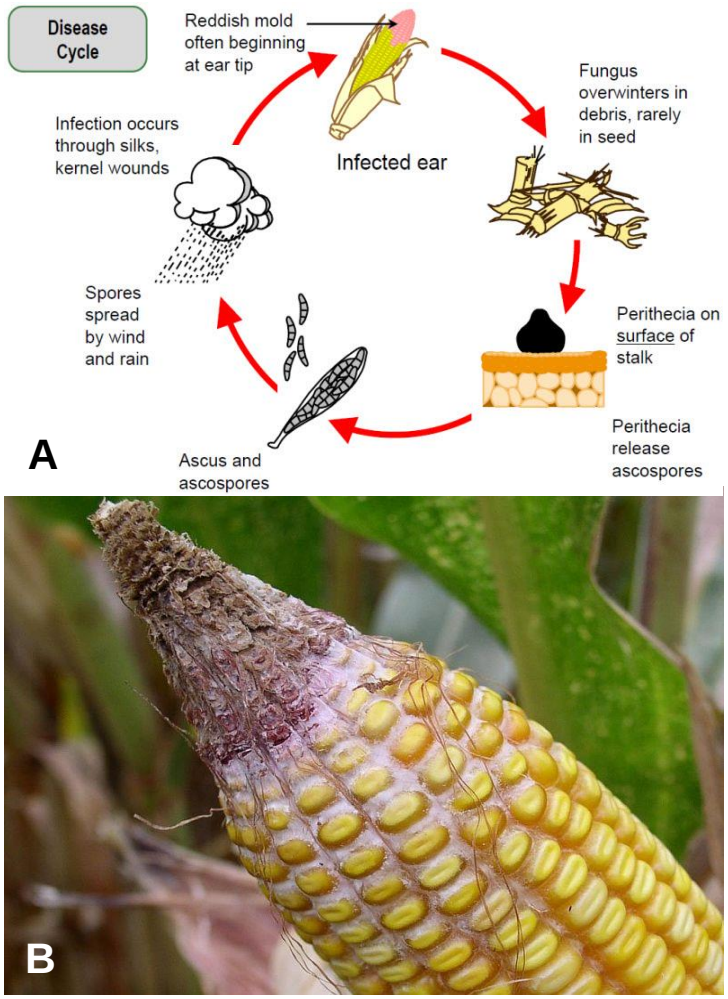


Figure 4. Gibberella ear rot caused by the pathogens in the *F. graminearum* species complex. A: Disease cycle, B: white to pink mould starting at the tip of the ear characteristic of Gibberella ear rot (Source https://www.pioneer.com/home/site/us/agronomy/crop-management/corn-insect-disease/gibberella-ear-rot/).

CHAPTER 2

In vitro assessment of phenolic compounds on the growth and mycotoxin production of *Fusarium* species associated with maize ear rot

ABSTRACT

Fusarium ear rot (FER) and Gibberella ear rot (GER) are of the most important fungal diseases that affect maize in South Africa. *Fusarium verticillioides*, *F. proliferatum* and *F. subglutinans* are the causal agents of FER and *F. boothii* and *F. graminearum* sensu stricto are the causal agents of GER. These pathogens produce mycotoxins such as fumonisins (FUM), zearalenone (ZEA) and trichothecenes (TCT) which can have detrimental effects on humans and animals. Enhanced plant defense is considered the most feasible disease and mycotoxin management strategy. Phenolic compounds, produced by plants, have been associated with defence mechanisms and potentially contribute to the resistance response against mycotoxigenic fungi. This study, therefore, investigated the effect of phenolic compounds on the growth, fungal biomass and mycotoxin production of FER causal pathogens and GER causal pathogens. Six phenolic compounds, vanillic, ferulic, caffeic, coumaric, chlorogenic and sinapic acid were evaluated at three concentrations (0.5, 1.5 and 2.5 mM) *in vitro*. The effect of phenolic compounds on fungal growth was determined by phenolic-amended media while fungal biomass was evaluated using a microtiter-based assay. The effect of phenolics on mycotoxin production was assessed by a phenolic-amended rice and media assay, respectively. Phenolic compounds inhibited the fungal growth of all the *Fusarium* species evaluated when compared to the control. Ferulic acid (2.5 mM) was the most effective phenolic compound in reducing the fungal growth when compared to the other phenolic compounds for most of the isolates tested. Ferulic acid (0.5 mM) and chlorogenic acid (2.5 mM) in combination had a synergistic effect and resulted in a significantly greater reduction in fungal growth when compared to the control and the individual compounds. Phenolic compounds only reduced the biomass for isolates of *F. verticillioides, F. proliferatum* and *F. graminearum* s.s. All phenolic compounds (at different concentrations) reduced the production of FUM, ZEA and TCT, with chlorogenic acid reducing all three mycotoxins when using the rice protocol. This study demonstrated the ability of phenolic compounds to reduce the fungal growth and mycotoxin accumulation of pathogens associated with FER and GER of maize *in vitro*. Phenolic compounds could be incorporated into disease management strategies to prevent infection by mycotoxigenic fungi and subsequent contamination with mycotoxins.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important staple foods grown worldwide (Boutigny *et al*., 2009). In Southern Africa, maize is consumed by approximately 200 million individuals and the consumption of maize is as high as 300-500 grams per person daily (Du Plessis, 2003). The commercial average production of maize in 2017 was approximately 16.7 million tons, of which 8 million tons was used for human consumption and animal feed, while the surplus grain was exported thereby earning significant foreign income (South African DAFF, 2017). Maize production is threatened by biotic and abiotic factors which reduces the crop yield and grain quality (Cao *et al*., 2014). Maize ear and kernel rot are of the most important fungal diseases that affect maize worldwide (Munkvold and Desjardins, 1997). In South Africa, two of these important fungal diseases are Fusarium ear rot (FER) and Gibberella ear rot (GER).

FER is predominantly caused by *Fusarium verticillioides* (Saccardo) Nirenberg (syn = *F. moniliforme* Sheldon) as well as *F. proliferatum* (Matsushima) Nirenberg and *F. subglutinans* (Wollenweber and Reinking) Nelson, Toussoun and Marasas. These *Fusarium* species are known to produce secondary metabolites collectively known as mycotoxins, which have harmful effects on human and animal health upon the consumption of contaminated grain (Marasas *et al*., 2004). *Fusarium verticillioides* produces fumonisin (FUM) (FB_1, FB_2 and FB_3), fusaric C and trace amount beauvericin and moniliformin (Leslie and Summerell, 2006), *F. proliferatum* produces FB_1 and moniliformin (Leslie and Summerell, 2006), while *F. subglutinans* is known to produce trace amounts of FUM but produces moniliformin (Leslie and Summerell, 2006). GER is caused by the *Fusarium graminearum* species complex (FGSC). In South Africa, species within the FGSC commonly associated with GER is *F. boothii* (O'Donnell, Aoki, Kistler and Geiser) but *F. graminearum* sensu stricto (Schwabe) may also cause GER under rotational systems (Boutigny *et al*., 2011; Gokul, 2015). The mycotoxins produced by *F. boothii* and *F. graminearum* sensu stricto include zearalenone (ZEA) and type B trichothecenes (TCT), which is further divided into nivalenol (NIV) and deoxynivalenol (DON). Mycotoxins cause serious health risks in humans and animals and could even result in death (Pitt, 2000; Zain, 2011).

The ingestion of fumonisin-contaminated grain has been linked to oesophageal cancer in humans (Marasas *et al*., 1981) and the ingestion of grain contaminated with TCT may cause vomiting, diarrhoea and gastrointestinal haemorrhages, however in higher dosages it could eventually lead to death (Pestka and Smolinski, 2005). FUM and TCT are heat stable and are not completely eliminated during food processing. Recently, South Africa introduced a maximum allowable level of FUM and DON in maize and maize-based foods. The limit for FUM is 4000 µg kg^{-1} for further processing and 2000 µg kg^{-1} for human consumption while the maximum allowable level for DON is 2000 µg kg^{-1} for further processing and 1000 µg kg^{-1} for human consumption (South African Department of Health, 2016).

Currently, no maize cultivars are completely resistant to FER, GER and the associated mycotoxins. The infection and disease establishment of these pathogens do occur pre-harvest and even though storage conditions may aggravate disease severity, the emphasis should be on pre-harvest management. Numerous management strategies have been investigated but there is little to no success when the environmental conditions for the pathogens are favourable. Therefore, enhancing host-plant resistance could be seen as a feasible management strategy.

Recently, the use of naturally occurring plant compounds to manage mycotoxigenic fungi has been of interest (Dixon, 2002; Boutigny *et al.*, 2008; Arnason and Bernards, 2010; Bednarek, 2012). Plant secondary metabolites, such as phenolic compounds, form part of the induced defence response to fungal infection and may result in fungal growth inhibition and the prevention of mycotoxins accumulation (Beekrum *et al*., 2003; Boutigny *et al.*, 2008). Pre-existing phenolic compounds occur constitutively in the plant and can act as a physical barrier to pathogens (Sampietro *et al.*, 2013), while induced phenolic compounds have been known to accumulate in response to biotic stress or fungal infections (Nicholson and Hammerschmidt, 1992; Santiago *et al.*, 2007; Lattanzio *et al*., 2008). Phenolic compounds have been shown to inhibit the fungal growth of several fungal species (Purushothaman, 1976; Baranowski *et al.*, 1980; Santiago *et al.*, 2007) including *Fusarium* species (Kasenberg and Traquair, 1988; Assabgui *et al.*, 1993; McKeehen *et al.*, 1999; Santiago *et al.*, 2007). Phenolic compounds have also been known to significantly reduce the production of FUM and DON *in vitro* (Boutigny *et al.*, 2009; Ponts *et al.*, 2011, Cassiem, 2015).

Therefore, the aim of the study was to evaluate the efficacy of selected phenolic compounds in reducing the growth and mycotoxin production of FER and GER causal pathogens.

MATERIALS AND METHODS

Fungal isolates

The FER causal pathogens used in this study included *F. verticillioides* (isolates MRC 826, MRC 8267 and MRC 8559), *F. proliferatum* (isolates MRC 2301, MRC 6908 and MRC 7140) and *F. subglutinans* (isolates MRC 0115, MRC 2293 and MRC 6194). The FER isolates were obtained from the Medical Research Council (PROMEC, Tygerberg, South Africa). The GER causal pathogens evaluated in this study were *F. boothii* isolates (M0100, M0010 and M0002) obtained from a study by Boutigny *et al*. (2011) and *F. graminearum* s.s. isolates (M14.24 and M14.55) obtained from a study by Gokul (2015). All cultures are maintained in the culture collection of the Department of Plant Pathology, Stellenbosch University, South Africa.

Efficacy of phenolic compounds to restrict fungal growth

Phenolic compounds namely ferulic, coumaric, chlorogenic, caffeic, vanillic and sinapic acid (Sigma-Aldrich, St. Louis, USA) were used in this study. Each phenolic compound was dissolved in 100% ethanol or methanol to a stock concentration of 100 mM. Potato dextrose agar (PDA) was amended with the six phenolic compounds, respectively, at four different concentrations (0, 0.5, 1.5 and 2.5 mM). Each Petri dish contained 20 mL of amended PDA and a mycelial plug of a 7-day old culture was placed in the centre of the plate. After 5 days incubation, in a 12-hour light and dark cycle at 25°C, the colony diameter was measured using a digital calibre (Mitutoyo Crop. Model no. CD-6°C). Three replicates per treatment was used in this study. The colony diameter was used to calculate the surface area which was subjected to statistical analysis.

Efficacy of phenolic compounds in combination to restrict fungal growth

Ferulic acid in combination with chlorogenic acid or caffeic acid was tested against isolates of *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, *F. boothii* and *F. graminearum* s.s. The phenolics were evaluated independently as well as in every possible concentration (0, 0.5, 1.5, 2.5 mM) combination representing a 4x4 grid design. Control treatments were represented by non-amended PDA while the PDA was amended according to each treatment and the trial was executed as mentioned above.

Efficacy of phenolic compounds on fungal biomass

Optimisation of microtiter assay

A microtiter-based assay was used to determine the effect of phenolic compounds on the fungal biomass of isolates evaluated in this study. Two types of media namely Fusarium complete media (Leslie and Summerell, 2006) and complete media (10 g glucose, 2 g peptone, 1 g yeast extract, 1 g casein acid, 1 mL trace elements, and 1 mL of vitamin stock, 5% of mixture was removed and replaced with 5% of 20x nitrate salt, pH was adjusted to 6.5 before autoclaving) in combination with three different spore concentrations ($1x10^5$, $1x10^4$ and $1x\ 10^3$ spores ml^{-1}) were tested to determine the logarithmic phase of each fungal species (Figs. 1 and 2).

Fusarium verticillioides, *F. proliferatum* and *F. subglutinans* were grown on PDA for two weeks and mycelium of each isolate was inoculated in 40 mL of Armstrong media (Booth, 1971), respectively. These were incubated at 25°C in an incubator shaker (Labcon, Instrulab) at 100 revolutions per minute (rpm) for 4-5 days to induce sporulation. The 5-day old liquid culture was filtered through two layers of sterile cheesecloth and centrifuged at 3220 relative centrifugal force (rcf) for 10 min at 4°C. The supernatant was discarded and the conidial pellet was washed twice with deionised, autoclaved water (dH_2O) with volumes equivalent to that of the original suspension. Spore production for *F. boothii* and *F. graminearum* s.s. was achieved

using carnation leaf agar (CLA). The plates were incubated at 25°C for approximately 35-40 days. Five millilitres of autoclaved, deionised water was added to each plate and spores were gently dislodged using a sterilised hockey stick. The water was filtered through one layer of sterile cheesecloth. The spore concentration of all isolates was determined using a haemocytometer and the final spore concentration was adjusted to either $1x10^5$, $1x10^4$ or $1x\ 10^3$ spores ml^{-1}.

A 96- well micro-plate assay was performed with each well containing 100 µL of media (Complete media and Fusarium complete media), 80 µL of spores ($1x10^5$, $1x10^4$ or $1x\ 10^3$ spores ml^{-1}) and 20 µL of resazurin dye. Resazurin is an indicator of metabolic functions and is an intermediate electron acceptor that is visualised by a colour change. Electrons released by fungal cellular metabolism is used to oxidise resazurin (blue; non-fluorescent compound) to resofurin (pink; fluorescent compound) which can be measured by absorbance (Rampersad, 2012). Resofurin can furthermore be reduced to hydroresofurin, a non-fluorescent compound. Additional wells containing containing media and water and media and dye, respectively, were used to remove background absorbance. A Fluostar optima micro-plate reader (BMG Labtech), employing a well-point scan, was used to read the micro-plates at the logarithmic phase for the fungal species at the wavelengths 570 nm and 600 nm. Greater fungal activity over the logarithmic phase and the variability between species can be found in the logarithmic phase (Meletiadis *et al*., 2002; Rampersad, 2011).

Microtiter assay to evaluate phenolic compounds

Fungal biomass in the presence of the six phenolic compounds, respectively, at three concentrations (0.5, 1.5 and 2.5 mM) including a control was evaluated. Each phenolic concentration and fungal pathogen combination was replicated three times. A 96- well micro-plate assay was performed with each well containing 100 µL of amended complete media, 80 µL of $1x10^4$ spores ml^{-1} and 20 µL of resazurin dye. A Fluostar optima micro-plate reader, employing a well point scan, was used to read the micro-plates at the logarithmic phase for the fungal species at the wavelengths 570 nm and 600 nm. The logarithmic phase for *F. verticillioides* was reached at 30 hours after incubation while *F. proliferatum* and *F. subglutinans* absorbance readings were taken at 27 hours after incubation. Readings for *F. boothii* and *F. graminearum* s.s. were taken at 15 hours (Figs. 1 and 2).

Efficacy of phenolic compounds on mycotoxin production

Mycotoxin production using phenolic-amended rice

A amened rice-based protocol was used to determine the effect of phenolic compounds on mycotoxin production (Szécsi *et al*., 2005). Fifty grams of rice was soaked overnight in 25 mL of dH_2O. The rice was autoclaved at 121˚C for 15 min and allowed to cool to room temperature. The autoclaved rice was amended with the different phenolic compounds (n=6) at three

concentrations (0.5, 1.5 and 2.5 mM) including a control. Each phenolic compound by concentration by fungal isolate experiment was replicated three times. Thereafter, the rice was inoculated with a single mycelial plug from 7-day old PDA culture and left at room temperature for 7-15 days before mycotoxins were extracted.

Mycotoxin extractions were performed by adding 100 mL of extraction solvent (methanol: H_2O, 70:30) to the 50 g of rice. The rice was macerated, dislodged and placed in an incubator shaker (Labcon, Instrulab) for 60 min at 25°C and 200 rpm. The aqueous solution was filtered through filter paper (Whatman™ No. 4) into a 50-mL Falcon tube and centrifugation at 4°C for 10 min at 50 rcf. The supernatant was filtered through a 0.22 µm syringe filter into 2-mL tubes and refrigerated overnight. Following centrifugation for 10 min at 20 817 rcf, the extracts were transferred to vials and sent to the Mass spectrometry unit at Central Analytical Facility (CAF), Stellenbosch University.

Mycotoxin production using phenolic-amended malt extract agar (MEA)

For mycotoxins quantification from MEA, the media was amended with the phenolic compounds at three concentrations (0.5, 1.5 and 2.5 mM) including an unamended control. Each plate consisted of 20 mL of phenolic-amended MEA inoculated with a mycelial plug from 7-day old PDA culture. Cultures were incubated for 14 days at 25ºC. Each isolate by phenolic compound by concentration combination was replicated three times.

Mycotoxin extractions were performed on 15 randomly selected agar plugs using 7.5 mL extraction buffer (methanol: H_2O, 70:30). The samples were placed in a hot water bath at 65ºC for 30 min and then sonicated for 1 hour. The aqueous solution was filtered through a 0.22 µm syringe filter into 2-mL tubes and refrigerated overnight. The samples were then transferred to vials and sent to the Mass spectrometry unit at CAF, Stellenbosch University, for analysis by ultra-performance liquid chromatography (UPLC). A serial dilution of fumonisins consisting of concentrations ranging from 0.05 mg kg^{-1} to 20 mg kg^{-1} for FB_1 and FB_2 and from 0.005 mg kg^{-1} to 2 mg kg^{-1} for FB_3 was used while a serial dilution of TCT was from 0.032 mg kg^{-1} to 100 mg kg^{-1} and from 0.64 mg kg^{-1} to 200 mg kg^{-1} for ZEA.

Statistical analysis

To determine the effect of the phenolic compounds on fungal growth, the radius of each colony was calculated by taking the average of the two diameter readings. The surface area (SA) was calculated by the formula: $\mathrm{SA} = \pi r^2$ and the average of the three replicates was obtained. For the fungal biomass, absorbance values were obtained at 570 and 600 nm, the resazurin (RZ) reduction values were calculated using the following formula: $\frac{(\varepsilon_{ox})\lambda_2 A\lambda_1 - (\varepsilon_{ox})\lambda_1 A\lambda_2}{(\varepsilon_{RED})\lambda_1 A'\lambda_2 - (\varepsilon_{ox})\lambda_2 A'\lambda_1} \times 100$ (Vega *et al*., 2012). The percentage of growth compared to the control was calculated by: $\left(\frac{SA*100}{SA(control)}\right)$, while biomass compared to the control was calculated by: $\left(\frac{Biomass*100}{Biomass(control)}\right)$. All

data produced was subjected to analysis of variance (ANOVA) using the general linear models (GLM) procedures of the SAS software. Normality tests were done using Shapiro-Wilk test and Students t-least significant differenced (LSD), using a level of 5%, was used to compare the means.

RESULTS

Inhibition of fungal growth by phenolic compounds

A significant ($P<0.001$) fungal isolate by phenolic compound by concentration interaction was observed in this study, thus the results are presented for each isolate. All the phenolic compounds at 2.5 mM significantly reduced the growth of *F. verticillioides* isolates with the exception of chlorogenic acid (MRC 826; 8559), sinapic acid (MRC 826; 8559) and vanillic acid (MRC 8559) when compared to the control (Table 1). The most significant growth inhibition was observed in the presence of ferulic acid (2.5 mM) for MRC 826 (34.3%), MRC 8267 (42.4%) and MRC 8559 (46.2%) compared to all other phenolic compounds. Furthermore, coumaric acid (2.5 mM) also significantly reduced MRC 826 (42.1%), MRC 8267 (55.4%) and MRC 8559 (57.5%) compared to their controls. Conversely, chlorogenic acid (2.5 mM) stimulated fungal growth of MRC 826 (106.0%) and MRC 8559 (111.0%) but these did not differ from the controls (Table 1).

Most of the phenolic compounds reduced the growth of the *F. proliferatum* isolates at all three concentrations, however, the growth did not differ significantly from the controls (Table 2). The fungal growth of MRC 2301 for chlorogenic acid 1.5 mM (172.5%), 0.5 mM sinapic acid (137.3%) and 2.5 mM vanillic acid (133.2%) was significantly more compared to the control (Table 2). The greatest reduction in growth for MRC 2301 was in the presence of coumaric acid at 2.5 mM (64.0%) followed by ferulic acid at 1.5 mM (70.0%). The most significant growth inhibition of MRC 6908 was observed in the presence of 2.5 mM ferulic acid (39.5%), and similarly reduced the growth of MRC 7140 (57.0%) compared to all other phenolic compounds.

The most significant growth inhibition of *F. subglutinans* MRC 0115 (56.9%) and MRC 6194 (46.6%) was observed in the presence of 2.5 mM coumaric acid when compared to the control (Table 3). Ferulic acid (2.5 mM) significantly inhibited the growth of MRC 2293 (54.7%) when compared to the control. All the other phenolic compounds, at different concentrations, either inhibited or stimulated the growth of *F. subglutinans* MRC 0115 and MRC 2293, however, these did not differ significantly from the respective controls.

All phenolic compounds and concentrations reduced the growth of *F. boothii* isolates, with the exception of coumaric acid at 0.5 mM for M0010 (109.5%) (Table 4). All the phenolic compounds at 2.5 mM significantly reduced the growth of *F. boothii* isolates with the exception of sinapic acid and vanillic acid for isolate M0010 and coumaric acid for isolate M0002. The

most significant inhibition of M0100 (21.7%) and M0002 (39.7%) was at 2.5 mM ferulic acid, while M0010 was most significantly reduced by 2.5 mM caffeic acid (50.3%).

All phenolic compounds and concentrations reduced the growth of *F. graminearum* s.s. isolates (Table 5). The most significant growth inhibition of M14.24 (28.5%) and M14.55 (28.1%) was in the presence of ferulic acid at 2.5 mM. Caffeic acid (2.5 mM) for M14.24 (41.6%) and vanillic acid (2.5 mM) for M14.55 (36.1%) also significantly reduced fungal growth but did not differ significantly to the growth inhibition observed for 2.5 mM ferulic acid, respectively.

Efficacy of ferulic and chlorogenic acid in combination to restrict fungal growth

Significant differences in the response of fungal isolates were observed when ferulic and chlorogenic acid was evaluated in combination (Table 6–10). Ferulic acid (0.5 mM) in combination with chlorogenic acid (2.5 mM) resulted in the greatest growth inhibition for *F. verticillioides* isolates MRC 826 (8.1%), MRC 8267 (6.7%) and MRC 8559 (5.5%) when compared to an unamended control (100%) (Table 6). This result was consistent for all *Fusarium* species evaluated (Table 7-10). The growth inhibition of *F. proliferatum* isolates was MRC 2301 (12.9%), MRC 6908 (10.5%), MRC 7140 (10.1%) (Table 7) while *F. subglutinans* growth inhibition was MRC 0115 (12.3%), MRC 2293 (4.6%) and MRC 6194 (7.6%) (Table 8). GER pathogens *F. boothii* M0100 (14.5%) and M0002 (7.6%) was most significantly reduced by ferulic acid (0.5 mM) combined with 2.5 mM chlorogenic acid while isolate M0010 (9.2%) was reduced by ferulic acid (2.5 mM) combined with 2.5 mM chlorogenic acid (Table 9). *Fusarium graminearum* s.s. M14.24 (10.2%) and M14.55 (15.8%) (Table 10) was also the most significantly reduced in the presence of ferulic acid (0.5 mM) combined with chlorogenic acid (2.5 mM).

Efficacy of ferulic and caffeic acid in combination to restrict fungal growth

The combination of ferulic acid (2.5 mM) with different concentrations of caffeic acid resulted in the highest growth inhibition of FER causal pathogens (Table 11-13). This combination inhibited the growth of *F. verticillioides* isolates MRC 826 (33.1%), MRC 8267 (20.6%) and MRC 8559 (19.0%) (Table 11). The growth inhibition as a result of the combination was significantly higher when compared to the inhibition of the individual compounds at 2.5 mM. The only exception was observed for MRC 8559 where the most effective combination did not differ significantly from 2.5 mM ferulic acid (22.2%) (Table 11).

Ferulic acid (2.5 mM) in combination with different concentrations of caffeic acid inhibited the growth of *F. proliferatum* isolates MRC 2301 (28.0%), MRC 6908 (28.5%) and MRC 7140 (14.0%) (Table 12). The growth inhibition of ferulic and caffeic acid at 2.5 mM, respectively, was significantly less when compared to the best phenolic combination for each isolate with the exception of ferulic acid (2.5 mM) growth inhibition for MRC 6908 (39.6%) (Table 12).

Similarly, the aforementioned combination inhibited the growth of *F. subglutinans* isolates MRC 0115 (45.5%), MRC 2293 (22.8%) and MRC 6194 (31.2% for 0.5 mM; 31.6% for 2.5 mM) (Table 13). The growth inhibition as a result of the best combination for each isolate was significantly higher when compared to the inhibition of the individual compounds at 2.5 mM (Table 13).

Ferulic acid (0.5 mM) in combination with caffeic acid (2.5 mM) significantly reduced *F. boothii* isolate M0100 (17.5%) (Table 14). A combination of ferulic (2.5 mM) and caffeic (1.5 mM) significantly reduced M0100 (17.8%), M0010 (9.4%) and M0002 (15.2%) with the latter not being significantly different to a ferulic (2.5 mM) and caffeic acid (2.5 mM) combination that resulted in the greatest growth inhibition for M0002 (6.6%) (Table 14). The growth inhibition of ferulic and caffeic acid at 2.5 mM, respectively, was significantly less when compared to the best phenolic combination, with the exception of growth inhibition for M0100 (22.9%) at 2.5 mM ferulic acid (Table 14).

The combination of 2.5 mM ferulic acid combined with caffeic acid at 2.5 mM resulted in the most significant reduction for *F. graminearum* s.s. isolates M14.55 (12.6%) and M14.55 (22.9%) (Table 15). The growth inhibition as a result of the most effective combination was significantly higher when compared to the inhibition of the individual compounds at 2.5 mM, with the exception of 2.5 mM ferulic acid for M14.24 (22.9%) and M14.55 (25.6%) (Table 15).

Effect of phenolic compounds on the fungal biomass

All concentrations of caffeic, chlorogenic, sinapic and ferulic acid as well as vanillic acid (1.5 mM) significantly reduced the fungal biomass of MRC 826 when compared to the control (Table 16). The greatest reduction of MRC 826 (64.6%) growth was achieved by caffeic acid (2.5 mM). No significant differences in biomass was observed for MRC 8267 as ferulic acid at 1.5 mM (250%) and 2.5 mM (220%) had a significant stimulatory effect when compared to the control. Similar results of biomass stimulation were also observed for MRC 8559 with ferulic at 0.5 mM (246.3%), 1.5 mM (298.1%) and 2.5 mM (263.0%) (Table 16).

Caffeic (0.5 and 2.5 mM), coumaric (1.5 mM) and sinapic acid (1.5 mM) significantly reduced the fungal biomass of MRC 2301 when compared to the control (Table 17). The highest significant reduction was in the presence of 0.5 mM caffeic acid (1.7%). The biomass of MRC 2301 was, however, stimulated by chlorogenic (0.5 mM), coumaric (0.5 and 2.5 mM), sinapic (0.5 and 2.5 mM) and ferulic acid, at all concentrations evaluated, as well as vanillic acid (2.5 mM) but these did not differ significantly from the control. MRC 6908 was significantly reduced by 0.5 mM caffeic acid (61.0%) as well as 2.5 mM chlorogenic acid (44.4%). The biomass of MRC 6908 was significantly more in the presence of 1.5 mM ferulic acid when compared to the control. The biomass of MRC 7140 could not be determined as the dye was oxidised to hydroresofurin (a non-fluorescent stage).

Caffeic, chlorogenic and sinapic acid, at all concentrations evaluated, as well as ferulic acid (2.5 mM) significantly reduced the fungal biomass of MRC 0115 when compared to the control (Table 18). The highest, significant reduction in biomass for MRC 0115 was in the presence of 2.5 mM chlorogenic acid (71.1%) that did not differ significantly to 2.5 mM sinapic acid or ferulic acid, respectively. Biomass was significantly stimulated by coumaric acid (1.5 mM) and vanillic acid (1.5 and 2.5 mM) compared to the control. All phenolic compounds and concentrations reduced the biomass of MRC 2293 while the highest, significant reduction was in the presence of 0.5 mM caffeic acid (59.6%) followed by 2.5 mM (64.3%). This reduction did not differ significantly from a number of phenolic compounds at different concentrations such as 2.5 mM ferulic acid (69.4%) and 0.5 mM caffeic acid (71.0%). The biomass of MRC 6194 could not be determined at the dye was oxidised to hydroresofurin.

The biomass of *F. boothii* M0100 was only reduced by coumaric acid at 0.5 mM (85.7%) and 1.5 mM (89.3%) as well as vanillic acid at 0.5 mM (75.0%), 1.5 mM (82.1%) and 2.5 mM (82.1%) (Table 19). No significant differences in the reduction of fungal biomass could be determined for M0100 as all concentrations of caffeic, chlorogenic and sinapic acid as well as ferulic acid (1.5 and 2.5 mM) had a stimulatory effect when compared to the control. For M0010 the most significant reduction was in the presence of vanillic acid that had 64.9% reduction for all three concentrations. This reduction was not significantly different from other compounds at different concentrations such as coumaric acid (1.5 mM; 78.4% and 2.5 mM; 81.1%) and all concentrations of ferulic acid. The biomass of M0002 could not be determined since no spores could be produced for this isolate despite numerous attempts.

All concentrations of coumaric acid and vanillic acid (1.5 and 2.5 mM) reduced the biomass of M14.24 (Table 20). Vanillic acid at 2.5 mM (81.4%) resulted in the highest reduction of biomass. A stimulation in fungal biomass of M14.24 was observed by caffeic, chlorogenic, sinapic and ferulic acid, at all concentrations evaluated as well as vanillic acid (0.5 mM). Chlorogenic, coumaric, ferulic and vanillic acid, at all concentrations, including 2.5 mM sinapic reduced the biomass of M14.55. The highest, significant reduction in biomass was by vanillic acid at 1.5 mM (70.4%) and 0.5 mM (71.4%) but it did not differ significantly from several other phenolic compounds including 2.5 mM vanillic acid (77.6%), ferulic acid (0.5 and 2.5 mM) and 0.5 mM chlorogenic acid.

Inhibition of mycotoxin production

Inhibition of fumonisins

All phenolic compounds at 2.5 mM, except coumaric acid, significantly reduced FUM produced by MRC 826 when evaluated using the rice-based assay (Table 21). No significant differences in FUM were determined for *F. verticillioides* isolates when using amended-MEA as caffeic (0.5 and 1.5 mM), sinapic (2.5 mM) and vanillic acid (0.5 mM) had significantly stimulated FUM production relative to the control. Similar stimulation of FUM production was observed for MRC

8267 and MRC 8559 when amended-MEA was used. The least FUM produced by MRC 826 was in the presence of sinapic acid (2.5 mM), differing significantly from the control. FUM were the most reduced by coumaric acid (2.5 mM) for MRC 8267 (4.6 mg kg^{-1}) and sinapic acid (1.5 mM) for MRC 8559 (7.5 mg kg^{-1}) but these did not differ significantly from the controls when evaluated using the rice-based assay.

The highest, significant FUM reduction of MRC 2301 was by 1.5 mM chlorogenic acid (3.2 mg kg^{-1}) and sinapic acid at 2.5 mM (3.2 mg kg^{-1}) while several other compounds such as ferulic acid at 1.5 mM (7.1 mg kg^{-1}) and vanillic acid at all concentrations did not differ significantly when phenolics were assessed using a rice-based assay (Table 22). FUM production of MRC 2301 on amended-MEA was reduced by all compounds and concentrations when compared to the control (2896.5 mg kg^{-1}). None of the compounds significantly reduced FUM production of MRC 6908 when compared to the control (0.2 mg kg^{-1}) using the rice assay. Conversely, FUM production of MRC 6908 was reduced by most phenolic compounds, most notably 2.5 mM coumaric acid (0.0 mg kg^{-1}) on amended-MEA. The FUM production of MRC 7140 was the most reduced by 1.5 mM sinapic acid (45.3 mg kg^{-1}), 0.5 mM sinapic acid (46.2 mg kg^{-1}), ferulic acid at 0.5 mM (52.9 mg kg^{-1}) and 1.5 mM (51.1 mg kg^{-1}) and vanillic acid at 0.5 mM (60.2 mg kg^{-1}) but did not differ from the control (68.2 mg kg^{-1}) when rice-based assay was used. None of the compounds significantly reduced the FUM production of MRC 7140 compared to the control (411.4 mg kg^{-1}) on amended-MEA due to significant FUM stimulation by several compounds including ferulic acid (0.5 and 2.5 mM) and coumaric acid (2.5 mM) (Table 22).

Inhibition of trichothecenes and zearalenone

The effect on phenolic compounds on TCTs and ZEA produced by *F. boothii* and *F. graminearum* s.s. isolates could not be determined on amended-MEA as the mycotoxin quantities were below the limit of quantification (LOQ). Therefore, only the results of the rice-based assay are provided.

All phenolic compounds and concentrations, except caffeic acid at 1.5 mM (7.6 mg kg^{-1}) and 1.5 mM vanillic acid (9.2 mg kg^{-1}) reduced ZEA production of *F. boothii* M0100 when compared to the control (7.2 mg kg^{-1}) (Table 23). The highest, significant reduction of ZEA in M0100 was by ferulic acid at 1.5 mM (0.6 mg kg^{-1}) although the ZEA reduction by several compounds did not differ significantly to this. All phenolic compounds at 2.5 mM significantly reduced the production of ZEA for M0010 when compared to the control (5.6 mg kg^{-1}). The most significant reduction of ZEA was in the presence of 1.5 mM chlorogenic acid (0.0 mg kg^{-1}) although the reduction of numerous other compounds did not differ significantly from this. No TCTs were produced by isolates M0100 and M0010. None of the compounds significantly reduced the production of ZEA by M0002 compared to the control. The highest reduction in ZEA was ferulic acid at 1.5 mM (1.1 mg kg^{-1}) when compared to the control (4.6 mg kg^{-1}). The

production of TCTs for M0002 was significantly reduced only by 0.5 mM vanillic acid (4.5 mg kg^{-1}) while the reduction by other compounds such as 1.5 mM coumaric acid (6.2 mg kg^{-1}), 1.5 and 2.5 mM vanillic acid (6.5 mg kg^{-1}, respectively) and 1.5 mM ferulic acid (6.3 mg kg^{-1}) did not differ significantly to the reduction of 0.5 mM vanillic acid nor to the control (9.2 mg kg^{-1}).

None of the compounds significantly reduced the production of ZEA by M14.24 when compared to the control (Table 24). The greatest reduction in ZEA was by caffeic acid 2.5 mM (0.3 mg kg^{-1}) when compared to the control (1.6 mg kg^{-1}). The most significant reduction in TCTs produced by M14.24 was in the presence of chlorogenic acid at 1.5 mM (1.1 mg kg^{-1}) and caffeic acid (1.2 mg kg^{-1}) relative to the control (8.9 mg kg^{-1}) although several other compounds did not differ significantly from this reduction (Table 24). Several compounds reduced ZEA produced by M14.55 with the most significant reduction caused by chlorogenic acid 1.5 mM (0.3 mg kg^{-1}) when compared to the control (80.5 mg kg^{-1}). The production of ZEA was also stimulated by coumaric (2.5 mM), ferulic (1.5 and 2.5 mM) and vanillic acid (0.5 mM). The production of TCTs by M14.55 was also reduced by a number of compounds and the most significant reduction was in the presence of 1.5 and 2.5 mM chlorogenic acid (2.2 and 7.0 mg kg^{-1}, respectively) when compared to the control (120.9 mg kg^{-1}).

DISCUSSION

Phenolic compounds, naturally produced by plants, have been associated with defence mechanisms and continue to gain interest as a means to manage mycotoxigenic fungi and their associated mycotoxins. In this study, phenolic compounds were shown to reduce the fungal growth, biomass and mycotoxin production of specific *Fusarium* species associated with FER and GER of maize in South Africa. However, at low concentrations of certain compounds a stimulatory effect was observed.

Ferulic acid was the most effective phenolic compound in inhibiting the growth of all the species evaluated, followed by coumaric and caffeic acid. *Fusarium subglutinans* was the most sensitive fungal species while *F. boothii* and *F. graminearum* s.s. was seen to be the most tolerant fungal species against the phenolic compounds and concentrations evaluated. According to Ponts *et al*. (2011) ferulic acid and coumaric acid have a more toxic effect on fungal species because they both belong to the cinnamic-derived phenolic acids. As the concentrations of the phenolic compounds increased, the growth of the fungal pathogens decreased, showing that the inhibitory activity of the phenolic compounds on the fungal species is concentration dependent, this corresponds to findings by Samapundo *et al*. (2007). In this study, a significant isolate by compound by concentration interaction was observed. This demonstrates the importance of evaluating each phenolic compound against a representative number of fungal isolates of a particular species. However, in this study the highest concentration of most phenolic compounds did significantly reduce fungal growth indicating a

concentration threshold that could be applied to all isolates. In the study certain phenolic compounds at low concentrations had a stimulatory effect on fungal growth. This occurrence has previously been reported by Samapundo *et al*. (2007). However, it has also been reported that ferulic acid had no effect on the growth of *F. verticillioides* which contradicts the findings of this study (Beekrum *et al*., 2003).

A number of phenolic compounds are associated with maize grain and may be located in the pericarp (ferulic, coumaric and sinapic acid) or occur freely in plant cell (ferulic, vanillic and caffeic acid). Phenolic compounds in combination were shown to have a greater effect on all fungal species evaluated when compared to the individual compounds. This is in accordance to a study done by Lee and Lee (2010) where they found that the effects phenolic compounds in combination was significantly greater than the phenolic compounds individually when they tested it on the effects of *Salmonella enteritidis.*

The combination of ferulic and caffeic acid had the same effect on fungal growth when compared to ferulic acid at 2.5 mM individually. These results suggest that ferulic acid in this combination contributes significantly more to the inhibition of fungal growth. This was observed for several *Fusarium* species and isolates evaluated in this study. The combination of ferulic and chlorogenic acid resulted in the lowest mean surfaces area measured for all the fungal species and isolates evaluated in this study. This suggest that certain compounds, when in combination, can result in a greater inhibition of fungal growth as seen for of *F. verticillioides, F. proliferatum*, *F. subglutinans* (MRC 2293; 6194), *F. boothii* (M0100) as well as *F. graminearum* s.s. The growth study of the individual compounds could not be compared to the compounds in combination as trials were conducted at different times. Furthermore, compounds need to be evaluated in all possible combinations to determine their effect on fungal growth and mycotoxin production.

Caffeic acid reduced the biomass of FER pathogens while vanillic and coumaric reduced the biomass of the GER pathogens. Complete media was selected over Fusarium complete media simply because it has a stable pH after autoclaving. This is important to note since RZ dye is pH sensitive and it will allow for an optimal functioning of the RZ dye (Vega *et al*., 2012). The inability to detect differences, of the fungal species in the presence of the phenolic compounds, could be due to certain ingredients in that media that might be affecting the phenolic compounds. According to Pijls *et al*. (1994) specific compounds that make up the media might affect fungicides being tested. This technique could be optimised by reading the microtiter plate before and after the logarithmic growth phase of the fungal species selected. This ensures that the measurements taken for the effect of phenolic compounds on fungal biomass occurs during the change from resazurin to resofurin and not from resofurin to hydroresofurin. This is a non-fluorescent stage of the dye at which the fungal activity cannot be detected.

Mycotoxins were reduced by different phenolic compounds at different concentrations. However, at the higher concentrations such as 1.5 mM and 2.5 mM this reduction in mycotoxin production could be attributed to the reduction in fungal growth which in turn resulted in reduced the mycotoxin concentrations. While at the lower concentration of 0.5 mM, fungal growth did not differ significantly from the control but the reduction in mycotoxin production can be attributed to the presence of the phenolic compounds. Beekrum *et al*. (2003) showed that at low concentrations, where the growth was not affected, the mycotoxin production was significantly reduced. This occurrence was demonstrated in this study for ZEA and TCT on the rice-based assay. However, 0.5 mM phenolics did not significantly reduce fumonisins. Clarity is, however, still required on the exact mechanism by which phenolic compounds reduce mycotoxin production.

This study has provided insight into the potential role of phenolic compounds to the manage FER, GER and the mycotoxin accumulation. Phenolic compounds could potentially be a promising alternative and an environmentally safe strategy to manage ear rot causal pathogens. Phenolic compounds could be applied as a spray to increase the plants defence against the pathogen. The emphasis could be on the over-expression of genes that regulate phenolic compounds for the inhibition of fungal ingress and mycotoxin production. Therefore, the use of phenolic compounds as part of an integrative disease management strategy could ultimately reduce the health risk to humans and animals posed by mycotoxin contamination in maize-based foods.

REFERENCES

Arnason, J.T. and Bernards, M.A. 2010. Impact of constitutive plant natural products on herbivores and pathogens. Canadian Journal of Zoology 88: 615-627.

Assabgui, R.A., Reid, L.M., Hamilton, R.I. and Arnason, J.T. 1993. Correlation of kernel (E)-ferulic acid content of maize with resistance to *Fusarium graminearum*. Phytopathology 83: 949-953.

Atanasova-Penichon, V., Barreau, C. and Richard-Forget, F. 2016. Antioxidant secondary metabolites in cereals: Potential involvement in resistance to Fusarium and mycotoxin accumulation. Frontiers in Microbiology 7: 566.

Atanasova-Penichon, V., Bernillon, S., Marchegay, G., Lornac, A., Ponts, N., Zehraoui, E., Barreau, C. and Richard-forget, F. 2014. Bioguided isolation, characterization, and biotransformation by *Fusarium verticillioides* of maize kernel compounds that inhibit fumonisin production. Molecular Plant-Microbe Interactions 27: 1148-1158.

Baranowski, J.D., Davidson, P.M., Nagel, C.W. and Branen, A.L. 1980. Inhibition of *Saccharomyces cerevisiae* by naturally occurring hydroxycinnamates. Journal of Food Science 45: 592-594.

Bednarek, P. 2012. Sulfur-containing secondary metabolites from *Arabidopsis thaliana* and other brassicaceae with function in plant immunity. ChemBioChem 13: 1846-1859.

Beekrum, S., Govinden, R., Padayachee, T. and Odhav, B. 2003. Naturally occurring phenols: a detoxification strategy for fumonisin B1. Food Additives and Contaminants 20: 490-493.

Boutigny, A.L., Barreau, C., Atanasova-Penichon, V., Verdal-Bonnin, M.N., Pinson-Gadais, L. and Richard-Forget, F. 2009. Ferulic acid, an efficient inhibitor of type B trichothecene biosynthesis and *Tri* gene expression in *Fusarium* liquid cultures. Mycological Research 113: 746-753.

Boutigny, A.L., Beukes, I., Small, I., Zühlke, S., Spiteller, M., Van Rensburg, B.J., Flett, B. and Viljoen, A. 2011. Quantitative detection of *Fusarium* pathogens and their mycotoxins in South African maize. Plant Pathology 61: 522-531.

Boutigny, A.L., Richard-Forget, F. and Barreau, C., 2008. Natural mechanisms for cereal resistance to the accumulation of *Fusarium* trichothecenes. European Journal of Plant Pathology 121: 411-423.

Cao, A., Santiago, R., Ramos, A.J., Souto, X.C., Aquin, O., Malvar, R.A. and Butron, A. 2014. Critical environmental and genotypic factors for *Fusarium verticillioides* infection, fungal growth and fumonisin contamination in maize grown in northwestern Spain. International Journal of Food Microbiology 177: 63-71.

Cassiem, A. 2015. Determining the potential role of phenolic compounds in conferring resistance to *Fusarium* species and their associated mycotoxins in maize. BScHons mini-thesis, University of Stellenbosch, Stellenbosch, South Africa.

Dixon, R.A., Achnine, L., Kota, P., Liu, C.J., Reddy, M.S. and Wang, L., 2002. The phenylpropanoid pathway and plant defence - a genomics perspective. Molecular Plant Pathology 3: 371-390.

Du Plessis, J. 2003. Maize production. Resource Centre Directorate Agricultural Information Services Private Bag X144, Pretoria. pp. 1-38.

Ferruz, E., Atanasova-Pénichon, V., Bonnin-Verdal, M.N., Marchegay, G., Pinson-Gadais, L., Ducos, C., Lorán, S., Ariño, A., Barreau, C. and Richard-Forget, F. 2016. Effects of phenolic acids on the growth and production of T-2 and HT-2 toxins by *Fusarium langsethiae* and *F. sporotrichioides*. Molecules 21: 449.

Gokul, A. 2015. *Fusarium graminearum* species complex (FGSC) composition in South African wheat and maize grown in rotation. MScAgric thesis, University of Stellenbosch, Stellenbosch, South Africa.

Kasenberg, T.R. and Traquair, J.A. 1988. Effects of phenolics on growth of *Fusarium oxysporum* f.sp. *radicis-lycopersici in vitro.* Canadian Journal of Botany 66: 1174-1177.

Lattanzio, V., Kroon, P.A., Quideau, S. and Treutter, D., 2008. Plant phenolics - secondary metabolites with diverse functions. Recent Advances in Polyphenol Research 1: 1-35.

Lee, O.H. and Lee, B.Y., 2010. Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europaea* leaf extract. Bioresource Technology 101: 3751-3754.

Leslie, J.F. and Summerell, B.A. 2006. The Fusarium Laboratory Manual (J.F. Leslie and B.A. Summerell, eds.). Blackwell Publishing Professional, Iowa, USA.

Marasas, W.F.O., Riley, R.T., Hendricks, K.A., Stevens, V.L., Sadler, T.W., Gelineau-van Waes, J., Missmer, S.A., Cabrera, J., Torres, O., Gelderblom, W.C.A., Allegood, J., Martínez, C., Maddox, J., Miller, J.D., Starr, L. Sullards, M. C., Roman,A.V., Voss, K. A., Wang, E. and Merrill, A.H. Jr. 2004. Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. The Journal of Nutrition 134: 711-716.

Marasas, W.F.O., Wehner, F.C., Van Rensburg, S.J. and Van Schalkwyk, D.J. 1981. Mycoflora of corn produced in human esophageal cancer areas in Transkei, southern Africa. Phytopathology 71: 792-796.

McKeehen, J.D., Busch, R.H., and Fulcher, R.G. 1999. Evaluation of wheat (*Triticum aestivum* L.) phenolic acids during grain development and their contribution to *Fusarium* resistance. Journal of Agricultural and Food Chemistry 47: 1476-1482.

Meletiadis, J., Mouton, J.W., Meis, J.F.G.M., Bouman, B.A. and Verweij, P.E. 2002. Comparison of the Etest and the sensititre colorimetric methods with the NCCLS proposed standard for antifungal susceptibility testing of *Aspergillus* species. Journal of Clinical Microbiology 40: 2876-2885.

Munkvold, G.P. and Desjardins, A.E. 1997. Fumonisins in maize: Can we reduce their occurrence? Plant Disease 81: 556-565.

Nelson, P.E., Desjardins, A.E. and Plattner, R.D., 1993. Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry, and significance. Annual review of Phytopathology 31: 233-252.

Nicholson, R. L., and Hammerschmidt, R. 1992. Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology 30: 369-389.

Nicholson, R.L. and Hammerschmidt, R. 1992. Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology 30: 369-389.

Pestka, J.J. and Smolinski, A.T., 2005. Deoxynivalenol: toxicology and potential effects on humans. Journal of Toxicology and Environmental Health 8: 39-69.

Pijls, C.F.N., Shaw, M.W. and Parker, A. 1994. A rapid test to evaluate in vitro sensitivity of *Septoria tritici* to flutriafol, using a microtitre plate reader. Plant Pathology 43: 726-732.

Pitt, J.I. 2000. Toxigenic fungi and mycotoxins. British Medical Bulletin, Food Science Australia. New South Wales, Australia 56: 184-192.

Ponts, N., Pinson-Gadais, L., Boutigny, A.L., Barreau, C. and Richard-Forget, F. 2011. Cinnamic-derived acids significantly affect *Fusarium graminearum* growth and *in vitro* synthesis of type B trichothecenes. Phytopathology 101: 929-934.

Purushothaman, D. 1976. Changes in phenolic compounds in rice cultivars as influenced by *Xanthomonas oryzae*. Riso 25: 88-91.

Rampersad, S. N. 2012. Multiple applications of alamar blue as an indicator of metabolic function and cellular health in cell viability bioassays. Sensors 12: 12347-12360.

Rampersad, S.N. 2011. A rapid colorimetric microtiter bioassay to evaluate fungicide sensitivity among *Verticillium dahlia* isolates. Plant Disease Journal 95: 248-255.

Samapundo, S., De Meulenaer, B., Osei-Nimoh, D., Lamboni, Y., Debevere, J. and Devlieghere, F. 2007. Can phenolic compounds be used for the protection of corn from fungal invasion and mycotoxin contamination during storage? Food Microbiology 24: 465-473.

Sampietro, D.A., Fauguel, C.M., Vattuone, M.A., Presello, D.A. and Catalan, C.A.N. 2013. Phenylpropanoids from maize pericarp: resistance factors to kernel infection and fumonisin accumulation by *Fusarium verticillioides*. European Journal of Plant Pathology 135: 105-113.

Santiago, R., Reid, L.M., Arnason, J.T., Zhu, X., Martinez, N. and Malvar, R.A. 2007. Phenolics in maize genotypes differing in susceptibility to Gibberella stalk rot (*Fusarium graminearum* Schwabe). Journal of Agricultural and Food Chemistry 55: 5186-5193.

South African Department of Agriculture, Forestiers and Fisheries (DAFF). 2017. Final area planted and crop production figures of commercial maize, sunflower seed, soybeans, groundnuts and sorghum for 2017. Statistics and Economic Publications and Reports. http://www.daff.gov.za/daffweb3/Home/Crop-Estimates (June 2018).

South African Department of Health. 2016. Foodstuffs, cosmetics and disinfectants Act, 1972 (Act 54 of 1972). Regulations governing tolerance for fungus-produced toxins in foodstuffs: amendment. Government Gazette.

Szecsi, A., Bartók, T., Varga, M., Magyar, D. and Mesterházy, Á., 2005. Determination of trichothecene chemotypes of Fusarium graminearum strains isolated in Hungary. Journal of Phytopathology, 153: 445-448.

Vega, B., Liberti, D., Harmon, P.F. and Dewdney, M.M. 2012. A rapid resazurin-based microtiter assay to evaluate QoI sensitivity for *Alternaria alternata* isolates and their molecular characterization. Plant Disease 96: 1262-1270.

Zain, M.E. 2011. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society 15: 129-144.

Table 1. Growth of *Fusarium verticillioides* isolates by phenolic compounds.

		Growth of *F. verticillioides* isolates (%)[a]					
Treatment	**Concentration (mM)**	**MRC 826**		**MRC 8267**		**MRC 8559**	
Caffeic acid	0.5	86,1	a-d	80,4	b-d	88,3	b-e
	1.5	52,9	d-g	73,2	c-e	81,2	c-e
	2.5	62,6	d-g	63,6	d-f	60,8	ef
Chlorogenic acid	0.5	74,6	a-f	94,5	ab	98,6	c-d
	1.5	82,1	a-e	79,2	b-d	77,1	d-f
	2.5	106,0	a	76,8	cd	111,0	a-c
Coumaric acid	0.5	58,8	d-g	78,6	b-d	96,5	b-d
	1.5	47,8	e-f	70,5	c-f	63,7	ef
	2.5	42,1	fg	55,4	fg	57,5	ef
Sinapic acid	0.5	64,4	c-g	79,8	b-d	72,9	d-f
	1.5	65,2	b-g	68,5	d-f	117,1	ab
	2.5	98,5	a-c	72,1	c-f	86,8	b-e
Ferulic acid	0.5	66,7	b-g	80,4	b-d	78,0	de
	1.5	44,5	fg	57,9	e-g	60,1	ef
	2.5	34,3	g	42,4	g	46,2	f
Vanillic acid	0.5	72,8	a-f	87,6	a-c	134,4	a
	1.5	75,9	a-f	78,8	b-d	98,9	b-d
	2.5	67,6	b-g	56,3	e-f	79,8	de
Control[b]	0.0	100,0	ab	100,0	a	100,0	cd

[a]The percentage growth is represented by the average of three replicates
[b]The average of three replicates on PDA with *F. verticillioides* and in the absence of the phenolic compound

Table 2. Growth of *Fusarium proliferatum* isolates by phenolic compounds.

		Growth of *F. proliferatum* isolates (%)[a]					
Treatment	**Concentration**	**MRC 2301**		**MRC 6908**		**MRC 7140**	
Caffeic acid	0.5	90,7	b-d	86,3	a-d	82,5	a-e
	1.5	77,6	cd	99,4	a	71,1	b-e
	2.5	95,7	b-d	83,9	a-d	61,8	de
Chlorogenic acid	0.5	94,3	b-d	89,1	a-c	87,1	a-d
	1.5	172,5	a	93,6	ab	80,5	a-e
	2.5	103,5	b-d	78,2	b-d	76,4	a-e
Coumaric acid	0.5	102,6	b-d	84,8	a-d	88,5	a-c
	1.5	87,5	b-d	85,3	a-d	79,7	a-e
	2.5	64,0	d	67,8	df	80,4	a-e
Sinapic acid	0.5	137,3	ab	90,3	a-c	92,4	ab
	1.5	90,1	b-d	84,1	a-d	69,9	b-e
	2.5	88,8	b-d	79,8	b-d	70,5	b-e
Ferulic acid	0.5	104,2	b-d	87,4	a-c	77,2	a-e
	1.5	70,0	d	51,2	ef	62,6	de
	2.5	89,0	b-d	39,5	f	57,0	e
Vanillic acid	0.5	93,4	b-d	91,1	ab	86,0	a-d
	1.5	83,4	b-d	83,0	a-d	73,4	b-e
	2.5	133,2	a-c	72,1	cd	63,1	c-e
Control[b]	0.0	100,0	c-d	100,0	a	100,0	a

[a]The percentage growth is represented by the average of three replicates
[b]The average of three replicates on PDA with *F. proliferatum* and in the absence of the phenolic compound

Table 3. Growth of *Fusarium subglutinans* isolates by phenolic compounds.

		Growth of *F. subglutinans* isolates (%)[a]					
Treatment	**Concentration**	**MRC 0115**		**MRC 2293**		**MRC 6194**	
Caffeic acid	0.5	98,4	a-c	85,3	a-d	115,0	ab
	1.5	95,9	a-d	77,1	a-d	81,6	c-g
	2.5	117,5	ab	74,6	b-d	68,1	d-h
Chlorogenic acid	0.5	117,6	ab	95,6	a-d	85,8	c-f
	1.5	108,6	ab	105,7	ab	100,5	a-c
	2.5	86,9	b-d	76,8	a-d	80,1	c-g
Coumaric acid	0.5	96,0	a-d	88,5	a-d	86,1	b-f
	1.5	85,8	b-d	85,1	a-d	61,9	f-h
	2.5	56,9	d	62,9	b-d	46,6	h
Sinapic acid	0.5	125,8	ab	80,0	a-d	126,3	a
	1.5	97,1	a-d	70,9	b-d	85,5	c-f
	2.5	132,7	a	76,4	a-d	97,0	b-d
Ferulic acid	0.5	124,1	ab	85,9	a-d	73,5	c-h
	1.5	68,1	cd	59,7	cd	52,8	gh
	2.5	58,0	d	54,7	d	55,6	gh
Vanillic acid	0.5	119,1	ab	119,2	a	93,2	b-e
	1.5	106,6	a-c	67,7	b-d	70,7	d-h
	2.5	103,5	a-c	63,2	b-d	67,8	e-h
Control[b]	0.0	100,0	a-c	100,0	a-c	100,0	a-c

[a]The percentage growth is represented by the average of three replicates
[b]The average of three replicates on PDA with *F. subglutinans* and in the absence of the phenolic compound

Table 4. Growth of *Fusarium boothii* isolates by phenolic compounds.

		Growth of *F. boothii* isolates (%)[a]					
Treatment	**Concentration**	**M0100**		**M0010**		**M0002**	
Caffeic acid	0.5	93,5	a-c	89,0	a-d	79,5	a-d
	1.5	40,2	gh	71,5	b-f	82,8	a-c
	2.5	40,7	gh	50,3	f	51,7	de
Chlorogenic acid	0.5	94,0	a-c	75,8	b-f	96,0	ab
	1.5	73,1	b-e	69,7	b-f	84,5	a-c
	2.5	57,9	d-g	66,4	c-f	57,4	c-e
Coumaric acid	0.5	75,0	a-d	109,5	a	79,0	a-d
	1.5	71,5	c-e	90,7	a-d	65,5	b-e
	2.5	46,3	e-h	66,7	c-f	87,7	a-c
Sinapic acid	0.5	69,3	c-f	97,4	a-c	92,2	ab
	1.5	59,1	d-g	59,8	d-f	78,7	a-d
	2.5	70,1	c-e	79,5	a-f	67,0	b-e
Ferulic acid	0.5	87,2	a-c	94,5	a-c	96,0	ab
	1.5	58,8	d-g	66,6	c-f	68,3	b-e
	2.5	21,7	gh	54,3	ef	39,7	e
Vanillic acid	0.5	99,8	ab	99,2	ab	99,8	a
	1.5	70,0	c-e	85,8	a-e	83,2	a-c
	2.5	42,7	f-h	67,5	b-f	67,3	b-e
Control[b]	0.0	100,0	a	100,0	ab	100,0	a

[a]The percentage growth is represented by the average of three replicates
[b]The average of three replicates on PDA with *F. boothii* and in the absence of the phenolic compound

Table 5. Growth of *Fusarium graminearum* sensu stricto isolates by phenolic compounds.

Growth of *F. graminearum* s. s. isolates (%)[a]					
Treatment	**Concentration**	**M14.24**		**M14.55**	
Caffeic acid	0.5	74,6	a-c	89,6	ab
	1.5	60,2	b-d	67,9	b-e
	2.5	41,6	de	56,2	d-f
Chlorogenic acid	0.5	78,1	a-c	82,4	a-d
	1.5	65,0	b-d	75,5	a-e
	2.5	64,3	b-d	75,3	a-e
Coumaric acid	0.5	68,7	b-d	79,7	a-e
	1.5	72,0	a-d	94,6	ab
	2.5	56,1	c-e	75,4	a-e
Sinapic acid	0.5	84,9	a-c	91,6	ab
	1.5	60,7	b-d	86,7	a-c
	2.5	63,8	b-d	75,9	a-e
Ferulic acid	0.5	68,8	b-d	61,5	c-f
	1.5	69,0	b-d	75,8	a-e
	2.5	28,5	e	28,1	g
Vanillic acid	0.5	87,3	ab	98,7	a
	1.5	61,0	b-d	52,6	e-g
	2.5	57,0	b-e	36,1	fg
Control[b]	0.0	100,0	a	100,0	a

[a]The percentage growth is represented by the average of three replicates
[b]The average of three replicates on PDA with *F. graminearum* s.s. and in the absence of the phenolic compound

Table 6. The effect of ferulic and chlorogenic acid on the growth of *Fusarium verticillioides* isolates.

	Growth of *F. verticillioides* isolates (%)					
Treatment	**MRC 826**		**MRC 8267**		**MRC 8559**	
Chlorogenic (0.5 mM)	125,7	a	89,1	a	91,1	b
Ferulic (0.5 mM)	37,0	h	21,0	f	29,1	h
Ferulic (0.5 mM) + Chlorogenic (0.5 mM)	95,1	cd	59,5	c	79,0	cd
Ferulic (0.5 mM) + Chlorogenic (1.5 mM)	93,8	d	58,0	c	73,4	d
Ferulic (0.5 mM) + Chlorogenic (2.5 mM)	8,1	i	6,7	g	5,5	i
Chlorogenic (1.5 mM)	107,3	bc	71,8	b	75,0	cd
Ferulic (1.5 mM)	77,3	e	31,2	ef	60,6	e
Ferulic (1.5 mM) + Chlorogenic (0.5 mM)	69,8	ef	29,7	ef	49,8	f
Ferulic (1.5 mM) + Chlorogenic (1.5 mM)	57,1	fg	44,9	d	49,0	f
Ferulic (1.5 mM) + Chlorogenic (2.5 mM)	69,6	ef	57,4	c	48,3	f
Chlorogenic (2.5 mM)	108,5	b	72,2	b	82,1	c
Ferulic (2.5 mM)	56,2	g	37,4	de	38,4	g
Ferulic (2.5 mM) + Chlorogenic (0.5 mM)	48,4	gh	36,9	de	27,5	h
Ferulic (2.5 mM) + Chlorogenic (1.5 mM)	55,6	g	35,5	de	32,7	gh
Ferulic (2.5 mM) + Chlorogenic (2.5 mM)	50,3	g	34,9	de	32,8	gh
Control[a]	100,0	b-d	100,0	a	100,0	a

[a]The average of three replicates of *F. verticillioides* in the absence of the phenolic compound

Table 7. The effect of ferulic and chlorogenic acid on the growth of *Fusarium proliferatum* isolates.

Growth of *F. proliferatum* isolates (%)					
Treatment	**MRC 2301**		**MRC 6908**		**MRC 7140**	
Chlorogenic (0.5 mM)	110,9	a	111,1	a	87,0	b
Ferulic (0.5 mM)	29,7	i	47,1	fg	19,1	h
Ferulic (0.5 mM) + Chlorogenic (0.5 mM)	90,1	b-d	73,0	b-d	69,0	d
Ferulic (0.5 mM) + Chlorogenic (1.5 mM)	87,1	b-d	79,2	bc	58,4	e
Ferulic (0.5 mM) + Chlorogenic (2.5 mM)	12,9	j	10,5	i	10,1	i
Chlorogenic (1.5 mM)	97,3	a-c	82,9	b	84,1	b
Ferulic (1.5 mM)	85,1	b-e	61,6	de	52,0	ef
Ferulic (1.5 mM) + Chlorogenic (0.5 mM)	83,7	c-e	65,3	de	47,1	f
Ferulic (1.5 mM) + Chlorogenic (1.5 mM)	78,8	d-f	52,4	e-g	34,5	g
Ferulic (1.5 mM) + Chlorogenic (2.5 mM)	69,9	e-g	60,5	d-f	38,6	g
Chlorogenic (2.5 mM)	81,2	d-f	66,1	cd	76,7	c
Ferulic (2.5 mM)	60,3	gh	43,6	gh	33,5	g
Ferulic (2.5 mM) + Chlorogenic (0.5 mM)	53,1	h	42,7	gh	22,2	h
Ferulic (2.5 mM) + Chlorogenic (1.5 mM)	66,1	f-h	33,6	h	23,5	h
Ferulic (2.5 mM) + Chlorogenic (2.5 mM)	53,2	h	44,7	gh	23,1	h
Control[a]	100,0	ab	100,0	a	100,0	a

[a]The average of three replicates of *F. proliferatum* in the absence of the phenolic compound

Table 8. The effect of ferulic and chlorogenic acid on the growth of *Fusarium subglutinans* isolates.

	Growth of *F. subglutinans* isolates (%)					
Treatment	**MRC 0115**		**MRC 2293**		**MRC 6194**	
Chlorogenic (0.5 mM)	93,3	cd	60,3	b	106,0	a
Ferulic (0.5 mM)	101,0	bc	46,7	c	32,3	g
Ferulic (0.5 mM) + Chlorogenic (0.5 mM)	99,7	bc	46,5	c	77,3	b
Ferulic (0.5 mM) + Chlorogenic (1.5 mM)	95,2	c	44,4	cd	82,1	b
Ferulic (0.5 mM) + Chlorogenic (2.5 mM)	12,3	g	4,6	g	7,6	h
Chlorogenic (1.5 mM)	121,8	ab	64,9	b	79,0	b
Ferulic (1.5 mM)	70,2	d-f	39,9	c-e	57,0	c-e
Ferulic (1.5 mM) + Chlorogenic (0.5 mM)	88,5	c-e	38,8	c-e	58,5	cd
Ferulic (1.5 mM) + Chlorogenic (1.5 mM)	89,8	c-e	45,8	cd	55,8	de
Ferulic (1.5 mM) + Chlorogenic (2.5 mM)	80,1	c-f	36,4	d-f	46,7	d-f
Chlorogenic (2.5 mM)	129,2	a	47,8	c	70,0	bc
Ferulic (2.5 mM)	95,4	c	33,7	ef	44,1	e-g
Ferulic (2.5 mM) + Chlorogenic (0.5 mM)	57,6	f	28,1	f	39,3	fg
Ferulic (2.5 mM) + Chlorogenic (1.5 mM)	67,2	ef	26,8	f	40,8	fg
Ferulic (2.5 mM) + Chlorogenic (2.5 mM)	56,6	f	33,1	ef	38,8	fg
Control[a]	100,0	bc	100,0	a	100,0	a

[a]The average of three replicates of *F. subglutinans* in the absence of the phenolic compound

Table 9. The effect of ferulic and chlorogenic acid on the growth of *Fusarium boothii* isolates.

.

Growth of *F. boothii* isolates (%)						
Treatment	**M0100**		**M0010**		**M0002**	
Chlorogenic (0.5 mM)	188,6	a	138,6	a	180,5	a
Ferulic (0.5 mM)	27,8	fg	17,0	e-g	16,5	fg
Ferulic (0.5 mM) + Chlorogenic (0.5 mM)	52,7	cd	37,3	cd	21,6	e-g
Ferulic (0.5 mM) + Chlorogenic (1.5 mM)	67,0	c	47,7	c	17,5	fg
Ferulic (0.5 mM) + Chlorogenic (2.5 mM)	14,5	g	10,3	fg	7,6	g
Chlorogenic (1.5 mM)	47,0	de	26,6	de	24,9	e-g
Ferulic (1.5 mM)	49,2	cd	28,0	de	26,8	ef
Ferulic (1.5 mM) + Chlorogenic (0.5 mM)	43,9	d-f	26,1	de	39,3	de
Ferulic (1.5 mM) + Chlorogenic (1.5 mM)	26,9	fg	24,2	d-f	14,5	fg
Ferulic (1.5 mM) + Chlorogenic (2.5 mM)	65,2	c	46,3	c	78,1	c
Chlorogenic (2.5 mM)	39,2	d-f	23,9	d-f	23,5	e-g
Ferulic (2.5 mM)	26,2	fg	24,6	de	16,5	fg
Ferulic (2.5 mM) + Chlorogenic (0.5 mM)	17,0	g	22,4	e-g	18,9	fg
Ferulic (2.5 mM) + Chlorogenic (1.5 mM)	20,9	g	15,9	e-g	20,6	fg
Ferulic (2.5 mM) + Chlorogenic (2.5 mM)	29,6	e-g	9,2	g	45,3	d
Control[a]	100,0	b	100,0	b	100,0	b

[a]The average of three replicates of *F. boothii* in the absence of the phenolic compound

Table 10. The effect of ferulic and chlorogenic acid on the growth of *Fusarium graminearum* sensu stricto isolates.

Growth of *F. graminearum* s.s. isolates (%)				
Treatment	**M14.24**		**M14.55**	
Chlorogenic (0.5 mM)	133,0	a	131,0	a
Ferulic (0.5 mM)	34,7	e-g	49,9	de
Ferulic (0.5 mM) + Chlorogenic (0.5 mM)	50,2	c-e	57,4	cd
Ferulic (0.5 mM) + Chlorogenic (1.5 mM)	37,0	d-g	46,2	d-f
Ferulic (0.5 mM) + Chlorogenic (2.5 mM)	10,2	h	15,8	j
Chlorogenic (1.5 mM)	42,9	d-f	35,0	fg
Ferulic (1.5 mM)	52,0	cd	48,1	de
Ferulic (1.5 mM) + Chlorogenic (0.5 mM)	53,2	cd	28,2	g-j
Ferulic (1.5 mM) + Chlorogenic (1.5 mM)	35,3	e-g	38,4	e-g
Ferulic (1.5 mM) + Chlorogenic (2.5 mM)	61,1	c	69,2	c
Chlorogenic (2.5 mM)	39,7	d-g	52,6	d
Ferulic (2.5 mM)	29,8	fg	31,6	g-i
Ferulic (2.5 mM) + Chlorogenic (0.5 mM)	33,2	fg	20,2	ij
Ferulic (2.5 mM) + Chlorogenic (1.5 mM)	29,1	fg	22,1	h-j
Ferulic (2.5 mM) + Chlorogenic (2.5 mM)	23,5	gh	33,1	gh
Control[a]	100,0	b	100,0	b

[a]The average of three replicates of *F. graminearum* s.s. in the absence of the phenolic compound

Table 11. The effect of ferulic and caffeic acid on the growth of *Fusarium verticillioides* isolates.

Growth of *F. verticillioides* isolates (%)						
Treatment	**MRC 826**		**MRC 8267**		**MRC 8559**	
Caffeic (0.5 mM)	116,2	a	103,8	ab	62,7	bc
Ferulic (0.5 mM)	82,8	bc	100,8	a-c	72,0	b
Ferulic (0.5 mM) + Caffeic (0.5 mM)	74,6	cd	87,7	b-d	55,7	cd
Ferulic (0.5 mM) + Caffeic (1.5 mM)	75,2	cd	75,5	de	55,1	cd
Ferulic (0.5 mM) + Caffeic (2.5 mM)	64,0	c-f	63,4	e-g	53,3	cd
Caffeic (1.5 mM)	83,1	bc	115,5	a	57,5	c
Ferulic (1.5 mM)	58,9	d-g	71,2	de	56,9	c
Ferulic (1.5 mM) + Caffeic (0.5 mM)	52,2	f-h	62,7	e-g	44,8	de
Ferulic (1.5 mM) + Caffeic (1.5 mM)	47,1	f-h	43,4	g-i	56,8	c
Ferulic (1.5 mM) + Caffeic (2.5 mM)	53,9	e-g	48,9	f-h	34,7	ef
Caffeic (2.5 mM)	72,8	c-e	79,8	c-e	52,2	cd
Ferulic (2.5 mM)	59,2	d-g	70,7	d-f	22,2	g
Ferulic (2.5 mM) + Caffeic (0.5 mM)	56,2	d-g	20,6	j	24,4	fg
Ferulic (2.5 mM) + Caffeic (1.5 mM)	41,0	gh	26,8	ij	19,0	g
Ferulic (2.5 mM) + Caffeic (2.5 mM)	33,1	h	29,7	h-j	23,1	fg
Control[a]	100,0	ab	100,0	a-c	100,0	a

[a]The average of three replicates of *F. verticillioides* in the absence of the phenolic compound

Table 12. The effect of ferulic and caffeic acid on the growth of *Fusarium proliferatum* isolates.

	Growth of *F. proliferatum* isolates (%)					
Treatment	**MRC 2301**		**MRC 6908**		**MRC 7140**	
Caffeic (0.5 mM)	105,1	a	105,2	a	102,6	a
Ferulic (0.5 mM)	100,6	ab	95,5	ab	86,9	bc
Ferulic (0.5 mM) + Caffeic (0.5 mM)	81,1	b-e	82,9	bc	80,2	c
Ferulic (0.5 mM) + Caffeic (1.5 mM)	84,1	b-e	78,9	b-d	83,8	c
Ferulic (0.5 mM) + Caffeic (2.5 mM)	97,1	ab	67,2	cd	53,4	d
Caffeic (1.5 mM)	91,9	a-d	100,9	a	100,9	ab
Ferulic (1.5 mM)	87,4	a-e	72,7	cd	60,2	d
Ferulic (1.5 mM) + Caffeic (0.5 mM)	68,0	ef	62,2	d	54,5	d
Ferulic (1.5 mM) + Caffeic (1.5 mM)	83,7	b-e	82,4	bc	45,6	d-f
Ferulic (1.5 mM) + Caffeic (2.5 mM)	42,9	gh	67,1	cd	36,5	ef
Caffeic (2.5 mM)	95,7	a-c	105,8	a	77,0	c
Ferulic (2.5 mM)	73,9	de	39,6	e	50,6	de
Ferulic (2.5 mM) + Caffeic (0.5 mM)	28,0	h	62,9	d	52,6	d
Ferulic (2.5 mM) + Caffeic (1.5 mM)	77,4	c-e	69,5	cd	14,0	g
Ferulic (2.5 mM) + Caffeic (2.5 mM)	50,5	fg	28,5	e	31,3	f
Control[a]	100,0	ab	100,0	a	100,0	ab

[a]The average of three replicates of *F. proliferatum* in the absence of the phenolic compound

Table 13. The effect of ferulic and caffeic acid on the growth of *Fusarium subglutinans* isolates.

	Growth of *F. subglutinans* isolates (%)					
Treatment	**MRC 0115**		**MRC 2293**		**MRC 6194**	
Caffeic (0.5 mM)	93,5	d	123,5	a	109,7	a
Ferulic (0.5 mM)	116,0	bc	65,3	fg	103,8	ab
Ferulic (0.5 mM) + Caffeic (0.5 mM)	122,8	b	99,6	bc	106,4	a
Ferulic (0.5 mM) + Caffeic (1.5 mM)	92,5	d	103,1	bc	73,8	c-e
Ferulic (0.5 mM) + Caffeic (2.5 mM)	151,7	a	99,0	bc	56,4	ef
Caffeic (1.5 mM)	87,4	de	108,0	ab	85,6	bc
Ferulic (1.5 mM)	89,5	de	87,6	c-e	68,7	c-e
Ferulic (1.5 mM) + Caffeic (0.5 mM)	68,9	e	70,6	ef	56,8	ef
Ferulic (1.5 mM) + Caffeic (1.5 mM)	79,9	de	96,7	b-d	76,7	cd
Ferulic (1.5 mM) + Caffeic (2.5 mM)	82,1	de	61,9	fg	36,6	g
Caffeic (2.5 mM)	160,3	a	105,1	a-c	80,0	c
Ferulic (2.5 mM)	86,7	de	46,1	g	59,0	d-f
Ferulic (2.5 mM) + Caffeic (0.5 mM)	45,5	f	64,8	fg	31,2	g
Ferulic (2.5 mM) + Caffeic (1.5 mM)	95,4	cd	78,8	d-f	43,3	fg
Ferulic (2.5 mM) + Caffeic (2.5 mM)	94,0	d	22,8	h	31,6	g
Control[a]	100,0	cd	100,0	bc	100,0	ab

[a]The average of three replicates of *F. subglutinans* in the absence of the phenolic compound

Table 14. The effect of ferulic and caffeic acid on the growth of *Fusarium boothii* isolates.

	Growth of *F. boothii* isolates (%)					
Treatment	**M0100**		**M0010**		**M0002**	
Caffeic (0.5 mM)	50,4	d	58,4	c-e	75,4	c-e
Ferulic (0.5 mM)	77,3	b	88,3	ab	117,3	a
Ferulic (0.5 mM) + Caffeic (0.5 mM)	59,0	c	57,9	c-f	74,5	c-e
Ferulic (0.5 mM) + Caffeic (1.5 mM)	43,6	e-g	59,4	cd	91,0	bc
Ferulic (0.5 mM) + Caffeic (2.5 mM)	17,5	l	76,7	a-c	84,1	b-d
Caffeic (1.5 mM)	50,0	de	53,3	c-g	89,1	b-d
Ferulic (1.5 mM)	39,1	gh	67,4	bc	88,1	b-d
Ferulic (1.5 mM) + Caffeic (0.5 mM)	30,0	ij	39,0	d-h	79,1	c-e
Ferulic (1.5 mM) + Caffeic (1.5 mM)	46,3	d-f	77,9	a-c	83,5	b-d
Ferulic (1.5 mM) + Caffeic (2.5 mM)	41,0	f-h	31,9	g-i	69,6	de
Caffeic (2.5 mM)	28,8	i-k	41,3	d-h	76,5	c-e
Ferulic (2.5 mM)	22,9	kl	34,7	e-h	63,0	e
Ferulic (2.5 mM) + Caffeic (0.5 mM)	25,5	jk	33,7	f-i	39,8	f
Ferulic (2.5 mM) + Caffeic (1.5 mM)	17,8	l	9,4	i	15,2	g
Ferulic (2.5 mM) + Caffeic (2.5 mM)	35,5	hi	19,8	hi	6,6	g
Control[a]	100,0	a	100,0	a	100,0	ab

[a]The average of three replicates of *F. boothii* in the absence of the phenolic compound

Table 15. The effect of ferulic and caffeic acid on the growth of *Fusarium graminearum* sensu stricto isolates.

Growth of *F. graminearum* s.s. isolates (%)				
Treatment	**M14.24**		**M14.55**	
Caffeic (0.5 mM)	84,6	bc	84,8	a
Ferulic (0.5 mM)	96,2	ab	83,7	ab
Ferulic (0.5 mM) + Caffeic (0.5 mM)	72,9	c-e	62,7	cd
Ferulic (0.5 mM) + Caffeic (1.5 mM)	64,8	ef	63,2	b-d
Ferulic (0.5 mM) + Caffeic (2.5 mM)	79,5	cd	55,8	de
Caffeic (1.5 mM)	60,0	fg	82,3	a-c
Ferulic (1.5 mM)	60,6	e-g	37,3	e-g
Ferulic (1.5 mM) + Caffeic (0.5 mM)	44,9	hi	33,3	fg
Ferulic (1.5 mM) + Caffeic (1.5 mM)	72,0	d-f	55,1	de
Ferulic (1.5 mM) + Caffeic (2.5 mM)	21,2	j	28,1	g
Caffeic (2.5 mM)	48,4	gh	51,3	d-f
Ferulic (2.5 mM)	22,9	j	25,6	g
Ferulic (2.5 mM) + Caffeic (0.5 mM)	36,2	hi	24,5	g
Ferulic (2.5 mM) + Caffeic (1.5 mM)	35,8	i	36,0	e-g
Ferulic (2.5 mM) + Caffeic (2.5 mM)	12,6	j	22,9	g
Control[a]	100,0	a	100,0	a

[a]The average of three replicates of *F. graminearum* s.s. stricto in the absence of the phenolic compound

Table 16. Resazurin reduction of *Fusarium verticillioides* by phenolic compounds.

		***F. verticillioides* isolates (%)**					
Treatment	**Concentration**	**MRC 826**		**MRC 8267**		**MRC 8559**	
Caffeic acid	0.5	70,6	ef	52,5	ef	66,7	gh
	1.5	70,6	ef	77,5	c-f	90,7	e-h
	2.5	64,6	f	107,5	c-f	125,9	d-h
Chlorogenic acid	0.5	83,8	b-e	50,0	ef	114,8	d-h
	1.5	83,6	b-e	25,0	f	127,8	d-h
	2.5	85,2	b-d	30,0	f	77,8	f-h
Coumaric acid	0.5	93,9	ab	90,0	c-f	44,4	h
	1.5	93,7	ab	80,0	c-f	159,3	d-f
	2.5	91,7	a-c	140,0	b-d	194,4	b-d
Sinapic acid	0.5	78,4	c-f	37,5	f	92,6	e-h
	1.5	78,2	c-f	85,0	c-f	113,0	d-h
	2.5	71,8	d-f	122,5	c-e	107,4	e-h
Ferulic acid	0.5	81,8	b-e	147,5	bc	246,3	a-c
	1.5	83,4	b-e	250,0	a	298,1	a
	2.5	82,4	b-e	220,0	ab	263,0	ab
Vanillic acid	0.5	86,1	a-d	92,5	c-f	109,3	d-h
	1.5	70,3	ef	62,5	d-f	166,7	c-e
	2.5	87,8	a-c	157,5	bc	150,0	d-g
Control[a]	0.0	100,0	a	100,0	c-f	100,0	e-h

[a]The average of three replicates of *F. verticillioides* in the absence of the phenolic compound

Table 17. Resazurin reduction of *Fusarium proliferatum* by phenolic compounds.

		***F. proliferatum* isolates (%)**		
Treatment	**Concentration**	**MRC 2301**	**MRC 6908**	**MRC 7140**
Caffeic acid	0.5	1,7 d-f	61,0 de	-
	1.5	-	101,4 bc	-
	2.5	13,6 d-f	75,3 c-e	-
Chlorogenic acid	0.5	115,3 a-f	76,0 c-e	-
	1.5	-	75,3 c-e	-
	2.5	-	44,4 e	-
Coumaric acid	0.5	139,0 a-d	93,8 b-d	-
	1.5	50,8 c-f	79,5 b-d	-
	2.5	135,6 a-d	82,9 b-d	-
Sinapic acid	0.5	106,8 a-f	90,4 b-d	-
	1.5	35,6 d-f	102,1 bc	-
	2.5	120,3 a-e	104,8 bc	-
Ferulic acid	0.5	140,7 a-d	95,9 bc	-
	1.5	213,6 a-c	180,1 a	-
	2.5	123,7 a	111,6 b	-
Vanillic acid	0.5	72,9 b-f	89,0 b-d	-
	1.5	74,6 b-f	95,2 b-d	-
	2.5	132,2 a-e	97,9 bc	-
Control[a]	0.0	100,0 ab	100,0 bc	-

[a]The average of three replicates of *F. proliferatum* in the absence of the phenolic compound
-Resazurin reading too low

Table 18. Resazurin reduction of *Fusarium subglutinans* by phenolic compounds.

		***F. subglutinans* isolates (%)**				
Treatment	**Concentration**	**MRC 0115**		**MRC 2293**		**MRC 6194**
Caffeic acid	0.5	85,8	f-h	71,0	e-g	-
	1.5	85,6	f-h	59,6	g	-
	2.5	86,9	f-h	64,3	fg	-
Chlorogenic acid	0.5	84,8	f-h	86,5	a-e	-
	1.5	77,4	hi	82,3	b-e	-
	2.5	71,7	i	79,2	b-f	-
Coumaric acid	0.5	99,9	de	91,3	a-c	-
	1.5	112,7	ab	93,0	ab	-
	2.5	102,0	cd	93,0	ab	-
Sinapic acid	0.5	84,3	f-h	71,2	e-g	-
	1.5	82,2	f-h	74,7	c-g	-
	2.5	81,3	g-i	72,2	d-g	-
Ferulic acid	0.5	91,9	ef	80,8	b-f	-
	1.5	91,2	e-g	79,2	b-f	-
	2.5	80,5	hi	69,4	e-g	-
Vanillic acid	0.5	105,3	b-d	89,3	a-d	-
	1.5	120,6	a	93,2	ab	-
	2.5	110,7	bc	92,7	ab	-
Control[a]	0.0	100,0	de	100,0	a	-

[a]The average of three replicates of *F. subglutinans* in the absence of the phenolic compound
-Resazurin reading too low

Table 19. Resazurin reduction of *Fusarium boothii* by phenolic compounds.

		***F. boothii* isolates (%)**			
Treatment	**Concentration**	**M0100**		**M0010**	
Caffeic acid	0.5	146,4	a	118,9	a-e
	1.5	146,4	a	121,6	a-d
	2.5	146,4	a	129,7	ab
Chlorogenic acid	0.5	117,9	a-e	94,6	c-g
	1.5	114,3	b-f	89,2	e-g
	2.5	117,9	a-d	100,0	b-f
Coumaric acid	0.5	85,7	d-g	81,1	fg
	1.5	89,3	d-g	78,4	fg
	2.5	100,0	d-g	81,1	fg
Sinapic acid	0.5	135,7	a-c	110,8	a-f
	1.5	135,7	a-c	132,4	a
	2.5	135,7	ab	127,0	a-c
Ferulic acid	0.5	100,0	c-g	86,5	fg
	1.5	107,1	b-g	86,5	fg
	2.5	107,1	b-g	91,9	d-g
Vanillic acid	0.5	75,0	g	64,9	g
	1.5	82,1	e-g	64,9	g
	2.5	82,1	fg	64,9	g
Control[a]	0.0	100,0	c-g	100,0	b-f

[a]The average of three replicates of *F. boothii* in the absence of the phenolic compound

Table 20. Resazurin reduction of *Fusarium graminearum* sensu stricto by phenolic compounds.

		***F. graminearum* s.s. isolates (%)**			
Treatment	**Concentration**	**M14.24**		**M14.55**	
Caffeic acid	0.5	132,6	ab	108,2	a
	1.5	125,6	ab	106,1	a
	2.5	148,8	ab	101,0	a-d
Chlorogenic acid	0.5	114,0	b	83,7	f-h
	1.5	107,0	b	89,8	c-g
	2.5	125,6	ab	90,8	b-g
Coumaric acid	0.5	83,7	b	89,8	d-g
	1.5	88,4	b	85,7	e-g
	2.5	95,3	b	89,8	c-g
Sinapic acid	0.5	141,9	ab	105,1	ab
	1.5	116,3	b	104,1	a-c
	2.5	137,2	ab	88,8	d-g
Ferulic acid	0.5	116,3	b	79,6	gh
	1.5	100,0	b	83,7	f-h
	2.5	118,6	b	94,9	a-f
Vanillic acid	0.5	186,0	a	71,4	h
	1.5	90,7	b	70,4	h
	2.5	81,4	b	77,6	gh
Control[a]	0.0	100,0	b	100,0	a-e

[a]The average of three replicates of *F. graminearum* s.s. in the absence of the phenolic compound

Table 21. Total fumonisins produced by *Fusarium verticillioides* isolates on phenolic-amended media.

Total fumonisins produced by *F. verticillioides* isolates (mg kg^{-1})											
Treatment	**Concentration**	**MRC 826**				**MRC 8267**				**MRC 8559**			
		Rice		**MEA**		**Rice**		**MEA**		**Rice**		**MEA**	
Caffeic acid	0.5	91,3	a	191,8	a-c	9,5	fg	47,5	b	33,9	a	2,7	g
	1.5	3,5	de	302,3	ab	20,2	d-g	52,0	b	12,6	a-c	4,4	fg
	2.5	1,5	e	4,1	d	14,2	d-g	71,3	b	13,4	a-c	0,4	g
Chlorogenic acid	0.5	82,2	ab	0,3	d	11,3	e-g	105,9	b	15,2	a-c	53,7	fg
	1.5	3,3	de	34,6	cd	15,2	d-g	61,4	b	29,9	ab	1326,0	d-f
	2.5	1,4	e	40,0	cd	56,0	a	35,9	b	21,0	a-c	473,0	fg
Coumaric acid	0.5	0,5	e	10,2	d	33,5	b-d	541,5	a	20,7	a-c	4162,3	ab
	1.5	49,0	a-e	0,8	d	24,4	c-g	468,9	a	14,7	a-c	956,1	e-g
	2.5	40,5	b-e	12,4	d	4,6	g	131,6	b	13,7	a-c	94,5	fg
Sinapic acid	0.5	22,6	c-e	133,0	cd	17,3	d-g	19,5	b	21,7	a-c	649,3	fg
	1.5	57,0	a-c	39,2	cd	7,6	g	70,4	b	7,5	c	2123,4	c-e
	2.5	0,4	e	299,4	ab	7,5	g	85,9	b	20,4	a-c	2545,2	cd
Ferulic acid	0.5	86,6	ab	0,6	d	42,1	a-c	43,0	b	29,4	a-c	3442,6	bc
	1.5	4,3	de	38,8	cd	30,3	b-e	163,8	b	25,5	a-c	2270,6	c-e
	2.5	6,0	de	27,8	cd	20,8	d-g	74,5	b	31,5	ab	3184,9	bc
Vanillic acid	0.5	45,3	a-e	329,8	a	28,0	b-f	21,2	b	20,6	a-c	212,8	fg
	1.5	52,3	a-d	27,2	cd	45,0	ab	0,0	b	10,0	bc	2210,6	c-e
	2.5	2,3	e	46,0	cd	30,1	b-e	102,9	b	14,7	a-c	5166,8	a
Control[a]	0.0	59,8	a-c	161,5	b-d	7,2	g	13,4	b	19,21	a-c	599,8	fg

[a]The average of three replicates of *F. verticillioides* in the absence of the phenolic compound

Table 22. Total fumonisins produced by *Fusarium proliferatum* isolates on phenolic-amended media.

Fumonisin produced by *F. proliferatum* isolates (mg kg^{-1})											
Treatment	**Concentration**	**MRC 2301**				**MRC 6908**				**MRC 7140**			
		Rice		**MEA**		**Rice**		**MEA**		**Rice**		**MEA**	
Caffeic acid	0.5	12,6	d-g	0,0	d	0,2	bc	788,3	cd	122,6	c-f	469,5	de
	1.5	25,3	ab	0,2	d	0,6	a	648,1	c-e	154,6	b-e	100,9	e
	2.5	23,4	a-c	0,9	d	0,3	a-c	890,4	bc	92,0	c-f	3520,6	a
Chlorogenic acid	0.5	14,7	c-f	37,6	d	0,1	c	89,7	fg	85,2	d-f	1112,0	c-e
	1.5	3,2	h	0,4	d	0,5	a-c	169,9	e-g	232,1	ab	1180,7	c-e
	2.5	6,5	f-h	0,1	d	0,3	a-c	121,7	fg	172,3	b-d	1700,5	b-d
Coumaric acid	0.5	29,6	a	6,7	d	0,1	bc	247,5	e-g	174,2	b-d	1500,1	b-e
	1.5	23,5	a-c	20,4	d	0,4	a-c	66,3	fg	179,5	a-c	2572,3	a-c
	2.5	11,1	d-h	0,6	d	0,2	bc	0,0	g	104,1	c-f	495,2	de
Sinapic acid	0.5	26,0	ab	3,1	d	0,2	a-c	525,0	c-f	46,2	f	65,3	e
	1.5	4,2	gh	0,4	d	0,3	a-c	555,1	c-f	45,3	f	255,3	de
	2.5	3,2	h	0,6	d	0,2	bc	1443,6	a	96,9	c-f	509,4	de
Ferulic acid	0.5	15,7	c-e	2525,0	ab	0,4	a-c	307,3	d-g	52,9	f	2942,4	ab
	1.5	7,1	e-h	0,2	d	0,1	bc	301,6	d-g	51,1	f	454,2	de
	2.5	26,1	ab	8,4	d	0,2	a-c	467,2	c-g	110,4	c-f	3384,8	a
Vanillic acid	0.5	5,5	gh	1615,1	bc	0,5	ab	969,5	a-c	60,2	f	165,8	e
	1.5	7,8	e-h	179,1	d	0,3	a-c	213,3	e-g	87,3	d-f	3315,9	a
	2.5	8,7	e-h	512,6	cd	0,1	bc	143,0	e-g	267,9	a	1359,0	c-e
Control[a]	0.0	17,9	b-d	2896,5	a	0,2	bc	1346,4	ab	68,2	ef	411,4	de

[a]The average of three replicates of *F. proliferatum* in the absence of the phenolic compound

Table 23. Zearalenone and trichothecenes produced by *Fusarium boothii* isolates on phenolic-amended rice.

Zearalenone (ZEA) and trichothecenes (TCT) produced by *F. boothii* isolates (mg kg^{-1})								
Treatment	**Concentration**	**M0100**		**M0010**		**M0002**		
		ZEA		**ZEA**		**ZEA**		**TCT**
Caffeic acid	0.5	2,2	d-f	0,3	c	2,7	ab	9,2 a-e
	1.5	7,6	ab	0,3	c	4,1	ab	8,7 b-e
	2.5	5,6	a-e	1,4	c	6,8	a	10,0 a-d
Chlorogenic acid	0.5	1,7	d-f	1,2	c	5,8	ab	9,1 a-e
	1.5	2,2	d-f	0,0	c	4,2	ab	10,5 a-c
	2.5	3,2	b-f	0,1	c	5,6	ab	9,8 a-e
Coumaric acid	0.5	1,6	d-f	7,5	a	6,5	a	8,0 c-f
	1.5	0,9	ef	0,7	c	2,2	ab	6,2 ef
	2.5	1,5	d-f	2,0	bc	6,5	a	12,0 ab
Sinapic acid	0.5	2,4	c-f	5,9	a	7,5	a	12,4 a
	1.5	1,9	d-f	9,4	a	4,3	ab	10,0 a-d
	2.5	3,4	b-f	0,1	c	6,6	a	9,8 a-e
Ferulic acid	0.5	1,2	d-f	7,7	a	3,9	ab	7,4 c-f
	1.5	0,6	f	6,4	a	1,1	b	6,3 ef
	2.5	2,7	b-f	0,7	c	6,6	a	7,8 c-f
Vanillic acid	0.5	1,0	ef	8,3	a	2,3	ab	4,5 f
	1.5	9,2	a	7,3	a	2,5	ab	6,5 d-f
	2.5	6,0	a-d	0,4	c	7,5	a	6,5 d-f
Control[a]	0.0	7,2	a-c	5,6	ab	4,6	ab	9,2 a-e

[a]The average of three replicates of *F. boothii* in the absence of the phenolic compound

Table 24. Zearalenone and trichothecenes produced by *Fusarium graminearum* sensu stricto isolates on phenolic-amended rice.

Zearalenone (ZEA) and trichothecenes (TCT) produced by *F. graminearum* s.s. isolates (mg kg^{-1})							
Treatment	**Concentration**	**M14.24**			**M14.55**		
		ZEA		**TCT**	**ZEA**		**TCT**
Caffeic acid	0.5	5,2 a		2,3 ef	75,1 b-d		133,2 a-c
	1.5	0,8 e		1,2 f	55,5 d		116,3 a-e
	2.5	0,3 e		1,7 ef	10,6 e		42,7 e-h
Chlorogenic acid	0.5	0,6 e		2,0 ef	4,0 e		12,6 h
	1.5	1,0 de		1,1 f	0,3 e		2,2 h
	2.5	2,0 b-e		9,9 b-d	1,9 e		7,0 h
Coumaric acid	0.5	1,6 c-e		1,8 ef	5,8 e		17,8 gh
	1.5	1,1 de		1,4 ef	4,2 e		11,9 h
	2.5	0,9 e		2,5 ef	87,8 bc		161,8 ab
Sinapic acid	0.5	0,8 e		7,7 b-e	62,0 cd		95,2 b-f
	1.5	1,8 c-e		18,7 a	62,4 cd		98,3 b-f
	2.5	1,1 de		19,3 a	11,3 e		45,8 d-h
Ferulic acid	0.5	4,5 ab		12,0 b	10,5 e		47,1 d-h
	1.5	3,8 a-c		11,6 b	86,8 bc		96,8 b-f
	2.5	3,4 a-d		5,9 b-f	119,0 a		189,8 a
Vanillic acid	0.5	1,6 c-e		4,2 d-f	100,7 ab		93,0 b-g
	1.5	2,2 b-e		4,4 c-f	80,1 b-d		71,3 c-h
	2.5	2,3 b-e		10,6 bc	20,7 e		26,4 f-h
Control[a]	0.0	1,6 c-e		8,9 b-d	80,5 b-d		120,9 a-d

[a]The average of three replicates of *F. graminearum* s. s. in the absence of the phenolic compound

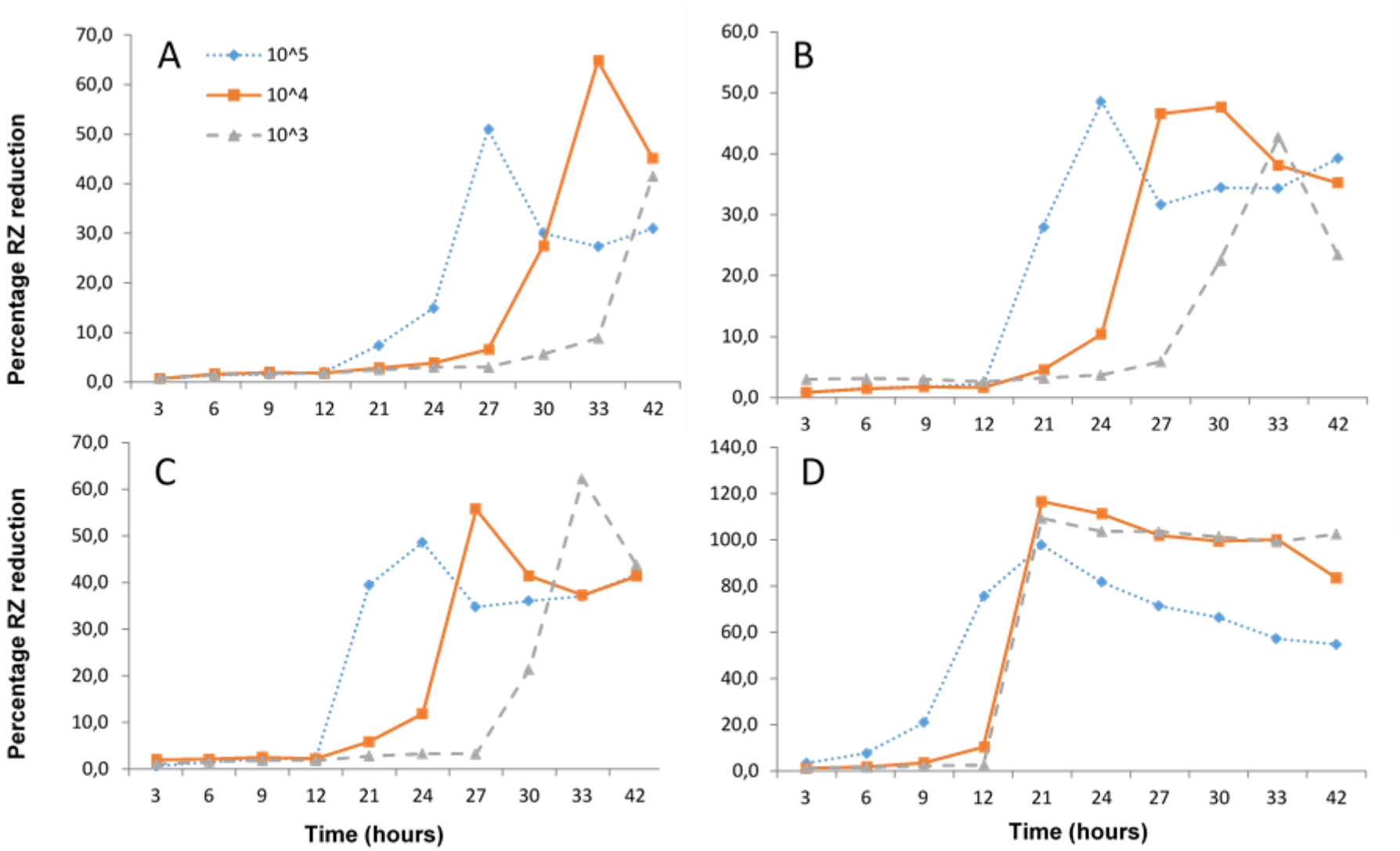


Figure 1. Percentage reduction over 42 hours in Fusarium complete media to determine the logarithmic growth phase of *Fusarium* species.
A: *F. verticillioides*, B: *F. proliferatum*, C: *F. subglutinans*, D: *F. graminearum*.

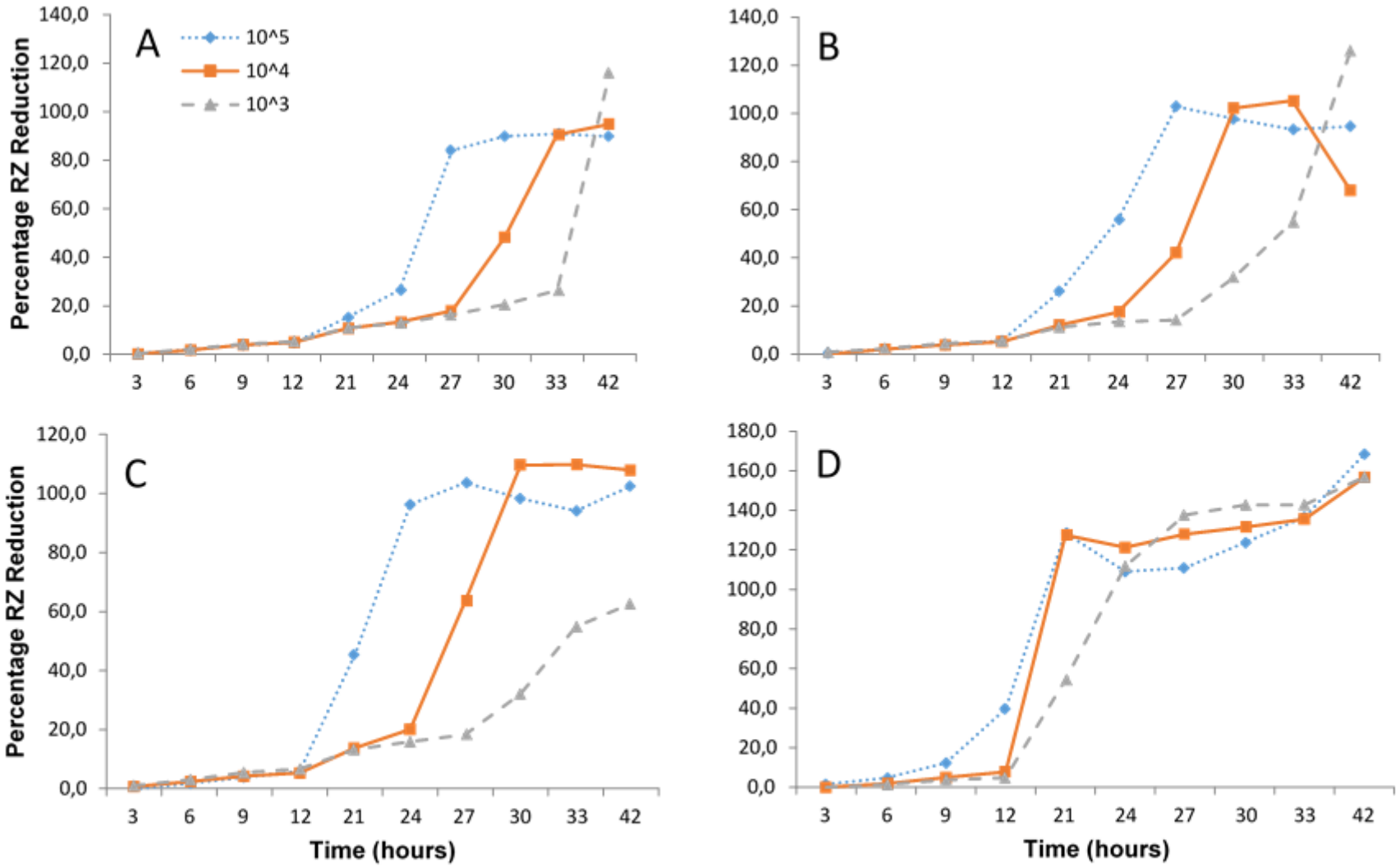


Figure 2. Percentage reduction over 42 hours in complete media to determine the logarithmic growth phase of *Fusarium* species. A: *F. verticillioides*, B: *F. proliferatum*, C: *F. subglutinans*, D: *F. graminearum*.

CHAPTER 3

Phenolic acid composition in maize kernels resistant or susceptible to *Fusarium verticillioides*

ABSTRACT

Fusarium ear rot (FER) of maize is predominantly caused by *Fusarium verticillioides* and is of economic importance as it reduces the crop yield and affects grain quality. *Fusarium verticillioides* produces mycotoxins that may have detrimental effects on humans and animals, most notably fumonisins. Cultural practises, to manage FER and fumonisins, have been identified but there is little to no success when environmental conditions favour the pathogen. Naturally occurring plant metabolites, such as phenolic compounds, have been shown to limit the growth and toxin production of *F. verticillioides in vitro*. In this study, the phenolic compounds of a resistant (CML 390) and susceptible (R2565y) maize inbred line, at different kernels maturation stages, was evaluated. The lines were inoculated with *F. verticillioides* and maize kernels were harvested at 7, 14, 28, 42 and 52 days after inoculation (dai). The fungal contamination was determined by quantitative PCR while fumonisins and phenolic compounds were quantified by liquid chromatography tandem mass spectrometry. Additionally, the expression of the phenylalanine ammonia lyase (*PAL*) 3 gene, key in the biosynthesis of phenolic compounds, was determined by reverse transcription quantitative PCR. The fungal content of grain samples from the resistant and susceptible line did not differ significantly, except at 42 dai when the susceptible line had significantly more fungal contamination as compared to the resistant line. Fumonisin levels were also significantly higher in the susceptible line at 42 and 52 dai. Phenolic acid concentrations increased over time in both lines, while ferulic and caffeic acid was found at higher levels in the inoculated susceptible line. A correlation between fungal contamination and fumonisin levels with ferulic and caffeic acid content, respectively, in the resistant line was determined. Prior to inoculation, the basal expression of *PAL3* was up-regulated in the susceptible line but down-regulated 7 and 14 dai. The expression of *PAL3* was only up-regulated from day 28 onwards. The expression of *PAL3* was up-regulated from 7 to 42 dai in the resistant line indicating an induction of this gene from early to late infection when compared to the susceptible line. Phenolic compounds represent a promising alternative to manage FER and fumonisins as it may act directly on the fungus, to limit infection and mycotoxin production or indirectly reduce mycotoxin contamination.

INTRODUCTION

Fusarium ear rot (FER) of maize is predominantly caused by *Fusarium verticillioides* (Saccardo) Nirenberg (syn = *F. moniliforme* Sheldon) in South Africa (Boutigny *et al*., 2011) but it can also be caused by *F. proliferatum* (Matsushima) Nirenberg as well as *F. subglutinans* (Wollenweber and Reinking) Nelson, Toussoun and Marasas. The disease is of economic importance as it reduces the crop yield and affects the grain quality. The causal pathogens of FER produce secondary toxic metabolites, mycotoxins, which may have detrimental effects on humans and animals once consumed. The mycotoxins produced by *F. verticillioides* is fumonisin B1 (FB_1), B2 (FB_2) and B3 (FB_3), fusaric C as well as trace amounts of moniliformin and beauvericin, however, fumonisins are the most important mycotoxins produced. The consumption of fumonisin-contaminated food has been linked to oesophageal cancer (Marasas *et al*., 1981) and the consumption of contaminated feed causes leukoencephalomalacia in horses (Marasas *et al*., 1988) as well as pulmonary oedema in pigs (Harrison *et al*., 1990).

Fumonisins are heat-stable molecules and are not fully eliminated during food processing (Sydenham *et al.*, 1995). In 2016 South Africa implemented a regulation stipulating the maximum level of fumonisin for food processing (4000 µg kg^{-1}) and human consumption (2000 µg kg^{-1}) (South African Department of Health, 2016). The effects of favourable environmental conditions on infection and disease development can deem cultural practises inadequate. An environmentally safe management strategy could be enhancing host plant resistance. Of late, naturally occurring plant compounds, such as phenolic compounds, has been widely studied *in vitro* to limit the growth and toxin production of *Fusarium* spp. (Beekrum *et al*., 2003; Samapundo *et al*., 2007; Pont *et al*., 2011; Atanasova-Penichon *et al*., 2014).

Phenolic compounds, produced by the phenylpropanoid pathway, have been shown to reduce the growth rate of *F. verticillioides* (Samapundo *et al*., 2007; Cassiem, 2015) as well as limit the production of fumonisins (Beekrum *et al*., 2003; Samapundo *et al*., 2007; Cassiem, 2015). These natural, secondary metabolites may act as a physical barrier against insects or as antimicrobial substances produced in response to fungal infections (Santiago *et al*., 2007). It has been previously illustrated that the content of phenolic compounds and phytoalexins differs between lines and might vary at different developmental stages (Santiago *et al*., 2007; Atanasova-Penichon *et al*., 2012; Veenstra *et al*., 2018). In the maize pericarp, an increased level of phenylpropanoid compounds has been associated with a decrease in disease severity and fumonisins by *F. verticillioides* (Sampietro *et al*., 2012; Lanubile *et al*., 2014). According to Lanubile *et al*. (2014) the enzyme phenylalanine ammonia-lyase (PAL), which is involved in phenolic compound biosynthesis, was highly expressed in an inoculated sample when compared to the control.

In this study, the aim was to correlate the phenolic composition and the relative expression of *PAL3* to a resistant and susceptible maize inbred line, evaluated at different kernel developmental stages.

MATERIALS AND METHODS

Greenhouse trial

Two maize inbred lines, R2565y (ARC-GCI, South Africa) and CML 390 (CIMMYT-Zimbabwe) was used in this study. These lines were previously characterised as tolerant (CML 390) and susceptible (R2565y) to FER and fumonisin contamination (Rose *et al*., 2016). The maize plants were grown in a greenhouse at the Welgevallen experimental farm at Stellenbosch University, Stellenbosch. Organic coconut coir growth medium (Greenhouse Technologies (Pty) Ltd., Johannesburg, South Africa) was prepared by adding 5 kg of the dry block in 20 L of water that was amended with 250 g $CaNO_3$. The organic coconut coir was soaked overnight before being used to fill 15-L plant bags. Before the seeds were planted, the electrical conductivity (EC) of the coconut coir was reduced to below 0.5 by rinsing it several times with water. An automated irrigation system was used to irrigate plants while simultaneously providing nutrients.

The primary maize ears were closed with a plastic bag before silk emergence to prevent any cross pollination. When the silks were fully emerged, self-pollinations of the primary maize ear was performed. Pollen, collected from the tassels of a maize plant, was deposited onto the silks of the primary ear of the same maize plant. The ears were covered following pollination to prevent cross pollination. Self-pollination was performed over a period of 2 weeks. A completely randomised design for maize lines were used for the trial layout.

Artificial inoculation

Maize ears were inoculated with a conidial suspension of the isolate *F. verticillioides* MRC 826 (Medical Research Council - PROMEC, Tygerberg, South Africa). A spore suspension was made by adding 40 mL of Armstrong media (Booth, 1971) in a 250 mL Erlenmeyer flask with fungal mycelia. The flask was incubated at 25°C in an orbital shaker platform (LAB smart) at 100 revolutions per minute (rpm) for 4-5 days. The liquid culture was filtered through two layers of sterile cheesecloth into a 50 mL tube. The tube was centrifuged for 10 min at 4°C at 3220 relative centrifugal force (rcf) after which the supernatant was discarded and the conidial pellet was washed twice with autoclaved, deionised water (with equal volumes to that of the original suspension). A haemocytometer was used to determine and adjust the final spore concentration to $2x10^6$ spores mL^{-1}.

Maize primary ears were artificially inoculated (two weeks after pollination) with 1 mL of the spore suspension using a sterile needle and syringe. The spore suspension was injected

directly into the primary maize ear though the silk channel. Control plants were inoculated with autoclaved, deionised water (dH_2O) only and three plants per treatment, of each cultivar, served as biological replicates.

Kernel harvesting and processing

Primary maize ears were harvested according to kernel developmental stages coinciding with 7, 14, 28, 42 and 52 days after inoculation (Table 1). Once the maize ears were harvested they were immediately submerged in liquid nitrogen to freeze-dry. The kernels were removed, ground into a fine powder using liquid nitrogen and stored at -80°C.

Fumonisin extraction and analysis

Fumonisin extraction and analysis was performed according to Rose *et al.* (2016). Briefly, five grams of the maize flour samples were combined with 20 mL of the extraction buffer (Methanol:dH_2O, 70:30). The samples were shaken vigorously to suspend all the grain in the extraction buffer and further mixed by placing in an incubator shaker for 30 min at 25 ºC and 200 rpm. The samples were centrifuge at 4ºC for 10 min at 500 rcf. A sterile syringe was used to remove approximately 2 mL of the clear extract after which the extract was filtered through a 0.20 µm regenerated cellulose filter and placed in the fridge overnight. The samples were centrifuged for 10 min at 20 817 rcf and was sent to the Central Analytical Facility (CAF) Mass spectrometry unit at Stellenbosch University, Stellenbosch, where free fumonisins were quantified using liquid chromatography tandem mass spectrometry (LC-MS/MS).

***Fusarium verticillioides* DNA quantification in maize kernels**

DNA extraction

Two grams of maize flour from each sample was combined with 10 mL of CTAB buffer (1.4 M NaCl, 2% w/v $[N(CH_3)_3]Br$, 0.1 M Trich-HCL, 0.02 M Ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA)) containing 1% polyvinylpyrrolidone (PVP). Thereafter 40 µL of proteinase K (10 mg mL^{-1}) was added. The samples were then shaken at 65°C for 2 h at 200 rpm in an incubator shaker. The samples were centrifuged at 3220 rcf for 10 min at 25°C. In a new 1.5-mL tube, 1 mL of the supernatant was transferred and 30 µL of RNase (10 mg mL^{-1}) was added. Samples were incubated for 15 min at 65°C after which they were centrifuged at 11 400 rcf for 10 min. The DNeasy Plant mini kit (QIAGEN, Hilden, Germany) was further used, according to the manufacturer's recommendations, to complete the DNA isolation.

Quantitative PCR (qPCR) assay and analysis

For the quantification of fungal DNA in plant material, a dilution series of fungal DNA, in maize DNA, was prepared. A four times dilution factor was used to provide a range of fungal DNA standards: undiluted (30.4 ng μL^{-1}), 4, 16, 64, 256, 1024 and 4096 fold dilution of *F.*

verticillioides 826 DNA in clean maize (*F. verticillioides*-free; 10 ng uL^{-1}). Samples were analysed in duplicate while standards were analysed triplicate in a CFX-96 Touch™ (Bio-Rad, Hercules, USA) machine. The total volume of each reaction was 20 µL using the KAPA SYBR® FAST qPCR kit (Lasec SA (Pty) Ltd., Cape Town, South Africa). The primers (200 nM) Fver356 forward and Fver412 reverse (Nicolaisen *et al*., 2009) were used and amplified the elongation factor (*EF-1α*) gene region of *F. verticillioides*. Two µL of the template DNA (10 ng uL^{-1}) was added to each reaction. The cycling conditions used were as follows: 5 min at 95°C, 35 cycles of; 5 sec at 95°C, 35 sec at 65°C and 1sec at 75°C. The analysis of a melt curve was included from 72°C to 95°C as the temperature increased by 1°C for each step. The qPCR assays adhered to the MIQE guidelines to ensure that the results obtained were reliable (Bustin *et al*., 2009). The correlation coefficient (R^2) was greater than 0.99, the efficiency (E-value) was between 1.0 to 1.03 and the slope (M-value) was between -3.2 to -3.3.

Phenolic profiling of maize inbred lines

Extraction of free phenolic compounds

One gram of the maize flour sample was used to specifically detect and quantify ferulic, sinapic, caffeic and coumaric acid. The sample was stirred in 5 mL of hexane (1:5 ratio w/v) for 10 min at ambient temperature and centrifuged for 5 min at 3220 rcf. The supernatant was discarded and the hexane extraction was repeated. Samples were dried in the fume hood for 30 to 60 min. To each tube, 10 mL of 80% methanol was added and the tubes were shaken for 30 min at room temperature. The samples were centrifuged for 10 min at 3220 rcf. Eight millilitres of the supernatant was transferred to a new 15-mL tube while the pellet was used for the extraction of the bound phenolics. The sample was concentrated to 4 mL under a nitrogen stream at 40˚C and water added to a 10 mL volume. The solution was acidified to pH 2-3 with HCl and transferred into a new 50-mL tube. Ethyl acetate (10 mL) was added and the samples were shaken for 5 min at room temperature then centrifuged for 5 min at 3220 rcf. Eight millilitres of the supernatant was transferred to a new 15-mL tube and dried under a steam of nitrogen. The samples were kept at -20˚C for 1-2 weeks and then re-constituted in 200 µL of 50% methanol (methanol: dH_2O, 50:50) and vortexed for 1 min. The samples were transferred to glass vials and submitted to CAF at Stellenbosch University for analysis.

Extraction of ester bound phenolic compounds

The tube containing the ester bound phenolic compounds was dried in a fume hood overnight and 100 mg was weighed off in a 15-mL tube. The dried pellet was hydrolysed with 4 mL of NaOH 2N. The tube was shaken in a nitrogen atmosphere (blow nitrogen in tube before closing it) for 2 h. The pH was adjusted to 2-3 with 12 N HCl (900 µL) followed by 1 N HCl. To each tube, 5 mL of ethyl acetate was added and shaken for 5 min. The tubes were then centrifuged for 5 min at 3220 rcf. Four millilitres of the upper phase was collected and transferred to a new

15-mL tube. The step was repeated and the upper phase was pooled to give a final volume of 8 mL. The samples were dried under a steady stream of nitrogen and kept at -20˚C for 1-2 weeks. The samples were reconstituted in 200 µL of 50% methanol and vortexed for 1 min. The samples were transferred to glass vials and submitted to CAF at Stellenbosch University for analysis.

Phenolic analysis

Phenolic acids were quantified using ultra performance liquid chromatography (UPLC) and photodiode array detection. Solvent A was 0.1% formic acid and solvent B was acetonitrile. The gradient applied was as follows: 100% of solvent A for one min as it gradually changes in a linear way to 50% of solvent B in 22 min. It then reached 100% solvent B after 23 min where it remained until 25 min 30s. The post-run reconditioning to obtain the initial conditions was reached after 4 min. The sample injection volume used was 3 µL with a flow rate of 0.25 mL min^{-1} while the column was maintained at 60ºC. The detector adjusted to scan from 220-600 nm. A standard dilution series was made up with the range 0.01-200 mg kg^{-1} using an external calibration with commercial phenolic compounds purchased from Sigma-Aldrich (St. Louis, USA).

Reverse transcription quantitative PCR (RT-qPCR)

RNA extraction

Maize kernels were course and then fine milled using liquid nitrogen. Maize flour (0.2 g) was used for RNA isolation and combined with 0.4 mL of the RNA extraction buffer (Tris-HCl-121.14 g mol^{-1}; pH 9). The sample was vortexed and incubated at room temperature for 10 min. Twenty microliters of 20% sodium dodecyl sulphate (SDS; 288.4 g mol^{-1}; 0.1 M) was added and incubated for 5 min. The tube was centrifuged at 11 000 rcf for 10 min at 4ºC and 0.2 mL of the aqueous phase was transferred to a new 1.5-mL Eppendorf tube. In the same tube 0.4 mL TRIzol (Invitrogen, Carlsbad, USA) was added. The tube was then vortexed and incubated for 10 min at room temperature after which 120 µL chloroform was added and the tube was vortexed again. The tube was centrifuged at 11 000 rcf at 4ºC for 10 min. In a new 1.5-mL tube 300 µL of the aqueous phase was transferred and 300 µL of isopropanol was added and mixed by pipetting. The tube was then placed at -20ºC for 20 min for precipitation of the RNA.

After the precipitation step the tube was centrifuged at 4ºC for 10 min at 12000 rcf. The supernatant was discarded and the pellet was re-suspended in 400 µL of diethyl pyrocarbonate (DEPC-1 mL L^{-1}) treated H_2O and an equal volume at phenol:chloroform was added and mixed by pipetting. The tube was centrifuged once again at 12 851 rcf for 10 min at 4ºC. In a new tube 200 µL of the aqueous phase was transferred as well as an equal volume of chloroform, the sample was vortexed and centrifuged at 12 851 rcf at 4ºC for 10 min. In a new 1.5-mL

Eppendorf tube, 100 µL of the aqueous phase, 10 µL of 3 M sodium acetate (82.034 g mol^{-1}) and 200 µL of 100% ethanol was added and mixed by pipetting after which it was kept at -80ºC for 30 min to allow the RNA to precipitate. After the precipitation step the tubes were centrifuged for 20 min at 12 851 rcf at 4ºC. The supernatant was removed with a pipette and washed with 500 µL 70% ethanol. The tube was vortexed and centrifuged for 5 min at 4ºC at 12 851 rcf. The supernatant was removed with a pipette and the sample was washed again. After the supernatant was discarded, the pellet was left to dry at room temperature for 15-30 min. Once the sample was completely dried the pellet was re-suspended with 43.75 µL of DEPC-treated. A DNase treatment was performed using the RNase-free DNase set (QIAGEN, Hilden, Germany) according to the manufacturer's recommendations.

cDNA synthesis

Following the isolation of total RNA, complementary (c)DNA was synthesised using the iScript cDNA Synthesis Kit protocol (Bio-Rad, Hercules, USA) according to the manufacturer's protocol and 100 fg – 1 µg of total RNA. Using the Applied Biosystems Veriti™ Thermal Cycler (Applied Biosystems, California, United States), the following conditions were used: 5 min at 25ºC; 20 min at 46ºC and 1 min at 95ºC.

RT-qPCR assay and analysis

Quantification of relative gene expression was conducted by reverse transcription quantitative PCR (RT-qPCR) according to Lanubile *et al.* (2010; 2014). Four genes of interest were tested namely; *PAL1* and *PAL2* (Starr *et al*., 2014) as well as *PAL3* and *PAL6* (Lanubile *et al*., 2017). Only *PAL3* was used due to non-specific binding of the other genes evaluated. The elongation factor (*EF*)-1α was used as the internal control (reference gene) to normalise all data (Lanubile *et al*., 2017). Validation experiments were performed to demonstrate that the efficiencies of *PAL3* and the reference gene amplifications were equal (Livak and Schimittgen, 2001; Bustin *et al.*, 2009). The reactions contained 20 ng of single strand cDNA, 2×iQ SYBR Green Supermix (Bio-Rad, CA, USA), and 0.4 µM of each primer. The analysis was performed using the CFX-96 device (Bio-Rad) under the following conditions: 95°C for 3 min and 44 cycles at 95°C 10 s, 60°C 25 s. A melt curve analysis, ranging from 60 to 95°C, was used to ensure amplification of the correct products. Three technical replicates, within each biological replicate, were employed for each sample and template-free samples served as the no template control. Relative expression of *PAL3* was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schimittgen, 2001). The standard deviation of the Ct values between replicates was less than 5%.

Statistical analysis

For fungal quantification and fumonisin content analysis of variance (ANOVA) was performed using the general linear models (GLM) procedures of the SAS software. Homogeneity of variance was tested by assessing the control and inoculated plants as separate trials. To test for normality the Shapiro-Wilk test was performed. The data was then transformed Ln(x+1) to stabilise the variance. Student's t-least significant difference (LSD) was used to compare the means at a level of 5%. A probability of 5% was considered significant for the significant tests performed. Free and bound phenolics were combined to represent total phenolics. Analysis of total phenolic compounds were performed by using a split-plot design with the main-plot factors being treatment (control and inoculated) and a subplot factor being the time-points (stages of kernel development). ANOVA was performed using the SAS software and the Shapiro-Wilk test was performed to test for normality. Fisher's least significant difference (LSD) was calculated at 5% to compare treatment means and a probability of 5% was considered significant for the significant tests performed. ANOVA of free and bound phenolics were performed, however, no significant difference were determined and, therefore, phenolic compounds were represented as the total of both forms.

RESULTS

Fungal and fumonisin contamination

Significant differences in fungal contamination was determined at 7 days after inoculation (dai) between R2565y control (0.012 ng mL^{-1}) when compared to CML 390 control (0.008 ng mL^{-1}). However, the R2565y inoculated (0.007 ng mL^{-1}) and CML 390 inoculated (0.005 ng mL^{-1}) did not differ significantly from each other (Fig 1). No significant differences were observed between the inoculated and control samples of each line, respectively, nor between the lines at 14 and 28 dai. At 42 dai grain from R2565y inoculated (0.201 ng mL^{-1}) had a significantly higher fungal content when compared to R2565y control (0.034 ng mL^{-1}). The fungal target DNA measured in the control (0.014 ng mL^{-1}) and inoculated (0.009 ng mL^{-1}) grain of CML 390 did not differ significantly from each other. The fungal content of R2565Y inoculated was significantly higher than that of CML 390 inoculated. At 52 dai the control (0.052 ng mL^{-1}) and inoculated of R2565y (0.437 ng mL^{-1}) did not differ significantly from each other as well as the control (0.050 ng mL^{-1}) and inoculated (0.006 ng mL^{-1}) of CML 390 and no significant differences between inbred lines were determined.

No significant differences in fumonisin levels were observed between the inoculated and control samples of each line, respectively, nor between the two lines from 7 to 28 dai (Fig. 2). At 42 dai R2565Y inoculated (0.127 mg kg^{-1}) had significantly higher fumonisins levels when compared to all other samples. The fumonisins measured in R2565y control (0.003 mg kg^{-1}), CML 390 control (0.003 mg kg^{-1}) and CML 390 inoculated (0.003 mg kg^{-1}), however, did not

differ significantly from each other. At 52 dai R2565y inoculated (0.528 mg kg^{-1}) and control (0.241 mg kg^{-1}) still accumulated more fumonisins compared to CML 390 inoculated (0.007 mg kg^{-1}) and control of CML 390 (0.001 mg kg^{-1}) but the levels were not significantly different.

Phenolic profiles of maize inbred lines

Phenolic acid concentrations differed significantly between fungal and water-inoculated grain of an inbred line, over the kernel developmental stages evaluated (Figs. 3-6).

Ferulic acid

No significant differences in ferulic acid was determined between inoculated and control grain of R2565y and CML 390 as well as no significant differences between the inbred lines (Fig. 3). Significantly more ferulic acid was measured in grain from control plants at 14 (15.9 mg kg^{-1}) and 28 dai (5.2 mg kg^{-1}) when compared to fungal-inoculated grain of R2565y at the same time-points (14 dai: 7.4 mg kg^{-1}; 28 dai: 8.2 mg kg^{-1}) (Fig. 3). Furthermore, no significant differences in ferulic acid were determined between the inoculated and control samples of each line, respectively, nor between the two lines from 42 to 52 dai.

Sinapic acid

No significant differences in sinapic acid were determined between inoculated and control grain of R2565y and CML 390, except at 42 dai when the level of sinapic acid in control plants of CML 390 (9.46 mg kg^{-1}) was significantly higher when compared to fungal-inoculated CML 390 (2.78 mg. kg^{-1}) (Fig. 4). Furthermore, the concentration of sinapic acid in fungal-inoculated grain from R2565y and CML 390 did not differ significantly from each other over all the time-points with the exception of 42 dai where sinapic acid for R2565y (10.3 mg kg^{-1}) was significantly higher than that of CML 390 (12.6 mg kg^{-1}).

Caffeic acid

No significant differences in caffeic acid was determined between inoculated and control grain of R2565y and CML 390 as well as no significant differences between the inbred lines (Fig. 5).

Coumaric acid

Significant differences in coumaric acid was determined between R2565y inoculated (4.07 mg kg^{-1}) and control grain (121.4 mg kg^{-1}) at 7 dai, while coumaric acid in CML 390 inoculated (73.2 mg kg^{-1}) also differed from the control grain (9.90 mg kg^{-1}) at 52 dai (Fig. 6). Significant differences in inbred inoculated lines were determined at 14 (R2565y - 81.1 mg kg^{-1}; CML 390- 2.7 mg kg^{-1}) and 42 dai (R2565y - 60.9 mg kg^{-1}; CML 390- 0.8 mg kg^{-1}).

Correlations

A strong, significant correlation (R = 0.97; P < 0.0001) was observed between coumaric acid and total phenolics in grain samples of R2565y inoculated with *F. verticillioides*. Total fumonisins and fungal content had a strong, positive correlation of R = 0.99 (P < 0.0001) in these samples as well (Table 2).

A good, positive correlation (R = 0.77; P = 0.001) was determined between ferulic and caffeic acid in inoculated grain of CML 390 (Table 3). Additionally, caffeic acid correlated with other phenolic compounds as well including coumaric (R = 0.65; P = 0.009), sinapic (R = 0.53; P = 0.043) and the total phenolic content (R = 0.71; P = 0.003). Ferulic acid (R = 0.63; P = 0.013) and caffeic acid (R = 0.58; P = 0.024) content correlated with total fumonisins measured. Additionally, ferulic (R = 0.78; P = 0.001) and caffeic acid (R = 0.63; P = 0.012) also correlated with fungal content measured. A strong correlation of R = 0.95 (P < 0.0001) was observed between fungal content and total fumonisins.

Reverse transcription quantitative PCR

The melting temperature (T_m) for *PAL3* was at 87ºC with the C_t (cycling threshold cycle) values ranging from 26.0-36.7. The E-value was 0.97 with a R^2 of 0.91 and a M-value of -3.41. Before inoculation R2565y had a 2.02±0.3-fold for *PAL3*. At 7dai there was a 0.38±0.1-fold decrease followed by day 14 with a 0.57±0.5-fold decrease (Fig. 7). At day 28 there was a 2.69±3.64-fold increase followed by a 3.85±6.4-fold increase at 42 dai and a 1.43±1.4-fold increased at 52 dai relative to the control (Fig. 7).

Before inoculation the expression of *PAL3* in CML 390 was 0.30±0.14-fold decrease relative to the control. At 7 dai a 2.06±2.02-fold increase was followed by a 2.59±0.77-fold increase at 14 dai. At 28 dai the expression of *PAL3* increased (2.81±4.67) resulting in an increase compared to the control. At 42 dai *PAL3* expression increased (3.45±5.9), however, at 52 dai (0.87±0.1) there is a decrease in the expression of *PAL3.*

DISCUSSION

The predominant attempts to clarify the contribution of cereal secondary metabolites, including phenolic compounds, to the *in planta* control of *Fusarium* and mycotoxin accumulation have evaluated mature grains (Atanasova-Penichon *et al*., 2016). Studies to evaluate phenolics in response to *F. verticillioides*, as kernels mature, are limited. A study by Giordano *et al*. (2017) showed that free phenolic acid had a negative correlation with DON while no significant correlations were observed for FUM, indicating that resistance to FUM could be due to other factors. Similar studies have been reported for other important mycotoxigenic fungi such as *F. graminearum*, *F. langsethiae*, *F. sporotrichioides* (Ferruz *et al*., 2016; Atanasova-Penichon *et al*., 2012). In this study, a direct comparison of phenolic acid composition was reported, during

kernel maturation, between a maize inbred line resistant or susceptible to *F. verticillioides* and fumonisins.

An increase in the accumulation of the post-infection phenolic content in cell walls has been reported in several other host parasite interactions, and it was often associated with plant resistance (Santiago *et al.,* 2007). In this study, phenolic compounds were elevated, prior to inoculation with *F. verticillioides*; and continued to increase despite the initial low levels of fungal and fumonisin contamination in both inbred lines. Even though the fungal-inoculated, susceptible line (R2565y) had significantly higher levels of fungal and fumonisin contamination at harvest, this did not coincide with significantly higher levels of any of the phenolic compounds evaluated. Thus, the result suggests that these phenolic compounds do not primarily contribute to the resistant phenotype observed for line CML 390.

Ferulic and caffeic acid was found at a higher concentration in R2565y than CML 390. Ferulic acid has been shown to reduce fungal growth and mycotoxin contamination (Beekrum *et al*., 2003; Boutigny *et al*., 2009; Ponts *et al*., 2011). **Chapter 2** illustrates the ability of ferulic acid to reduce the fungal growth of all *Fusarium* species evaluated. Bernardi *et al*. (2018) stated that compounds such as hydroxycinnamic was found at higher concentrations in the susceptible maize line than in the resistant maize line. According to literature ferulic and coumaric are found at higher concentrations while caffeic and sinapic are found at lower levels (Atanasova-Penichon *et al*., 2016). Similarly, coumaric acid was found at a higher concentration while caffeic and sinapic were found at lower levels.

No significant differences in phenolic content were determined between fungal-inoculated grain of the resistant CML 390 and susceptible R2565y lines during the early stages of infection (0-14 dai) with the exception of significantly higher coumaric acid in R2565y at 14 dai. Similarly, the phenolic content of inoculated grain did not differ significantly as kernels matured (28 to 52 dai) considering that the dent-kernel stage (42 dai) has been proposed as the most conducive kernel developmental stage for mycotoxin contamination (Picot *et al*., 2013). These results suggest that the inbred lines evaluated do not induce phenolic compounds to slow fungal ingress or inhibit fumonisin accumulation in mature maize kernels. The processes during plant defense are, however, not rigid and phenolic measurements at different time-points may provide more information regarding its role during infection by *F. verticillioides.*

The expression of the *PAL3* gene, a global regulator of the phenylpropanoid pathway further supports this finding. The expression of *PAL3* in R2565y was down-regulated upon infection (at blister and milk/dough stages) suggesting that *F. verticillioides* has the ability to modulate the expression of this gene. According to Lanubile *et al.* (2017) *F. verticillioides* has the ability to detoxify plant secondary metabolites. Only at early dent stage (28 dai) did the plant induce the expression of *PAL3* indicating a delayed response to infection when compared to the resistant line in which the gene was up-regulated from 7 dai, indicating a much stronger

and faster defence response. Differential gene response by a resistant or susceptible maize line to *F. verticillioides* has revealed that pathogen surveillance and defence-related genes are transcribed at higher levels in resistant lines compared to susceptible lines (Lanubile *et al*., 2012; 2014).

In the susceptible line coumaric acid was shown to be a good indicator of total phenolics but could not be correlated with fungal progression or fumonisin contamination. In the resistant line ferulic and caffeic acid correlated with fungal target DNA and fumonisin contamination. Ferulic acid was better correlated specifically with fungal DNA. This result demonstrates as fungal and fumonisin content increased, ferulic acid also increased in CML 390 indicating a role in retarding fungal ingress. A very strong, positive correlation between the amount of *F. verticillioides* and fumonisin content was observed for lines R2565y and CML 390 in this study. Such correlations have previously been reported when studies have quantified fungal and fumonisin content in maize grain (Small *et al*., 2012; Wu *et al*., 2013; Rose *et al*., 2016).

This study has demonstrated that R2565y was more easily colonised by *F. verticillioides* over the time-points and accumulated significantly more fumonisins in the latter stages of infection. These lines have been well characterised (Small *et al*., 2012; Rose *et al*., 2016; 2017) and their contrasting phenotypes in this study provide further support of the stability of their response to *F. verticillioides* infection. The infection by *F. verticillioides* was associated with increased concentrations of all phenolic compounds evaluated for both lines. Higher phenolic concentrations was seen in the susceptible inbred lines, however, no significant differences could be determined when compared to the resistance line.

Studies evaluating the effect of phenolics on mycotoxigenic fungi have also looked at the gene expression of mycotoxin biosynthetic genes. Boutigny *et al*. (2010) suggested that natural phenolic acids from wheat bran inhibit *Fusarium culmorum* type B trichothecene biosynthesis *in vitro* by repressing *Tri* gene expression. Kulik *et al*. (2017) showed that sinapic acid resulted in more efficient reduction of mycotoxin accumulation in the media and that *Tri* genes, responsible for trichothecene biosynthesis, was inhibited at a transcriptional level in the presence of sinapic acid. Similar results were found by Ferruz *et al*. (2016) who indicated that mycotoxin inhibition can be regulated at a transcriptional level. However, studies that relate plant gene and fungal gene expression during *F. verticillioides*-maize interaction are required to understand this complex process. Nonetheless, research has indicated the possibility of engineering-selective changes in this phenylpropanoid pathway by the overexpression of a single early pathway gene (Howles *et al*., 1996).

In conclusion, the analysis of a resistant or susceptible maize line did not reveal a clear correlation between resistance/susceptibility to *F. verticillioides* and the content of phenolic compounds at different kernel maturation stages. Disease resistance to *F. verticillioides* and fumonisins consists of multifaceted mechanisms in cereals, and many other components could contribute the overall resistance phenotype observed in CML 390. Phenolic compounds can

be used to reduce fungal and mycotoxin contamination while a greater understanding of their regulatory genes would provide key targets for genetic engineering approaches towards enhanced resistance in maize to mycotoxigenic fungi.

REFERENCES

Atanasova-Penichon, V., Pons, S., Pinson-Gadais, L., Picot, A., Marchegay, G., Bonnin-Verdal, M.-N., Ducos, C., Barreau, C., Roucolle, J., Sehabiague, P., Carolo, P. and Richard-Forget, F. 2012. Chlorogenic acid and maize ear rot resistance: a dynamic study investigating Fusarium graminearum development, deoxynivalenol production, and phenolic acid accumulation. Molecular Plant-Microbe Interactions 25: 1605-1616.

Atanasova-Penichon, V., Bernillon, S., Marchegay, G., Lornac, A., Ponts, N., Zehraoui, E., Barreau, C. and Richard-forget, F. 2014. Bioguided isolation, characterization, and biotransformation by Fusarium verticillioides of maize kernel compounds that inhibit fumonisin production. Molecular Plant-Microbe Interactions 27: 1148-1158.

Beekrum, S., Govinden, R., Padayachee, T. and Odhav, B. 2003. Naturally occurring phenols: a detoxification strategy for fumonisin B1. Food Additives and Contaminants 20: 490-493.

Bernardi, J., Stagnati, L., Lucini, L., Rocchetti, G., Lanubile, A., Cortellini, C., De Poli, G., Busconi, M. and Marocco, A., 2018. Phenolic profile and susceptibility to *Fusarium* infection of pigmented maize cultivars. Frontiers in plant science, 9: 1189.

Beukes, I., Rose, L. J., Shephard, G.S., Flett, B. C. and Viljoen, A. 2017. Mycotoxigenic *Fusarium* species associated with grain crops in South Africa – A review. South African Journal of Science. 113: 12 p. DOI: https://doi.org/10.17159/sajs.2017/20160121

Boutigny, A.L., Atanasova-Pénichon, V., Benet, M., Barreau, C. and Richard-Forget, F., 2010. Natural phenolic acids from wheat bran inhibit *Fusarium culmorum* trichothecene biosynthesis in vitro by repressing Tri gene expression. European journal of plant pathology 127: 275-286.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J. and Wittwer, C.T. 2009. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry 55: 611-622.

Gelderblom, W.C.A., Jaskiewicz, K., Marasas, W.F.O., Thiel, P.G., Horak, R.M., Vleggaar, R. and Krick, N.P.J. 1988. Fumonisins - Novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Applied and Environmental Microbiology 54: 1806-1811.

Harrison, L.R., Colvin, B.M., Green, J.T., Newman, L.E. and Cole, J.R. 1990. Pulmonary edema and hydrothorax in swine produced by fumonisin B1, a toxic metabolite of *Fusarium moniliforme*. Journal of Veterinary Diagnostic Investigation 2: 217-221.

Howles, P.A., Sewalt, V.J., Paiva, N.L., Elkind, Y., Bate, N.J., Lamb, C. and Dixon, R.A., 1996. Overexpression of L-phenylalanine ammonia-lyase in transgenic tobacco plants

reveals control points for flux into phenylpropanoid biosynthesis. Plant Physiology 112: 1617-1624.

Kulik, T., Stuper-Szablewska, K., Bilska, K., Buśko, M., Ostrowska-Kołodziejczak, A., Załuski, D. and Perkowski, J., 2017. Sinapic acid affects phenolic and trichothecene profiles of *F. culmorum* and *F. graminearum* sensu stricto. Toxins 9: 264.

Lanubile, A., Ferrarini, A., Maschietto, V., Delledonne, M., Marocco, A. and Bellin, D. 2014. Functional genomic analysis of constitutive and inducible defense responses to *Fusarium verticillioides* infection in maize genotypes with contrasting ear rot resistance. BMC Genomics 15:710.

Lanubile, A., Maschietto, V., Borrelli, V.M., Stagnati, L., Logrieco, A. F. and Marocco, A. 2017. Molecular basis of resistance to Fusarium ear rot in Maize. Frontiers in plant science, 8: 1774.

Livak, K.J. and Schimittgen, T.D. 2001. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Nature Methods 25: 402-408.

Marasas, W.F.O., Kellerman, T.S., Gelderblom, W.C.A., Coetzer, J.A.W., Thiel, P.G. and Van der Lugt, J.J. 1988. Leukoencephalomalacia in a horse induced by fumonisin B1 isolated from Fusarium moniliforme. Journal of Veterinary Research 55: 197-203.

Marasas, W.F.O., Wehner, F.C., Van Rensburg, S.J. and Van Schalkwyk, D.J. 1981. Mycoflora of corn produced in human esophageal cancer areas in Transkei, southern Africa. Phytopathology 71: 792-796.

Ponts, N., Pinson-Gadais, L., Boutigny, A.L., Barreau, C. and Richard-Forget, F. 2011. Cinnamic-derived acids significantly affect *Fusarium graminearum* growth and in vitro synthesis of type B trichothecenes. Phytopathology 101: 929-934.

Rose, L. J., Mouton, M., Beukes, I., Flett, B. C., van der Vyver, C. and Viljown, A. 2016. Multi-environment evaluation of maize inbred lines for resistance to Fusarium ear rot and fumonisins. Plant disease 100: 2134-2144.

Samapundo, S., De Meulenaer, B., Osei-Nimoh, D., Lamboni, Y., Debevere, J. and Devlieghere, F., 2007. Can phenolic compounds be used for the protection of corn from fungal invasion and mycotoxin contamination during storage? Food Microbiology 2: 465-473.

Small, I.M., Flett, B.C., Marasas, W.F.O., McLeod, A., Stander, M.A. and Viljoen, A. 2012. Resistance in maize inbred lines to *Fusarium verticillioides* and fumonisin accumulation in South Africa. Plant Disease 96: 881-888.

South African Department of health. 2016. Foodstuffs, cosmetics and disinfectants Act, 1972 (Act 54 of 1972) Regulations governing tolerance for fungus-produced toxins in foodstuffs: amendment. Government Gazette.

Sydenham, E.W., Stockenström, S., Thiel, P.G., Shephard, G.S., Koch, K.R. and Marasas, W.F.O. 1995. Potential of alkaline hydrolysis for the removal of fumonisins from contaminated corn. Journal of Agricultural Food Chemistry 43: 1198-1201.

Wu, L., Wang, X., Xu, R. and Li, H., 2013. Difference between resistant and susceptible maize to systematic colonization as revealed by DsRed-labeled *Fusarium verticillioides*. The Crop Journal 1: 61-69.

Table 1. Kernel developmental stages and harvesting timepoints based on days after inoculation.

Time-points	Kernel developmental stage	Days after inoculation
T1	Blister (R2)	7
T2	Milk/ dough (R3)	14
T3	Early dent (R4)	28
T4	Late dent/ early physiological maturity (R5)	42
T5	Biological maturity (R6)	52

Table 2. Correlation of phenolic compounds and total fumonisins and *Fusarium verticillioides* target DNA for inoculated grain of R2565y.

Variables	Ferulic	Caffeic	Coumaric	Sinapic	Total phenolics	Total fumonisins	Fungal content
ferulic	**1**	0.077	0.314	0.047	0.474	0.041	0.012
caffeic	0.077	**1**	0.093	0.490	0.191	0.150	0.129
coumaric	0.314	0.093	**1**	0.256	0.971 **< 0,0001***	0.184	0.229
Sinapic	0.047	0.490	0.256	**1**	0.397	0.488	0.469
Total phenolics	0.474	0.191	0.971 **< 0,0001***	0.397	**1**	0.247	0.277
Total fumonisins	0.041	0.150	0.184	0.488	0.247	**1**	0.993 **< 0,0001***
Fungal content	0.012	0.129	0.229	0.469	0.277	0.993 **< 0,0001***	**1**

***** Significant *P*-value with a 95% confidence level

Table 3. Correlation of phenolic compounds and total fumonisins and *Fusarium verticillioides* target DNA for inoculated grain of CML 390.

Variables	Ferulic	Caffeic	Coumaric	Sinapic	Total phenolics	Total fumonisins	Fungal content
ferulic	**1**	0.774 **0.001***	0.345	0.251	0.431	0.625 **0.013***	0.775 **0.001***
caffeic	0.774 **0.001***	**1**	0.645 **0.009***	0.529 **0.043***	0.705 **0.003***	0.580 **0.024***	0.631 **0.012***
coumaric	0.345	0.645 **0.009***	**1**	0.699 **0.004***	0.993 **< 0,0001***	0.027	0.146
Sinapic	0.251	0.529 **0.043***	0.699 **0.004***	**1**	0.744 **0.001***	-0.162	-0.092
Total phenolics	0.431	0.705 **0.003***	0.993 **< 0,0001***	0.744 **0.001***	**1**	0.071	0.199
Total fumonisins	0.625 **0.013***	0.580 **0.024***	0.027	-0.162	0.071	**1**	0.951 **< 0,0001***
Fungal content	0.775 **0.001***	0.631 **0.012***	0.146	-0.092	0.199	0.951 **< 0,0001***	**1**

***** Significant *P*-value with a 95% confidence level

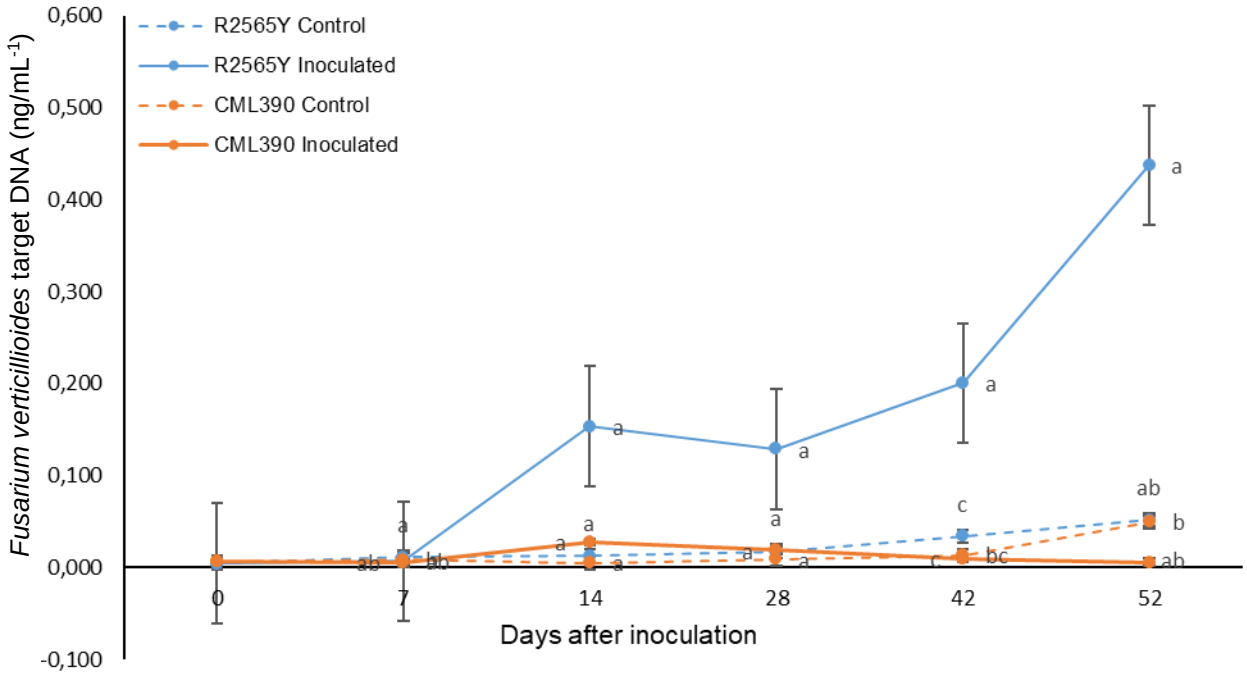


Figure 1. *Fusarium verticillioides* target DNA in maize kernels of resistant (CML 390) and susceptible (R2565y) inbred lines, at different kernel developmental stages, after inoculation.

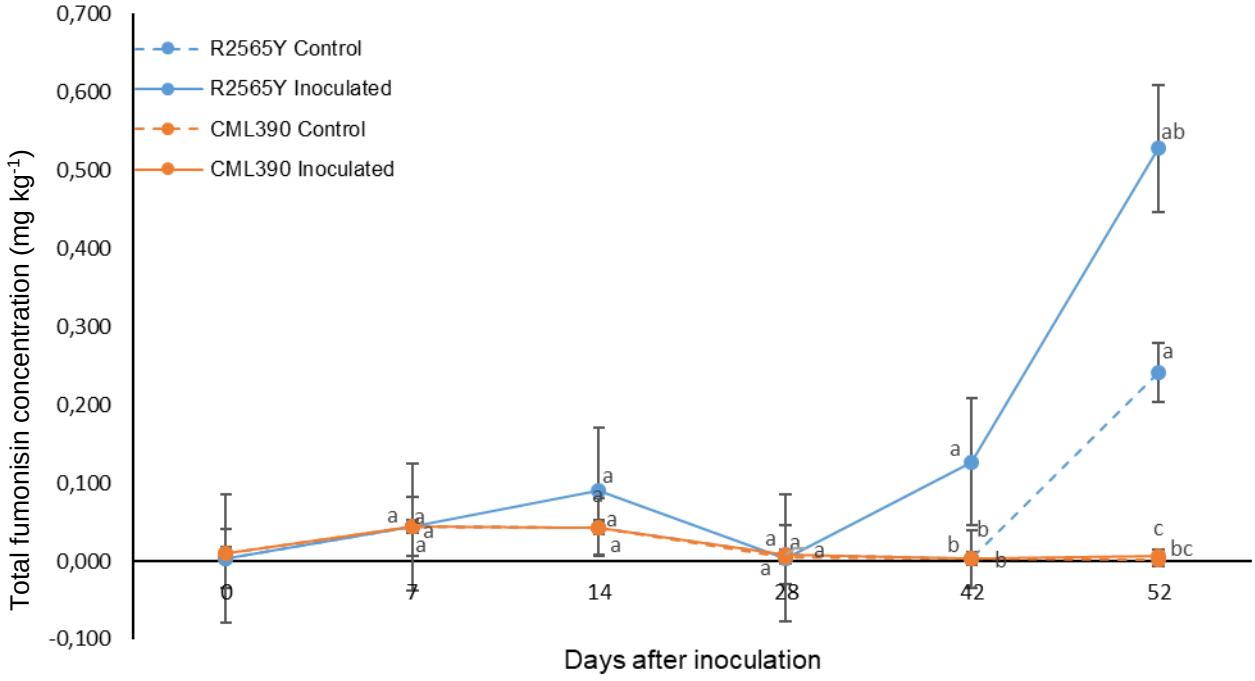


Figure 2. Fumonisin concentration in maize kernels of resistant (CML 390) and susceptible (R2565y) inbred lines, at different kernel developmental stages, after inoculation.

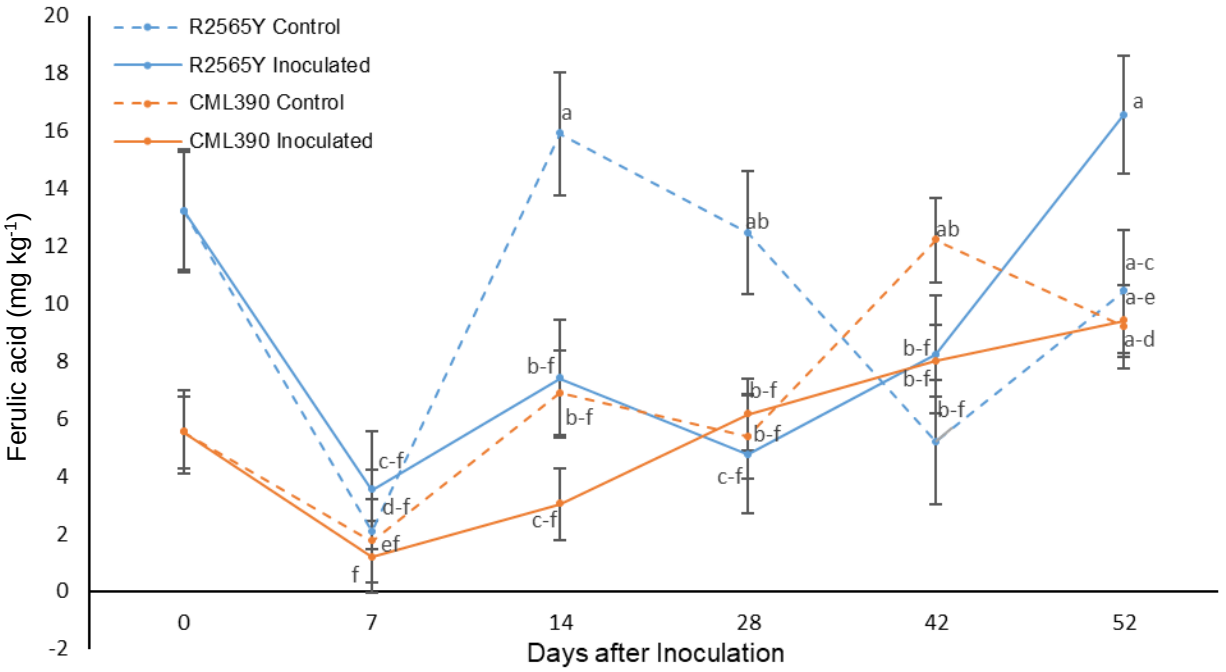


Figure 3. Ferulic acid in maize kernels of resistant (CML 390) and susceptible (R2565y) inbred lines, at different developmental kernel stages, after inoculation.

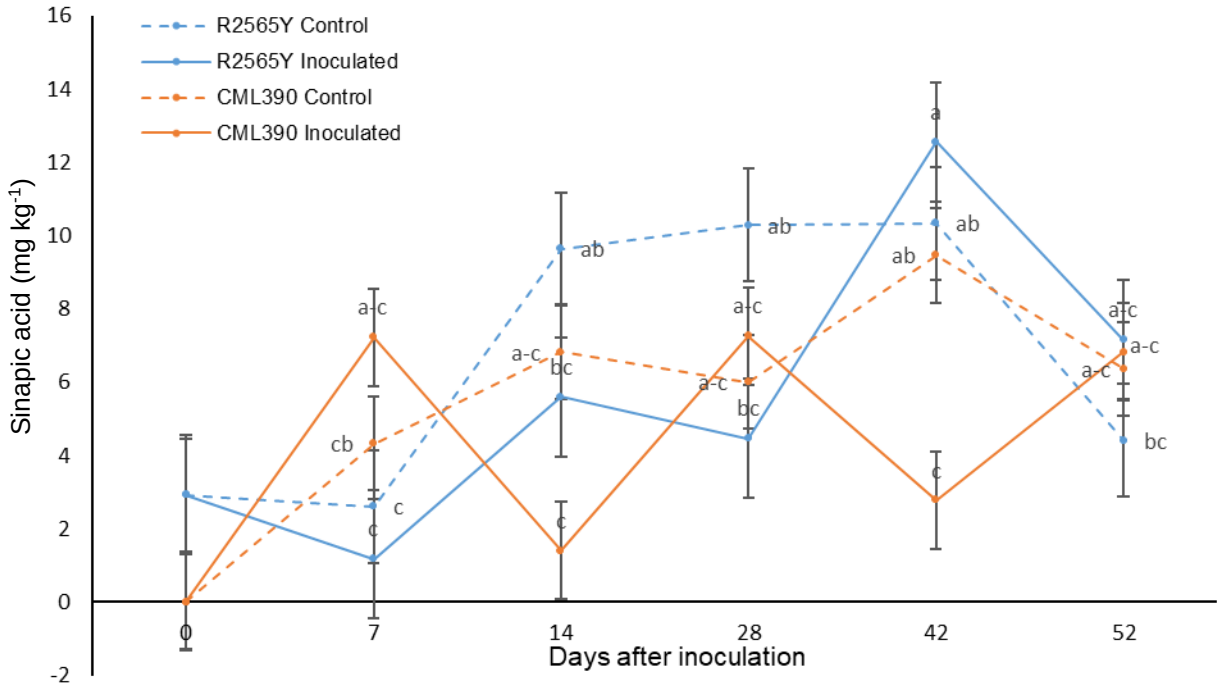


Figure 4. Sinapic acid in maize kernels of resistant (CML 390) and susceptible (R2565y) inbred lines, at different kernel developmental stages, after inoculation.

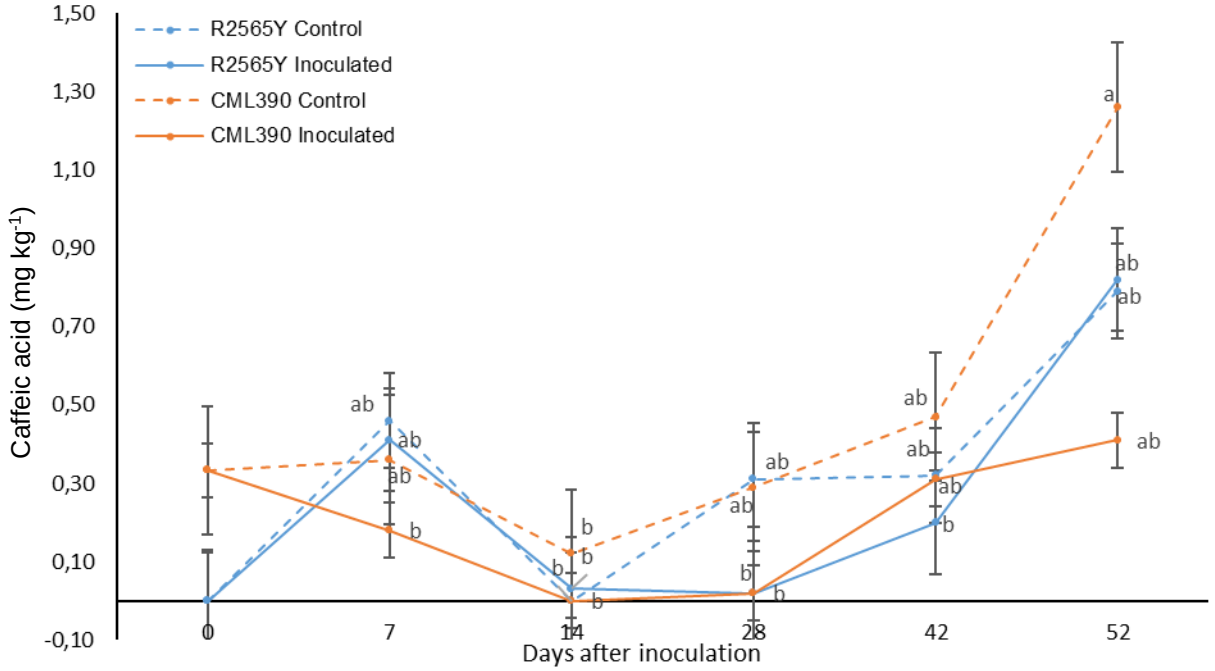


Figure 5. Caffeic acid in maize kernels of resistant (CML 390) and susceptible (R2565y) inbred lines, at different kernel developmental stages, after inoculation.

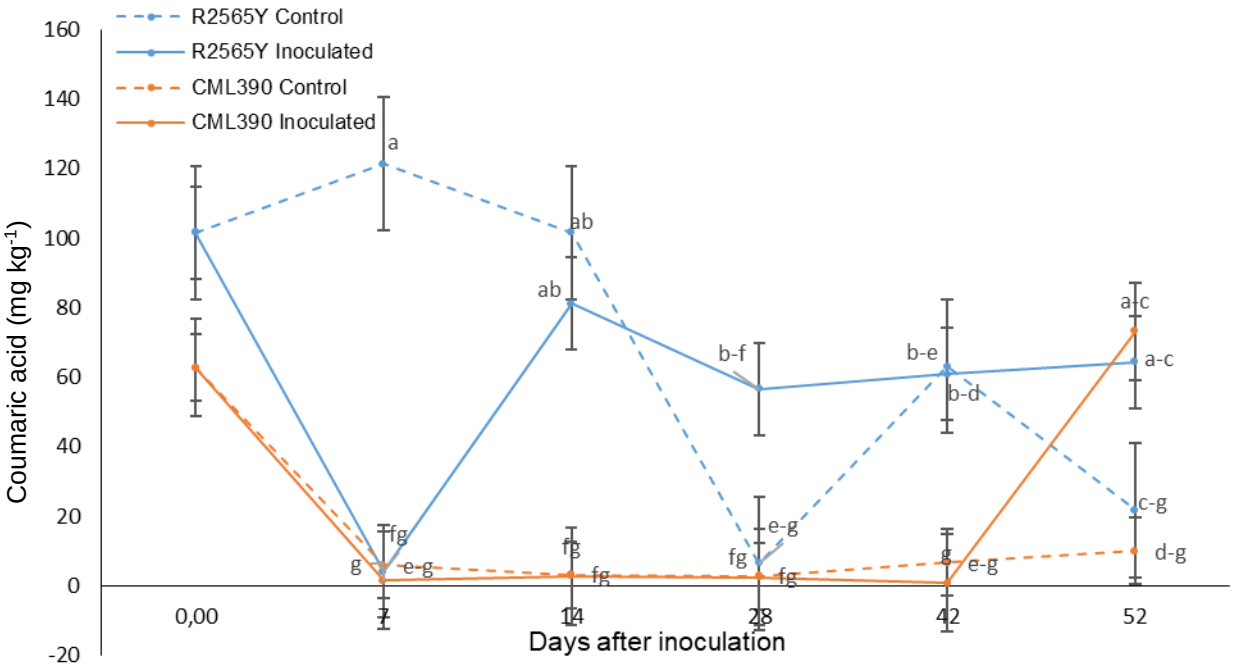


Figure 6. Coumaric acid in maize kernels of resistant (CML 390) and susceptible (R2565y) inbred lines, at different kernel developmental stages, after inoculation.

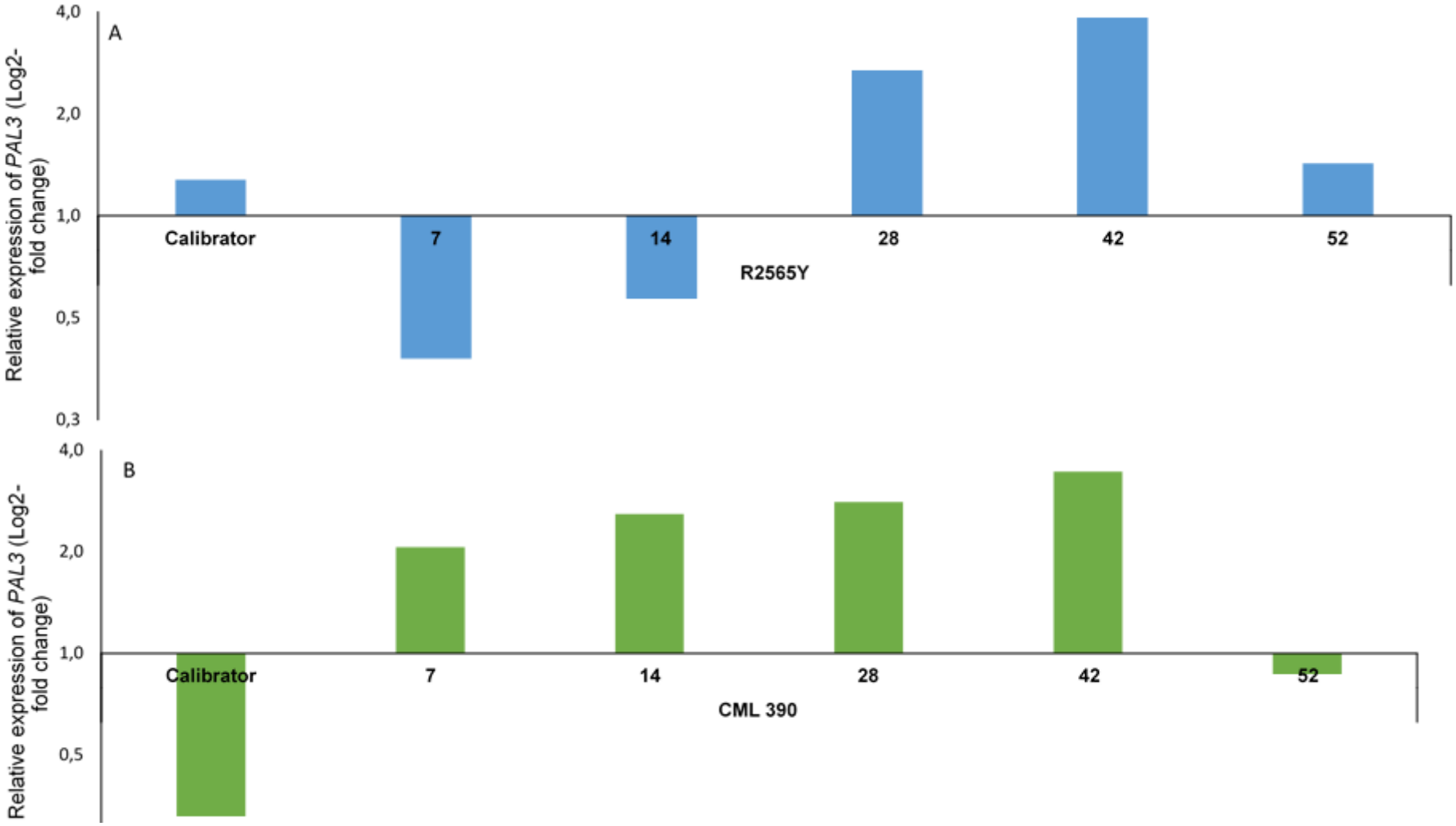


Figure 7. Expression of *PAL3* relative to the reference gene elongation factor 1α (*EF-1α*) in maize kernels of resistant (CML 390) and susceptible (R2565y) maize inbred lines, at different kernel developmental stages, after inoculation. A: R2565y, B: CML 390.