Fusarium verticillioides infection and fumonisin production during maize kernel maturation

by

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SUMMARY

Maize (*Zea mays*. L), a cereal crop readily consumed by humans and animals globally, is subject to infection by fungal pathogens such as *Fusarium verticillioides*. This pathogen is found wherever maize is grown, causes Fusarium ear rot (FER) and is also capable of producing harmful secondary metabolites known as mycotoxins. Fumonisins are the most important class of mycotoxins. Fumonisin contaminated maize has been associated with irreversible, nocuous effects in humans and animals. For this reason, fumonisins are of great concern to farmers and researchers. More recently, attention has been given to hidden fumonisins. These are fumonisin molecules trapped within the maize kernel that are not detected during mycotoxin screening and are potentially liberated upon ingestion, thus contributing to a greater risk of exposure.

Fusarium verticillioides can be managed using cultural practices and controlling maize-feeding insects. However, once conditions that favour the growth of the pathogen occur, these practices are no longer adequate to prevent fungal infection in the field. Not only is the use of chemicals not feasible, but there are also no registered chemicals available for the control of *F. verticillioides* in South Africa. Incorporating host resistance into locally adapted maize serves as the most environmentally friendly means of managing *F. verticillioides*. This requires a better understanding of factors that may contribute to disease development and progression. As the physical and biochemical composition of maize contribute to resistance to FER, understanding the relationship between structural and physico-chemical factors and fungal infection as well as fumonisin contamination would provide pivotal knowledge for breeding resistant maize cultivars.

In this study, we utilised locally adapted maize inbred lines with known response to FER and/or fumonisin contamination to investigate the role that structural traits, such as husk coverage, silk length, silk detachment and silk browning, may have on FER and/or fumonisin and hidden fumonisin contamination. We also investigated physico-chemical properties of the maize kernel, such as pH, moisture, total carbon and nitrogen, fatty acids and starch in the form of amylopectin. Maize ears were inoculated at 7 days after pollination (dap) while another independent set of maize plants were inoculated 35 dap and grain was subsequently harvested at 7, 28, 42 and 52 days after inoculation (dai). Infection indicators (FER disease severity, *F. verticillioides* target DNA and fumonisin contamination) as well as hidden fumonisins were correlated with the physico-chemical properties.

Fusarium verticillioides growth and fumonisins increased progressively over time after inoculation, reaching a maximum at 52 dai for both inoculation events with significant differences between inoculated and control maize grain. Inoculated grain of resistant lines accumulated lower levels of *F. verticillioides* target DNA and fumonisins when inoculated 7 dap, however, when inoculated 35 dap resistant lines showed an increase in fumonisin

contamination. The susceptible line accumulated high levels of fungal DNA and fumonisins in both inoculation events. Of the physico-chemical properties evaluated, pairwise correlations revealed that carbon and nitrogen had strong significant association with fungal DNA at both inoculation events. Silk browning, carbon, nitrogen and C/N were also significantly correlated with infection indicators. Moisture content had a significant negative association with fungal DNA at both inoculation events. Amylopectin increased over time in Inoculation Event 1 and remained constant in Inoculation Event 2 while no significant associations were observed between amylopectin and infection indicators. The fatty acid profile showed a synchronised increase and decrease over time, however, no significant associations with infection indicators or hidden fumonisins were noted. Hidden fumonisins extracted using alkaline hydrolysis was significantly higher when only free hydrolysed fumonisins was extractedusing a standard fumonisin extraction. Furthermore, hidden fumonisins followed a similar trend as free fumonisins, increasing over time after inoculation and peaking at the mature stages of kernel development. Inoculated resistant maize grain accumulated significantly less hidden fumonisins. Lastly, there were no significant correlations between hidden fumonisins and any of the physico-chemical factors evaluated.

This study demonstrated that *F. verticillioides* growth and fumonisin accumulation in maize grain is dependent on the timing of infection and is not triggered by a specific kernel developmental stage. Response of maize lines should also be assessed by artificially inoculating early stages of kernel maturation to determine accurate plant response in matured grain. Physico-chemical factors, such as carbon, nitrogen and C/N, may serve as indicators of potential resistance to *F. verticillioides* and/or fumonisins. The maize inbred lines used in this study can now be further classified according to their ability to accumulate both free fumonisins and hidden fumonisins.

OPSOMMING

Mielies (*Zea mays*. L), 'n graangewas wat grootskaals deur die mens en diere wêreldwyd verbruik word, is onderhewig aan infeksie deur swampatogene soos *Fusarium verticillioides*. Hierdie patogeen word gevind waar mielies ook al verbou word, veroorsaak Fusarium kop vrot (FER) en is ook in staat om skadelike sekondêre metaboliete, bekend as mikotoksiene, te produseer. Fumonisiene is die belangrikste klas van mikotoksiene. Fumonisien-gekontamineerde mielies is al met onomkeerbare, giftige effekte in mense en diere geassosieer. Om hierdie rede is fumonisiene rede tot groot kommer vir produsente en navorsers. Aandag is meer onlangs aan versteekte fumonisiene gegee. Hierdie is fumonisien molekules wat in die mieliekern vasgevang is en wat nie gedurende mikotoksien toetsing waargeneem word nie, en potensieel gedurende inname vrygestel word, en sodoende tot 'n groter blootstellingsrisiko bydra.

Fusarium verticillioides kan deur verbouingspraktyke en beheer van mielie-vretende insekte bestuur word. Sodra toestande wat die groei van die patogeen bevorder voorkom, is hierdie toestande egter nie meer voldoende om swam-infeksie in die veld te voorkom nie. Nie net is die gebruik van chemikalieë onuitvoerbaar nie, maar daar is ook geen geregistreerde chemikalieë beskikbaar vir die beheer van *F. verticillioides* in Suid-Afrika nie. Die inkorporering van gasheerweerstand in plaaslik-aangepaste mielies, dien as die mees omgewingsvriendelike wyse van bestuur van *F. verticillioides*. Dit vereis beter kennis van faktore wat tot siekte-ontwikkeling en -vordering kan bydra. Aangesien die fisiese en biochemiese samestelling van mielies tot weerstand teen FER bydra, kan kennis oor die verhouding tussen strukturele en fisiek-chemiese faktore en swam-infeksie, asook fumonisien kontaminasie, sleutel kennis tot die teel van weerstandbiedende mielie kultivars bydra.

In hierdie studie is plaaslik-aangepaste mielie inteellyne met bekende reaksie tot FER en/of fumonisien kontaminasie gebruik om die rol wat strukturele kenmerke, soos mieliekop blaar bedekking, mieliekop baard lengte, baard losmaking en baard verbruining, op FER en/of fumonisien en versteekte fumonisien kontaminasie het, ondersoek. Ons het ook fisiek-chemiese eienskappe van die mieliekern, soos pH, vog, totale koolstof en stikstof, vetsure en stysel in die vorm van amilopektien, ondersoek. Mielie koppe is op 7 dae ná bestuiwing (dap) geïnokuleer, terwyl 'n ander onafhanklike stel van mielieplante op 35 dap geïnokuleer is, en graan is gevolglik op 7, 28, 42 en 52 dae ná inokulasie (dai) geoes. Infeksie indikators (FER siekte intensiteit, *F. verticillioides* teiken DNS en fumonisien kontaminasie) asook versteekte fumonisiene is met die fisiek-chemiese eienskappe gekorreleer.

Fusarium verticillioides groei en fumonisiene het progressief oor tyd ná inokulasie toegeneem, en het 'n maksimum op 52 dai vir beide inokulasie gebeurtenisse bereik, met betekenisvolle verskille tussen geïnokuleerde en kontrole mieliegraan. Geïnokuleerde graan van weerstandbiedende lyne het laer vlakke van *F. verticillioides* teiken DNS en fumonisiene

geakkumuleer wanneer geïnokuleer op 7 dap, maar wanneer egter geïnokuleer op 35 dap, het weerstandbiedende lyne 'n toename in fumonisien kontaminasie getoon. Vatbare lyne het hoë vlakke van swam DNS en fumonisiene in beide inokulasie gebeurtenisse geakkumuleer. Van die fisiek-chemiese eienskappe wat geëvalueer is, het paarsgewys korrelasies getoon dat koolstof en stikstof sterk betekenisvolle assosiasie met swam DNS by beide inokulasie gebeurtenisse gehad het. Mieliekop baard verbruining, koolstof, stikstof en C/N was ook betekenisvol met infeksie indikators gekorreleer. Vog-inhoud het 'n betekenisvolle negatiewe assosiasie met swam DNS by beide inokulasie gebeurtenisse gehad. Amilopektien het met tyd in Inokulasie Gebeurtenis 1 toegeneem en het konstant gebly in Inokulasie Gebeurtenis 2, terwyl geen betekenisvolle assosiasies tussen amilopektien en infeksie indikators waargeneem is nie. Die vetsuur profiel het 'n gesinkroniseerde toename en afname oor tyd getoon, maar geen betekenisvolle assosiasies is egter met infeksie indikators of versteekte fumonisiene waargeneem nie. Versteekte fumonisiene wat geëkstraheer is, deur gebruik te maak van alkaliese hidrolise, was betekenisvol hoër as vry gehidroliseerde fumonisiene verkry, deur gebruik te maak van 'n standaard fumonisien ekstraksie. Versteekte fumonisiene het verder 'n soortgelyke tendens gevolg as vry fumonisiene, deur oor tyd ná inokulasie toe te neem, en te piek by die volwasse stadia van korrel ontwikkeling. Geïnokuleerde weerstandbiedende mieliegraan het betekenisvol minder versteekte fumonisiene geakkumuleer. Laastens, daar was geen betekenisvolle korrelasies tussen versteekte fumonisiene en enige van die fisiek-chemiese faktore wat geëvalueer is nie.

Hierdie studie demonstreer dat *F. verticillioides* groei en fumonisien akkumulasie in mieliegraan, afhanklik is van die tyd van infeksie en word nie aangeskakel deur 'n spesifieke korrel ontwikkelingsstadium nie. Reaksie van mielielyne moet ook vasgestel word deur kunsmatige inokulasie van vroeë stadia van korrel rypwording, ten einde akkurate plantreaksie in volwasse graan te bepaal. Fisiek-chemiese faktore, soos koolstof, stikstof en C/N, kan as indikators van potensiële weerstand dien. Die mielie inteellyne wat in hierdie studie gebruik is, kan nou verder geklassifiseer word volgens hul vermoë om beide vry fumonisiene en versteekte fumonisiene te akkumuleer.

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CONTENTS

CHAPTER 1

Reviewing maize kernel infection and fumonisin deposition by *Fusarium verticillioides*

INTRODUCTION

Maize (*Zea mays* L.) is an important crop grown for food-security in many countries worldwide (Ranum *et al.*, 2014). It feeds more than 1.2 billion people in sub-Saharan Africa and Latin America collectively, with over 300 million Africans dependent on maize grain as a staple diet (IITA, 2018). Of the maize produced in Southern Africa, two thirds are used for local consumption, with humans and animals consuming a calculated 50% and 40% respectively and the remaining 10% used as seed (DAFF, 2017). The dependency of the world population on cereals such as maize as a staple food can only be accentuated in future as food demand is expected to increase over 50% by 2050 due to rising population numbers (Borlaug, 2007). Therefore, efforts to enhance the continued production of maize has become vital in order to address biotic and abiotic stresses that leads to crop losses. This includes crop losses caused by viruses, bacteria and fungi (Oerke and Dehne, 2004; Savary *et al.*, 2012).

Fusarium verticillioides (Saccardo) Nirenberg is associated with maize wherever the crop is grown (Bacon *et al.*, 2008). The fungus causes Fusarium ear rot (FER) and is capable of producing harmful secondary metabolites known as mycotoxins (Bezuidenhout *et al.*, 1988; Gelderblom *et al.*, 1988). Kernels infected with *F. verticillioides* can be asymptomatic, yet still have significant levels of mycotoxins (Foley, 1962). These contaminated maize kernels are not discarded during sorting and contribute to the mycotoxin load within maize products destined for exportation, sale to local markets and processing. (Mukanga *et al.*, 2011; Matumba *et al.*, 2015). Mycotoxins produced by *F. verticillioides* have also been associated with adverse health effects in humans and animals (Marasas *et al.*, 1988; Voss *et al.*, 2001; Waes *et al.*, 2005; Hahn *et al.*, 2015; Kamle *et al.*, 2019).

Levels of mycotoxins are regulated on a global scale, with maize lots that do not meet the mycotoxin criteria set for import/export to a specific country being potentially discarded. As a result, economic loss occurs for farmers, processors/handlers and consumers alike (Schmale and Munkvold, 2009). Regulations are necessary to prevent exposure and adverse health effects of the consumer, however these regulations often do not impact subsistence farmers who produce maize for livelihoods, local communities and villages (Segal *et al.*, 1988; Mukanga *et al.*, 2011; Degraeve *et al.*, 2016). Reports of high incidences of oesophageal

cancers in rural communities, such as those in the former Transkei region of South Africa (Marasas *et al.*, 1981; Rheeder *et al.*, 1992; Makaula *et al.*, 1996), are thus accumulating.

Fusarium verticillioides is managed in the field by removing maize stubble that serve as primary inoculum (Smith and White, 1988) and by improving the general health of the maize plant through the use of cultural practices, soil amendments, pest and weed management (Flett *et al.*, 1998; Miller, 2001; Dowd, 2003; Munkvold, 2003a, b; Blandino *et al.*, 2008). These strategies, however, have a limited efficacy under environmental conditions that favour pathogen growth and proliferation (Munkvold, 2003a, b). Chemical control of *F. verticillioides* has to date been unsuccessful in managing *F. verticillioides* in the field (Nayaka *et al.*, 2008). The most reliable, cost effective and eco-friendly means of managing FER is host resistance in locally-adapted maize cultivars (Munkvold, 2003b; Clements *et al.*, 2004). Yet, no cultivars are available that are immune to *F. verticillioides* and mycotoxin contamination, even though cultivars and inbred lines had been identified that show varying degrees of resistance to *F. verticillioides* (Janse van Rensburg, 2012; Small *et al.*, 2012; Rose, 2016).

The objectives of this review chapter are to summarise existing literature on the importance of maize as a food crop on a local and international level, the FER pathogen and its management, and fumonisins as well as fumonisin derivatives produced in maize grain. Structural and physico-chemical properties that contribute to FER and fumonisin resistance will also be reviewed to gain a better understanding of the maize-*F. verticillioides* interaction and the factors that influence their relationship.

THE MAIZE PLANT

Origin

Maize belongs to the *Poaceae* family in the order *Poales* (ITIS, 2019). It is grouped within the taxonomic class *Lillianae* that typically consists of monocotyledonous plants, predominantly grasses such as wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.) and sugar cane (*Saccharum officinarum* L.) (Motley *et al.*, 2006). The genus *Zea* consists of annual and perennial plants that evolved in Mexico and Central America (Motley *et al.*, 2006) that includes the teosinte (*Z. mays* ssp. *parviglumis* Itis and Doebley), a wild relative of maize (Mammadov *et al.*, 2018). *Zea mays* ssp. *mays* is the modern, domesticated version of teosinte (Eubanks, 2001; Mammadov *et al.*, 2018). This domestication is a consequence of farmers selecting maize landraces for superior traits, such as fewer branches and larger ears (cobs) able to carry more kernels (Yang *et al.*, 2019). Over time this led to an increase in alleles associated with these desired traits, while non-beneficial alleles decreased in frequency (Eubanks, 2001; Motley *et al.*, 2006). Modern maize, therefore, lacks diversity, but

is rich in genetic variation which has allowed it to adapt to a range of environments over time (Yang *et al.*, 2019).

Morphology of the maize plant

Maize is an annual plant with a root system consisting of adventitious and brace roots, a main stem (stalk) with leaves arranged in opposing rows, and a male and female inflorescence that produce the fruit (ear) (Fig. 1) (Du Plessis, 2003; Bennetzen and Hake, 2009; Edwards, 2009). Adventitious roots (Fig. 1A) found below the soil surface are fine in structure and vital for the uptake of water and nutrients, whereas thicker brace roots (Fig. 1B) form in a crown above the soil surface to provide the maize plant with support (Edwards, 2009). Leaves emerge from the main stem of the plant in an alternating fashion (Fig. 1C) and consist of a sheath, ligule and auricle (Fig. 1D) (Edwards, 2009). The plant height is determined by the maize genotype in conjunction with prevailing environmental conditions and can range from 0.6-5.0 m (Bennetzen and Hake, 2009). The stem is a cylindrical, hard structure divided into nodes and internodes (Fig. 1) (Edwards, 2009). Male and female inflorescence are found separately on the maize plant, and are referred to as tassels (Fig. 1E) and ears (Fig. 1F), respectively (Nickerson, 1945; Nielsen, 2010). The ears are enclosed by leaves, referred to as husks, and hold the silks (Fig. 1G) (Nickerson, 1945). Pollen is produced by the male tassels in pollen sacs (Fig. 1H), and fertilises the silks when it comes into contact with their moist surface (Bennetzen and Hake, 2009; Edwards, 2009). The pollen grain germinates on the silk and forms a germ tube that extends towards the ovule found on the maize ear (Nielsen, 2010). The maize kernel is a consequence of fertilisation that occurs once the germ tub reaches the ovule (Nielsen, 2010). These kernels are carried on the ear of the maize plant beneath the husks and are the seed used to propagate the next generation (Fig. 1I) (Du Plessis, 2003).

Growth and development of the maize plant

Growth of the maize plant can be divided into 10 stages (Fig. 2) (Du Plessis, 2003). Seeds are planted at a depth of 25-40 mm below the soil surface, and germinate at temperatures of 20-30˚C (Hanway, 1966). Growth Stage 1 commences once the seedling emerges and continues until four leaves are present (Hanway, 1966; Du Plessis, 2003). Tiller formation and ear initiation is also triggered at this stage (Bennetzen and Hake, 2009). The first true leaves develop during Growth Stage 2, and the leaf area and stem mass increase rapidly (Hanway, 1966; Du Plessis, 2003). The tassels emerge from the growth point during Stage 3, while lateral shoots emerge to bear ears from nodes 6-8 (Bennetzen and Hake, 2009). By Stage 4 the tassels are nearly developed and silks begin to lengthen, marking the start of the reproductive phase (Nielsen, 2010). Stage 5 consists of a nearly mature maize plant, while ovules within the female reproductive parts continue to enlarge (Hanway, 1966). Pollen that

forms on the tassels are shed and caught by the silks for fertilisation (Bennetzen and Hake, 2009; Nielsen, 2010). The kernel begins to develop at Stage 6 and matures through the accumulation of sugar, starch and other nutrient precursors from Stages 6-8 (Nielsen, 2013). During the process of kernel maturation, the maize kernels become the sink for photosynthate allocation. This shift within the plant metabolism results in the senescence of the maize plant over time (Nielsen, 2013). The maize kernels soon reach physiological maturity at Stage 9, followed by biological / harvest maturity at Stage 10 (Nielsen, 2013).

The maize kernel

The maize kernel (caryopsis) comprises of several structures (Fig. 3). These include the pericarp, germ, endosperm and the tip cap (Du Plessis, 2003; Gwirtz and Garcia-Casal, 2014). The endosperm makes up the largest portion of the kernel and accounts for 80-85% of the total kernel weight (Gwirtz and Garcia-Casal, 2014). The germ has a high fat content (33.3%) as well as enzymes and nutrients to aid growth and development of the plant after germination (Gwirtz and Garcia-Casal, 2014). The pericarp is high in fibre (87%) and covers the endosperm and germ, but not the tip cap (Gwirtz and Garcia-Casal, 2014). The tip cap is the dead tissue found at the tip of the kernel where the kernel attaches to the ear, and is a passage for the movement of moisture out of the kernel as it matures making up 1% of the total kernel composition (Gwirtz and Garcia-Casal, 2014).

After fertilisation, maize kernel maturation can be classified into six stages, namely blister (R2), milk (R3), dough / early dent (R4), late dent (R5), physiological (R6) and biological / harvest maturity (Table 1) (Nielsen, 2013). These stages are characterised by their moisture content and the number of nutrient precursors. Stages R2-R4 have less starch, carbon and nitrogen with high amounts of free amino acids and sugars (Bluhm and Woloshuk, 2005), whereas Stages 5-6 have less free amino acids and sugars, and are high in starch, carbon and nitrogen (Bluhm and Woloshuk, 2005). The kernels also lose moisture as they mature and are, as a result, higher in moisture at the initial stages of kernel development (Table 1) (Hanway, 1966; Nielsen, 2013).

Economic value of maize

Maize is one of the most produced and consumed agricultural commodities in the world (McCormick, 2020). It is a widely grown and traded agricultural products due to its ease of cultivation, plasticity and versatility (Fandohan *et al.*, 2003). Not only is maize used as human food and animal feed, but it also serves as raw material in the production of oil, starch, fuel, sweeteners and alcoholic beverages (Ranum *et al.*, 2014).

The USA followed by China and Brazil, are the top maize-producing countries (McCormick, 2020). The largest exporters of maize are the USA, Argentina, Ukraine and Brazil

(McCormick, 2020), with the USA exporting approximately 15% of their total maize production (Ranum *et al.*, 2014). Maize in the USA has previously been used mainly as an animal feed, but the crop's use for fuel production has increased significantly in the past (Ranum *et al.*, 2014; Wu and Guclu, 2013). Consumption of maize in Mexico is the highest in the Americas, with daily consumption levels reaching 267 g/person, while in other parts of the Americas consumption can range from 50-187 g/person/day (Ranum *et al.*, 2014).

In Southern Africa maize is utilised predominantly by the poor. Here, it is the second largest crop produced after sugar cane (DAFF, 2017), and is grown mainly in the Free State, Mpumalanga and North West provinces (DAFF, 2017). Local production reached a record high in the 2017/18 season with 16 769 million tons harvested from over 2 628 600 hectares. Of this, approximately 4.8 million tons was exported (GrainSA, 2018). Maize consumption in rural parts of South Africa is generally higher than in cities, with consumption levels estimated to reach 632 g/person/day for males and 440 g/person/day for females (Shephard *et al.*, 2019).

South Africa contributes only 2.2% to global maize exportation (Kapuya and Sihlobo, 2013). The country exports maize mainly to African countries such as Botswana, Lesotho, Namibia, Swaziland, Zimbabwe, Kenya, Mozambique, Mauritius and Zambia, and is in fact the 8th largest exporter of maize in the world (Kapuya and Sihlobo, 2013). More recently South Africa also exported to Japan, while maize is imported from the Americas, Europe, Asia and Africa (DAFF, 2017). In the 2018/19 season, 539 588 tons of white maize and 1 520 636 tons of yellow maize were exported (SAGIS, 2019). Furthermore, the Agbiz 2017 quarterly report owed the growth in agricultural gross domestic product (GDP) of 22.2% to maize and soybean harvests, with maize harvest reaching 15.63 million tons that season (Sihlobo, 2017).

Biotic and abiotic stressors of maize

Sustainable production of maize is threatened by various abiotic and biotic stressors. Abiotic stressors include drought, temperature extremities, soil salinity or changes in precipitation (Suzuki *et al.*, 2014). Biotic stressors, on the other hand, are caused by viruses, bacteria, fungi, nematodes and insects (Suzuki *et al.*, 2014). Maize production is continuously hampered by crop losses that occur annually from weeds, fungal and bacterial pathogens and viruses (Oerke and Dehne, 2004). Plants experience combinations of these factors in the field (Atkinson and Urwin, 2012), often leading to a weakened defence response within the plant and susceptibility to infection by microorganisms (Suzuki *et al.*, 2014). Fungi affecting maize considered most important include species of *Fusarium*, *Penicillium*, *Telletia*, *Aspergillus* and *Rhizopus* (Suleiman *et al.*, 2013). Of these, the most problematic are the *Fusarium* spp. (Thompson and Raizada, 2018).

THE FUSARIUM EAR ROT PATHOGEN *FUSARIUM VERTICILLIOIDES*

Amongst the diseases caused by *Fusarium* spp. on maize, FER is considered the most significant (Munkvold and Desjardins, 1997). This disease is caused by several *Fusarium* spp., of which *F. verticillioides* is most important due to its natural abundance (Munkvold and Desjardins, 1997). This fungal pathogen is capable of infecting more than half of field grown maize prior to harvest (Robledo-Robledo, 1991; Fandohan *et al.*, 2003) at any stage of kernel development, resulting in yield losses of 10% and 30-50% in mildly and severely affected areas, respectively (Gai *et al.*, 2018).

Biology and epidemiology

Fusarium verticillioides, the causal agent of FER is a member of the *F. fujikuroi* species complex (FFSC) (Blacutt *et al.*, 2017). The monophyletic FFSC consists of several morphologically diverse species, which are divided into American, African and Asian clades. *Fusarium verticillioides* groups within the African clade (Blacutt *et al.*, 2017). The fungus is heterothallic and has two mating types, *MAT 1-1* or *MAT 1-2* (Li *et al.*, 2006). Sexual reproduction between the two mating types lead to genetic recombination and the production of ascospores in sexual fruiting bodies, called perithecia (Li *et al.*, 2006).

Fusarium verticillioides is an ascomycetous fungus that overwinters in soil and maize stubble (Smith and White, 1988; Cotten and Munkvold, 1998), as well as on other host crops such as sorghum, rice, rye, millet, soy and peas (Glenn *et al.*, 2001). This fungus can produce large numbers of asexual spores, called microconidia (Fig. 4A and 4B) and macroconidia (Fig. 4C) (Li *et al.*, 2006). These spores infect the ears, stalks and seedlings of the maize plant and cause ear rot (Fig. 5A), stalk rot (Fig. 5B) and seedling blight, respectively. The asexual spores are more commonly found in nature than the ascospores produced in perithecia (Li *et al.*, 2006). Micro- and macroconidia produced on maize stubble serve as primary inoculum that infects maize plants in the new crop cycle (Fig. 6A) (Smith and White, 1988; Cotten and Munkvold, 1998). The conidia are produced from thick hyphae that survive harsh climatic and environmental conditions (Smith and White, 1988), with microconidia being produced in larger quantities than macroconidia and are more frequently dispersed (Rossi *et al.*, 2009). *Fusarium verticillioides* thrives in warm, dry areas with optimum temperatures of approximately 30˚C (Munkvold, 2003b; Rossi *et al.*, 2009). The fungus has a hemi-biotrophic lifestyle, and initially gains its nutrients from living cells (Bacon *et al.*, 2008). Once the plant becomes vulnerable to environmental stresses, or favourable conditions occur for fungal growth, the pathogen becomes necrotrophic and causes FER (Bacon *et al.*, 2008).

Infection and Fusarium ear rot symptoms

Several infection pathways have been identified, namely silk infection (Thompson and Raizada, 2018), insect / animal injury (Warfield and Davis, 1996; Dowd, 2003; Yates and Sparks, 2008), and systemic infection through the roots (Fig. 6B) (Desjardins, 2002), where the fungus then colonises the plant systemically and survives as an endophyte during asymptomatic infection, and in contaminated seed (Foley, 1962). Silk infection is the most common infection court (Jones *et al.*, 1980; Headrick and Pataky, 1991; Munkvold and Desjardins, 1997) and does not require maize kernels to be damaged, as the silk channel delivers the fungus directly into the kernel microenvironment (Cao *et al.*, 2013). Once the spore germinates, mycelial growth proceeds down the silk using free water (Reid and Sinha, 1998; Duncan and Howard, 2010). The spores of *F. verticillioides* are also airborne and can be spread by wind, water splashing and with insect vectors that carry the spores on their body surfaces (Fig. 6C) (Ooka and Kommedahl, 1977). As mentioned, *Fusarium verticillioides* gains entry into maize plants through wounds (Fig. 6C), natural openings such as the stylar canal (Fig. 6D) and roots when cracks form during root emergence (Duncan and Howard, 2010). Seed is considered a minor source of inoculum (Fig. 6F) (Munkvold, 2003a).

Kernels infected with *F. verticillioides* are characterised by a white to light pink fungal growth occurring randomly on kernels and/or groups of kernels (Koehler, 1959) (Fig. 7A). These symptoms differ from those caused by other *Fusarium* species such as *F. graminearum* (Schwabe), where infected kernels are clustered together at the tip of the ear (Fig. 7B) (Dragich and Nelson, 2014). Kernels infected with *F. verticillioides* also have white streaks radiating across the kernel, called “starburst symptoms”, which originate from the silk attachment region (Fig. 8) (Duncan and Howard, 2010). Infected maize kernels are discarded during sorting after harvest, or are reallocated and used as animal feed (Afolabi *et al.*, 2006; Mukanga *et al.*, 2011; Matumba *et al.*, 2015).

FUMONISINS AND MODIFIED FUMONISINS

Fusarium verticillioides produces several different mycotoxins such as beauvericin, fucarin C, , fumonisins and moniliformin, with the main class of mycotoxins being the fumonisins (Nelson *et al.*, 1993). Fumonisins was first described in 1988 by Gelderblom *et al*. (1988), and its chemical structure revealed by Bezuidenhout *et al.* (1988). Fumonisins can be present as free fumonisins in maize kernels, or it can be bound to macromolecular constituents such as starch and fatty acids (Kim *et al.*, 2003; Seefelder *et al.*, 2003; Mangia, 2009; Dall’Asta *et al.*, 2012; Lazzaro *et al.*, 2012; Falavigna *et al.*, 2013). These forms of fumonisins are then referred to as “masked” or “hidden” fumonisins (Rychlik *et al.*, 2014; Berthiller *et al.*, 2016). The reason

for the production of these bound forms of fumonisins are unknown, but some sources suggest that it may be part of a defence mechanism by the plant to detoxify free fumonisins (Berthiller *et al.*, 2013, 2016).

Free fumonisins

Free fumonisins are polyketide-derived compounds with several homologous forms that can be classified into the A, B, C and P series based on structural differences (Musser and Plattner, 1997). The B series are the most infamous due to their potential carcinogenic properties in humans and animals (Gelderblom *et al.*, 1988; Rheeder *et al.*, 1992; Nelson *et al.*, 1993; Yoshizawa *et al.*, 1994; Marasas, 1996; Voss *et al.*, 2001). The toxicological significance of the other fumonisin groups are largely unknown (Blacutt *et al.*, 2017). Fumonisin B_1 (FB_1) (Fig. 9) is more abundant in nature compared to FB_2, FB_3 and FB_4, and is, therefore, more documented (Rheeder *et al.*, 2002). Fumonisin B_1 has a 20-carbon backbone with an amine group at carbon 2 (C^2) and esterified tricarballylic acid (TCA) groups at C^{14} and C^{15} with hydroxyl groups at C^5 and C^{10} (Fig. 9) (Proctor *et al.*, 2006). The biosynthesis of fumonisins are encoded by the fumonisin biosynthesis (*FUM*) gene cluster comprising of 16 co-regulated genes, namely *FUM1* and *FUM6* through *FUM19* (Proctor *et al.*, 2003) and *FUM21* (Brown *et al.*, 2007). These genes encode for a variety of enzymes, transport and regulatory proteins (Brown *et al.*, 2007; Rocha *et al.*, 2016).

Modified fumonisins

Modified forms of mycotoxins have been documented, recently and in the past (Dall'Asta *et al.*, 2012, 2015; Falavigna *et al.*, 2012; Lazzaro *et al.*, 2012; Berthiller *et al.*, 2016; Bryla *et al.*, 2016; Freire and Sant'Ana, 2018; Ekwomadu *et al.*, 2020). A myriad of terms has been used to describe these molecules, including masked, hidden, modified, cryptic and bound mycotoxins. The term "masked mycotoxins" has been formally defined by the International Life Science Institute as "mycotoxin derivatives that are undetectable using conventional analytical techniques due to changes in their molecular structure within the plant tissue" (Rychlik *et al.*, 2014). According to this definition, mycotoxins exist as their "parent forms" or they can have an altered chemical structure and are termed "cryptic mycotoxins" (Fig. 10). Cryptic mycotoxins can further be matrix-associated or have their structure altered chemically or biologically, and are termed "modified mycotoxins" (Fig. 10) (Rychlik *et al.*, 2014; Wallace *et al.*, 2018). Modified mycotoxins can also be characterised as those that were biologically transformed in the fungus, infested plant or mammalian organism (Fig. 10) (Rychlik *et al.*, 2014; Wallace *et al.*, 2018). Mycotoxins modified by chemical or enzymatic means during processing are referred to as "chemically modified mycotoxins" (Fig. 10) (Rychlik *et al.*, 2014; Wallace *et al.*, 2018).

The terms “masked mycotoxins” and “hidden mycotoxins” are often used interchangeably in the literature and has created much confusion, especially where fumonisins are concerned (Kim *et al.*, 2003; Dall’Asta *et al.*, 2012; Berthiller *et al.*, 2016; Dall’Asta and Battilani, 2016). The key difference is related to the stability of the molecule within the kernel matrices. Both “masked” and “hidden” toxin molecules are entrapped through molecular interactions with the kernel matrices, but mycotoxins are classified as “hidden” if the parental forms can be released upon ingestion, while “masked fumonisins” are stable under gastrointestinal conditions (Falavigna *et al.*, 2013). What tends to create a lot of confusion in the naming of these compounds is that the same compound can be named from different points of reference. For example, mycotoxins can be bio-transformed and then occur as phase I or phase II metabolites. Phase I metabolites are oxidised, reduced or hydrolysed versions of the parent mycotoxin, while phase II metabolites are formed through conjugation with polar molecules (Rychlik *et al.*, 2014; Wallace *et al.*, 2018). Furthermore, modified mycotoxins can also occur as a result of thermal or chemical processing and can be covalently or non-covalently linked to macromolecules within the kernel tissue and are referred to as “bound” and “hidden” fumonisins, respectively (Rychlik *et al.*, 2014; Wallace *et al.*, 2018). Lastly, they can be covalently or non-covalently linked to kernel matrices before processing occurs with non-covalently linked forms, also referred to as “hidden fumonisins” (Rychlik *et al.*, 2014; Wallace *et al.*, 2018). Thus, we see that the use of the term “hidden”, “masked” and “bound” are dependent on the degree of affinity that the respective mycotoxin has with the matrices in question as well as the information available about the specific compound at a specific point in time.

To date, most studies have focused on the conjugation of deoxynivalenol-3-glucoside (D3G), zearalenone-14-glucoside (Z14G) and Z14-sulphate, as these hidden / masked mycotoxins are commonly found in raw and processed maize-based commodities (Ekwomadu *et al.*, 2020). More recently the modification of FB_1 has become of major concern as it is the principle contaminant of maize in the field (Ekwomadu *et al.*, 2020). Apart from the presence of modified fumonisins in processed maize (Kim *et al.*, 2003; Humpf and Voss, 2004; Scott, 2012; Bryla *et al.*, 2016), there are also reports of modified fumonisins in raw maize (Mangia, 2009; Dall’Asta *et al.*, 2012; Dall’Asta *et al.*, 2015). The nature of the mechanism underlying the modification of fumonisins has been related to the covalent or non-covalent bond formation between TCA groups in fumonisins and the hydroxyl group of starch, as well as the amine group of fumonisin molecules and sulfidryl groups of proteins (Mangia, 2009). Seefelder *et al.* (2003) demonstrated the ability of fumonisins to bind to polysaccharides and proteins by means of their TCA side chains using model experiments (Seefelder *et al.*, 2003). From this study it was deduced that FB_1 is able to bind to both starch and proteins *in vitro* with FB_1 having a higher binding affinity for starch (Seefelder *et al.*, 2003). Some studies suggest that

biopolymers of starch may form inclusion complexes with FBs, facilitating the entrapment of these molecules within kernel matrices (Mangia, 2009; Dall'Asta and Battilani, 2016).

In vitro studies have been carried out to assess the ability of different strains of *F. verticillioides* to produce both fumonisins and their modified forms on synthetic media such as malt agar and maize-based media (Lazzaro *et al.*, 2012). Masked / hidden fumonisins were only detected on maize-based media and not in cultures of *F. verticillioides* grown on malt agar, indicating that the maize kernel macromolecular constituents may play a role in the masking phenomenon (Lazzaro *et al.*, 2012). Furthermore, Dall'Asta *et al*. (2012; 2015) demonstrated that the chemical composition of different hybrids played a significant role in the level of masked / hidden fumonisins as masked / hidden fumonisin content was higher in hybrids with a higher oleic to linoleic fatty acid ratio, and higher free fumonisins in hybrids with increased linoleic acid content. Their findings suggest that the fatty acid composition of the hybrids play a role in plant-pathogen communication and results in modification of the fumonisins within the maize kernels (Dall'Asta *et al*., 2012; 2015). Falavigna *et al*. (2013) also reported the presence of fatty acid esters on malt agar and cornmeal- based medium as well as in raw maize. Their study found FB_1 to mainly form esters with linoleic rather than oleic acids (Falavigna *et al.*, 2013). Moreover, esterification on synthetic media only occurred significantly on cornmeal-based media and provides more evidence for the potential association of the kernel matrix with the modification of fumonisins (Falavigna *et al.*, 2013).

The underlying mechanism of fumonisin biosynthesis, as well as the ability of resistant plant varieties being able to metabolise fumonisins by binding them to macromolecules as a means of detoxification, may be at play (Berthiller *et al.*, 2013, 2016; Dall'Asta and Battilani, 2016). Therefore, by analysing the amount of masked / hidden fumonisins in known resistant and susceptible maize genotypes, a better understanding of the factors governing resistance or susceptibility to *F. verticillioides* and/or fumonisins in maize inbred lines may be gained. Consequently, the quantification of free and masked / hidden fumonisins will better aid in risk and exposure assessment studies.

The quantification of free and modified fumonisins

Free fumonisins are extracted for quantification using an aqueous methanol or acetonitrile extraction buffer solution, and are quantified with mass-spectrometry and commercially available standards (Dall'Asta *et al.*, 2008). Masked / hidden fumonisins, however, cannot be quantified directly (Berthiller *et al.*, 2013; Rychlik *et al.*, 2014; Dall'Asta and Battilani, 2016; Wallace *et al.*, 2018), since the free carboxyl groups in their TCA side chains (Fig. 9) can potentially react with sugars, starch, proteins and fatty acids within the kernel matrix (Wallace *et al.*, 2018). These "trapped" fumonisins should be analysed using a modified extraction protocols and liquid chromatography with the appropriate standards (Dall'Asta *et al.*, 2008;

Cirlini *et al.*, 2012; Berthiller *et al.*, 2013; Rychlik *et al.*, 2014; Dall'Asta and Battilani, 2016; Wallace *et al.*, 2018). The modified extraction protocol for masked / hidden fumonisins requires the use of a strong basic solution that chemically hydrolyses the entrapped fumonisins to a form that lacks one or both of the TCA side chains, termed hydrolysed fumonisins (HFs) (Fig. 11) (Dall'Asta and Battilani, 2016). Partially hydrolysed fumonisins can also form if only one of the side chains are removed (Wallace *et al.*, 2018). The hydrolysis liberates all molecules within the kernel matrix, thereby making it impossible to differentiate the free fumonisins from the once masked / hidden fumonisins. The value obtained after hydrolyses is thus referred to as "total fumonisins" (Dall'Asta *et al.*, 2012; Lazzaro *et al.*, 2012; Falavigna *et al.*, 2013). Masked / free fumonisins are then calculated as the difference between total fumonisins after hydrolysis and free fumonisins quantified using the standard protocol (Dall'Asta *et al.*, 2012; Lazzaro *et al.*, 2012; Falavigna *et al.*, 2013). Due to the altered chemical structure of the modified fumonisin molecules after hydrolysis, they have often escaped routine analysis methods developed for their parent forms, leading to an underestimation of the true mycotoxin load (Rychlik *et al.*, 2014; Ekwomadu *et al.*, 2020). Due to this escape from conventional analysis, the modified mycotoxins are broadly referred to as "masked" or "hidden" mycotoxins in literature (Rychlik *et al.*, 2014; Wallace *et al.*, 2018). The term "hidden fumonisins" will henceforth be used to describe products of alkaline hydrolysis.

The significance of fumonisin

The biological significance of fumonisins is unclear, although it is postulated that it could provide the FER fungus with a competitive advantage to survive in the presence of other microorganisms (Reid *et al.*, 1999). Kernels isolated from the five major maize growing regions in SA demonstrated that *Fusarium verticillioides* is able to successfully outcompete microbes such as *F. graminearum*, *Stenocarpella maydis*, *Sutton maydis* (Berk.) Sutton and *F. subglutinans* (Rheeder *et al.*, 1990). The relationship between *F. verticillioides* and maize has also been described as mutualistic as secondary metabolites produced by the fungus, such as fusaric acid and gibberellins, are beneficial to the growth of the maize plant (Wicklow, 1995; Miller, 2001). Fumonisins have been reported to play a role in fungal virulence through the regulation of pathogenesis related (PR) proteins (Sánchez-Rangel *et al.*, 2012), however other studies demonstrated that non-fumonisin producing strains of *F. verticillioides* were as virulent as their wild-type strains (Desjardins and Plattner, 2000; Desjardins *et al.*, 2002).

Fumonisins are produced by *F. verticillioides* in response to changes within the kernel environment (Picot *et al.*, 2010, 2011; Parsons and Munkvold, 2012). It may help the fungus to adapt to different environmental conditions (Schmidt-Heydt *et al.*, 2008) and to respond to oxidative stress (Ferrigo *et al.*, 2014). It is postulated that, after pathogen infection and colonisation, plants produce reactive oxygen species that are toxic to the fungal growth within

the plant tissue (Ferrigo *et al.*, 2014). This not only deprives the fungus of oxygen, but also breaks down its mycelial components (Shetty *et al.*, 2006). Fungal spread and proliferation are thus prevented due the aerobic nature of *F. verticillioides* (Shetty *et al.*, 2006). The effects of oxidative stress are countered by the fungus through the production of mycotoxins to create an environment that is more conducive for fungal growth and colonisation. This allows the pathogen to survive within plant tissue (Shetty *et al.*, 2006). FB_1 is also able to inhibit ß-1,3-glucanase, an enzyme involved in the plant defence, thereby allowing for fungal colonisation (Sánchez-Rangel *et al.*, 2012).

The harmful effects of free and hidden fumonisins on human and animal health

Soon after fumonisin were described in 1988 (Gelderblom *et al.*, 1988), its ability to cause harmful effects to humans and animals was demonstrated. The mycotoxin was shown to cause tumour formation in rats, equine leukoencephalomalacia (ELEM) in horses (Marasas *et al.*, 1988), porcine pulmonary oedema (PPE) in swine (Harrison *et al.*, 1990) and hepatocarcinoma in rats (Gelderblom *et al.*, 1988; Pozzi *et al.*, 2000). In 1989, high levels of fumonisin contamination caused an outbreak of ELEM in horses and PPE in pigs in the US (Marasas, 2001). Autopsies indicated two types of histopathological consequences of ELEM that included the accumulation of clear fluid in the cerebral hemisphere as well as pitting of the white matter of the right cerebral hemisphere of the brain, swelling of the liver and, in severe cases, fibrotic lesions (Marasas *et al.*, 1988; Dutton, 1996). Pigs have also developed cardiovascular abnormalities (Haschek *et al.*, 1992), and renal injury have been reported in sheep, rats and rabbits (Marasas, 2001).

The consumption of mouldy maize contaminated with fumonisins has been linked to human oesophageal cancers in Pordenone (Italy), Transkei (South Africa), Golestan (Iran),Santa Catrina (Brazil) and several part of China (Segal *et al.*, 1988; Rheeder *et al.*, 1992; Chu and Li, 1994; Yoshizawa *et al.*, 1994; Braun and Wink, 2018). Neural tube defects have also been associated with fumonisin intake (Waes *et al.*, 2005; Missmer *et al.*, 2006). This occurs when the brain and spinal cord fusion fails during early embryonic development due to reduced folate uptake by the foetus. Studies at a cellular level revealed that fumonisins can have an effect on several biologically important pathways (Merrill *et al.*, 1996). Fumonisin B_1 are able to alter cell morphology, cell-to-cell interactions, cell-surface protein behaviour, lipid metabolism and general cell growth as a result of sphingolipid metabolism disruption (Merrill *et al.*, 1996).

Modified mycotoxins are said to be released from maize grain into the gastrointestinal tract upon ingestion (Dall'Asta *et al.*, 2012; Dall'Erta *et al.*, 2013). The intestinal environment then transforms the modified mycotoxins by cleaving them from their bound matrices and releasing their parent forms (Wallace *et al.*, 2018). Although this has not directly been studied

in humans, Dall'Erta *et al*. (2013) demonstrated that maize conjugated Z14S, Z14G and D3G can be released by colonic microbiota *in vitro* (Dall'Erta *et al.*, 2013). Studies have also shown that the exposure of rats to elevated levels of hydrolysed fumonisins resulted in increased sphinganine-to-sphingosine ratio after 1 week of exposure (Hahn *et al.*, 2015). However, fumonisin derivatives (hydrolysed and partially hydrolysed fumonisins) were lower in toxicity compared to fumonisins (Hahn *et al.*, 2015). Moreover, *in vitro* digestion assays showed that covalently-bound fumonisins (masked fumonisins) were stable under digestion and alkaline hydrolysis conditions, whereas non-covalently bound fumonisins (hidden fumonisins) that formed as a consequence of thermal and enzymatic processing, were able to release their parent forms and contribute to the mycotoxin load (Falavigna *et al.*, 2012).

Legislation to control free and modified fumonisin contamination of maize grain

The association of fumonisins with noxious effects in humans and animals resulted in their classification as a "Group 2B carcinogen" by International Agency for Research on Cancer. This has led the Codex Alimentarius Commission to regulate their occurrence on a global level. Regulations were set as follows: Maximum levels (MLs) of 4 000 µg kg^{-1} of FB_1, FB_2 and FB_3 in raw unprocessed maize, and 2 000 µg kg^{-1} for maize intended for processing (Eskola *et al.*, 2019). These limits are merely to ensure that fumonisin exposure falls below the provisional maximum tolerable daily intake of 2 µg kg^{-1} bodyweight/day (Shephard *et al.*, 2019). The European Union (EU) as well as the US have similar regulations of 4 000 µg kg^{-1} of FB_1 for unprocessed maize, while the EU also regulates for maize intended for human consumption at 1 000 µg kg^{-1} (Eskola *et al.*, 2019). Previously, only restrictions were set for aflatoxin and patulin in SA, but these have been amended to include fumonisins and deoxynivalenol as part of act no. 54 of 1972 (Government Gazette, September 2016). These restrictions were set at 4 and 2 mgkg^{-1} of fumonisins (FB_1 and FB_2) for raw maize grain intended for further processing and for processed maize products for human, respectively. For animal feed in South Africa, legislative guidelines set by the USA food and drug administration are used to meet the mycotoxin criteria worldwide (Grain SA, 2018).

Modified mycotoxins are not considered by many countries when setting legislative boundaries for mycotoxins in food and feed (Ekwomadu *et al.*, 2020). Literature indicates that of all maize producers worldwide, only Italy, Canada and the USA consider modified mycotoxins when doing random sample screening for potential market exposure (Braun and Wink, 2018). It has been approximated that hidden fumonisins may contribute an additional 60% to an exposure assessment in the EU, and that it can be even higher in areas of South America (Knutsen *et al.*, 2018). This equates to a factor of 1.6 derived from calculations based on data obtained from research groups located in Italy, Poland and Brazil (Knutsen *et al.*, 2018). The values obtained for standard fumonisin analysis during risk assessments should,

therefore, be multiplied by this factor to account for the possible presence of hidden fumonisins within the given sample. This calculation may, however, be inaccurate as several factors such as differences in environmental conditions, moisture and host genotype of a region determine the level of fumonisin contamination (Munkvold, 2003a; Picot *et al.*, 2010).

Factors affecting fumonisin and hidden fumonisin production

Several factors affect the production of fumonisins in maize kernels. These can include physico-chemical and environmental changes that take place as the kernels mature. These changes can either supress or induce the production of fumonisins by making the environment more conducive or unfavourable for the growth of *F. verticillioides* and/or fumonisin contamination.

Drought stress and low water availability has been associated with increased fumonisin production (Shelby *et al.*, 1994; Schmidt-Heydt *et al.*, 2008; Picot *et al.*, 2011). High temperatures and low precipitation before pollination results in high fumonisin accumulation compared to cooler temperatures with high precipitation (Parsons and Munkvold, 2012). This may be due to the role of water activity in fungal metabolism and the triggering of the *FUM* gene in response to water stress (Desjardins *et al.*, 2002). Insufficient fertilizer application was shown to increase FER incidence and severity (Blandino *et al.*, 2008). Insects such as the European corn borer (*Ostrinia nubilalis*)(Hübner) vector *F. verticillioides* spores that may infect the kernels through wounds caused by wind, hail, feeding of other herbivorous animals or mechanical injury caused in the field (Munkvold, 2003b; Duncan and Howard, 2009).

Physico-chemical changes within the maize kernels include altered pH, carbon, nitrogen, sugar and starch as well as fatty acid substrates (Warfield and Gilchrist, 1999; Bluhm and Woloshuk, 2005; Schmidt-Heydt *et al.*, 2008; Picot *et al.,* 2011; Dall'Asta *et al*., 2012; Smith *et al.*, 2012). These changes influence the survival and ability of *F. verticillioides* to produce free fumonisins (van Zyl, 2015; Links, 2019), but their effect on hidden fumonisins has not yet been established. The maize genotype also contributes to FER and/or fumonisin contamination (Lanubile *et al.*, 2010, 2017; Maschietto *et al.*, 2015; Rose *et al.*, 2016; Links *et al.*, 2020). It determines the overall macronutrient constitution of maize kernels, and could have an impact on hidden fumonisins (Dall'Asta *et al.*, 2012, 2015).

MANAGEMENT OF FER AND FUMONISINS

Effective management of FER and fumonisin contamination is best achieved using an integrated disease management approach (Munkvold, 2003b). This is a multi-disciplinary tactic involving cultural, chemical, biological and genetic practices to make maize production,

harvest, storage and transport as non-conducive for pathogen development and spread as possible (Munkvold, 2003b).

Cultural practices

Pre-harvest: Inoculum of *F. verticillioides* that survives on the previous crop's residues (Smith and White, 1988; Cotten and Munkvold, 1998) are generally managed using soil tillage and crop rotation (Skoglund and Brown, 1988; Mabuza *et al.*, 2018). These strategies aim to break the disease cycle of the pathogen by removing the primary source of inoculum. The pathogen may also be seed-borne in which case these techniques have little effect on disease development. Plant stress during cultivation generally increases plant susceptibility (Ferrigo *et al.*, 2014; Suzuki *et al.*, 2014). Proper irrigation, as well as nutrient supplementation by means of soil amendments are typically required to increase plant vigour (Alakonya *et al.*, 2008; Blandino *et al.*, 2008). Yet, a significant increase in fumonisin production with nitrogen soil supplementation have been reported (Marocco *et al.*, 2007; Blandino *et al.*, 2008). Planting maize earlier and at lower densities, and reducing competition for water and nutrients, have also reduced fumonisin contamination of maize grain (Blandino *et al*., 2008a, Parsons and Munkvold, 2010, 2012). Unfortunately, the use of cultural practices to combat *F. verticillioides* is limited as environmental conditions favourable for pathogen growth and proliferation reduces the efficacy thereof and under these conditions are no longer sufficient to prevent high levels of fumonisin contamination (Munkvold, 2003b). Early harvesting of maize also reduces the levels of mycotoxin contamination by shortening the period where possible fungal infection can occur (Bush *et al.*, 2004). The wounding of maize grain by insects can be prevented by using insecticides and the planting of transgenic maize varieties such as *Bt*-maize (Munkvold *et al.*, 1997, 1999; Munkvold, 2000; Bakan *et al.*, 2002; Dowd, 2003; Weaver *et al.*, 2017).

Harvest and drying: Maize kernels can be physically damaged during harvesting and transportation, which could result in fungal infection and subsequent mycotoxin contamination (Munkvold, 2003b). Harvesting methods and transportation should thus be refined to lower the risk of physical damage (Munkvold, 2003b). The moisture content of maize kernels can be reduced by artificial drying to reduce fungal infection (Munkvold, 2003b), but this approach is not feasible to all (Mukanga *et al.*, 2011). Where the infrastructure for artificial drying in unavailable such as in rural communities, maize is left to dry in the field or on roofs where losses may occur from feeding by birds, rats or monkeys (Mukanga *et al.*, 2011). Stockpiling maize on the soil surface in rural tropical areas may also lead to an increase in *Aspergillus flavus* (Link.) and aflatoxin contamination (Mukanga *et al.*, 2011). Maize should generally be dried to a kernel moisture content of 25% before storage, where after the kernels should be

further dried to a moisture content of less than 15% before being stored or processed (Bush *et al*., 2004).

Post-harvest: The sorting and washing of maize kernels after harvest can significantly reduce fumonisin contamination in grain and is actively taught and utilised in African rural maize-farming communities (Afolabi *et al.*, 2006; Mcpherson and Stroebel, 2017; Matumba *et al.*, 2015; GrainSA, 2017). Fumonisin levels in maize that had been hand-sorted with a kernel density separation technique using clean drinking water to submerge kernels and remove floating ones have reduced fumonisins by 84% (van der Westhuizen *et al.*, 2011). The infrastructure of storage facilities of maize varies between the developed and developing world (Munkvold, 2003b). Developed countries are able to afford the infrastructure that allows grain to be properly stored, contributing to a higher food-security status compared to developing countries (Munkvold, 2003b). It is crucial that containers used for storage of maize grain be cleaned prior to the storage of new crops, and that the temperature for storage be set between 1 and 4˚C, with 10-15˚C being more appropriate for storage in summer (Munkvold, 2003b).

Chemical control

Chemical control of *F. verticillioides* has thus far been unsuccessful (Pietri *et al.*, 2011). Maize ears are tightly covered by husks, which prevents protection with contact fungicides (Janse van Rensburg, 2012). No significant differences were found in *Fusarium* spp. and fumonisin contaminated maize kernels in maize ears treated with strobilurin, triazole and benzimidozole fungicides and those not treated with such fungicides (Janse van Rensburg, 2012). Benomyl (Cruz *et al.*, 2014) and tebuconazole (Marín *et al.*, 2013) reduced the growth of *F. verticillioides* and fumonisin biosynthesis *in vitro*. Since fumonisin production was also significantly affected by temperature and water fluctuations, the efficacy of these fungicides under field conditions may only be suitable for certain climates (Cruz *et al.*, 2014). Formenti *et al*. (2012) found that Folicur® (Active ingredient: Tenbuconazole), Proline® (Prothioconazole) and Sportak 45EW® (Prochloraz) effectively inhibited *F. verticillioides* growth and fumonisin production *in vitro* compared to the control treatment. Fungicides such as thiram and carbendazim have been used as seed treatments in India to control *F. verticillioides*, but their cost and acute residue levels were of concern (Nayaka *et al.*, 2008) To date, no fungicides are registered internationally for the management of FER.

Biological control

Biological control holds some promise for managing *F. verticillioides* in the field (Pal and Gardener, 2006). The effectivity of several biological agents, such as *Pseudomonas fluorescens* (Trevisan) Migula, has been assessed against *F. verticillioides* on maize (Nayaka

et al., 2008). Bacteria such as *Bacillus subtilis* (Ehrenberg) Cohn may be more desirable as a biological control agent against *F. verticillioides* due to their favourable survival capabilities by forming endospores, and the wide variety of antibiotics they produce (Bevivino *et al*., 1998). Formenti *et al*. (2012) found that *Bacillus subtilis* reduced *F. verticillioides* growth and fumonisin production *in vitro* by 70-75%. However, *B. subtilis* is sensitive to low water activity and can, therefore, be influenced by environmental changes (Formenti *et al.*, 2012).

Plant resistance

Host resistance to *F. verticillioides* is the most affordable and environmentally sound way of managing FER and fumonisin contamination of maize (Munkvold, 2003b). Host resistance can be introduced into plants using conventional or unconventional breeding methods (Acquaah, 2007). The former involves the introgression of disease resistance into locally adapted and agronomically-desirable plants. Unconventional breeding for disease resistance includes genetic modification, gene-editing to mutation breeding.

Conventional breeding involves screening maize populations for desirable characteristics on a phenotypic level using infection indicators (FER disease severity, fumonisin and/or fungal contamination) as done by van Zyl (2015) and Links (2018). Screening can also be done on a genotypic level (Acquaah, 2007) as confirmed by recent studies (Links *et al.*, 2020). The selected plants from a population are used to create inbred lines by means of pedigree selection and artificial pollination (Acquaah, 2007). This form of breeding is a lengthy process and, in some cases, can take up to 10 years to reach a marketable result. Furthermore, traits conferring resistance to mycotoxigenic fungi are polygenic and quantitative in nature, with moderate to high heritability, making the selection and breeding for these traits challenging (Pérez-Brito *et al.*, 2001; Eller *et al.*, 2008; Lanubile *et al.*, 2017) and are difficult to integrate without losing valuable agronomic traits. Trials to assess potential resistance or susceptibility also require labour-intensive inoculation techniques that are costly and time consuming (Reid *et al.*, 2002; Pereira *et al.*, 2011).

Previous studies have successfully evaluated F1 hybrids created using parental lines resistant to FER/fumonisin and *Aspergillus flavus* (Netshifhefhe *et al.*, 2018). Resistance between parental and F1 hybrids did not differ significantly, indicating that improved resistance in maize lines can be achieved using single crosses. Furthermore, locally adapted maize varieties should be investigated for resistance to FER and/or fumonisin contamination in order to ensure optimal performance and stability of maize genotypes to a range of environments (Rose *et al.*, 2016; Netshifhefhe *et al.*, 2018). This will facilitate a timely and efficient means of resistant cultivar development (Rose *et al.*, 2016). Quantitative trait loci (QTLs) that confer resistance to FER and fumonisin contamination have been identified by Pérez-Brito *et al.* (2001), Robertson-Hoyt *et al.* (2006), Ding *et al.* (2008) and Li *et al.* (2011). These QTLs can

be used to develop DNA markers that allow for the identification of desired traits in a more timely manner without the need for prolonged field trials using marker assisted selection (MAS) (Robertson-Hoyt *et al.*, 2006).

Unconventional breeding methods such as mutation breeding and genetic modification allow breeders to introduce traits that facilitate resistance to FER and/or fumonisins into maize varieties while still maintaining desirable agronomic traits (Acquaah, 2007). An example would be the genetically modified (GM) *Bt*-maize created using the *cry*1A(b) gene from *Bacillus thuriengiensis* (Berliner). Expression of this gene in maize leads to the production of insecticidal compounds toxic to maize-feeding insects (Bakan *et al.*, 2002). The level of fumonisin contamination in *Bt*-maize was significantly lower than the non-modified hybrids when assessed under natural field conditions owing to a reduction in insect feeding wounds that allow for fungal infection and/or fumonisin contamination of maize grain (Bakan *et al.*, 2002). Disease resistance genes that were introduced into the maize genome to degrade fumonisins include those from black yeast, *Exophialia spinifera* (H.S. Nielsen and Conant) McGinnis, which is responsible for the production of fumonisins esterases and amine oxide (Duvick, 2001; Munkvold, 2003b). Herbicide tolerant maize, another example of GM maize, and insect resistant maize are commercially available in SA (SAASTA, 2014). In fact, 86% of maize grown in SA are GM with herbicide resistant maize occupying almost 49% of all GM maize cultivated (SAASTA, 2014). In 2008, the development of Water Efficient Maize for Africa (WEMA) was founded with the aim of developing drought-tolerant, insect-resistant African maize varieties for sub-Saharan Africa using both conventional breeding methods in the form of MAS, and unconventional breeding methods in the form transgenic modifications (GM) (SAASTA, 2014).

PLANT RESISTANCE MECHANISMS

Plants are able to defend themselves from pathogen attack using structural barriers that prevent infection (Freeman and Beattie, 2008), or by activating biochemical defence responses that inhibit pathogen damage (Bennet and Wallsgrove, 1994). These structural and biochemical barriers can be pre-existing or induced after the recognition of the pathogen by the plant (Bennet and Wallsgrove, 1994; Freeman and Beattie, 2008; Lanubile *et al.*, 2017). In maize, plant resistance is governed at a structural, physico-chemical and genetic level (Links *et al.*, 2020).

Structural defence

Structural traits; such as silk length, husk coverage and kernel and pericarp thickness; may play an important role in resistance to FER and fumonisin contamination in maize (Blandino

and Reyneri, 2008; Sampietro *et al.*, 2013). Longer and dryer silks (Stroshine *et al.*, 1986), tighter husks (Warfield and Davis, 1996), thicker pericarps (Hoenisch and Davis, 1994) and harder kernels (Blandino and Reyneri, 2008) are strongly associated with reduced infection by *F. verticillioides* and the production of fumonisin. Longer silks (Fig. 12) are thought to provide a longer distance for the fungus to travel before gaining entry into the kernel, and is thus associated with improved resistance to *F. verticillioides* infection (Reid and Sinha, 1998). As silks age they become dryer (Fig. 13), thereby providing a less favourable environment for pathogen progression as *F. verticillioides* makes use of free water to travel toward the silk attachment point (Thompson and Raizada, 2018). Maize genotypes that dry out quicker are, therefore, seen as being more resistant to *F. verticillioides* infection (Headrick *et al.*, 1990). The detachment of the silk from the husk after pollination (Fig. 14) has also been associated with resistance to *F. verticillioides* (Thompson and Raizada, 2018).

The husk leaves of maize plants prevent kernels from drying out and provide protection from insects that may wound the kernels and spread fungal spores (Nickerson, 1945). Tighter husks that completely enclose the maize ear (Fig. 15A) are seen as more resistant than husks that are open (Fig. 15B) (Warfield and Davis, 1996). Pericarp thickness may also contribute to resistance as it is the first fraction of the kernel that is encountered by the fungus (Sampietro *et al.*, 2009). Kernels with thicker pericarps are less prone to infection by *F. verticillioides* (Sampietro *et al.*, 2009). Kernel hardness, a highly heritable trait, could thus play a significant role in host resistance (Fox and Manley, 2009). When structural traits such as silk length, husk coverage, kernel mass, kernel hardness and pericarp thickness were evaluated in inbred lines and commercial cultivars, no correlation was found between structural characteristics and infection, suggesting that structural traits are not sufficient to determine resistance or susceptibility to *F. verticillioides* (Links *et al.*, 2020).

Physico-chemical defence

Temperature and water availability (a_w) affect FB_1 production by isolates of *F. verticillioides* and *F. proliferatum* (Marin *et al*., 1999). Fumonisin production was induced at a low a_w (0, 97), as observed in mature maize kernels (Marin *et al*., 1999). The expression of the *FUM* gene in *F. verticillioides* is triggered during water stress (Jurado *et al.*, 2008). As a results, low a_w during the later stages of kernel development has been correlated with high levels of fumonisins (Jurado *et al.*, 2008; Schmidt-Heydt *et al.*, 2008). It should also be noted that the range of a_w tends to be narrower for fumonisin production compared to fungal growth, with less fungal growth leading to less fumonisin production (Marın *et al.*, 1999).

Fumonisin production by *F. verticillioides* is strongly associated with changes in pH (Keller *et al.*, 1997; Flaherty *et al.*, 2003; Bluhm and Woloshuk, 2005; Schmidt-Heydt *et al.*, 2008; Smith *et al.*, 2012). There is, however, uncertainty whether kernel pH directly affects

fumonisin production, or whether the fungus manipulates the kernel environment to make it more conducive for fumonisin production (Bluhm and Woloshuk, 2005). van Zyl (2015) and Links (2018) detected a significantly lower pH in kernels inoculated with *F. verticillioides* compared to the uninoculated control. Keller *et al.* (1997) also demonstrated that the growth of *F. verticillioides* and FB_1 production *in vitro* are influenced by pH and oxygen under nitrogen-limiting conditions. Acidic conditions thus tend to enhance FB_1 production while alkaline conditions supress the production thereof (Keller *et al.*, 1997).

Mature maize kernels consist of a protein and lipid-rich germ layer and a starchy endosperm (Bluhm and Woloshuk, 2005). Starch within the maize kernel is composed of linear and branched polymers, respectively called amylose and amylopectin (Manners, 1989). *Fusarium verticillioides* growth, conidiation and mycotoxin production was assessed in response to different carbon sources, such as amylose, amylopectin, starch and maltose on culture medium (Achimón *et al.*, 2019). Low lag phases were noted when amylose, amylopectin and starch were used as a carbon source (Achimón *et al.*, 2019). Furthermore, amylose and maltose stimulated fungal growth and conidiation (Achimón *et al.*, 2019). Previous studies have implicated amylopectin in the production of FB_1 *in vitro* (Bluhm and Woloshuk, 2005; Falavigna *et al.*, 2013) and *in planta* (Picot *et al.*, 2011). Disruption of the *AMY1* gene, a gene regulating starch metabolism, in *F. verticillioides* created an α-amylase-deficient mutant that produce low levels of FB_1 in maize kernels, which demonstrates the potential role of amylopectin in fumonisin production (Bluhm and Woloshuk, 2005). Yet, van Zyl (2015) found no significant correlation between total starch and fumonisin production. Instead, van Zyl (2015) observed a significant positive correlation between the sugar levels of the maize kernels and fungal target DNA, and a negative correlation between sugar content and fumonisin production. In *F. oxysporum*, sugar molecules activate the H+-ATPase proton pump located in fungal cell membranes to cause extracellular acidification (Brandao *et al.*, 1992). This may provide a potential theory for kernel acidification and how it could be linked to sugar metabolism in the maize kernel by the fungus (Brandao *et al.*, 1992).

Warfield and Gilchrist (1999) and Picot *et al.* (2011) previously reported that fumonisin production was highest at the dent stage of kernel development. The dent stage is characterised by a high amylopectin content and low kernel pH. However, when maize ears were inoculated 30 days after flowering, similar levels of fungal growth and fumonisins were found at physiological and biological stages of kernel development (van Zyl, 2015). The timing of infection may, therefore, play a crucial role in fumonisin contamination of maize kernels, rather than the kernel developmental stage (Warfield and Gilchrist, 1999; Picot *et al.*, 2011; van Zyl, 2015). More than one inoculation should be performed in future trials to elucidate the role of timing of infection on fumonisin contamination.

Carbon and nitrogen are important for the growth of *F. verticillioides* and for fumonisin production (Jiménez *et al.*, 2003). The fungus hydrolyses sugar and starch molecules to obtain carbon for growth and metabolism (Kim *et al*., 2011). The ratio of carbon and nitrogen (C/N) may also be very important for the colonisation of kernels with *F. verticillioides*. Links (2019) found that the pH and C/N were negatively correlated with fungal target DNA and fumonisin production in physiologically mature maize kernels. Prior *in vitro* studies also demonstrate that the C/N ratio regulates both fumonisin production as well as fungal biomass *in vitro* (Kim and Woloshuk, 2008). These studies show that the growth of *F verticillioides* is negatively associated with a high pH and high C/N ration during the early stages of kernel maturation, while acidic conditions and a lower C/N have been linked to an increase in fumonisin production (Kim and Woloshuk, 2008). The expression of the nitrogen metabolism regulator gene, *AREA*, has also been linked to fumonisin biosynthesis (Kim and Woloshuk, 2008). Activation of this gene takes place under nitrogen-limiting conditions, thereby allowing the pathogen to make use of alternative nitrogen sources (Kim and Woloshuk, 2008).

The fatty acid content of maize hybrids has been associated with free fumonisin and hidden fumonisin accumulation (Dall'Asta *et al.*, 2012, 2015). This process may involve oxylipins, which are oxidised forms of lipids, more specifically polyunsaturated fatty acids (PUFAs), produced by both plants and pathogens as signalling metabolites (Battilani *et al.*, 2018). Oxylipins have been found to regulate a variety of processes such as growth and development of fungi, as well as spore production and mycotoxin biosynthesis (Battilani *et al.*, 2018). Previous studies showed that oxylipins in plants and fungi control the interaction between the pathogen and host plant by acting as defence or virulence response modifiers (Battilani *et al.*, 2018). Giorni *et al*. (2015) evaluated the relationship between sphingolipids and oxylipins in maize after infection with *F. verticillioides* at different stages of development and observed an increase in fungal growth with a reduced level of oxylipins. This confirmed a close association between the lipid profile of the maize kernel and fumonisin accumulation. Yet, it is unclear whether the plant lipid profile is changed as a result of fumonisin production, or whether the lipid profile itself modulates fumonisin production (Giorni *et al.*, 2015).

Phenolic compounds are secondary metabolites with antifungal properties against *F. verticillioides* and that can reduce fumonisin levels *in vitro* (Shaw *et al.*, 1990). They are produced via the shikimate pathway to produce aromatic amino acids by the plant in response to pathogen attack. Maize genotypes with high levels of phenolic acids called phenylpropanoids in the maize pericarp was previously linked to lower disease severity and fumonisin contamination by *F. verticillioides* (Sampietro *et al.*, 2013). A more recent study, however, showed no correlation between the level of free, bound or total phenolics in maize kernels and FER symptoms, fungal or fumonisin contamination (Links *et al.*, 2020).

Molecular defence

The defence mechanism of plants to pathogens consists of a double-layer system comprising of two signalling pathways (Katagiri and Tsuda, 2010; Hou *et al.*, 2011; Thomma *et al.*, 2011; Lanubile *et al.*, 2017). The first signalling pathway utilises pattern recognition receptors (PRRs) that detect pathogen/molecular associated molecular patterns (PAMPs/MAMPs) that triggers PAMP/MAMP-triggered immunity (PTI/MTI) (Lanubile *et al.*, 2017). This signalling pathway recognises conserved microbial molecules (Lanubile *et al.*, 2017) that contributes to general microbial fitness (Thomma *et al.*, 2011). The second pathway comprises of resistance gene (*R*-gene) receptors that recognise pathogen effector molecules, resulting in effector-triggered immunity (ETI). These effector molecules are species, strain and race specific, and can be in the form of proteins and secondary metabolites such as fumonisins (Sánchez-Rangel *et al.*, 2012). Thus, effector molecules serve as an attempt at overcoming PTI/ MTI (Thomma *et al.*, 2011; Lanubile *et al.*, 2017).

The maize plant responds to infection by *F. verticillioides* through the up- or down-regulation of genes involved in plant defence (Lanubile *et al.*, 2010, 2017; Links *et al.*, 2020). These genes are called pathogenesis-related (*PR*) genes that result in the activation of *PR*-gene transcripts (*PR* proteins, detoxification enzymes and ß-glucosidases). Lanubile *et al.* (2010) demonstrated that *PR*-genes were up-regulated in resistant maize lines before infection and provided a form of basal defence against the fungus, whereas susceptible maize responded only after infection, from a basal level, to *F. verticillioides* (Lanubile *et al.*, 2010). Resistant lines can, therefore, be seen as being primed with the ability to respond to infection sooner and are thus more resistant. The production of these *PR*-related gene transcripts, however, works in conjunction with a range of other plant defence mechanisms, such as those previously mentioned, to limit pathogen infection within the maize plant. Resistance on a genetic, phenotypic and physico-chemical level should be investigated in locally adapted maize varieties in order to gain a better understanding of factors that contribute to resistance to FER and/or fumonisins in maize.

CONCLUSION

FER of maize, caused by *F. verticillioides*, is an economically important fungal disease in South Africa. The fungus causes a reduction in grain quality and yield and produces toxic secondary metabolites such as fumonisins with irreversible health effects in humans and animals. The planting of resistant maize plants is the most environmentally friendly and cost-effective means of disease management globally. However, factors governing resistance to *F. verticillioides* on a physico-chemical and structural level are insufficiently studied and forms the basis of this thesis.

To better understand how *F. verticillioides* interacts with the plant at different stages of kernel development, **Chapter 2** determined whether fumonisin production by *F. verticillioides* is dependent on kernel maturation. Changes in moisture content, pH, carbon, nitrogen, starch and fatty acids were thus studied in four well-characterised, locally-adapted maize inbred lines inoculated at 7 and 35 days after pollination in a single trial on independent sets of maize plants. This will allow us to establish whether fumonisin production is associated with a specific stage in kernel developmental, or if it is dependent on the time of infection. The hypothesis that maize inbred lines resistant / partially resistant to free fumonisins may have higher levels of hidden fumonisins compared to free fumonisins will also be investigated. Physico-chemical properties will be quantified and correlated to the degree of visual fungal colonisation, free fumonisin contamination, *F. verticillioides* target DNA and the level of hidden and free fumonisins. The findings of this study will provide greater insight into how the kernel micro-environment influence fumonisin deposition by evaluating maize lines with contrasting response to the *F. verticillioides*.

REFERENCES

Achimón, F., Dambolena, J.S., Zygadlo, J.A. and Pizzolitto, R.P. 2019. Carbon sources as factors affecting the secondary metabolism of the maize pathogen *Fusarium verticillioides*. LWT-Food Science and Technology 115: 1–7.

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

Afolabi, C.G., Bandyopadhyay, R., Leslie, J.F. and Ekpo, E.J.A. 2006. Effect of sorting on incidence and occurrence of fumonisins and *Fusarium verticillioides* on maize from Nigeria. Journal of Food Protection 69: 2019–2023.

Alakonya, A.E., Monda, E.O. and Ajanga, S. 2008. Management of *Fusarium verticillioides* root infection court in maize using organic soil amendments. Plant Pathology 5: 161–170.

Atkinson, N.J. and Urwin, P.E. 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. Journal of Experimental Botany 63: 3523–3544.

Bacon, C.W., Glenn, A.E. and Yates, I.E. 2008. *Fusarium verticillioides*: managing the endophytic association with maize for reduced fumonisins accumulation. Toxin Reviews 27: 411–446.

Bakan, B., Melcion, D., Richard-Molard, D. and 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.

Battilani, P., Lanubile, A., Scala, V., Reverberi, M., Gregori, R., Falavigna, C., Dall'Asta, C., Park, Y., Bennet, J., Boreg, E.J. and Kolomiets, M.V. 2018. Oxylipins from both pathogen and host antagonize jasmonic acid-mediated defence via the 9-lipoxygenase pathway in *Fusarium verticillioides* infection of maize. Molecular Plant Pathology 19: 2162–2176.

Bennet, R.N. and Wallsgrove, R.M. 1994. Secondary metabolites in plant defence mechanisms. New Phytologist 127: 617–633.

Bennetzen, J. and Hake, S. 2009. Handbook of Maize: It's Biology. Springer Science, New York, 577 pp.

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S. De, Haesaert, G., Karlovsky, P., Oswald, I.P., Seefelder, W., Speijers, G. and Joerg, S. 2013. Masked mycotoxins: A review. Molecular Nutrition and Food Research 57: 165–186.

Berthiller, F., Maragos, C.M. and Dall'Asta, C. 2016. Introduction to masked mycotoxins. Pages 1-13 in: Masked mycotoxins in food: Formation, Occurrence and Toxicological

Relevance (Berthiller, F and Dall'Asta, C). Royal Society of Chemistry, London, United Kingdom.

Beukes, I. 2015. Pathogenicity and mycotoxin production of the *Fusarium graminearum* species complex in South African grains. MSc Agric thesis, University of Stellenbosch, Stellenbosch, South Africa, 66 pp.

Bevivino, A., Sarrocco, S., Dalmastri, C., Tabacchioni, S., Cantale, C. and Chiarini, L. 1998. Characterization of a free-living maize-rhizosphere population of *Burkholderia cepacia*: Effect of Seed Treatment on Disease Suppression and Growth Promotion of Maize. Federation of European Microbiological Societies: Microbiology Ecology 27: 225–237.

Bezuidenhout, C.S., Gelderblom, W., Gorst-Allman, C.P., Horak, M.R., Marasas, W.F., Spiteller, G. and Vleggaar, R. 1988. Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. Journal of Chemical Society, Chemical communications 1730: 743–745.

Blacutt, A.A., Gold, S.E., Voss, K.A., Gao, M. and Glenn, A.E. 2017. *Fusarium verticillioides*: advancements in understanding the toxicity, virulence, and niche adaptations of a model mycotoxigenic pathogen of maize. Phytopathology 108: 312–326.

Blandino, M. and Reyneri, A. 2008. Effect of maize hybrid maturity and grain hardness on fumonisin and zearalenone contamination. Italian Journal of Agronomy 3: 107–117.

Blandino, M., Reyneri, A. and Vanara, F. 2008a. Effect of plant density on toxigenic fungal infection and mycotoxin contamination of maize kernels. Field Crops Research 106: 234–241.

Blandino, M., Reyneri, A. and Vanara, F. 2008b. Influence of nitrogen fertilization on mycotoxin contamination of maize kernels. Crop Protection 27: 222–230.

Bluhm, B.H. and Woloshuk, C.P. 2005. Amylopectin induces fumonisin B1 production by *Fusarium verticillioides* during colonization of maize kernels. Molecular Plant-Microbe Interactions 18: 1333–1339.

Borlaug, N. 2007. Feeding a hungry world. Science 318: 359. Online publication https://science.sciencemag.org/content/318/5849/359/tab-pdf (20 November 2020).

Brandao, R., Castro, I.M., Passos, J.B., Nicoli, J.R. and Thevelein, J.M. 1992. Glucose-induced activation of the plasma membrane H+-ATPase in *Fusarium oxysporum*. Journal of General Microbiology 138: 1579–1586.

Braun, M.S. and Wink, M. 2018. Exposure, occurrence, and chemistry of fumonisins and their cryptic derivatives. Comprehensive Reviews in Food Science and Food Safety 17: 769–791.

Brown, D.W., Butchko, R.A.E., Busman, M. and Proctor, R.H. 2007. The *Fusarium verticillioides FUM* gene cluster encodes a Zn (II) 2Cys6 protein that affects *FUM* gene expression and fumonisin production. Eukaryotic Cell 6: 1210–1218.

Bryla, M., Roszko, M., Szymczyk, K., Renata, J. and Obiedzinski, M.W. 2016. Fumonisins and their masked forms in maize products. Food Control 59: 619–627.

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.

Campos-Bermudez, V.A., Fauguel, C.M., Tronconi, M.A., Casati, P., Presello, D.A. and Andreo, C.S. 2013. Transcriptional and metabolic changes associated to the Infection by *Fusarium verticillioides* in maize inbreds with contrasting ear rot resistance. PLoS ONE 8: 1-10.

Cao, A., Santiago, R., Ramos, Antonio, J., Marin, S., Reid, L.M. and Butron, A. 2013. Environmental factors related to fungal infection and fumonisin accumulation during the development and drying of white maize kernels. International Journal of Food Microbiology 167: 1–25.

Chu, S. and Li, G.1994. Simultaneous occurrence of fumonisin B1 and other mycotoxins in mouldy corn collected from the People's Republic of China in regions with high incidences of oesophageal cancer. Applied and Environmental Microbiology 60: 847–852.

Cirlini, M., Dall'Asta, C. and Galaverna, G. 2012. Hyphenated chromatographic techniques for structural characterization and determination of masked mycotoxins. Journal of Chromatography 1255: 145–152.

Clements, M.J., Maragos, C.M., Pataky, J.K. and White, D.G. 2004. Sources of resistance to fumonisin accumulation in grain and Fusarium ear and kernel rot of corn. Phytopathology 94: 251–260.

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.

Crop Protection Network: Fusarium ear rot of corn. Online publication: https://cropprotectionnetwork.org/resources/articles/diseases/fusarium-ear-rot-of-corn (14 December 2020).

Crop Protection Network: Fusarium stalk rot of corn. Online publication: https://cropprotectionnetwork.org/resources/articles/diseases/fusarium-stalk-rot-of-corn (14 December 2020).

Cruz, A., Marín, P., Magan, N. and González-jaén, M.T. 2014. Combined effects of benomyl and environmental factors on growth and expression of the fumonisin biosynthetic genes *FUM1* and *FUM19* by *Fusarium verticillioides*. International Journal of Food Microbiology 191: 17–23.

Dall'Asta, C. and Battilani, P. 2016. Fumonisins and their modified forms, a matter of concern in future scenario? World Mycotoxin Journal 9: 727–739.

Dall'Asta, C., Falavigna, C., Galaverna, G. and Battilani, P. 2012. Role of maize hybrids and their chemical composition in *Fusarium* infection and fumonisin production. Journal of Agricultural and Food Chemistry 60: 3800–3808.

Dall'Asta, C., Galaverna, G., Aureli, G., Dossena, A. and Marchelli, R. 2008. A LC/MS/MS method for the simultaneous quantification of free and masked fumonisins in maize and maize-based products. World Mycotoxin Journal 1: 237–246.

Dall'Asta, C., Giorni, P., Cirlini, M., Reverberi, M., Gregori, R., Ludovici, M., Camera, E., Fanelli, C., Batillani, P. and Scala, V. 2015. Maize lipids play a pivotal role in the fumonisin accumulation. World Mycotoxin Journal 8: 87–97.

Dall'Asta, C., Mangia, M., Berthiller, F., Molinelli, A., Sulyok, M., Schuhmacher, R., Krska, R., Galaverna, G., Dosenna, A. and Marchelli, R. 2009. Difficulties in fumonisin determination: the issue of hidden fumonisins. Analytical and Bioanalytical chemistry 395: 1335–1345.

Dall'Erta, A., Cirlini, M., Dall'Asta, M., Del Rio, D., Galaverna, G. and Dall'Asta, C. 2013. Masked mycotoxins are efficiently hydrolyzed by human colonic microbiota releasing their aglycones. Chemical Research in Toxicology 26: 305–312.

Degraeve, S., Madege, R.R., Audenaert, K., Kamala, A., Ortiz, J., Kimanya, M., Tiisekwa, B., De Meulenaer, B. and Haesaert, G. 2016. Impact of local pre-harvest management practices in maize on the occurrence of *Fusarium* species and associated mycotoxins in two agro-ecosystems in Tanzania. Food Control 59: 225–233.

Department of Agriculture, Forestry and Fisheries (DAFF). 2017. A Profile of the South African Maize Market Value Chain. Online publication: https://www.nda.agric.za/docs/AMCP/Citrusmvcp2011-12.pdf (14 June 2019).

Desjardins, A.E. and Plattner, R.D. 2000. Fumonisin B1-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., Munkvold, G.P., Plattner, R.D. and Proctor, R.H. 2002. *FUM1* - A gene required for fumonisin biosynthesis but not for maize ear rot and ear infection by *Gibberella moniliformis* in field tests. Molecular Plant-Microbe Interactions 15: 1157–1164.

Ding, J.Q., Wang, X.M., Chander, S., Yan, J.B. and Li, J.S. 2008. QTL mapping of resistance to Fusarium ear rot using a RIL population in maize. Molecular Breeding 22: 395–403.

Dowd, P.F. 2003. Insect management to facilitate preharvest mycotoxin management. Journal of Toxicology 22: 327–350.

Dragich, M. and Nelson, S. 2014. Gibberella and Fusarium ear rots of maize in Hawai'i. Plant Disease 102: 1-8.

Duncan, K.E. and Howard, R.J. 2010. Biology of Maize Kernel Infection by *Fusarium verticillioides*. Molecular Plant-Microbe Interactions 23: 6–16.

Du Plessis, J. 2003. Maize production. Online publication: https://www.arc.agric.za/arc-gci/Fact Sheets Library/Maize Production.pdf (06 June 2019).

Dutton, M. 1996. Fumonisins, mycotoxins of increasing importance: Their nature and their effects. Pharmacology Therapeutics 70: 137–161.

Duvick, J. 2001. Prospects for reducing fumonisin contamination of maize through genetic modification. Environmental Health Perspectives 1091: 337–342.

Edwards, J. 2009. Maize Growth & Development. Online publication: https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0007/516184/Procrop-maize-growth-and-development.pdf (23 November 2020).

Ekwomadu, T.I., Dada, T.A., Nleya, N., Gopane, R., Sulyok, M. and Mwanza, M. 2020. Mycotoxin metabolites in maize from agriculture regions of South Africa. Toxins 12: 1–19.

Eller, M.S., Robertson-Hoyt, L.A., Payne, G.A. and Holland, J.B. 2008. Grain yield and Fusarium ear rot of maize hybrids developed from lines with varying levels of resistance. Maydica 53: 231–237.

Eskola, M., Kos, G., Elliott, C.T., Hajšlová, J., Mayar, S. and Krska, R. 2019. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%. Critical Reviews in Food Science and Nutrition 1–17.

Eubanks, M.W. 2001. The mysterious origin of maize. Economic Botany 55: 492–514.

Eulgem, T. 2005. Regulation of the Arabidopsis defence transcriptome. Trends in Plant Science 10: 71–78.

Falavigna, C., Cirlini, M., Galaverna, G. and Dall'Asta, C. 2012. Masked fumonisins in processed food: Co-occurrence of hidden and bound forms and their stability under digestive conditions. World Mycotoxin Journal 5: 325–334.

Falavigna, C., Lazzaro, I., Galaverna, G., Battilani, P. and Dall'Asta, C. 2013. Fatty acid esters of fumonisins: First evidence of their presence in maize. Food Additives and Contaminants - Part A 30: 1606–1613.

Falavigna, C., Lazzaro, I., Battilani, P., Galaverna, G. and Dall'Asta, C. 2013. Cornmeal and starch influence the dynamic of fumonisin B, A and C production and masking in *Fusarium verticillioides* and *F. proliferatum*. International Journal of Food Microbiology 166: 21–27.

Falavigna, C., Lazzaro, I., Galaverna, G., Dall'Asta, C. and Battilani, P. 2016. Oleoyl and linoleoyl esters of fumonisin B1 are differently produced by *Fusarium verticillioides* on maize and rice-based media. International Journal of Food Microbiology 217: 79–84.

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.

Dealing with Fusarium ear rots. 2017. Farmer's Weekly. Online publication: https://www.farmersweekly.co.za/farm-basics/how-to-crop/dealing-fusarium-ear-rot/ (14 December 2020).

Ferrigo, D., Raiola, A. and Causin, R. 2014. Plant stress and mycotoxin accumulation in maize. Agrochimica 58: 116–127.

Flaherty, J.E., Pirttila, A.M., Bluhm, B.H. and Woloshuk, C.P. 2003. *PAC1*, a pH-regulatory gene from *Fusarium verticillioides*. Applied and Environmental Microbiology 69: 5222–5227.

Flett, B.C., Mclaren, N.W. and Wehner, F.C. 1998. Incidence of ear rot pathogens under alternating corn tillage practices. Plant Disease 82: 781–784.

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

Formenti, S., Magan, N., Pietri, A. and Battilani, P. 2012. *In vitro* impact on growth, fumonisins and aflatoxins production by *Fusarium verticillioides* and *Aspergillus flavus* using anti-fungal compounds and a biological control agent. Phytopathologia Meditterranea 51: 247–256.

Fox, G. and Manley, M. 2009. Hardness methods for testing maize kernels. Journal of Agricultural and Food Chemistry 57: 5647–5657.

Freeman, B.C. and Beattie, G.A. 2008. An overview of plant defences against pathogens and herbivores. The Plant Health instructor 94: 1-12.

Gai, X., Dong, H., Wang, S., Liu, B., Zhang, Z., Li, X. and Gao, Z. 2018. Infection cycle of maize stalk rot and ear rot caused by *Fusarium verticillioides*. PLoS ONE 13: 1–11.

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

Giorni, P., Dall'Asta, C., Reverberi, M., Scala, V., Ludovici, M., Cirlini, M., Galaverna, G., Fanelli, C. and Batillani, P. 2015. Open field study of some *Zea mays* hybrids, lipid compounds and fumonisins accumulation. Toxins 7: 3657–3670.

Glenn, A.E., Hinton, D.M., Yates, I.E. and Bacon, C.W. 2001. Detoxification of corn antimicrobial compounds as the basis for isolating Fusarium verticillioides and some other Fusarium species from corn. Applied and Environmental Microbiology 67: 2973–2981.

GrainSA. 2017. Methods to reduce mycotoxin exposure. Online publication: https://www.grainsa.co.za/methods-to-reduce-mycotoxin-exposure (6 March 2020).

GrainSA. 2018. New season prospects for maize 2018. Online publication: https://www.grainsa.co.za/new-season-prospects-for-maize (21 January 2019).

Gwirtz, J.A. and Garcia-Casal, N.M. 2014. Processing maize flour and corn meal food products. Annals of the New York Academy of Sciences 1312: 66–75.

Hahn, I., Nagl, V., Schwartz-Zimmermann, H.E., Varga, E., Schwarz, C., Slavik, V., Reisinger, N., Malachová, A., Cirlini, M., Generotti, S., Dall'Asta, C., Krska, R., Moll, W. D. and Berthiller, F. 2015. Effects of orally administered fumonisin B1 (FB1), partially hydrolysed FB1, hydrolysed FB1 and N-(1-deoxy-D-fructos-1-yl) FB1 on the sphingolipid metabolism in rats. Food and Chemical Toxicology 76: 11–18.

Hanway, J.J. 1966. How a corn plant develops. Online publication: https://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1045&context=specialreports (3 February 2019).

Harrison, L.R., Colvin, B.M., Greene, 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.

Haschek, W., Motelin, G., Ness, D., Harlin, K., Hall, W., Vesonder, R., Peterson, R., and Beasley, V. 1992. Characterization of fumonisin toxicity in orally and intravenously dosed swine. Mycopathologia 117: 83–96.

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.

Headrick, J.M., Pataky, J.K. and Juvik, J.A. 1990. Relationships among carbohydrate content of kernels, condition of silks after pollination, and the response of sweet corn inbred lines to Infection of kernels by *Fusarium moniliforme*. Phytopathology 80: 487-494.

Hinton, D.M. and Bacon, C.W. 1995. *Enterobacter cloacae* is an endophytic symbiont of corn. Mycopathologia 129: 117–125.

Hoenisch, R., and Davis, R. 1994. Relationship between kernel pericarp thickness and susceptibility to Fusarium ear rot in field corn. Plant Disease 78: 517–519.

Hou, S., Yang, Y., Wu, D. and Zhang, C. 2011. Evolutionary insights from PBS1, Pto and RIN4 plant immunity. Plant Signaling and Behavior 6: 794–799.

Howard, R.J. 1997. Breaching the outer barriers: Cuticle and Cell Wall Penetration. Pages 43-60 in: The Mycota V, Part A. Plant Relationships (G.C. Carroll and P. Tudzynski, eds.). Springer-Verlag, Berlin, Germany.

Humpf, H.U. and Voss, K.A. 2004. Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins. Molecular Nutrition and Food Research 48: 255–269.

The international Institute of Tropical Agriculture (IITA). 2018. Maize. Online publication: https://www.iita.org/cropsnew/maize/#:~:text=Maize%20production%20in%20Africa%20was,2%20tons%2Fhectare%2Fyear (28 August 2019).

Integrated Taxonomic Information System (ITIS). 2019. Online publication: https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=42269#null (24 June 2019).

Janse van Rensburg, B. 2012. Modelling the incidence of *Fusarium* and *Aspergillus* toxin producing species in maize and sorghum in South Africa. PhD dissertation, University of the Free State, Bloemfontein, South Africa, 148 pp.

Jiménez, M., Mateo, J.J., Hinojo, M.J. and Mateo, R. 2003. Sugars and amino acids as factors affecting the synthesis of fumonisins in liquid cultures by isolates of the *Gibberella fujikuroi* complex. International Journal of Food Microbiology 89: 185–193.

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 859–863.

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

Kachroo, A. and Kachroo, P. 2009. Fatty acid-derived signals in plant. Annual Reviews of Phytopathology 47:153-76.

Kamle, M., Mahato, D.K., Devi, S., Lee, K.E., Kang, S.G. and Kumar, P. 2019. Fumonisins: Impact on agriculture, food, and human health and their management strategies. Toxins 11: 328-351.

Kapuya, T. and Sihlobo, W. 2013. South Africa's maize exports: A Strategic Export Market Analysis Model Approach. Online publication: https://agbiz.co.za/uploads/documents/trade-intelligence/publications/14_05_20_maize_exports_article.pdf (03 November 2019).

Katagiri, F. and Tsuda, K. 2010. Understanding the plant immune system. Molecular Plant-Microbe Interactions 3: 1531–1536.

Keller, S.E., Sullivan, T.M. and Chirtel, S. 1997. Factors affecting the growth of *Fusarium proliferatum* and the production of fumonisin B1: Oxygen and pH. Journal of Industrial Microbiology and Biotechnology 19: 305–309.

Keyser, Z., Vismer, H.F., Klaasen, J.A., Snijman, P.W. and Marasas, W.F. 1999. The antifungal effect of fumonisin B1 on *Fusarium* and other fungal species. South African Journal of Science 95: 455–458.

Kim, H., Smith, J.E., Ridenour, J.B., Woloshuk, C.P. and Bluhm, B.H. 2011. HXK1 regulates carbon catabolism, sporulation, fumonisin B1 production and pathogenesis in *Fusarium verticillioides*. Microbiology 157: 2658-2669.

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.

Kim, E.K., Scott, P.M. and Lau, B.P.Y. 2003. Hidden fumonisin in corn flakes. Food additives and contaminants 20: 161–169.

Knutsen, H.K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L., Nebbia, C. S., Petersen, A., Rose, M., Roudot, A. C., Schwerdtle, T., Vleminckx, C., Vollmer, G., Wallace, H., Dall'Asta, C., Eriksen, G. S., Taranu, I., Altieri, A., Roldán-Torres R. and Oswald, I.P. 2018. Risks for animal health related to the presence of fumonisins, their modified forms and hidden forms in feed. European Food and Safety Authority Journal 16: 1-75.

Koehler, B. 1942. Natural mode of entrance of fungi into Corn ears and some symptoms that indicate infection. Journal of Agricultural Research 64: 421-442.

Lanubile, A., Luca, P. and Adriano, M. 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.

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.

Lazzaro, I., Falavigna, C., Dall'Asta, C., Proctor, R.H., Galaverna, G. and Battilani, P. 2012. Fumonisins B, A and C profile and masking in *Fusarium verticillioides* strains on fumonisin-inducing and maize-based media. International Journal of Food Microbiology 159: 93–100.

Li, S., Myung, K., Guse, D., Donkin, B., Proctor, R.H., Grayburn, W.S. and Calvo, A.M. 2006. *FvVE1* regulates filamentous growth, the ratio of microconidia to macroconidia and cell wall formation in *Fusarium verticillioides*. Molecular Microbiology 62: 1418–1432.

Li, Z.M., Ding, J.Q., Wang, R.X., Chen, J.F., Sun, X.D., Chen, W., Song, W. Bin, Dong, H.F., Dai, X., Xia, Z. L. and Wu, J. Y. 2011. A new QTL for resistance to Fusarium ear rot in maize. Journal of Applied Genetics 52: 403–406.

Links, S. 2019. Phenotypic, physico-chemical and genetic responses of maize associated with resistance to *Fusarium Verticillioides*. MSc Agric thesis, University of Stellenbosch, Stellenbosch, South Africa, 76 pp.

Links, S., van Zyl, K., Cassiem, A., Flett, B.C., Viljoen, A. and Rose, L.J. 2020. The association of maize characteristics with resistance to *Fusarium verticillioides* and fumonisin accumulation in commercial maize cultivars. World Mycotoxin Journal 13:1–14.

Mabuza, L.M., Janse van Rensburg, B., Flett, B.C. and Rose, L.J. 2018. Accumulation of toxigenic *Fusarium* species and *Stenocarpella maydis* in maize grain grown under different cropping systems. European Journal of Plant Pathology 152: 297–308.

Magan, N. and Aldred, D. 2007. Post-harvest control strategies minimizing mycotoxins in the food chain. International Journal of Food Microbiology 119: 131–139.

Mangia, M. 2009. Free and hidden fumonisins in maize and gluten-free products. PhD dissertation, University of Parma, Emilia-Romagna, Italy, 171 pp.

Makaula, N.A., Marasas, W.F.O., Venter, F.S., Badenhorst, C.J., Bradshaw, D. and Swanevelder, S. 1996. Oesophageal and other cancer patterns in four selected districts of Transkei, Southern Africa: 1985-1990. African Journal of Health Sciences 3: 11-15.

Mammadov, J., Buyyarapu, R., Guttikonda, S.K., Parliament, K., Abdurakhmonov, I.Y. and Kumpatla, S.P. 2018. Wild relatives of maize, rice, cotton, and soybean: Treasure Troves for Tolerance to Biotic and Abiotic stresses. Frontiers in Plant Science 9: 1–21.

Manners, D.J. 1989. Recent developments in our understanding of amylopectin structure. Carbohydrate Polymers 11: 87–112.

Marasas, W.F.O. 1996. Fumonisins: History, world-wide occurrence and impact. Fumonisins in Food 392: 1988–1989.

Marasas, W.F.O. 2001. Discovery and occurrence of the fumonisins: A historical perspective. Environmental Health Perspectives 109: 239–243.

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

Marasas, W.F.O., Small, I.M., Viljoen, A., Flett, B.C., Stander, M.A. and McLeod, A. 2011. Resistance in maize inbred lines to *Fusarium verticillioides* and fumonisin accumulation in South Africa. Plant Disease 96: 881–888.

Marasas, W.F.O., Wehner, F.C., Janse van Rensburg, S. and Schalkwyk, D.J. van. 1981. Mycoflora of corn produced in human esophageal areas in Transkei, Southern Africa. Postharvest Pathology and Mycotoxins 71:792-796.

Marín, P., Ory, A. De, Cruz, A., Magan, N. and González-jaén, M.T. 2013. Potential effects of environmental conditions on the efficiency of the antifungal tebuconazole controlling *Fusarium verticillioides* and *Fusarium proliferatum* growth rate and fumonisin biosynthesis. International Journal of Food Microbiology 165: 251–258.

Marın, S., Magan, N., Bellı, N., Ramos, A.., Canela, R. and Sanchis, V. 1999. Two-dimensional profiles of fumonisin B1 production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modelling toxin formation in maize grain. International Journal of Food Microbiology 51: 159–167.

Marocco, A., Gavazzi, C., Pietri, A. and Vincenzo, T. 2007. On fumonisin incidence in monoculture maize under no-till, conventional tillage and two nitrogen fertilisation levels. Journal of the Science of Food and Agriculture 1243: 1237–1243.

Maschietto, V., Marocco, A., Malachova, A. and Lanubile, A. 2015. Resistance to *Fusarium verticillioides* and fumonisin accumulation in maize inbred lines involves an earlier and enhanced expression of lipoxygenase (*LOX*) genes. Journal of Plant Physiology 188: 9–18.

Matumba, L., Van Poucke, C., Njumbe Ediage, E., Jacobs, B. and De Saeger, S. 2015. Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. Food Additives and Contaminants - Part A 32: 960–969.

McCormick. 2020. All the latest data on maize production around the world. Online publication: https://www.mccormick.it/za/all-the-latest-data-on-maize-production-around-the-world/#:~:text=Maize%20is%20one%20of%20the,other%20substances%20for%20industrial%20uses (23 November 2020).

Mcpherson, J. and Stroebel, L. 2017. Pula Imvula, GrainSA. Online publication: https://www.grainsa.co.za/upload/article_pdfs/Pula-September-English.pdf (05 June 2019).

Merrill, A.H., Liotta, D.C. and Riley, R.T. 1996. Fumonisins: fungal toxins that shed light on sphingolipid function. Trends in Cell Biology 6: 218–223.

Miller, J.D. 2001. Factors that affect the occurrence of fumonisin. Environmental health perspectives 109: 321–324.

Missmer, S.A., Suarez, L., Felkner, M., Wang, E., Jr, A.H.M., Rothman, K.J. and Hendricks, K.A. 2006. Exposure to fumonisins and the occurrence of neural tube defects along the Texas – Mexico Border. Environmental Health Perspectives 114: 237–241.

Motley, T.J., Zerega, N. and Cross, H. 2006. Maize origins, domestication and selection. Pages 67-90 in: Darwin's Harvest: New approaches to the origins, evolution, and conservation of crops (Motley, T. J., Zerega, N. and Cross, H). Columbia University Press, New York.

Mrema, G.C. 2011. Grain crop drying, handling and storage. Pages 363-386 in: Rural Structures in the Tropics: Design and Development (Mrema, C., Gumbe, L.O., Chepete, H.J. and Agullo, J.O). FAO, Rome, Italy.

Mukanga, M., Derera, J., Tongoona, P. and Laing, M.D. 2011. Farmers' perceptions and management of maize ear rots and their implications for breeding for resistance. African Journal of Agricultural Research 6: 4544–4554.

Munkvold, G. 2000. Genetically modified, insect resistant maize: Implications for ear and stalk diseases. Plant Health Progress 1: 1-10.

Munkvold, G.P. 2003a. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. European Journal of Plant Pathology 109: 705–713.

Munkvold, G.P. 2003b. 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 Showers, W.B. 1997. Reduced fusarium ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. Phytopathology 87: 1071–1077.

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

Musser, S.M. and Plattner, R.D. 1997. Fumonisin Composition in Cultures of *Fusarium moniliforme*, *Fusarium proliferatum*, and *Fusarium nygami*. Journal of Agricultural and Food Chemistry 45: 1169–1173.

Nankam, C. and Pataky, J.K. 1996. Resistance to kernel infection by *Fusarium moniliforme* in the sweet corn inbred IL125b. Plant Disease 80: 593–598.

Nayaka, S.C., Udaya Shankar, A.C., Reddy, M.S., Niranjana, S.R., Prakash, H.S., Shetty, H.S. and Mortensen, C.N. 2008. Control of *Fusarium verticillioides*, cause of ear rot of maize, by *Pseudomonas fluorescens*. Pest Management Science 65: 769–775.

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 233–252.

Netshifhefhe, N.E.I., Flett, B.C., Viljoen, A. and Rose, L.J. 2018. Inheritance and genotype by environment analyses of resistance to *Fusarium verticillioides* and fumonisin contamination in maize F1 hybrids. Euphytica 214: 1–20.

Nickerson, N. 1945. Morphological Analysis of the Maize Ear. American Journal of Botany 41: 87–92.

Nielsen, B. 2010. Tassel Emergence & Pollen Shed (Agronomy Deptartment, Purdue University, West Lafayette). Online publication: https://www.agry.purdue.edu/ext/corn/news/timeless/Tassels.pdf (3 July 2019).

Nielsen, B. 2013. Grain filling stages in corn (Agronomy Deptartment, Purdue University, West Lafayette). Online publication: https://www.agry.purdue.edu/ext/corn/news/articles.04/grainfill-0705.pdf (21 November 2020).

Oerke, E.C. and Dehne, H.W. 2004. Safeguarding production - Losses in major crops and the role of crop protection. Crop Protection 23: 275–285.

Okoth, S., Rose, L.J., Ouko, A., Beukes, I., Sila, H., Mouton, M., Flett, B.C., Makumbi, D. and Viljoen, A. 2017. Field evaluation of resistance to aflatoxin accumulation in maize inbred lines in Kenya and South Africa. Journal of Crop Improvement 31: 862–878.

Ooka, J.J. and Kommedahl, T. 1977. Wind and rain dispersal of *Fusarium moniliforme* in corn fields. Phytopathology 67: 1023–1026.

Pal, K.K. and Gardener, B.M. 2006. Biological control of plant pathogens. The Plant Health Instructor 2: 1–25.

Pacific North West Pest Management Handbooks: Corn (*Zea mays*)-Stalk Rots. Online publication: https://pnwhandbooks.org/plantdisease/host-disease/corn-zea-mays-stalk-rots (14 December 2020).

Parsons, M.W. and Munkvold, G.P. 2010. Associations of planting date, drought stress, and insects with Fusarium ear rot and fumonisin B1contamination in California maize. Food Additives and Contaminants - Part A 27: 591–607.

Parsons, M.W. and Munkvold, G.P. 2012. Effects of planting date and environmental factors on Fusarium ear rot symptoms and fumonisin B1 accumulation in maize grown in six North American locations. Plant Pathology 61: 1130–1142.

Pascale, M., Visconti, A. and Chelkowski, J. 2002. Ear rot susceptibility and mycotoxin contamination of maize hybrids inoculated with *Fusarium* species under field conditions. European Journal of Plant Pathology 108: 645–651.

Pereira, P., Ibáñez, S.G., Agostini, E. and Etcheverry, M. 2011. Effects of maize inoculation with *Fusarium verticillioides* and with two bacterial biocontrol agents on seedlings growth and antioxidative enzymatic activities. Applied Soil Ecology 51: 52–59.

Pérez-Brito, D., Jeffers, D., González-de-León, D., Khairallah, M., Cortés-Cruz, M., Velázquez-Cardelas, G., Azpíroz-Rivero, S. and Srinivasan, G. 2001. QTL mapping of *Fusarium moniliforme* ear rot resistance in highland maize, Mexico. Agrocencia 35: 181–196.

Picot, A., Barreau, C., Pinson-Gadais, L., Caron, D., Lannou, C. and Richard-Forget, F. 2010. Factors of the Fusarium verticillioides -maize environment modulating fumonisin production. Critical Reviews in Microbiology 36: 221–231.

Picot, A., Barreau, C., Pinson-Gadais, L., Piraux, F., Caron, D., Lannou, C. and Richard-Forget, F. 2011.The dent stage of maize kernels is the most conducive for fumonisin

biosynthesis under field conditions. Applied and Environmental Microbiology 77: 8382–8390.

Pietri, A., Mazzoni, E., Battilani, P., Giorni, P. and Scandolara, A. 2011. Field control of Fusarium ear rot, *Ostrinia nubilalis* (Hübner), and fumonisins in maize kernels. Pest Management Science 67: 458–465.

Pozzi, C.R., Corrêa, B., Xavier, J.G., Direito, G.M., Orsi, R.B. and Matarazzo, S. V. 2000. Effects of prolonged oral administration of fumonisin B1 and aflatoxin B1 in rats. Mycopathologia 151: 21–27.

Presello, D.A., Botta, G., Iglesias, J. and Eyhérabide, G.H. 2008. Effect of disease severity on yield and grain fumonisin concentration of maize hybrids inoculated with *Fusarium verticillioides*. Crop Protection 27: 572–576.

Proctor, R.H., Brown, D.W., Plattner, R.D. and Desjardins, A.E. 2003. Co-expression of 15 contiguous genes delineates a fumonisin biosynthetic gene cluster in *Gibberella moniliformis*. Fungal Genetics and Biology 38: 237–249.

Proctor, R.H., Plattner, R.D., Desjardins, A.E., Busman, M. and Butchko, R.A.E. 2006. Fumonisin production in the maize pathogen *Fusarium verticillioides*: Genetic basis of naturally occurring chemical variation. Journal of Agricultural and Food Chemistry 54: 2424–2430.

Ranum, P., Pablo Peña-Rosas, J. and Garcia-Casal, M.N. 2014. Global maize production, utilization, and consumption. Annals of the New York Academy of Sciences 1312: 105–112.

Reid, L.M. and Sinha, R.C. 1998. Maize maturity and the development of Gibberella ear rot symptoms and deoxynivalenol after inoculation. European Journal of Plant Pathology 104: 147–154.

Reid, L.M., Nicol, R.W., Quellet, 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.

Reid, L.M., Woldemariam, T., Zhu, X., Stewart, D.W. and Schaafsma, A.W. 2002. Effect of inoculation time and point of entry on disease severity in *Fusarium graminearum*, *Fusarium verticillioides*, or *Fusarium subglutinans* inoculated maize ears. Canadian Journal of Plant Pathology 24: 162–167.

Rheeder, J.P., Marasas, W.F.O., Thiel, P.G., Sydenham, E.W., Shephard, G.S. and Van Schalkwyk, D.J. 1992. *Fusarium moniliforme* and fumonisins in corn in relation to human oesophageal cancer in Transkei. Postharvest Pathology and Mycotoxins 82: 353–357.

Rheeder, J.P., Marasas, W.F.O. and Vismer, H.F. 2002. Production of fumonisin analogs by *Fusarium* species. Applied and Environmental Microbiology 68: 2101–2105.

Robertson-Hoyt, L.A., Jines, M.P., Balint-Kurti, P.J., Kleinschmidt, C.E., White, D.G., Payne, G.A., Maragos, C.M., Molnár, T.L. and Holland, J.B. 2006. QTL mapping for fusarium ear rot and fumonisin contamination resistance in two maize populations. Crop Science 46: 1734–1743.

Robledo-Robledo, E. 1991. Fungi and mycotoxins in stored products: Proceeding of an international conference held at Bangkok, Thailand, 23-26 April 1991.

Rocha, L.O., Barroso, V.M., Andrade, L.J., Pereira, G.H.A., Ferreira-Castro, F.L., Duarte, A.P., Michelotto, M.D. and Correa, B. 2016. *FUM* gene expression profile and fumonisin production by *Fusarium verticillioides* inoculated in *Bt* and non-*Bt* maize. Frontiers in Microbiology 6: 1–10.

Rose, L.J. 2016. Investigating maize inbred line response following infection by the mycotoxigenic fungus *Fusarium verticillioides*. PhD dissertation, University of Stellenbosch, Stellenbosch, South Africa, 210 pp.

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

Rossi, V., Scandolara, A. and Battilani, P. 2009. Effect of environmental conditions on spore production by *Fusarium verticillioides*, the causal agent of maize ear rot. European Journal of Plant Pathology 123: 159–169.

Rychlik, M., Humpf, H.U., Marko, D., Dänicke, S., Mally, A., Berthiller, F., Klaffke, H. and Lorenz, N. 2014. Proposal of a comprehensive definition of modified and other forms of mycotoxins including “masked” mycotoxins. Mycotoxin Research 30: 197–205.

Sihlobo, W. 2017. SA agricultural economy now showing signs of recovery (06 June 2017). Agbiz Research. Online publication: https://agriorbit.com/wp-content/uploads/2017/06/SA-agricultural-economy-now-showing-signs-of-recovery.pdf (20 November 2020).

South African Agency for Science and Technology Advancement (SAASTA). 2014. GMOs in South African Agriculture. Online publication: https://www.pub.ac.za/wp-content/uploads/2015/06/Factsheet-Pub-GMOPRINT.pdf (November 2020).

South African Grain Information services (SAGIS). 2019. Maize imports and exports during the 2018 / 2019 marketing season. Online publication: https://sagl.co.za/wp-content/uploads/Reports/Maize/2017.2018/Page-99.pdf (7 September 2019).

Samapundo, S., Devliehgere, F., Meulenaer, B.D.E. and Debevere, J. 2005. Effect of water activity and temperature on growth and the relationship between fumonisin production

and the radial growth of *Fusarium verticillioides* and *Fusarium proliferatum* on corn 68: 1054–1059.

Sampietro, D.A., Vattuone, M.A., Presello, D.A., Fauguel, C.M. and Catalán, C.A.N. 2009. The pericarp and its surface wax layer in maize kernels as resistance factors to fumonisin accumulation by *Fusarium verticillioides*. Crop Protection 28: 196–200.

Sampietro, D.A., Marín, P., Iglesias, J., Presello, D.A., Vattuone, M.A., Catalan, C.A.N. and Gonzalez Jaen, M.T. 2010. A molecular based strategy for rapid diagnosis of toxigenic *Fusarium* species associated to cereal grains from Argentina. Fungal Biology 114: 74–81.

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

Sánchez-Rangel, D., Sánchez-Nieto, S. and Plasencia, J. 2012. Fumonisin B1, a toxin produced by *Fusarium verticillioides*, modulates maize β-1,3-glucanase activities involved in defence response. Planta 235: 965–978.

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

Schmale, D.G. and Munkvold, G.P.2009. Mycotoxins in crops: A Threat to Human and Domestic Animal Health. The Plant Health Instructor 3: 340-353.

Schmidt-Heydt, M., Magan, N. and Geisen, R. 2008. Stress induction of mycotoxin biosynthesis genes by abiotic factors. Federation of European Microbiological Societies Microbiology Letters 284: 142–149.

Scott, P.M. 2012. Recent research on fumonisins: A Review. Food Additives and Contaminants - Part A 29: 242–248.

Seefelder, W., Knecht, A. and Humpf, H.U. 2003. Bound fumonisin B1: Analysis of fumonisin-B1 glyco and amino acid conjugates by liquid chromatography-electrospray ionization-tandem mass spectrometry. Journal of Agricultural and Food Chemistry 51: 5567–5573.

Segal, I., Reinach, S.G. and De Beer, M. 1988. Factors associated with oesophageal cancer in Soweto, South Africa. British Journal of Cancer 58: 681–686.

Shaw, N.M., Bolwell, G.P. and Smith, C. 1990. Wound-induced phenylalanine ammonia-lyase in potato (*Solanum tuberosum*) tuber discs. Significance of glycosylation and immunolocalization of enzyme subunits. Biochemical Journal 267: 163–170.

Shelby, R.A., White, D.G. and Bauske, E.M. 1994. Differential fumonisin production in maize hybrids. Plant Disease 78: 582–584.

Shephard, G.S., Burger, H.M., Rheeder, J.P., Alberts, J.F. and Gelderblom, W.C.A. 2019. The effectiveness of regulatory maximum levels for fumonisin mycotoxins in commercial and subsistence maize crops in South Africa. Food Control 97: 77–80.

Shetty, N.P., Mehrabi, R., Lütken, H., Haldrup, A., Kema, G.H., Collinge, D.B. and Jørgensen, H.J.L. 2006. Role of hydrogen peroxide during the interaction between the hemibiotrophic fungal pathogen *Septoria tritici* and wheat. New Phytologist 174: 637–647.

Skoglund, L. and Brown, W.M. 1988. Effects of tillage regimes and herbicides on *Fusarium* species associated with corn stalk rot. Canadian Journal of Plant Pathology 10: 332–338.

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. Diseases of Corn: Corn and Corn Improvement 18: 687–766.

Smith, J.E., Lay, J.O. and Bluhm, B.H. 2012. Metabolic fingerprinting reveals a new genetic linkage between ambient pH and metabolites associated with desiccation tolerance in *Fusarium verticillioides*. Metabolomics 8: 376–385.

Statista, 2019. Global corn production 2019/2020, by country. Online publication: https://www.statista.com/statistics/254292/global-corn-production-by-country/ (24 June 2019).

Stroshine, R.L., Kirleis, A.W., Tuite, J.F., Bauman, L.F. and Emam, A. 1986. Differences in grain quality among selected corn hybrids. Cereal Foods World 31: 311–316.

Suleiman, R.A., Rosentrater, K.A. and Bern, C.J. 2013. Effects of deterioration parameters on storage of maize: A Review. American Society of Agricultural and Biological Engineers 3: 147-165.

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

Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E. and Mittler, R. 2014. Abiotic and biotic stress combinations. New Phytologist 203: 32–43.

Thomma, B.P.H.J., Nu, T. and Joosten, M.H.A.J. 2011. Of PAMPs and effectors: The Blurred PTI-ETI Dichotomy. American Society of Plant Biologists 23: 4–15.

Thompson, M. and Raizada, M. 2018. Fungal pathogens of maize gaining free passage along the silk road. Pathogens 7: 81.

van der Westhuizen, L., Shephard, G.S., Rheeder, J.P., Burger, H.M., Gelderblom, W.C.A., Wild, C.P. and Gong, Y.Y. 2011. Optimising sorting and washing of home-grown maize to reduce fumonisin contamination under laboratory-controlled conditions. Food Control 22: 396–400.

van Zyl, K. 2015. Resistance in maize to infection and toxin production. MSc Agric thesis, University of Stellenbosch, Stellenbosch, South Africa, 142 pp.

Voss, K.A., Howard, P.C., Riley, R.T., Sharma, R.P., Bucci, T.J. and Lorentzen, R.J. 2001. Carcinogenicity and mechanism of action of fumonisin B_1: a mycotoxin produced by *Fusarium moniliforme* (= *F. verticillioides*). Cancer Detection and Prevention 26: 1–9.

Waes, J.G., Starr, L., Maddox, J., Aleman, F., Voss, K.A., Wilberding, J. and Riley, R.T. 2005. Maternal fumonisin exposure and risk for neural tube defects: Mechanisms in an *in vivo* mouse model. Birth Defects Research- Part A 73: 487–497.

Wallace, H., Dall'Asta, C., Gutleb, A.C., Humpf, H., Galli, C., Metzler, M., Oswald, I.P., Parent-massin, D., Binaglia, M., Steinkellner, H. and Alexander, J. 2018. Appropriateness to set a group health - based guidance value for fumonisins and their modified forms. European Food and Safety Authority 16: 51-72

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.

Warfield, C.Y. and Gilchrist, D.G. 1999. Influence of kernel age on fumonisin B_1 production in maize by *Fusarium verticillioides*. Applied and Environmental Microbiology 65: 2853–2856.

Weaver, M.A., Abbas, H.K., Brewer, M.J., Pruter, L.S. and Little, N.S. 2017. Integration of biological control and transgenic insect protection for mitigation of mycotoxins in corn. Crop Protection 98: 108–115.

Wicklow, D.T. 1995. The mycology of stored grain: an ecological perspective. Stored Grain Ecosystems 197–249.

Wu, F. and Guclu, H. 2013. Global maize trade and food security: Implications from a social network Model. Risk Analysis 33: 1–18.

Yang, C.J., Samayoa, L.F., Bradbury, P.J., Olukolu, B.A., Xue, W., York, A.M., Tuholski, M.R., Wang, W., et al. 2019. The genetic architecture of teosinte catalyzed and constrained maize domestication. Proceedings of the National Academy of Sciences of the United States of America 116: 5643–5652.

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., Hiett, K.L., Kapczynski, D.R., Smart, W., Glenn, A.E., Hinton, D.M., Bacon, C.W., Meinersmann, R., Liu, S. and Jaworski, A.J. 1999. GUS transformation of the maize fungal endophyte Fusarium moniliforme. Mycological Research 103: 129–136.

Yoshizawa, T., Yamashita, A. and Luo, Y. 1994. Fumonisin occurrence in corn from high- and low-risk areas for human oesophageal cancer in China. Applied and Environmental Microbiology 60: 1626–1629.

Table 1. The reproductive growth stages of the maize kernel.

Kernel development stage	Physical appearance	Days after pollination/silking	Approximate kernel moisture	Approximate nutrient composition
Blister (R2)		10-12	85%	Clear fluid consisting mostly of amino acids with the starch content increasing slowly over time
Milk (R3)		18-20	80%	Kernel is soft and increasing amount of starch with a slow decline in the amount of protein precursors
Dough/early dent (R4)		24-26	70%	Kernel is dough-like with approximately 50% dry matter
Late dent (R5)		31-33	60%	Kernel becomes dent at the crown of the kernel and maximum starch turnover takes place
Physiological maturity (R6)		64-66	35%	Kernel is completely hard with maximum dry matter content
Biological/harvest maturity		<64-66	<35%	Kernels dry to below 35% moisture

Sources : https://extension.entm.purdue.edu/newsletters/pestandcrop/article/grain-fill-stages-in-corn-3/ ; https://site.extension.uga.edu/stephenscoag/2020/04/growing-sweet-corn-in-north-georgia/ ; https://www.agry.purdue.edu/ext/corn/news/timeless/GrainFill.html ; https://www.farms.com/ag-industry-news/amount-u-s-corn-in-the-dough-stage-jumps-by-almost-20-percent-in-one-week-150.aspx and https://www.pioneer.com/CMRoot/International/Australia_Intl/Publications/Corn_Workshop_Book.pdf

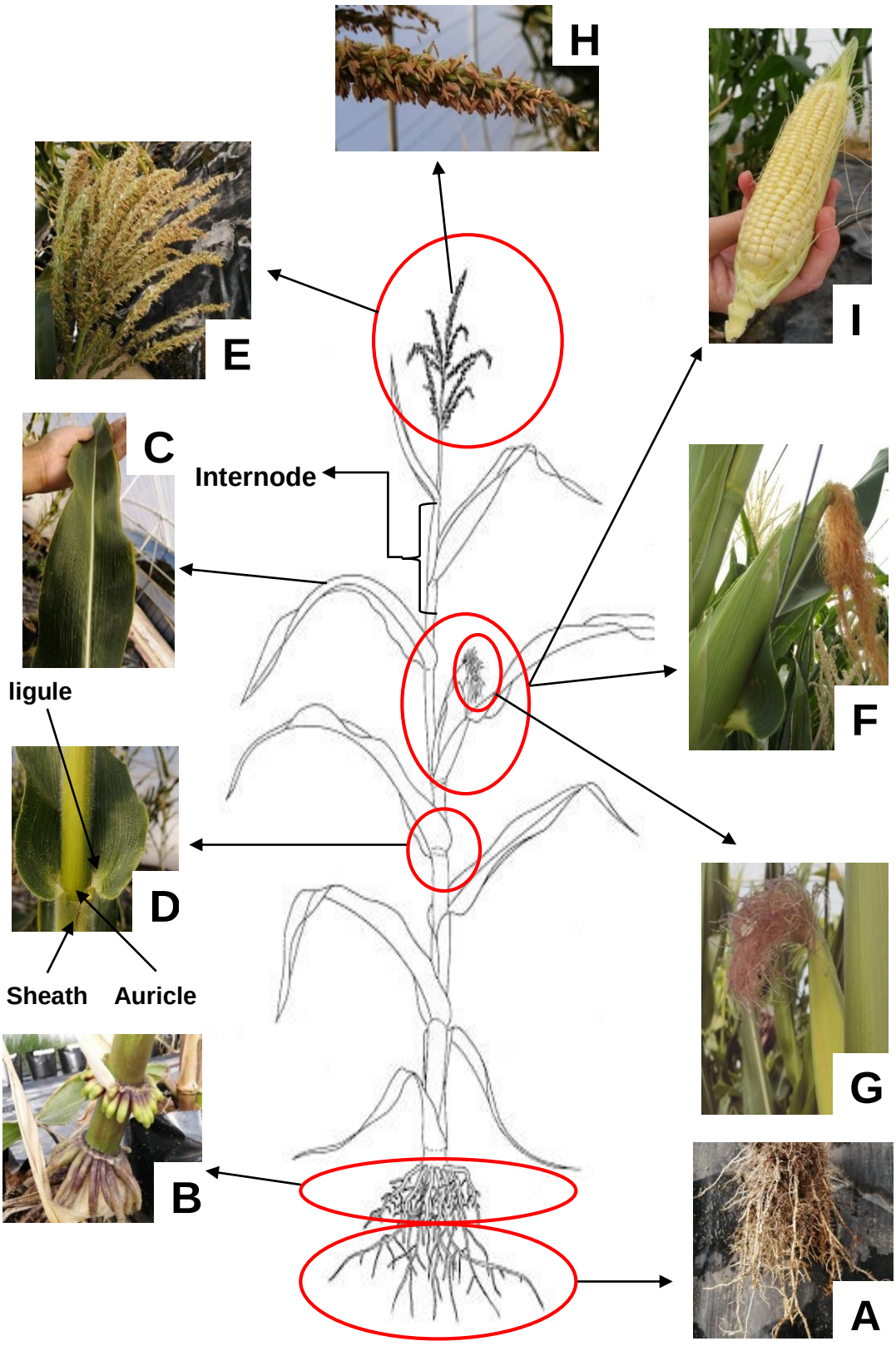

Figure 1. Morphology of the maize plant **A**: Adventitious root system found below the soil surface, **B**: Brace roots that form above the soil, **C**: Leaf blade, **D**: Sheath, ligule and auricle that join the leaf blade to the main stem, **E**: Male inflorescence (tassels), **F**: Female inflorescence (ear), **G**: Silks found at the tip of the ear, **H**: Pollen sacs on the tassels containing pollen, **I**: Maize kernels formed after successful fertilisation (Source: http://www.biology-resources.com/drawing-plant-flower-15-maize-plant.html).

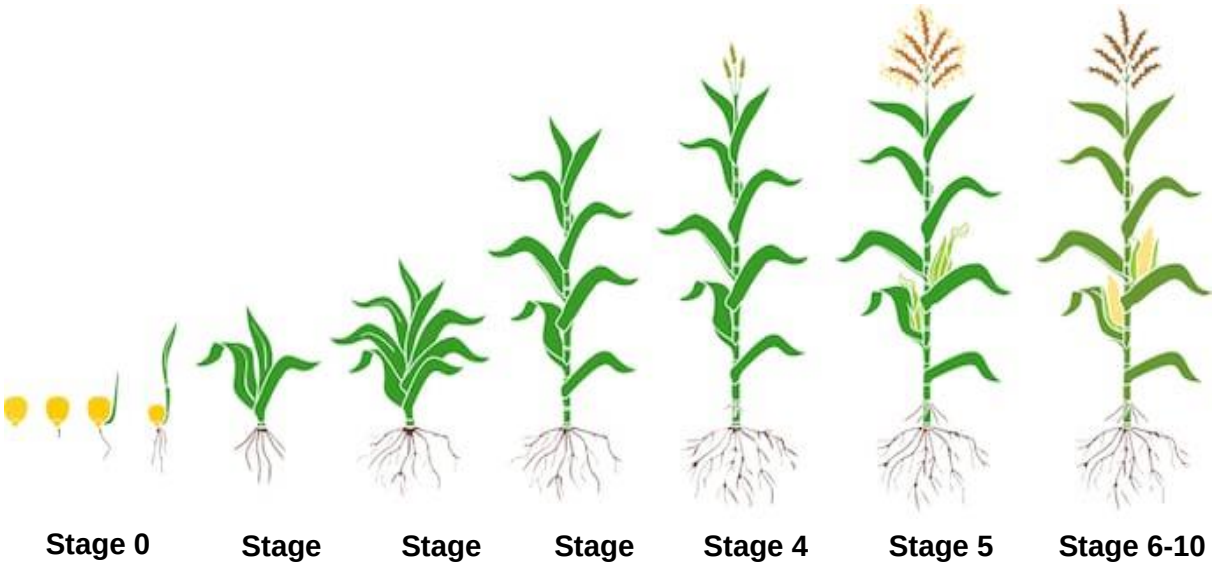

Figure 2. Growth of the maize plant from planting to biological maturity (Source: https://www.shutterstock.com/image-vector/life-cycle-corn-maize-plant-growth-1388972957).

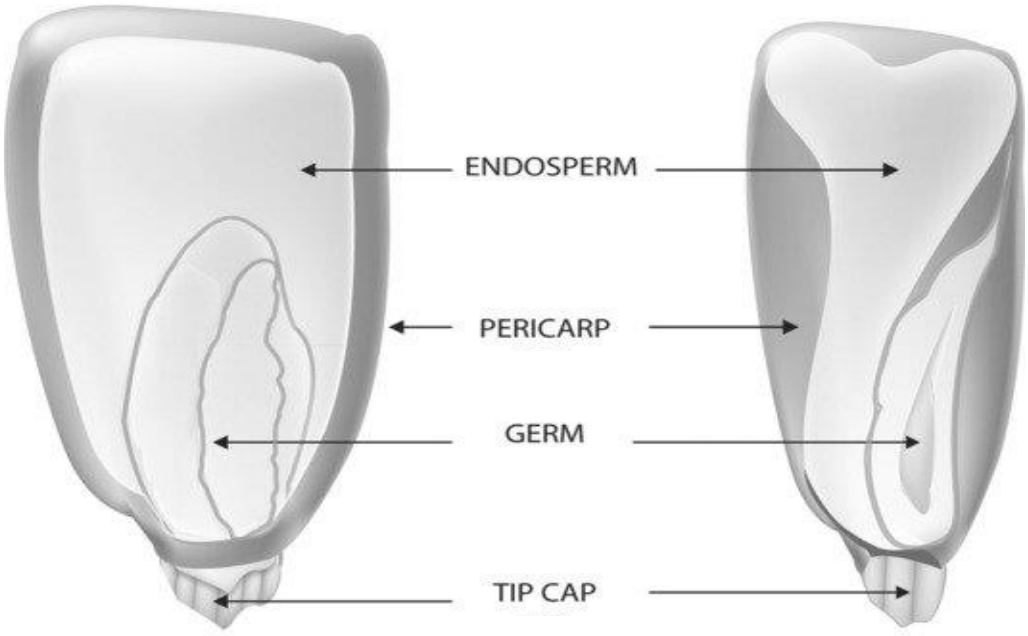

Figure 3. Structure of the maize kernel (Gwirtz and Garcia-Casal, 2014).

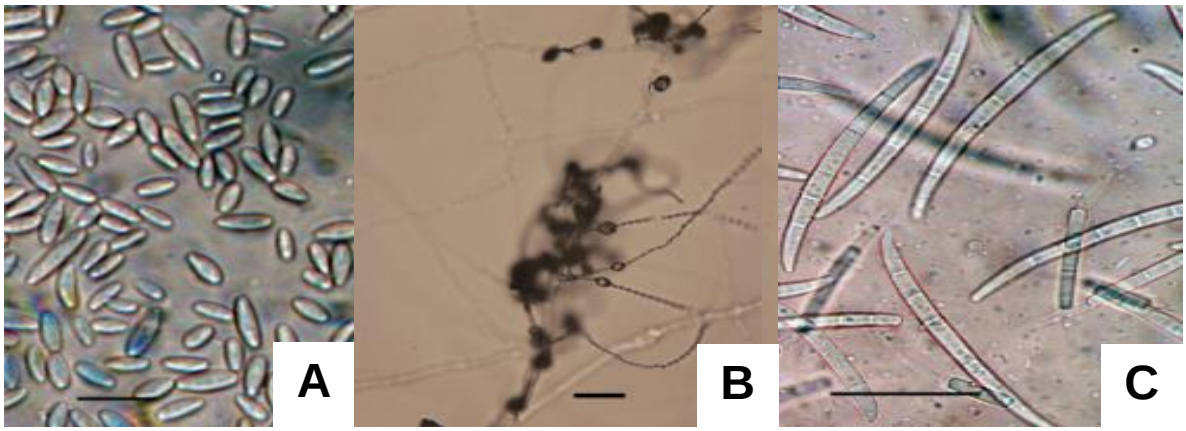

Figure 4. Sexual spores produced by *Fusarium verticillioides*. **A**: Microconidia, **B**: Microconidia in chains, **C**: Macroconidia (Pavlović *et al*., 2016).

Figure 5. Symptoms of *F. verticillioides* infection**. A**: Ear rot with varying degrees of disease severity**, B:** Internal and external stalk rot symptoms in maize caused by *F. verticillioides*.

(Sources: https://cropprotectionnetwork.org/resources/articles/diseases/fusarium-ear-rot-of-corn ; https://pnwhandbooks.org/plantdisease/host-disease/corn-zea-mays-stalk-rots and https://cropprotectionnetwork.org/resources/articles/diseases/fusarium-stalk-rot-of-corn)

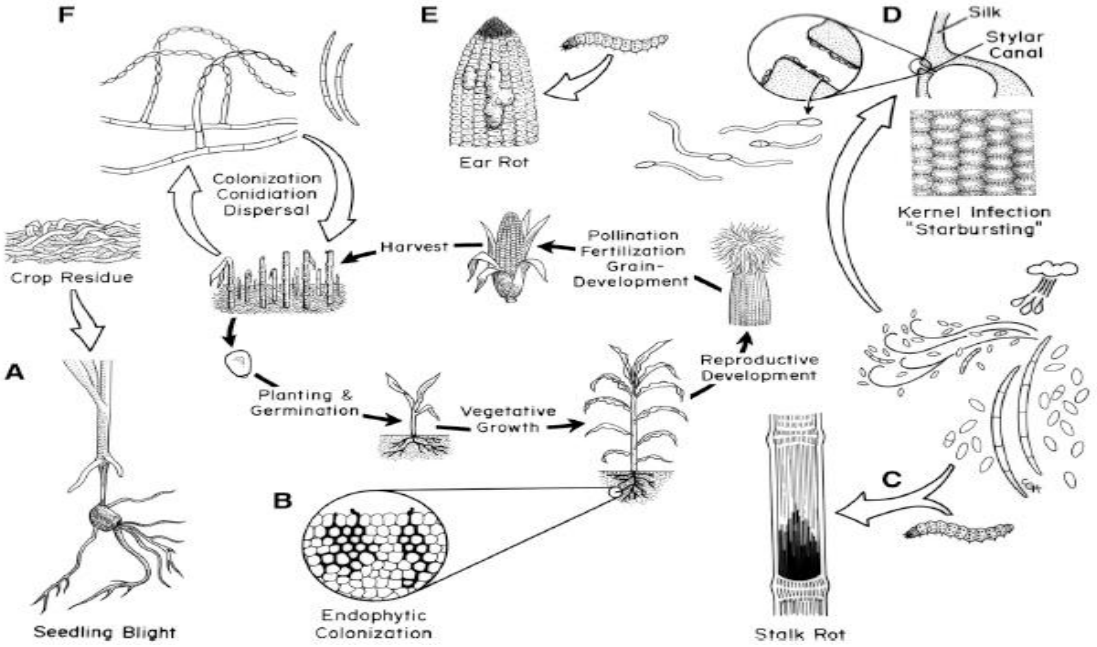

Figure 6. Life cycle of *Fusarium verticillioides*. **A**: Soil residues act as the primary source of inoculum for *F. verticillioides*, **B**: Entry of *F. verticillioides* through the roots of the maize plant causing asymptomatic infection, **C**: Spore dispersal from soil residues onto maize plants by wind, splashing of water and maize feeding insects, **D**: Spore germination and infection by means of the silk channel to cause “starburst symptoms”, **E**: Spores germinate and infect maize ears through wounds caused by insect feeding, **F**: Infected maize kernels can become sources of inoculum giving rise to plants with no visible symptoms (Blacutt *et al*., 2017).

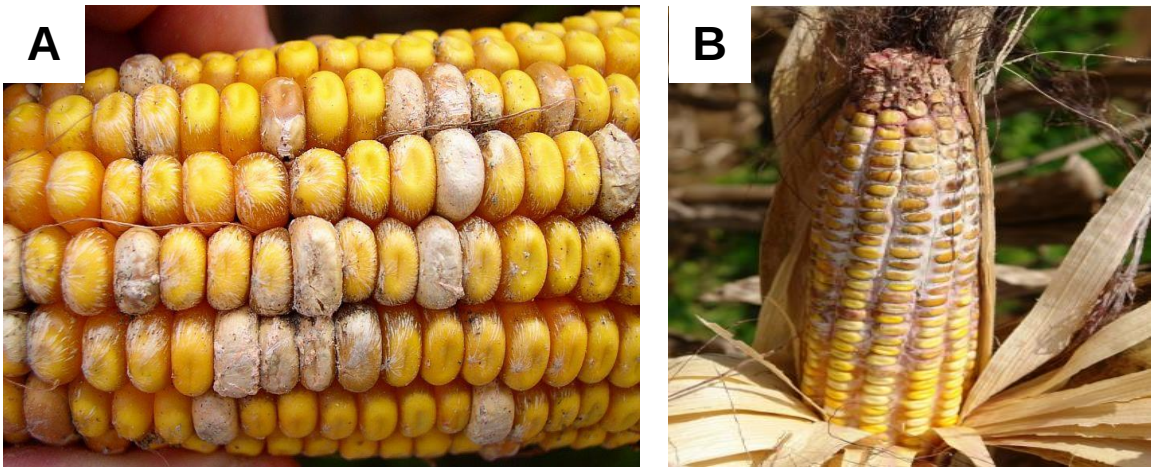

Figure 7. Symptoms and signs of maize kernel infection. **A**: Kernels infected with *Fusarium verticillioides* showing the random discolouration and contamination of kernels, **B**: Kernels infected with *Fusarium graminearum* showing fungal growth at the distal end of the ear (Source: https://www.farmersweekly.co.za/farm-basics/how-to-crop/dealing-fusarium-ear-rot/ and Dragich and Nelson, 2014).

Figure 8. Maize kernels infected with *Fusarium verticillioides*. The white streaks extending radially along the kernel are referred to as “starburst symptoms”.

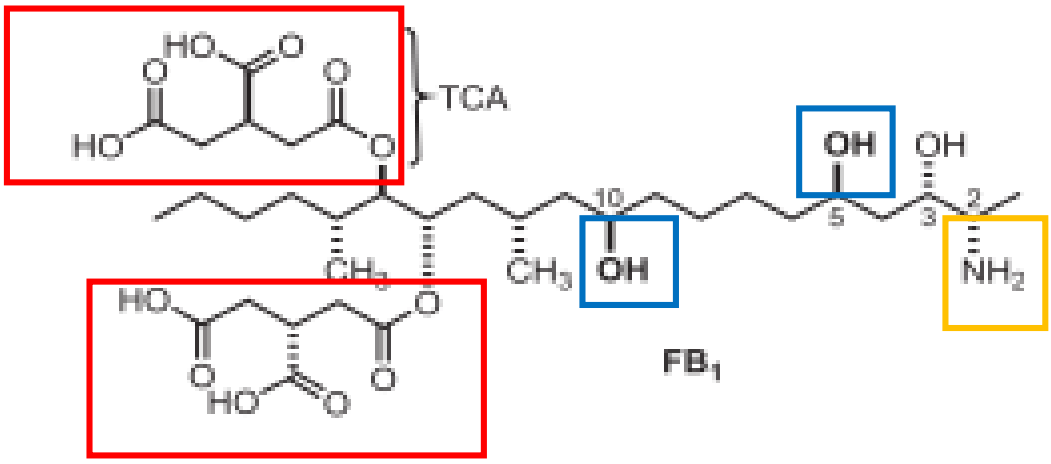

Figure 9. Molecular structure of fumonisin B_1 (FB_1) produced by *Fusarium verticillioides*. The molecule consists of a 20-carbon backbone, an amine group (yellow) at carbon 2 (C^2), two tricarballylic acid side chains (red) at C^{14} and C^{15} and two hydroxyl groups (blue) at C^5 and C^{10} (Wallace *et al*., 2018).

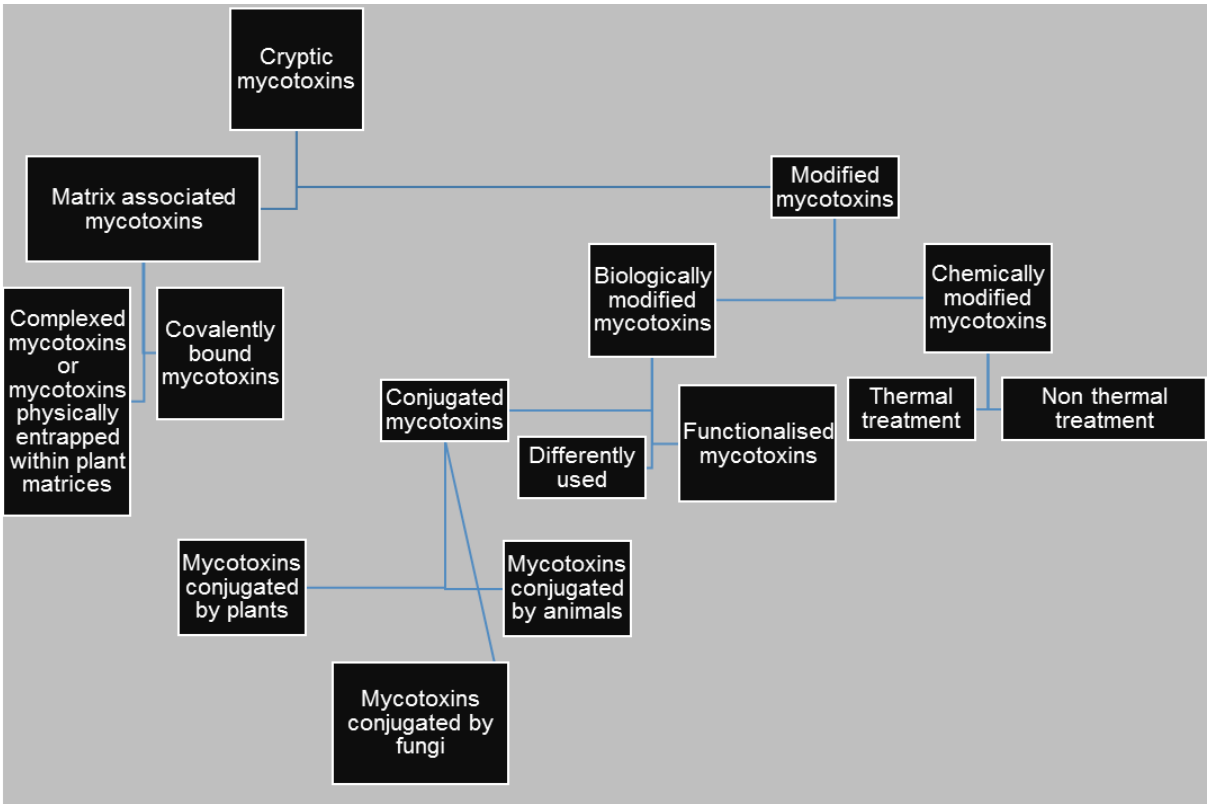

Figure 10. Classification of modified mycotoxins proposed by Rychlick *et al*. (2014) adapted from Braun and Wink (2018).

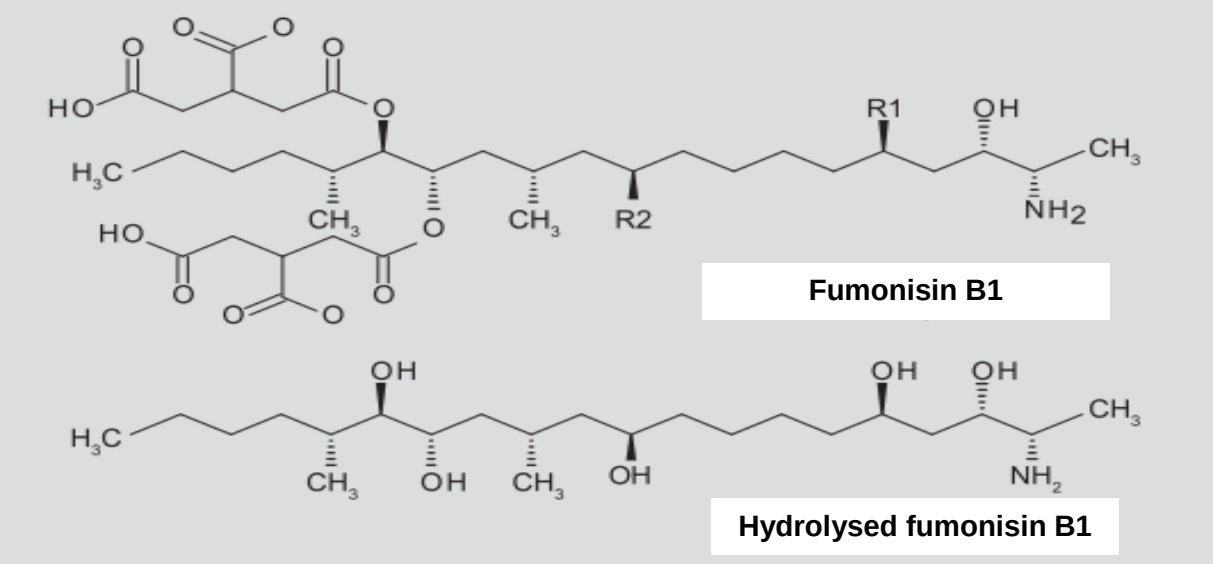

Figure 11. Molecular structure of fumonisin B_1 (FB_1) produced by *Fusarium verticillioides* and hydrolysed fumonisin B_1 (HFB_1) (Dall'Asta and Battilani, 2016).

Figure 12. Varying silk lengths that potentially contribute to resistance or susceptibility to *Fusarium verticillioides* in maize. **A**: Short silks (potentially susceptible), **B**: Long silks (potentially resistant).

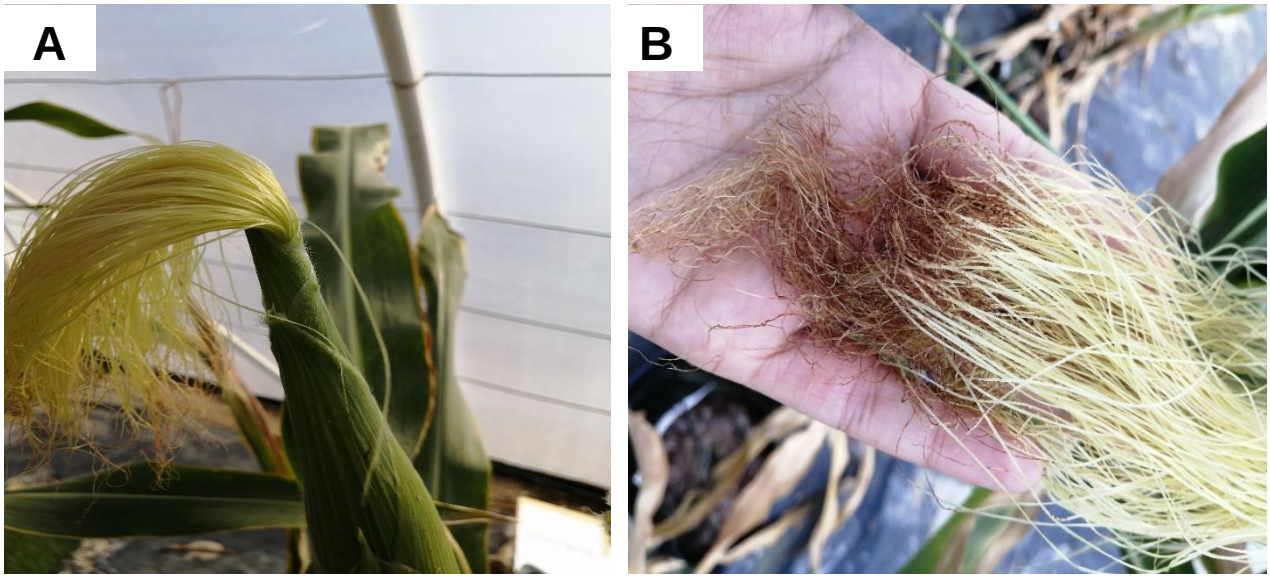

Figure 13. Silk maturity that potentially contribute to resistance or susceptibility to infection by *Fusarium verticillioides* in maize. **A**: Green silks (potentially susceptible), **B**: Brown silks as a result of drying (potentially resistant).

Figure 14. Detachment of silk from the maize ear.

Figure 15. Husk coverage of the maize ear as a potential indicator for resistance or susceptibility to *Fusarium verticillioides*. **A**: closed husks (potentially resistant), **B**: open husks (potentially susceptible).

CHAPTER 2

The relationship between structural and physico-chemical properties of maize and *Fusarium verticillioides* infection during maize kernel maturation.

ABSTRACT

Fusarium verticillioides is a widely distributed fungal pathogen that causes Fusarium ear rot (FER) of maize. The disease reduces grain quality and yield by causing rotting of the kernels. The fungus also produces harmful secondary metabolites known as mycotoxins with fumonisins being the principal mycotoxin contaminant associated with a range of health complications in humans and animals. More recently, fumonisins trapped within the maize kernel, termed hidden fumonisins, have also become a concern as they may contribute to the mycotoxin exposure of humans and animals. Host resistance is the most cost-effective and eco-friendly means of managing this pathogen and its toxins sustainably. In this study, structural characteristics of maize ears and kernel physico-chemical properties associated with resistance were investigated in maize lines with known response to FER and/or fumonisins. Self-pollinated maize plants were artificially inoculated at 7 days after pollination (dap; Inoculation Event 1) and another set of maize plants inoculated at 35 dap (Inoculation Event 2). Maize ears were then harvested at 7, 28, 42 and 52 days after inoculation (dai) for each inoculation event. During the trial structural properties including husk coverage, silk length, silk detachment, and silk browning were recorded. Following harvest, infection indicators (FER disease severity, *F. verticillioides* target DNA and fumonisins) as well as hidden fumonisins were analysed. Physico-chemical traits, such as kernel pH, moisture, total carbon and nitrogen, carbon:nitrogen (C/N), fatty acids and starch, were analysed in maize grain and correlated to infection indicators and hidden fumonisins. The trend in infection indicators were consistent for all lines, increasing progressively and peaking at approximately 52 dai, irrespective of the inoculation event. Maximum fumonisin contamination corresponded to the physiological (Inoculation Event 1) and biological (Inoculation Event 2) kernel maturation stages. Silk browning and physico-chemical factors such as carbon, nitrogen and C/N had a significant positive association with infection indicators at both inoculation events. Kernel moisture had a significant negative association with fungal target DNA while no significant associations were observed for fatty acids, however, the trend in fatty acid fluctuations over time may be indicative of plant response to *F. verticillioides* infection. Amylopectin increased over time in Inoculation Event 1, but was not significantly associated with fumonisin

accumulation. Hidden fumonisins were significantly higher in susceptible lines (I-B and R2565y) yet no physico-chemical properties were significantly associated with it. This work expands on the knowledge surrounding factors that influence *F. verticillioides* infection and fumonisin contamination over time in locally adapted maize lines and will allow breeders to identify and improve resistance on a structural and physico-chemical level.

INTRODUCTION

Maize (*Zea mays* L.) plays a vital role in providing food security to people in Africa and internationally (Ranum *et al.*, 2014; DAFF, 2017; GrainSA, 2018). However, significant losses in maize production occur annually due to biotic and abiotic factors (Savary *et al.*, 2012). Biotic factors include diseases caused by fungi, bacteria and viruses, which often lead to a reduction in grain quality and yield (Oerke and Dehne, 2004; Juroszek and von Tiedemann, 2013; Subedi, 2016). Of the pathogenic fungi, *Fusarium verticillioides* (Saccardo.) Nirenberg is considered one of the most important maize pathogens, as it causes Fusarium ear rot (FER) a disease that results in a rotting of maize kernels.

Fusarium verticillioides is a ubiquitous fungus that survives in plant residues and in the soil (Cotten and Munkvold, 1998). It gains entry into the plant through a multitude of pathways, of which silk infection is considered the most common (Jones *et al*., 1980; Headrick and Pataky, 1991; Munkvold *et al*., 1997). After infection the fungus follows a hemi-biotrophic lifestyle by first obtaining nutrients from living cells as a biotroph, which later becomes necrotrophic when the plant is stressed and conditions favour pathogen growth (Bacon *et al.*, 2008). This is when *F. verticillioides* causes severe kernel rot (Foley, 1962). Infected ears often have a white to light pink mould on the surface of the maize kernels or there may be white streaks radiating from the silk attachment region, commonly known as “starburst symptoms”(Koehler, 1942). The FER fungus typically thrives in warm, dry climates at temperatures of approximately 30˚C (Munkvold, 2003b).

Fusarium verticillioides can also produce harmful secondary metabolites, called mycotoxins, in maize kernels (Gelderblom *et al.*, 1988). The most important mycotoxins produced by *F. verticillioides* are the fumonisins, which have been associated with a variety of adverse health effects in humans and animals. In humans, fumonisins may cause neural tube defects and oesophageal cancers (Marasas *et al.*, 1981; Waes *et al.*, 2005; Missmer *et al.*, 2006), and are known to disrupt sphingolipid metabolism (Wang *et al.*, 1991; Merrill *et al.*, 1996; Voss *et al.*, 2001). In animal it causes mycotoxicosis such as equine leukoencephalomalacia (Marasas *et al.*, 1988) and porcine pulmonary oedema (Harrison *et al.*, 1990). Fumonisins can be found as either free fumonisins, which are the toxin molecules anaylse during standard mycotoxin screening procedures, or as hidden fumonisins, which are fumonisin molecules trapped within the kernel matrices (Berthiller *et al.*, 2016; Dall’Asta and Battilani, 2016; Freire and Sant’Ana, 2018). Hidden fumonisins are not detected using laboratory analyses optimised for free fumonisin molecules and are potentially released into the gastrointestinal environment upon ingestion (Dall’Erta *et al.*, 2013). This implies that the mycotoxin exposure for humans and animals may be far higher than estimated as hidden fumonisins are not included with risk assessment analyses.

Fusarium verticillioides and fumonisins in maize can be partially managed through cultural, chemical and biological means (Munkvold, 2003a; Bacon *et al.*, 2008; Nayaka *et al.*, 2008; Alberts *et al.*, 2016). Cultural practices such as soil tillage and crop rotation aim to reduce soil residues where *F. verticillioides* typically survives (Skoglund and Brown, 1988), however, the pathogen can be seed-borne, in which case these management strategies will no longer be sufficient (Foley, 1962). Early harvesting has also shown to reduce fumonisin accumulation in maize (Bush *et al.*, 2004) as well as controlling maize-feeding insects using chemical prays and transgenic varieties such as *Bt*-maize genetically engineered using the *cry*1A(b) gene from *Bacillus thuriengiensis* that allows for the production of toxic insecticidal compounds upon expression (Bakan *et al.*, 2002). There are also no registered chemicals available on the market to control *F. verticillioides* in the field. Research for potential biological control agents to combat *F. verticillioides* has been conducted in the past, however, most organisms are affected by changes in the environmental conditions and will not be reliable in the field (Nayaka *et al.*, 2008; Formenti *et al.*, 2012). With this said, the most promising strategy to deal with this fungus involves the planting of resistant maize varieties.

In maize resistance to *F. verticillioides* can occur on a structural, physico-chemical and genetic level (Links *et al.*, 2020). Structural traits such as husk coverage, silk length, silk age, kernel hardness and pericarp thickness have been associated with resistance. Ears with closed husks (Warfield and Davis, 1996), longer and dryer silks (Stroshine *et al*., 1986), and harder and thicker pericarps (Sampietro *et al.*, 2009) were found to be more resistant than plants with contrasting traits. These structural traits either prevent the initial entry of the pathogen into the plant or it may slow the rate at fungal colonisation, thus contributing to the overall resistance of the maize plant to *F. verticillioides*. In a recent study, however, Links *et al.* (2020) found no association between visual FER symptoms and/or fumonisin contamination of maize and structural barriers.

Physico-chemical traits have also influenced fungal growth and fumonisin production in maize kernels. These include factors such as kernel moisture content, pH, total carbon and nitrogen, fatty acid and starch content. Some studies have shown that the expression of the *FUM* gene in the fungal pathogen is triggered in response to stressful water limiting conditions (Jurado *et al.*, 2008). The low availability of water during the later stages of kernel development is, therefore, correlated with high levels of fumonisins contamination. Acidic conditions have also been found to favour fumonisin production (Keller *et al.*, 1997; Flaherty *et al.*, 2003) while the total carbon and nitrogen content serves as an essential aspect of fungal growth and toxin synthesis (Kim *et al*., 2011). The ratio of these elements (C/N) may also affect kernel suitability for fungal colonisation *in planta* (Links *et al.*, 2020). Kernel amylopectin content was shown to favour the production of fumonisins *in vitro* (Bluhm and Woloshuk, 2005; Falavigna *et al.*, 2013), with *in planta* studies indicating that the dent stage of kernel development most

conducive for fumonisin production (Warfield and Gilchrist, 1999; Picot *et al.*, 2011). van Zyl (2015) later showed that that the timing of infection, rather than the kernel developmental stage, determines levels of fumonisin contamination.

The contribution of the structural and physico-chemical factors previously mentioned and the role that they may play collectively in the resistance and susceptibility maize to FER and/or fumonisin contamination is rather lacking. The present study, therefore, aims to determine the structural and physico-chemical factors that influence *F. verticillioides* infection and fumonisins at different kernel developmental stages.. Specific objectives of the study include: 1) To determine whether fumonisin production is dependent on compositional changes associated with kernel maturation, 2) To determine whether hidden fumonisins can be used as indicators of potential resistance mechanisms and 3) To correlate fatty acid and starch content with free and hidden fumonisins. The information obtained would assist with the identification of potentially FER and/or fumonisin resistant lines in future and possibly expand the criteria used for the evaluation of resistance.

MATERIALS AND METHODS

Plant material

Four maize inbred lines were planted in a greenhouse at the Welgevallen Research Farm, Stellenbosch University in November 2018 (Trial 1) (Table 1). These included a fumonisin/FER-susceptible line (R2565y), a FER-resistant line (I-B), a fumonisin/FER-resistant line (CML 444) and a FUM-resistant genotype (CB 222). Seeds were planted in 15-L planting bags arranged in a randomised complete block design with three replications per treatment. Positive controls included fungus-inoculated plants, and the negative control included water-inoculated plants for each inbred line with three replications for each treatment. The plants were supplemented by means of the irrigation water twice per day. The irrigation water contained 1.4 kg KNO_3, 1.2 kg K_2SO_4, 1.0 kg MKP, 4.3 kg $CaNO_3$ and 2.2 kg $MgSO_4$ per litre. Once the plants reached maturity, the ears were covered with clear polyethylene bags to avoid cross pollination. The primary ears were manually pollinated by collecting the pollen in brown paper bags from male tassels and placing it onto the silks of the pant from which the pollen was collected. The brown paper bag was left on the pollinated ear and stapled closed until harvest. Only ears that were pollinated 7-10 after maturity were used for subsequent inoculation.

A second trial was planted in November 2019 (Trial 2) using lines CML 444, R2565y and I-B with the addition of FER/FUM-resistant line CML 390 for the evaluation of structural traits only and not for artificial inoculation. Approximately 60 seeds were planted for each line

in 15-L planting bags, and the plants were irrigated and fertilised as previously described. Pollinations were also carried out as described and allowed to dry naturally to biological maturity.

Structural trait assessment

Twenty-five maize plants of each inbred line were used to assess structural traits in both greenhouse trials. Husk coverage was scored as either open or closed at anthesis (flowering) and again at harvest. Silk length was measured only at anthesis from the point of silk emergence at the maize ear tip to the end of the longest silk of each ear and rounded off to the nearest centimetre (cm). Silk browning and silk detachment was recorded as either 'yes' or 'no' for silks 7-10 days after pollination.

Artificial inoculations

The pollinated maize ears of Trial 1 were artificially inoculated with *F. verticillioides* MRC 826 (PROMEC-MRC; Tygerberg, South Africa) (Fig. 1). Inoculations were done by injecting the inoculum into the silk channel, as described by Reid *et al*. (1999), using a 21 gauge (0.8 x 38 mm thin-walled) needle and a 5-mL syringe. The fungus was grown on potato dextrose agar (PDA) (39 g PDA; 1 L deionized autoclaved deionised water (dH_2O)) for approximately 4-5 days at room temperature, after which the hyphae was transferred into 100 mL of Armstrong media in an erlenmeyer flask (Booth, 1971) (Fig. 1). The spore suspension was then incubated at 25˚C on a shaking-incubator at 100 revolutions per minute (rpm) (Fig. 1). After 4-5 days, the suspension was passed through sterile cheesecloth and the resulting filtrate was centrifuged at 3500 rpm at 25˚C for 10 min and the supernatant discarded. The spores were washed twice with 20 mL sterile dH_2O and centrifuged as previously described. The spores were resuspended in autoclaved dH_2O and the spore concentration determined using a haemocytometer. The spore concentration was adjusted to 2 x 10^6 spores mL^{-1} of which 1 mL was used to inoculate the primary ear of each plant (Fig. 1). Autoclaved dH_2O was used to inoculate the control plants. The remaining inoculum after inoculation was plated onto PDA to ensure that the suspension was viable at the time of inoculation.

Artificial inoculation was carried out 7 days after pollination (dap), representing the blister kernel developmental stage (Inoculation Event 1) (Table 2). A second, independent set of plants were inoculated 35 dap, representing the early dent kernel developmental stage (Inoculation Event 2) (Table 2).

Grain sampling and processing

Fungus and water-inoculated maize ears were sampled by hand at 0, 7, 28, 42 and 52 days after inoculation (dai), for each inoculation event (Table 2), and stored at -20˚C. Kernels were

stripped from the ear and ground into flour using an IKA A11 basic analytical mill (IKA Laboratory Technology, Staufen, Germany). The resulting maize flour was transferred to sterile falcon tubes and stored at -80˚C until further analyses were performed.

Infection indicators

Infection indicators are traditional parameters used to assess the response of the maize plant to *F. verticillioides*. These include the rating of FER disease severity on maize ears, standard free fumonisins quantification of FB_1, FB_2 and FB_3, and the molecular quantification of *F. verticillioides* target DNA (Beukes, 2015; Links *et al.*, 2020).

Disease severity

Fusarium ear rot disease severity was rated at harvest as a percentage of the discolouration of the maize ear, with zero indicating no visual symptoms, 50% implying that approximately half of the maize ear was mouldy, and 100% indicating that the entire maize ear was colonised (Fig. 2). The presence of insect feeding damage on the ear was also recorded (Fig. 3).

Free fumonisin extraction and quantification

Free fumonisins (FB_1, FB_2 and FB_3) were extracted according to Rose *et al*. (2016) and Dall'Asta *et al*. (2012; 2015). Five grams of maize flour was suspended in 20 mL methanol/water (70:30 vol/vol) extraction buffer (Millipore, Bedford, MA, USA) and placed in a shaking-incubator (Labcon™, California, USA) at 200 rpm for 30 min at 25˚C. The suspension was then centrifuged at 500 rpm at 4˚C for 10 min. The resulting supernatant was transferred to a micro-centrifuge tube by passing the supernatant through a 0.22 µm regenerated cellulose syringe filter (Bonna-Agela Technologies Inc., Tianjin, China). The extract was allowed to precipitate overnight at 4˚C, and was subsequently centrifuged at 14 000 rpm for 10 min. Three hundred and fifty mL of the extract was transferred to a glass vial and submitted to the Central Analytical Facility (CAF) Mass Spectrometry Unit at Stellenbosch University to be analysed by means of liquid-chromatograph tandem mass-spectrometry (LC-MS/MS). A six-point standard curve was prepared, ranging from 0.05-20.2 mg kg^{-1} for FB_1 and FB_2, and 2.08 mg kg^{-1} for FB_3, using fumonisin standards for analytical analysis prepared as described by Small *et al*.(2012).

Fungal DNA extraction and quantification using quantitative PCR

Total DNA (Plant and fungal) was extracted from 2-g samples of milled maize grain using the DNeasy® Plant Mini Kit (QIAGEN, Hilden, Germany), as described by Boutigny *et al.* (2012). The maize flour was suspended in 5 mL CTAB/PVP extraction buffer (1.4 N NaCl, 0.1 N Tris/EDTA, 1% Polyvinylpyrrolidone (PVP), pH8) and 40 µL proteinase K (10 mg mL^{-1})

(Invitrogen™, USA, California). The suspension was placed in a shaking-incubator set to 65˚C at 200 rpm for 2 hrs. Once the incubation step was completed, the samples were centrifuged at 4 000 rpm set to 25˚C or 10 min. One mL of the resulting supernatant was transferred to a new micro-centrifuge tube and 30 µL RNase (QIAGEN) (10 mg mL^{-1}) was added. Samples were subsequently incubated in a water bath (Memmert, Schwabach, Germany) at 65˚C for 15 min where after they were centrifuged at 14 000 rpm for 10 min. Four hundred µL of the suspension was then transferred to a new micro-centrifuge tube, and the DNA isolation was completed using the DNeasy® Plant Mini kit (QIAGEN) commencing at Step 9 according to the manufacturer's instructions. The quality and quantity of total DNA was then assessed using a ND-1000 NanoDrop Spectrophotometer (Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa). The extracted DNA was diluted to 10 ng μL^{-1} for quantitative PCR (qPCR) and stored at 4˚C.

Fungal DNA was extracted from freeze-dried mycelia of *F. verticillioides* isolate MRC 826 using the DNeasy® Plant Mini kit and the CTAB/PVP lysis method (Boutigny *et al.,* 2012), with additional purification steps as described by (Boutigny *et al.*, 2012). A five-point matrix-matched dilution series was created by diluting *F. verticillioides* DNA with pathogen-free maize DNA extracted from clean maize and diluted to 10 ng uL^{-1}. The standard curve was established by running each dilution in triplicate and including a no template control (NTC). The standard curve was accepted as sufficient once the specifications indicated in the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) Guidelines were met (Bustin *et al.*, 2009). The specifications were a correlation coefficient (R^2) >0.98, a slope (M)-value of between -3.2 and -3.4, and a reaction efficiency (E-value) of 0.98-1.05. The standard curve selected for fungal DNA determination was achieved with the following specifications: R^2=0.99946, M=-3.4, E-value=0.97.

The quantity of *F. verticillioides* DNA in the samples was determined by quantitative PCR (qPCR) using the Fver356 forward and Fver412 reverse primers (Nicolaisen *et al.,* 2009). qPCR reactions for each dilution standard were performed in duplicate in 25-µL reaction volumes on a Rotor-geneTM 6000 (Corbett Life Science, Whitehead Scientific (Pty) Ltd., South Africa) (Boutigny *et al.*, 2012). The total reaction volume consisted of 12.5 µL SensiMix™ SYBR® No-ROX Kit (Bioline, London, UK), 1 µL of each primer (10 µM) and 2 µL of template DNA (10 ng μL^{-1}). Conditions for qPCR were set as follows: 95˚C for 10 min, followed by 35 cycles of 95˚C for 15 sec, 68˚C for 15 sec, 72˚C for 15 sec and a melting curve analysis from 72˚C to 95˚C, rising by 1˚C each step. Quantification of *F. verticillioides* DNA from the total DNA in maize samples was performed by means of qPCR in duplicate for each biological replicate, and a standard included with each run in triplicate at 10 ng μL^{-1}. An NTC was also included and the obtained Ct values of the samples evaluated were converted to DNA concentrations using the Rotor-Gene™ 2.0.2.4 software.

Extraction and quantification of hidden fumonisins

Free hydrolysed fumonisins

Free hydrolysed fumonisin (HFB) concentrations were determined using the same extraction protocol as for free fumonisins. Once extracted, it was also submitted to CAF, Stellenbosch University, to be analysed by means of LC-MS/MS. Hydrolysed FB_1 standards, obtained from Cape Peninsula University of Technology (CPUT), Institute of Biomedical and Microbial Biotechnology (IBMB), were included for analysis ranging from 0.03-616.95 mg kg^{-1}. The purpose of this analysis was to demonstrate the analytical differences between the standard fumonisin extraction method and the extraction with alkaline hydrolysis for the determination of hidden fumonisins.

Quantification of hidden fumonisins

Total hydrolysed fumonisin analysis was carried out as described by Dall'Asta *et al*. (2012; 2015), with slight modifications. Two aliquots containing 1.25 g of maize flour was amended with 25 mL of 2 M KOH and placed in a shaking-incubator at 25˚C for 60 min. Twenty-five mL of acetonitrile was then added to each sample and vortexed for 15 sec. The solution was subsequently centrifuged at 3 500 rpm for 15 min. One mL of the acetonitrile-rich upper layer from each tube of the sample was then combined in a 15-mL falcon tube and evaporated under a liquid nitrogen stream. The residue was re-suspended in water/methanol (30:70 v/v) buffer and filtered through a 0.22-µm cellulose syringe filter and transferred to a 1.5-mL glass vial for analysis by LC-MS/MS at CAF, Stellenbosch University. Hydrolysed FB_1 (HFB_1) standards (CPUT, IBMB) were included for analysis ranging from 0.039-636.92 mg kg^{-1}. Hidden fumonisins were then calculated as the difference between the total hydrolysed fumonisin concentration and only the free FB_1 concentration of a given sample as analytical standards for only HFB_1 were included for analysis due to lack of availability of standards for HFB_2 and HFB_3.

$$Hidden\ fumonisins = \text{Total hydrolysed fumonisins} - \text{Free FB1}$$

Where:

Total hydrolysed fumonisins = Total hydrolysed fumonisins obtained using the extraction with alkaline hydrolysis by Dall'Asta *et al*. (2012; 2015).

Free FB_1 = The free FB_1 concentration obtained using the standard fumonisin extraction method described by Rose *et al*. (2016) and Dall'Asta *et al*. (2012;2015).

Evaluation of physico-chemical properties

pH determination

The pH of the maize grain was determined as described by Links *et al*. (2020). Five mL of dH_2O was added to 500 mg ground maize and mixed thoroughly. The pH of each sample was measured in triplicate with a Jenway 3510 pH meter (Bibby Scientific Limited, Staffordshire, UK).

Moisture content

The moisture content of the grain for each sample was measured using the vacuum oven method (AOAC Official Method 925.09). Empty metal moisture dishes were dried in a conventional oven for 60 min and cooled in a desiccator for a minimum of 30 min before use. Maize flour (2 g) was then placed in the metal moisture dishes with the weight of the dish before (W1) and after (W2) the addition of the sample recorded. The metal dish containing the sample was then placed in a vacuum oven set at 99˚C for a maximum of 3-4 hrs. Once the samples were completely dried, the metal dishes were placed in a desiccator to cool overnight. The weight after drying (W3) was recorded and the percentage moisture content of the sample was calculated as follows:

$$\% \, Moisture = \frac{\mathrm{W2} - \mathrm{W3}}{\mathrm{W2} - \mathrm{W1}} \, X \, 100$$

Where:

W1= Weight of the moisture dish

W2= Weight of the moisture dish and sample before drying

W3=Weight of the moisture dish and sample after drying

Total carbon and nitrogen determination

Total carbon and nitrogen content of the maize flour was assessed by using the Vario EL Cube Elemental Analyzer (Elementar, Germany, Frankfurt) at CAF, Stellenbosch University. Maize flour (5-10 mg) was weighed into aluminium foil weighing boats, with approximately 5 mg tungstin trioxide powder acting as metal scavenger to facilitate optimal combustion. The sample was measured after combustion at 1050˚C within a column filled with tungsten oxide and oxygen at approximately 2 Bar. Gases such as carbon dioxide, water vapour, nitrogen, nitrogen dioxide, nitrogen oxide, sulphur dioxide and sulphur trioxide were formed from the sample and were selectively separated in chromatography tubes, absorbed and quantified. Argon 5.0 was used as the carrier gas at a pressure of approximately 1 Bar, moving the gasses through the reduction tube filled with copper conducting wire at 850˚C. The reduction column

reduces the nitrogen oxides to nitrogen gas and sulphur trioxide to sulphur dioxide. All the volatile halogen bound compounds produced during combustion were bound to the silver wool in the reduction column and the pure gasses were carried to the adsorption columns. The nitrogen was not adsorbed and, therefore, reached the thermal conductivity detector (TCD) first. The carbon dioxide, water vapour and sulphur dioxide were each adsorbed onto the adsorption columns at room temperature for effective separation. The adsorption columns were heated sequentially to desorb the different products and were carried through to the TCD. From this, the percentage (%) carbon and nitrogen were quantified. The carbon to nitrogen ratio was calculated as the % carbon quantified, divided by the % nitrogen.

Fatty acid analysis

Fatty acid profile determination was performed with slight modifications (Miquel and Browse, 1992). Approximately 250 mg of maize flour was weighed into a glass tube, and 1 mL of hexane added together with 25 µL heptadecanoic acid (C17) at 1000 ppm, 1 mL of 20% concentrated sulphuric acid and methanol. The mixture was vortexed and incubated in an oven at 80˚C for 1 hr. After incubation, the mixture was allowed to cool to room temperature and 2 mL of 20% (w/v) NaCl was added. The samples were vortexed again, followed by centrifugation at maximum rpm. The hexane phase above the separation layer (Fig. 4) was then transferred to a vial using a pasteur pipette and analysed by gas-chromatography mass-spectrometry (GC-MS/MS).

Separation was performed on a gas chromatograph (6890N, Agilent Technologies Inc., Palo Alto, CA) linked to an inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent Technologies Inc.). The GC-MS system was connected to a CTC Analytics PAL auto-sampler. Separation of fatty acids was performed on a non-polar ZB-5MS GUARDIAN (30 m, 0.25 mm ID, 0.25 µm film thickness) ZB 7HG-G010-11 capillary column. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The injector temperature was maintained at 250˚C. One µL of the sample was injected in a split ratio set at 5:1. The oven temperature was programmed as follows: 100˚C for 5 min, ramped up to 180˚C at a rate of 5˚C min^{-1} and held for 5 min, and finally ramped up to 330˚C at a rate of 8˚C min^{-1} and held for 5 min. The MSD was operated in a full scan mode, and the source and quad temperatures were maintained at 230˚C and 150˚C, respectively. The transfer line temperature was maintained at 280˚C. The mass spectrometer was operated under electron impact mode at ionization energy of 70 eV, scanning from 35 to 500 m/z.

Starch analysis

Starch analysis was conducted using the amylose/amylopectin kit (K-AMYL 06/18) (Megazyme, Ireland, Bray) according to the manufacturer's recommendations, with slight

modifications. Twenty-five mg of maize flour was weighed in a 2-mL Eppendorf tube for each sample in duplicate. Twenty-five mg of reference sample with a known amylose content was included in duplicate with each set of analyses. The samples were suspended in 1 mL dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Hilden, Germany), and heated in a boiling water bath with intermittent vortexing for approximately 15 min. Once the starch has dispersed completely the solution was allowed to cool at room temperature for 5 min. Two hundred µL of this solution was then transferred to a new 2-mL tube and re-suspended in 400 µL of 95% ethanol, with continuous vortexing. A further 800 µL ethanol was added, mixed by inversion, and allowed to precipitate at room temperature for 15 min. The sample was centrifuged at 2000 rpm for 5 min, and the resulting supernatant discarded. The residual ethanol was removed from the tube by blotting the tubes on sterile tissue paper, and the pellet re-suspended in 400 µL of DMSO followed by boiling of the solution for 15 min, with intermittent vortexing. After boiling, 800 µL Conanavlin A (Con A) buffer was added and the contents of the tube was quantitatively transferred to a 15-mL falcon tube and diluted to 5 mL using the Con A buffer. This final solution was referred to as solution A and used to quantify the total starch and amylose contents of the given maize sample.

Two-hundred µL of solution A was transferred to a 2-mL tube for the determination of amylose and amylopectin. One-hundred µL of the Con A solution was added to each tube and allowed to precipitate at room temperature for 1 hr. The samples were centrifuged at 14 000 rpm or 10 min at room temperature, and 200 µL of the resulting supernatant was transferred to a new 2-mL tube. Six-hundred µL of sodium acetate buffer (100 mM, pH 4.5) was added to each sample, boiled to denature the Con A for 5 min, and allowed to equilibrate in a water bath set to 40˚C. Twenty µL of amyloglucosidase/α-amylase was added and incubated at 40˚C for 30 min, where after the samples were centrifuged at 2000 rpm or 5 min.

For total starch determination, 100 µL of solution A was transferred to a new 2-mL tubes with 800 µL sodium acetate buffer (100 mM, pH 4.5). Twenty µL of amyloglucosidase/α-amylase solution was added to each sample and incubated for 10 min at 40˚C. Fifty µL of the solution was transferred to new 2-mL tube for all samples in duplicate and 200 µL of glucose determining agent added to each sample. These were incubated at 40˚C for 20 min together with the blank and D-glucose control made using the manufacturer's recommendations. Fifty µL of solution was used to determine total starch and amylose by measuring the absorbance of the solution at 510 nm in the FLUOstar OPTIMA Absorbance Microplate Reader (BMG Labtech, Offenburg, Germany) using a 96 well microplate reader plate (Sarstedt Inc., North Carolina, USA) (Fig. 5).

The absorbance values obtained for each sample were then used to calculate the percentage of amylose using the following calculation:

$$\text{Amylose \% (w/w)} = \frac{\text{Absorbance (Con A supernatant)}}{\text{Absorbance (Total starch)}} \text{ x } \frac{6.15}{9.2} \text{ x } \frac{100}{1}$$

$$= \frac{\text{Absorbance (Con A supernatant)}}{\text{Absorbance (Total starch)}} \text{ x } 66.8$$

Where 6.15 and 9.2 are dilution factors for Con A and total starch, respectively.

Amylopectin percentage was calculated as the average of the difference between the percentage total starch and the percentage of amylose for a specific sample. The analysis of the samples was only considered reliable if the amylose percentage of the reference sample was between 66 and 68%. In the event that the reference sample did not meet this requirement, the specific set of analyses was redone entirely to ensure that a reliable result was obtained.

Data analysis

The experimental design was a completely random 4x2x2x4 factorial for the inbred lines analysed in Trial 1 (CB 222, CML 444, IB and R2565Y), inoculation events, treatment (inoculated and control) and dai (7, 28, 42, 52). Each of the 64 treatment combinations were randomly replicated three times where an experimental unit consisted of three maize plants.

Observed variables were subjected to analysis of variance (ANOVA) using SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Since the four-factor interaction was significant ($P < 0.05$) for most variables, it was decided to also do separate ANOVAs for each inoculation event and day after inoculation separately to simplify interpretation. When line by treatment interactions were found to be significant, inoculated and control samples were assessed separately for the respective sampling times. Alternatively, when inbred line by treatment interactions were not significantly different, the inoculated and control samples were assessed in conjunction with one another for the respective sampling times. Shapiro-Wilk test was performed to test for deviation from normality (Shapiro, 1965). Variables that deviated from normality were Ln transformed to stabilize the variance and improve normality (Snedecor, 1980). Fisher's least significant difference (LSD) was calculated at the 5% level to compare means for significant effects (Ott, 1998). A probability level of 5% was considered significant for all significance tests. Pearson's correlation was performed to determine correlations between resistance characteristics and infection indicators. Pearson's correlation coefficients (r) were regarded as noteworthy when >0.60 and P < 0.0001.

Principal component analysis (PCA), employing the correlation matrix, was performed using XLStat (Version 2016, Addinsoft; New York, USA) to elucidate the associations amongst treatment combinations and observed variables. Multifactor analysis and principal component PCA were performed to determine whether significant correlations exist between infection

indicators and physico-chemical properties. Partial least squares (PLS) regression was performed to test correlations between individual factors of infection indicators and physico-chemical properties with one another. Both ln and non-log transformed data was included in these analyses.

The trial design, however, was not sufficient to subject the structural data recorded to ANOVA as it consisted of too few data points (four inbred lines evaluated). However, this data was used to identify trends between structural traits and the known genotype of the maize inbred lines. The structural data for the 2018/19 season could be subjected to multivariate analyses.

RESULTS

Structural traits of inbred lines before and at harvest

During the 2018/19 season, CB 222 (17.8 cm) had the highest silk length followed by R2565y (12.8 cm), CML 444 (10.9 cm) and I-B (9.8 cm) (Table 3). Line CB 222 had 88% closed husks at anthesis and harvest, where all other lines had 100% of maize ears closed at anthesis and harvest. Silk browning occurred on 44% and 28% of R2565y and I-B, respectively. Only R2565y had detached silks, with 16% of ears affected.

During the 2019/20 season, lines CML 444 and CML 390 had a similar silk length of ±13.6 cm followed by I-B (12.0 cm) and R2565y (9.5 cm) (Table 3). All lines had closed husks at both anthesis and harvest. Lines CML 390, I-B and R2565y experienced silk browning of 88%, 16% and 64%, respectively. Silk detachment was observed in 8% of R2565y, but not any of the other lines.

Infection indicators

FER disease severity

For Inoculation Event 1, FER disease severity increased over time for all inbred lines (Table 4 and Fig. 6). Line R2565y developed 10% FER symptoms at S0, while none of the other lines developed symptoms at this sampling time. No significant differences ($P \leq 0.05$) were observed among the inoculated lines at S7. R2565y developed significantly more FER symptoms than the other inbred lines at S28, S42 and S52. At S52, CML 444 had significantly less FER than I-B and R2565y, but not significantly less than CB 222. Only lines CB 222 and I-B had significant differences between the inoculated and control treatments at S52.

For Inoculation Event 2, FER disease severity also increased over time for all inbred lines (Table 4 and Fig. 6). No significant differences ($P \leq 0.05$) in FER development were observed among the lines at S0, S7 and S28. At S52, CML 444 developed significantly less

(*P ≤ 0.05*) FER symptoms than CB 222 and I-B, but not R2565y. Significant differences were observed between inoculated and control ears in lines CB 222 and I-B at S42 and S52.

Fungal target DNA

Fusarium verticillioides DNA levels in maize kernels remained low over time and increased at S42 in Inoculation Event 1 (Table 4 and Fig. 6). Significant differences (*P ≤ 0.05*) were observed between CML 444 (0.002 ng μL^{-1}) and R2565y, which had no fungal DNA at S0. At S7, inoculated maize grain of R2565y (0.000 ng μL^{-1}) contained significantly less fungal DNA compared to the other lines. Only CB 222 showed a significant difference between the inoculated and control material. Line I-B showed significant differences between inoculated and control kernels at S28, S42 and S52, while CB 222 and CML 444 showed significant differences between inoculated and control at S42 and S52, and R2565y at S52. Inoculated I-B had significantly more fungal DNA at S42 than CB 222 and CML 444. I-B had the most fungal DNA at S52, even though it was not significantly more compared to the other lines.

Fusarium verticillioides DNA increased in the kernels of maize lines for Inoculation Event 2 from S28 (Table 4 and Fig. 6). At S0, CML 444 (0.047 ng μL^{-1}) had significantly more (*P ≤ 0.05*) fungal DNA than the other lines. No significant differences were observed between the fungal DNA in the inoculated and control plants in the inbred lines at S7. However, inoculated CML 444 (0.160 ng μL^{-1}) had significantly more fungal DNA compared to all lines at this sampling time. Significant differences between inoculated and control were observed in CB 222 at S28, CML 444 and R2565y at S42 and CML 444 and I-B at S52. The fungal DNA of inoculated R2565y (0.086 ng μL^{-1}) was lowest at S28, but did not differ significantly when compared to the other lines. Inoculated R2565y (0.121 ng μL^{-1}) also contained significantly less fungal DNA than the other lines at S42. I-B had the most fungal DNA at S52, but this value did not differ significantly from the fungal DNA concentration in CB 222, R2565y and CML 444.

Free fumonisin contamination

Fumonisin levels increased over time in Inoculation Event 1, most notably in lines I-B and R2565y (Table 4 and Fig. 6). Fumonisin levels were highest in CML 444 at S0 (22.6 mg kg^{-1}), differing significantly (*P ≤ 0.05*) only from R2565y (0.1 mg kg^{-1}), which had the lowest fumonisin content. Inoculated I-B increased significantly from S7 (0.2 mg kg^{-1}) to S52 (76.1 mg kg^{-1}). Similarly, the fumonisin content of inoculated R2565y also increased significantly from S7 (0 mg kg^{-1}) to S52 (52.2 mg kg^{-1}). Inoculated I-B and R2565y contained significantly more fumonisins when compared to CB 222 and CML 444 at S42 and S52. However, inoculated I-B (76.1 mg kg^{-1}) and R2565y (52.2 mg kg^{-1}) did not differ significantly from each

other at S52. Significant differences were observed between the inoculated and control treatments for line R2565y at S7 as well as lines I-B and R2565y at S42.

For Inoculation Event 2 an increase in fumonisin levels was also observed over time (Table 4 and Fig. 6). No significant differences ($P \leq 0.05$) were found between the inoculated and control treatments in any of the inbred lines at S0, S28, S42 and S52, while I-B and R2565y showed significant differences between inoculated and control treatments at S7. Inoculated I-B (6.3 mg kg^{-1}) and R2565y (4.8 mg kg^{-1}) had significantly more fumonisins at S7 when compared to CB 222 (0.1 mg kg^{-1}) and CML 444 (0.2 mg kg^{-1}). No significant differences were observed between inoculated samples at S28 and S42. At S52, CB 222 (19.0 mg kg^{-1}) had the highest level of fumonisins, but the fumonisin content did not significantly differ from the other inbred lines.

Physico-chemical analysis of maize inbred lines

% Moisture

The moisture level in maize kernels decreased over time for both inoculation events (Table 5 and Fig. 7). In Inoculation Event 1, no significant differences ($P \leq 0.05$) were observed in the % moisture in any of the inbred lines at S0, nor were there significant differences between the inoculated and control treatments at S7, S28 and S42 (Table 5 and Fig. 7). Inoculated CML 444 was significantly drier than the other maize inbred lines at S7 (53.4%) and S28 (32.2%), except when compared to line CB 222 (42.2 %) at S28. Inoculated I-B had the lowest % moisture at S42 (28.3%), differing significantly from CB 222 (34.3%) and CML 444 (35.7%), but not from R2565y (30%). At S52, significant differences were observed between the inoculated and control treatments of CB 222 and I-B. Furthermore, the moisture in inoculated CB 222 kernels (19.7%) was significantly less than in CML 444 (31.8%) and R2565y (29.3%) kernels at the same sampling time, but it did not differ significantly from I-B (25.3%).

In Inoculation Event 2 (Table 5 and Fig. 7), CML 444 was significantly drier ($P \leq 0.05$) at S0 than the other maize lines (30.8%). Only CML 444 had significant differences between the inoculated and control treatments at S42 and S52. Inoculated kernels of CML 444 were also drier at S7 (29.5%) than at S42 (10.9%). At S7, inoculated CML 444 kernels were significantly drier than R2565y, and at S42 inoculated CML 444 kernels were significantly drier than all of the other lines. Inoculated I-B had the lowest % moisture at S28 (22.7%), which did not differ significantly from the inbred lines. At S42, inoculated CML 444 kernels (10.9%) had the least moisture, differing significantly from all the other lines. At S52, CB 222 (14.4%) was significantly drier compared to the other maize inbred lines.

Average pH

Maize kernel pH decreased over time in Inoculation Event 1, whereas in Inoculation Event 2 the pH was initially low and increased over time in most inbred lines (Table 5 and Fig. 7). In Inoculation Event 1 (Table 5 and Fig. 7), no significant differences ($P \leq 0.05$) were observed between the pH of the controls at S0, nor were there significant differences between inoculated and control at S7, S42 and S52 in any of the inbred lines. Kernel pH of inoculated R2565y was highest at S7 (pH=6.9) and S28 (pH=6.3), with significant differences between inoculated and control kernels at S28, but not S7. No significant differences were observed between inoculated samples at S42. At S52, inoculated CB 222 (pH=6.3) had a significantly higher pH than CML 444 (pH=6.1) and R2565y (pH=5.9) but did not differ significantly from I-B (pH=6.2).

In inoculation Event 2, no significant differences ($P \leq 0.05$) were observed between the control samples at S0 and the inoculated and control treatments at S42 (Table 5 and Fig. 7). The pH of CB 222 was significantly different between inoculated (pH=5.8) and control (pH=6.1) at S7. At S28, the pH of the CML 444 inoculated grain (pH=5.7) was significantly lower than that of CB 222 (pH=6.2) and I-B (pH=6.0), but not R2565y (pH=5.8). At S52, the pH of the inoculated CB 222 (pH=6.4) was significantly higher than that of CML 444 (pH=5.9), I-B (pH=6.2) and R2565y (pH=6.1).

Carbon %

Carbon % increased over time in both inoculation events (Table 5 and Fig. 7). In Inoculation Event 1, no significant differences ($P \leq 0.05$) were observed at S0 between any of the inbred lines, nor were there significant differences observed between the inoculated and control treatments in the inbred lines at S7, S28, S42 and S52 (Table 5 and Fig. 7). Inoculated CML 444 (26.9%) had a significantly higher carbon % at S7 when compared to CB 222 (16.4%) and R2565y (15.4%). Inoculated CML 444 (33.5%) also had significantly more carbon compared to R2565y (27.5%) at S28. Inoculated I-B (33.4%) had the highest carbon % at S42 but was not significantly different from that of the other inbred lines at this sampling time. At S52, inoculated CB 222 (37.4%) had the highest carbon %, but it did not differ significantly from the other lines at this sampling time.

At Inoculation Event 2, significantly more ($P \leq 0.05$) carbon was measured in R2565y (30.9%) and CML 444 (31.4%) at S0 compared to I-B (27.4%) and CB 222 (25.5%) (Table 5 and Fig. 7). No significant differences were observed between the inoculated and control treatments at S7 and S52. Significant differences between inoculated and control treatments were observed for CB 222 at S28 and R2565y and I-B at S42. The inoculated CML 444 (33.9%) had most carbon at S7, which differed significantly from R2565y (29.6%) with the lowest carbon % at this sampling time. Inoculated CML 444 also had the most carbon at S28 (40.9%), which differed significantly from all the other lines, as well as at S42 (41.9%) where its carbon levels differed significantly from that of I-B (32.8%) and R2565y (36.9%). The carbon

% in inoculated CB 222 grain (42.5%) was the highest at S52 and differed significantly from all other lines at this sampling time.

Nitrogen %

The nitrogen % increased over time for both inoculation events (Table 5; Fig. 7). At S0 in Inoculation Event 1, the nitrogen % of inoculated CML 444 (0.6%) differed significantly ($P \leq 0.05$) only from I-B (1.1%) (Table 5; Fig. 7). No significant differences were observed between inoculated and control at S28 to S52. Significant differences between inoculated and control were observed for CB 222 and I-B at S7. Inoculated CML 444 (1.3%) grain had the highest nitrogen % at S7, differing significantly from all lines at this sampling time. At S28 and S42, inoculated I-B (S28 – 1.7%; S42 – 1.8%) had the highest nitrogen % that differed significantly from CML 444 (1.3%) and R2565y (1.4%) at S28, and from all lines at S42. No significant differences were found between inoculated samples of all lines, or between inoculated and control kernels at S52.

At Inoculation Event 2, no significant differences ($P \leq 0.05$) were observed between the inbred lines at S0 or the inoculated and control kernels at S28 to S52 (Table 5 and Fig. 7). Nitrogen % of the inoculated (1.3%) and control grain (1.6%) differed significantly in line CB 222 at S7, while inoculated CML 444 (1.6%) had the highest nitrogen % at this sampling time, differing significantly from the other inbred lines. Inoculated CB 222 (1.8%) and CML 444 (1.9%) both had significantly more nitrogen than R2565y (1.3%) at S28. At S42, the nitrogen in the inoculated I-B (1.4%) and R2565y (1.5%) lines differed significantly from that in CB 222 (2.0%) and CML 444 (1.9%), but not from one another. At S52, inoculated CB 222 (2.1%) had the highest nitrogen %, differing significantly from the other lines.

C/N

The ratio of carbon to nitrogen (C/N) increased over time in all lines for both inoculation events (Table 5 and Fig. 7). In Inoculation Event 1, no significant differences ($P \leq 0.05$) were observed between the C/N ratio in any of the lines at S0, nor were there significant difference between inoculated and controls at S7 and S42 (Table 5 and Fig. 7). Significant differences between inoculated and control were observed for CML 444 at S28 and R2565y at S52. The C/N ratio was highest in the inoculated I-B line at S7 (20.5%), but it was not significantly higher than in the other lines. Inoculated CML 444 had the highest the C/N ratio at S28 (25.1%), which differed significantly from the other lines. At S42, CB 222 inoculated (22.2%) had the highest C/N ratio, which did not differ significantly from the other lines. Inoculated R2565y (23.9%) and I-B (23.9%) had the highest C/N ration at S52, both differing significantly from CML 444 (20.3%).

In Inoculation Event 2, no significant differences ($P \leq 0.05$) were observed in the C/N ratio between the control samples at S0, nor were there significant differences between the inoculated and control at S28 to S52 (Table 5 and Fig. 7). Inoculated CB 222 had the highest C/N ratio at S7, which was not significantly different from other lines. Line R2565y (27.2%) had the highest C/N ratio at S28 compared to CB 222 (21.6%) and CML 444 (21.6%), but it did not differ significantly from line I-B (23.4%). Similarly, inoculated R2565y (25.0%) had a significantly higher C/N ratio at S42 compared to CB 222 (21.1%) and CML 444 (22.0%), while it did not differ significantly from I-B (24.3%). At S52, inoculated I-B (25.6%) had the highest C/N ratio, differing significantly only from CB 222 (20.8%).

Amylose %

No consistent trends were observed for amylose % over time in Inoculation Events 1 and 2 (Table 5). In Inoculation Event 1, significantly more ($P \leq 0.05$) amylose was found in the kernels of CML 444 at S0 (64.5%), differing significantly from all other lines at this sampling time. Inoculated R2565y had the highest amylose % at S7 (62.4%), S28 (48.2%) and S42 (46.2%) (Table 5). At S7, amylose levels in the inoculated R2565y (62.4%) differed significantly from amylose % in the other lines, whereas at S28, R2565y (48.2%) differed significantly ($P \leq 0.05$) from CML 444 (36.7%) with no significant differences in amylose % at S42 between any of the inbred lines. The inoculated and control treatments of CB 222 differed significantly in amylose levels at S7 and S28, with CML 444 also differing significantly at S28. At S52, the inoculated and control treatments of I-B differed significantly in amylose %, with no significant differences between the inoculated samples of the other inbred lines at this sampling time.

In Inoculation Event 2, no significant differences ($P \leq 0.05$) were observed in amylose levels between the lines at S0, nor were there significant differences between inoculated and control treatments at S7, S28 and S52 (Table 5). Significant differences ($P \leq 0.05$) were observed between inoculated and control in line CB 222 at S42. Line I-B contained the highest amylose % at S0 (48.0 %) and inoculated samples at S7 (46.0 %), S28 (43.5 %) and S42 (50.0 %), differing significantly from CB 222 (40.3 %) at S7 and R2565y (41.0 %) at S42. At S52, inoculated grain of CML 444 (45.4 %) contained the highest amylose %, which was not significantly higher than in the other lines evaluated.

Amylopectin %

The % amylopectin increased from S0 to S52 in Inoculation Events 1 and 2 (Table 5 and Fig. 8). In Inoculation Event 1, CML 444 had the lowest amylopectin % before inoculation (S0) (35.5 %), differing significantly ($P \leq 0.05$) from the other lines at this sampling time (Table 5 and Fig. 8). Inoculated CML 444 had the highest amylopectin % at S7 (54.8 %) and S28 (63.3

%), differing significantly from I-B (46.6 %) and R2565y (37.6 %) at S7 and only R2565y (51.8 %) at S28. Significant differences were also observed between inoculated and control treatments of CB 222 at both S7 and S28. Significant differences between inoculated and control treatments were also observed for line CML 444 at S28 and I-B at S52. No significant differences were observed between the inoculated and control at S42 and S52, nor were there significant differences between the inoculated samples at these sampling times.

At Inoculation Event 2, inoculated CB 222 had the highest amylopectin % at S0 (56.7%), which was not significantly different ($P \leq 0.05$) from the other lines. Inoculated CB 222 also had the highest amylopectin % at S7 (59.7%) (Table 5 and Fig. 8). However, it was not significantly different to other lines at this sampling time. No significant differences were observed between the amylopectin % in inoculated samples of any of the inbred lines at S28, nor were there significant differences between the inoculated and controls at this sampling time. Significant differences in amylopectin levels between the inoculated (53.4%) and control (60.6%) treatments were observed only for CB 222 at S42. The inoculated maize line R2565y (58.9%) had the highest amylopectin % at S42, differing significantly from all lines. At S52, inoculated grain of CB 222 (59.5%) had the highest amylopectin %, which was not significantly different from the other lines.

Fatty acids

Several fatty acids (n=35) were simultaneously quantified in this study. These include palmitic acid (C16:0), oleic acid (C18:1n9), stearic acid (C18:0), linoleic (C18:2n6) and linolenic acid (C18:3n3) (Table 6).

Palmitic acid

No consisted trend was observed in palmitic acid levels over time for Inoculation Events 1 and 2 (Table 6 and Fig. 9). Higher levels of palmitic acid were also quantified at S0 in Inoculation Event 1 compared to Inoculation Event 2 (Table 6 and Fig. 9). The grain of line CML 444 (13.9 mg g^{-1}) had the highest level of palmitic acid at S0, which differed significantly only from I-B (4.2 mg g^{-1}). Palmitic acid in the inoculated grain of CB 222 (4.4 mg g^{-1}) differed significantly from that in CML 444 (12.8 mg g^{-1}) and R2565y (10.5 mg g^{-1}) at S7. At S42 and S52, CML444 (S42- 10.1 mg g^{-1}; S52- 7.5 mg g^{-1}) also had the highest palmitic acid level, differing significantly from CB 222 (S42- 3.7 mg g^{-1}; S52- 0.9 mg g^{-1}) and I-B (S42- 1.6 mg g^{-1}; S52- 1.9 mg g^{-1}). No significant differences were observed between the inoculated and control treatments of inbred lines at any of the sampling times.

For Inoculation Event 2 (Table 6 and Fig. 9), no significant difference ($P \leq 0.05$) were observed between the controls at S0, nor were there significant differences between the inoculated and control samples at S42 and S52. Significant differences between inoculated

and control treatments were observed at S7 for R2565y and CML 444 at S28. Palmitic acid was also not significantly different between inoculated samples in any of the lines over time, except at S28 in line R2565y (1.5 mg g^{-1}).

Oleic acid

The oleic acid quantified in Inoculation Event 1 was higher compared to Inoculation Event 2 (Table 6 and Fig. 9). For Inoculation Event 1, R2565y (9.2 mg g^{-1}) had the highest oleic acid content at S0, significantly differing ($P \leq 0.05$) only from I-B (0.7 mg g^{-1}) (Table 6 and Fig. 9). Inoculated I-B (5.0 mg g^{-1}) had the highest oleic acid level at S7, differing significantly from CB 222 (1.3 mg g^{-1}) and CML 444 (1.6 mg g^{-1}). At S28, oleic acid in the inoculated line R2565y (2.7 mg g^{-1}) differed significantly from that of lines I-B (0.9 mg g^{-1}) and CML 444 (0.4 mg g^{-1}), while at S52 (R2565y; 4.3 mg g^{-1}), oleic acid levels differed significantly from CB 222 (0.4 mg g^{-1}). No significant difference was observed between the oleic acid levels in the inoculated and control treatments at S7 and S28. Inoculated CB 222 (2.1 mg g^{-1}) had the highest oleic acid at S42, which differed significantly from CML 444 (0.5 mg g^{-1}). Significant differences were observed between inoculated (0.5 mg g^{-1}) and control grain (2.2 mg g^{-1}) of CML 444 at S42. At S52, R2565y had significant differences in oleic acid between the inoculated (4.3 mg g^{-1}) and control treatments (0.5 mg g^{-1}). Inoculated R2565y also had the highest oleic acid at this sampling time, differing significantly only from CB 222 (0.4 mg g^{-1}).

At Inoculation Event 2, R2565y had the highest level of oleic acid at S0 (0.2 mg g^{-1}), which did not differ significantly ($P \leq 0.05$) from the other inbred lines (Table 6 and Fig. 9). At S7, R2565y (2.0 mg g^{-1}) differed significantly from CML 444 (0.7 mg g^{-1}), while at S42 and S52, R2565y (S42- 2.0 mg g^{-1}; S52-2.1 mg g^{-1}) differed significantly from CML 444 (S42- 0.6 mg g^{-1}; S52- 0.7 mg g^{-1}) and CB 222 (S42- 0.7 mg g^{-1}; S52- 1.0 mg g^{-1}). Significant differences between inoculated and control were observed at S7 (R2565y), S28 (CML 444) and S42 (R2565y).

Stearic acid

Similar levels of stearic acid were observed between Inoculation Events 1 and 2 (Table 6 and Fig. 9). At Inoculation Event 1 (Table 6 and Fig. 9), no significant differences ($P \leq 0.05$) were observed between inoculated and control treatments at S7, S42 and S52. Inoculated CB 222 grain (0.1 mg g^{-1}) had significantly less stearic acid than I-B (0.3 mg g^{-1}) at S7. At S28, there were significant differences between the inoculated and control treatments of I-B and R2565y. At S42, inoculated CML 444 (0.3 mg g^{-1}) contained more oleic acid than the other lines, but this difference was not significant. At S52, oleic acid in the inoculated R2565y line (0.3 mg g^{-1}) was the highest, but not significantly different from the other lines.

At Inoculation Event 2, no significant differences ($P \leq 0.05$) were observed between inoculated and control samples at S7 and S52 (Table 6 and Fig. 9). Line CML 444 experienced significant differences in stearic acid between the inoculated (0.1 mg g^{-1}) and control (0.1 mg g^{-1}) samples at S28. Inoculated R2565y (0.2 mg g^{-1}) contained significantly more oleic acid than CML 444 (0.1 mg g^{-1}) at S42. Furthermore, R2565y contained significantly more stearic acid in the inoculated grain than in the control grain. At S52, inoculated R2565y (0.4 mg g^{-1}) grain had significantly higher levels of stearic acid compared to the other lines.

Linoleic acid

Linoleic acid levels were higher in Inoculation Event 1 than Inoculation Event 2 (Table 6 and Fig. 9). In Inoculation Event 1, no significant differences ($P \leq 0.05$) were observed in linoleic acid levels between the inoculated and control treatments at S7 to S52 (Table 6 and Fig. 9). Line CML 444 (1.8 mg g^{-1}) had the lowest level of linoleic acid at S0, differing significantly from line R2565y (17.3 mg g^{-1}), which had the highest level of linoleic acid. Inoculated grain of R2565y (9.3 mg g^{-1}) had the highest linoleic acid level at S7, differing significantly from CB 222 (3.7 mg g^{-1}) and CML 444 (1.9 mg g^{-1}). Inoculated R2565y (5.5 mg g^{-1}) also had the highest linoleic acid content at S28, differing significantly only from CML 444 (0.7 mg g^{-1}). Conversely, at S42, the inoculated CB 222 (4.2 mg g^{-1}) had the highest linoleic acid, differing significantly from I-B (1.4 mg g^{-1}) and R2565y (1.6 mg g^{-1}). At S52, inoculated R2565y (5.9 mg g^{-1}) had the highest linoleic acid level, but it didn't differ significantly to the when compared to other lines.

At Inoculation Event 2, no significant differences ($P \leq 0.05$) in linoleic acid were observed at S0, nor between the inoculated and control treatments at S7 and S52 (Table 6 and Fig. 9). Linoleic acid levels differed significantly between inoculated and control treatments at S28 for CML 444 and for CML 444 and R2565y at S42. At S7, inoculated CML 444 (0.8 mg g^{-1}) had significantly less linoleic acid compared to all other lines. Inoculated R2565y had the highest linoleic acid content at S7 (2.9 mg g^{-1}), S28 (1.8 mg g^{-1}), S42 (2.3 mg g^{-1}) and S52 (2.1 mg g^{-1}), but it was not significantly different than the other lines at S28 and S42 and only differed significantly from CML 444 (0.8 mg g^{-1}) at S7. There were no significant differences observed between the inoculated samples at S52.

Linolenic acid

The linolenic acid level in all lines declined over time in Inoculation Event 1, with higher levels found during Inoculation Event 1 compared to Inoculation Event 2 (Table 6 and Fig. 9). At Inoculation Event 1, no significant differences ($P \leq 0.05$) were observed between the inoculated and control treatments at S28 and S52 (Table 6 and Fig. 9). There were, however, significant differences in linolenic acid level between the inoculated and control treatments for

R2565y at S7 and S42. Line I-B (3.5 mg g^{-1}) had the highest linolenic acid level at S0, which was not significantly different from that in the other lines. Inoculated R2565y had the highest level of linolenic acid at S7 (1.9 mg g^{-1}), which differed significantly from the other lines. Inoculated CB 222 had the highest linolenic acid content at S28 (0.9 mg g^{-1}) and S42 (0.3 mg g^{-1}), differing significantly from CML 444 (S28; 0.1 mg g^{-1}) and I-B (S28; 0.1 mg g^{-1}), and for all other lines for S42. No significant differences were observed between the inoculated of all inbred lines at S52.

At Inoculation Event 2, no significant differences ($P \leq 0.05$) were observed between the controls at S0, nor were significant differences observed between the inoculated and control treatments at S7 and S52 (Table 6 and Fig. 9). Significant differences were found between inoculated and control treatments for line CML 444 and R2565y at S28 and S42, respectively. At S7, inoculated R2565y (0.2 mg g^{-1}) had significantly more linolenic acid than CML 444 (0.1 mg g^{-1}). No significant differences were observed between inoculated samples at S28, S42 and S52.

Correlations between physico-chemical variables and infection indicators

Pearson's correlations between infection indicators (FER disease severity, fungal target DNA and fumonisins) and physico-chemical properties were evaluated (Table. 7). At Inoculation Event 1, physico-chemical properties such as palmitic acid (r= -0.310; P= 0.026), moisture (r= -0.373; P= 0.005), pH (r= -0.334; P= -0.018) and carbon (r=0.325; P= 0.017) had a significant correlation with FER disease severity in inoculated maize samples. The level of association was, however, low. A significantly negative correlation was observed between fungal target DNA quantified in inoculated maize grain and their moisture levels (r= -0.709; P=<0.0001), while a significantly positive correlation was observed between fungal target DNA and carbon (r= 0.658; P=<0.0001) and nitrogen (r= 0.638; P=<0.0001) levels in the grain. Other significant associations with inoculated fungal target DNA were found, such as palmitic acid (r= -0.499; P=0.000), oleic acid (r=-0.308; P= 0.033), linoleic acid (r= -0.393; P= 0.006), linolenic acid (r= -0.3888; P= 0.006), pH (r= -0.516; P= 0.000), C/N (r=0.347; P= 0.160), amylose (r= -0.486; P= 0.000) and amylopectin (r= 0.486; P= 0.000). These associations were, however, low. Significantly negative correlations were also observed between inoculated free fumonisins and linolenic acid (r= -0.308; P= 0.033), moisture (r= -0.306; P= 0.034), pH (r= -292; P= 0.044) and amylose (r= -0.374; P= 0.009), whereas significantly positive correlations were found between inoculated free fumonisins and carbon (r=0.393; P= 0.006), C/N (r= 0.358; P= 0.013) and amylopectin (r= 0.374; P= 0.009). Again, the level of association was low.

At Inoculation Event 2 (Table 7), a significant, negative correlation was observed between FER disease severity and moisture (r= -0.479; P= 0.000) in the inoculated samples, as well as a significantly positive correlation with pH (r= 0.545; P=<0.0001), carbon (r= 0.445;

P=0.001) and nitrogen (r= 0.298; P= 0.038). These associations were poor. Inoculated fungal target DNA had a significantly positive correlation with pH (r= 0.336; P= 0.020), but this association was also poor. Inoculated fungal target DNA showed a significantly positive correlation with carbon (r= 0.663; P=<0.0001) and nitrogen (r= 0.673; P= 0.000) levels. These associations were strong and, therefore, regarded as noteworthy. A significant, but poor, positive correlation was observed between fungal target DNA in inoculated samples and C/N (r= 0.526; P= 0.131). No significant correlations were observed between free fumonisins and physico-chemical properties at this inoculation event

Modified mycotoxins in grain at different kernel maturation stages

Free hydrolysed fumonisins

Hydrolysed fumonisins were detected at S0 in Inoculation Event 1 (Table 8). Here, free hydrolysed fumonisin levels increased over time, with the most significant (*P ≤ 0.05*) increase observed in the inoculated samples of line I-B at S52 (0.0425 mg kg^{-1}), differing significantly (*P ≤ 0.05*) only from line R2565y (0.0231 mg kg^{-1}). No significant differences were observed at S0, S7 and S28. At S42, there were no significant differences in free hydrolysed fumonisins of the inoculated and control treatments. Inoculated I-B (0.0080 mg kg^{-1}) had the most free hydrolysed fumonisin at S42, but these levels did not differ significantly from that of other lines. At S52, the inoculated and control free hydrolysed levels of R2565y differed significantly from one another. Inoculated I-B had the highest free hydrolysed fumonisins at this sampling time (0.0425 mg kg^{-1}), differing significantly only from R2565y (0.231 mg kg^{-1}).

In Inoculation Event 2, a similar increase in free hydrolysed level was found over time (Table 8). However, inoculated CB 222 (0.0397 mg kg^{-1}) and CML 444 (0.0129 mg kg^{-1}) accumulated higher levels of free hydrolysed fumonisins at S52, but not significantly more than I-B (0.0078 mg kg^{-1}) and R2565y (0.0191 mg kg^{-1}). At S0, inoculated R2565y had the highest level of free hydrolysed fumonisins (0.0018 mg kg^{-1}), which did not differ significantly from the other lines. The inoculated and control treatment of R2565y differed significantly at S7, with the inoculated R2565y (0.0078 mg kg^{-1}) differing significantly from all other lines. No significant differences were observed at S28. At S42, the free hydrolysed fumonisin levels in the inoculated and control treatments of CB 222 differed significantly from each other, while the inoculated and control treatments at S52, differed significantly from lines CML 444, I-B and R2565y.

Hidden fumonisins

Hidden fumonisins were significantly (*P ≤ 0.05*) higher at the later stages of kernel development (S42 and S52) for both inoculation events (Table 8 and Fig. 10). However, the

degree of contamination differed between the inbred lines. In Inoculation Event 1, I-B plants inoculated with *F. verticillioides* had the highest level of hidden fumonisin contamination (95 mg kg^{-1}), followed by R2565y (63.5 mg kg^{-1}) at S52 (Table 8 and Fig. 10). Furthermore, hidden fumonisins in line I-B was not significantly more than that found in other lines, while hidden fumonisin in R2565y grain differed significantly from that in CML 444 (1.9 mg kg^{-1}). No significant differences in hidden fumonisin levels were observed between any of the treatments at S0, S7 and S28. In line I-B, hidden fumonisin levels differed significantly between inoculated and control treatments at S42, with the hidden fumonisin level highest in the inoculated grain (16.8 mg kg^{-1}), although it did not differ significantly from the other lines.

At Inoculation Event 2 (Table 8. and Fig. 10), hidden fumonisin levels in inoculated plants were highest in R2565y (25.0 mg kg^{-1}), which differed significantly ($P \leq 0.05$) from I-B (3.7 mg kg^{-1}) and CB 222 (6.6 mg kg^{-1}), but not CML 444 (4.3 mg kg^{-1}) at S52. No significant differences were observed at S0, S7, S28 and S42.

Correlations of modified fumonisins with infection indicators and fatty acids and starch

In Inoculation Event 1, significant positive correlations were observed between inoculated free-hydrolysed fumonisins and fungal target DNA (r=0.367; P= 0.010), as well as between free-hydrolysed fumonisins and total free fumonisins (r= 0.464; P= 0.001) (Table 9). The degree of association was, however, low. Inoculated hidden fumonisins had a significant, positive correlation with fungal target DNA (r= 0.575; P= 0.001) and total free fumonisins (r= 0.531; P=<0.0001), however, the degree of association was low. At Inoculation Event 2, significantly positive correlations were observed between the inoculated free hydrolysed fumonisins, FER disease severity (r= 0.450; P= 0.481) and fungal target DNA (r= 0.537; P= 0.449) (Table 9). The degree of association was low. Inoculated hidden fumonisins had a significant positive correlation with FER disease severity (r= 0.298; P= 0.014) and fungal target DNA (r= 0. 497; P= 0.000). Again, the level of association was low.

No significant associations were observed between inoculated free hydrolysed and hidden fumonisins with fatty acids, amylose and amylopectin in Inoculation Event 1 (Table 10). In Inoculation Event 2 (Table 10), no significant correlations were observed between free hydrolysed fumonisins and fatty acids, amylose and amylopectin. Significant correlations were observed for hidden fumonisins and some of the fatty acids such as oleic acid (r= 0.351; P= 0.015) and stearic acid (r= 0.402; P= 0.005). However, the degree of association was low.

Multivariate analyses of structural traits, physico-chemical properties and infection indicators

The PCA of structural traits, physico-chemical properties and infection indicators show a clear separation between fumonisin resistant lines (CB 222 and CML 444) and fumonisin

susceptible lines (R2565y and I-B) where susceptible lines also differed from one another (Fig. 11). The biplot accounted for 78.5% of the observed variation where F1 and F2 explained 51.3% and 27.2% of the variation, respectively. In the F1 axis, most of the variation observed was associated with FER disease severity, fumonisin production (Free fumonisins and fumonisin derivatives),oleic acid, stearic acid, linoleic acid, linolenic acid, average pH, carbon, nitrogen, ,husk coverage at anthesis and harvest and silk browning. . Target DNA, amylose, amylopectin and silk detachment were highly associated with the F2. Palmitic acid, moisture content, C/N and silk length were associated with the F3.

The multifactor biplot represented 79.0% of the observed variation with F1 representing 52.76% and F2 26.27% of the observed variation (Fig. 12). Only structural properties, as a group, could be significantly positively correlated with physico-chemical properties based on Pearson's correlation coefficients (RV) (RV=0.86). Partial least squares (PLS) analysis, however, showed significant correlations between certain factors within different groups and were reflected in the variable importance on projection (VIP) values (Fig. 13). The PLS again showed significant associations in both dimensions between certain structural traits, such as silk browning (VIP = 1.5), and physico-chemical properties, such as carbon (VIP = 1.24), nitrogen (VIP = 1.68) and C/N (VIP = 1.49) with infection indicators.

Multivariate analyses of physico-chemical properties and infection indicators only

The PCA of physico-chemical properties, infection indicators and inoculation events (Fig. 14A) and sampling time (Fig. 14B) show a clear separation between Inoculation Events 1 and 2 as well as the physico-chemical factors that contribute to each of the inoculation events. The biplots of inoculation events and sampling time accounted for 57.0% of the total variation where F1 explained approximately 39.0% and F2 18.0% of the observed variation. Infection indicators were closely associated and contributed more significantly to Inoculation Event 2 than Inoculation Event 1. Furthermore, physico-chemical properties such as carbon, nitrogen, C/N and amylopectin contributed significantly to Inoculation Event 2 whereas fatty acids, moisture content, pH and amylose had a more significant contribution to Inoculation Event 1. Modified mycotoxins (free hydrolysed fumonisins, total hydrolysed fumonisins and hidden fumonisins) were also closely associated with infection indicators at Inoculation Event 2. Similarly, certain physico-chemical factors had a more significant contribution at the early stages of kernel development while others contributed more significantly to the later stages of kernel development. Infection indicators are closely associated with the later stages of kernel development (S42 and S52) while fatty acids, pH, amylose and moisture were all closely associate with the early stages of kernel development.

DISCUSSION

Resistance to *F. verticillioides* and fumonisin contamination is complex and gaining more knowledge regarding the role of maize physical features and kernel physico-chemical properties during infection would be invaluable toward breeding resistant cultivars. This study clearly demonstrated that the trends in FER disease severity, fumonisin deposition and fungal target DNA was consistent for all lines, increasing progressively and peaking at approximately 52 dai, irrespective of whether early (Inoculation Event 1; 7 dap) or late (Inoculation Event 2; 35 dap) infection occurred. Maximum fumonisin contamination corresponded to the physiological (Inoculation Event 1) and biological (Inoculation Event 2) kernel maturation stages, which is in agreement with van Zyl (2015) who found that maximum fungal and fumonisin contamination in grain, inoculated 30 dap, increased from the physiological to biological kernel-stages. These results are, however, in contrast with Picot *et al*. (2011) where the most significant increase in fumonisin production was between 22-42 days after inoculation, corresponding to the dent stage of kernel development after inoculating maize ears 4-7 days after silk emergence. Based on the results of this study, *F. verticillioides* growth and subsequent fumonisin contamination appears to be dependent on the timing of infection rather than being triggered by a specific kernel maturation stage, with maximum contamination observed in matured grain.

The levels of fumonisin deposition between Inoculation Events 1 and 2 varied significantly for all inbred lines evaluated. Higher levels of fumonisins were observed in lines characterised as susceptible to fumonisins (R2565y and I-B) following inoculation during flowering, while those characterised as resistant (CB 222 and CML 444) had lower levels of fumonisins. However, later inoculation of more mature grain (Inoculation Event 2) resulted in elevated levels of fumonisins in the resistant lines compared to the susceptible lines. Bush *et al*. (2004) and Dall'Asta *et al*. (2015) obtained similar results when evaluating FER response and fumonisins in mature stages of kernel development. Bush *et al*. (2004) found that 9 weeks after pollination, the susceptible line still had high levels of fumonisins, but resistant lines had higher levels of fumonisins than at 4-6 weeks after pollination. These findings, together with that observed in the present study, indicate that the evaluation of the plant response to *F. verticillioides* infection needs to be assessed at the immature stages of kernel development to accurately determine resistance or susceptibility at maturity. It further supports studies that have shown that early harvesting reduces fumonisin accumulation in grain (Bush *et al.*, 2004; Blandino *et al*., 2009; Parsons and Munkvold, 2010). South African maize farmers, especially subsistence farmers, leave maize to dry within the field due to a lack of infrastructure to reduce the moisture content of the kernels in a controlled environment before being stored (Mukanga

et al., 2011). This practice extends the period in which maize kernels can be infected with *F. verticillioides*, leading to increased fumonisin contamination.

The close association between total carbon, nitrogen and C/N with infection indicators suggests that these factors mediate infection by potentially serving as food source for the fungus and/or required component for fumonisin production. Secondary metabolites inlcuding FB_1 are synthetised from precursors derived from primary metabolism, which is strongly affected by carbon sources (Achimón *et al.*, 2019). Links *et al*. (2020), who also analysed carbon, nitrogen and C/N and its association with FER and fumonisins in physiologically mature maize kernels of the same inbred lines achieved similar results. Furthermore, the increase in C/N from inoculation to biological maturity in the present study has been corroborated *in vitro* by Jiménez *et al.* (2003) where early stages of kernel maturation were characterised by a low C/N and low levels of fumonisin contamination compared to later stages of kernel development that had a high C/N and high levels of fumonisins contamination. Blister kernels have a high amino acid content and a low starch content, creating an overall low C/N not suited for fumonisin production (Jiménez *et al.*, 2003). A high C/N at the time of artificial inoculation in Inoculation Event 2 (35 dap) may have allowed the fungus to overcome plant resistance mechanisms, resulting in high levels of fumonisins.

The pH of the maize kernels never reached alkaline conditions, varying between a minimum of pH 4.8 and a maximum of pH 7.1. *In vitro* studies show that fumonisin production by *F. verticillioides* is favoured by acidic conditions (Flaherty *et al.*, 2003). Furthermore, fumonisin production has been associated with the dent stage of kernel development where kernel acidification is greatest (Warfield and Gilchrist, 1999; Picot *et al.*, 2011). Even though fumonisin production was greatest at the later stages of kernel development where maximum kernel acidification is typically found, there were no significant differences between the pH of inoculated and control maize kernels at any of the sampling times for both inoculation events in this study. This differs from van Zyl (2015) and Links (2019) who both observed that inoculated maize had a lower pH than their respective controls *in planta*. Previously, it was hypothesized that an acidic pH is a by-product of fungal metabolism (Marin *et al*., 2004). The results of this study, however, showed that fumonisin production is active even at a neutral pH *in planta* and that the fungus may be producing fumonisins in response to the pH instead of manipulating the kernel pH to suite fungal growth and proliferation.

Kernel moisture declines as it matures and accumulates starch, reducing the amount of water available for fungal metabolism (water activity), which induces fumonisin production by *F. verticillioides* (Jurado *et al*., 2008; Schmidt-Heydt *et al*., 2008). The moisture content of kernels had a significant negative correlation to fungal target DNA for both inoculation events. Generally a high kernel moisture facilitates fungal growth and fumonisin production (Samapundo *et al.*, 2005). However, *Fusarium verticillioides* is able to grow at low kernel

moisture and could explain the observed result (Marín *et al*., 2004). The increase in fungal DNA could also be linked to the notion that fumonisins are produced as an adaptive response to facilitate fungal colonisation (Schmidt-Heydt *et al*., 2008). Low water availability during the later stages of kernel maturation increases the expression of the fumonisin polyketide synthase gene (*FUM1*) for fumonisin biosynthesis (Desjardins *et al.*, 2002). Thus, water stress may be a contributing factor to the high levels of fumonisin contamination during the later stages of kernel development for both inoculation events.

Components of starch, amylose and amylopectin, play a role in the stimulation of fungal growth and fumonisin production when used as carbon sources by *F. verticillioides* (Achimón *et al.*, 2019). Amylose stimulated fungal growth *in vitro* (Achimón *et al.*, 2019) whereas amylopectin content has been implicated in the production of fumonisins *in vitro* (Bluhm and Woloshuk, 2005) and *in planta* (Picot *et al.*, 2011). Our study does not support these findings as no significant associations were observed between infection indicators and amylose at either of the inoculation events. This difference can be attributed to the fact that our study was conducted *in planta* or it does not act as part of the resistance mechanism in these lines evaluated, but could still play a role in FER and/or fumonisin resistance in other maize lines. We also observe that while amylose may be associated with some factors, such as target DNA, were associated with amylose in the PCAs, however, was not associated in the Pearson's correlations. This can be explained by the fact that the number of observations for these two different analyses varied. Where Pearson's correlations used each data point individually, this analysis only used the mean of each factor for each inbred line. Maize kernels accumulated amylopectin at the milk stage of kernel development and continued to increase to a maximum at physiological and biological maturity and fumonisins were also produced as early as the blister stage, whereas Picot *et al*. (2011) observed enhanced fumonisin production three weeks after amylopectin accumulation had started. Therefore, even though there is lack of association between amylopectin and fumonisin contamination, we cannot rule out the possibility that fumonisin production is influenced by amylopectin content as our results demonstrate that increasing levels of amylopectin coincides with fumonisin production and starts as early as the milk stage increasing to physiological maturity where fumonisin production in greatest. Amylopectin could, therefore, be indirectly involved in fumonisin biosynthesis.

Dall'Asta *et al*. (2015) noted that resistant maize lines accumulated lower levels of hidden fumonisins over time. Similarly, in the present study, fumonisin-resistant lines CB 222 and CML 444 both accumulated low levels of hidden fumonisins during the later stages of kernel development for both inoculation events compared to fumonisin-susceptible lines I-B and R2565y. Selecting for resistance to free fumonisins may also result in the simultaneous selection for resistance to hidden fumonisins in maize grain. Furthermore, the level of hidden

fumonisins itself, nor its content in relation to free fumonisin contamination, does not appear to be an indicator of detoxification as a potential resistance mechanism. The biochemical pathway of hidden forms of fumonisin production has not yet been elucidated, although a fumonisin biosynthetic pathways has been proposed (Bojja *et al.*, 2004). It is unclear which molecular form of fumonisins is produced by *F. verticillioides* and whether the plant may be involved in the conversion of the parent fumonisins to the hidden forms trapped within the kernel matrix. What is clear from literature thus far is that hidden fumonisins are produced when *F. verticillioides* is found in conjunction with the kernel matrix and never in cultures of *Fusarium* grown on media such as malt agar (Dall'Asta *et al.*, 2012; Lazzaro *et al.*, 2012). We also note that in this study hidden fumonisins were never produced in the absence of free fumonisins, demonstrating that the production of hidden fumonisins and free fumonisins could be a joint process.

Clear analytical differences were observed when detecting hydrolysed fumonisins using the standard fumonisins analysis and HFB standards versus the use of the modified protocol where alkaline hydrolysis is used to liberate the fumonisins from the kernel matrix. Total free fumonisins was significantly higher after hydrolysis compared to analysing for hydrolysed fumonisins using the standard fumonisin extraction. This demonstrates the efficacy of using alkaline hydrolysis for the detection of hidden fumonisins. The broader concern with regards to hidden fumonisins lies in the possibility that they can be released into the gastrointestinal tract by humans and animals (Kim *et al.*, 2003; Humpf and Voss, 2004; Dall'Erta *et al.*, 2013; Bryla *et al.*, 2016; Dall'Asta and Battilani, 2016; Dellafiora and Dall'Asta, 2016; Zhang *et al.*, 2019). The implication would then be that hidden fumonisins have an additive effect on free fumonisins upon ingestion. Moreover, the hidden fumonisins detected in this study are only those of fumonisin B_1 and subsequently HFB_1 due to a lack of available standards for other hydrolysed forms of fumonisins (HFB_2 and HFB_3). Although FB_1 is the most abundant in nature compared to its homologs, previous studies such as that of Dall'Asta *et al*. (2012; 2015) were able to quantify total fumonisins as the sum of all hydrolysed forms of fumonisins in raw maize, providing a more accurate representation of potential contamination. The development of analytical standards is crucial for accurate and reliable research efforts in mycotoxicology, especially where hidden mycotoxins are concerned to make risk assessments of potential human exposure more accurate.

This study could not determine any correlations between the levels of fatty acids or starch and the levels of free and hidden fumonisin contamination for any of the maize inbred lines. Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) have been consistently identified within the maize lipid profile (Dall'Asta *et al.*, 2012, 2015; Betancourt *et al.*, 2017). These fatty acids have a variety of different roles contributing to plant defence. Oleic and linoleic acids induce protein kinase c-mediated

activation of NADPH oxidase upon pathogen infection leading to the accumulation of reactive oxygen species that facilitate plant defence to pathogen infection (Kachroo and Kachroo, 2009). Linolenic acid is involved in protein modifications in heat stressed plants and elevated levels of stearic acid has been observed in soybean after colonisation by *Diaporthe phaseolorum* leading to enhanced resistance (Kachroo and Kachroo, 2009). Linoleic acid levels have also contributed to fungal colonisation, fungal development and mycotoxin accumulation by *Aspergillus* spp. (Kachroo and Kachroo, 2009). Fatty acids, therefore, play an important role in the plant-pathogen cross-talk directly or in the form of oxylipins and other signalling molecules (Walley *et al.*, 2013).

Previous studies have associated high levels of free and hidden fumonisins with high levels of fatty acids, specifically unsaturated fatty acids such as oleic (C18:1) and linoleic acid (C18:2) in maize harvested approximately 35 dap corresponding to the dent stage of kernel development (Dall'Asta *et al.*, 2012). More recently, the linoleic acid pathway had also been implicated in fumonisin accumulation in maize (Righetti *et al.*, 2019). Dall'Asta and co-workers (2012) found that hybrids with higher levels of free fumonisin contamination also had high levels of linoleic acid and that an abundance of hidden fumonisins was accompanied by a higher oleic to linoleic acid ratio. In another study, total fatty acids had a significantly positive association with fugal incidence and fumonisins (Dall'Asta *et al.*, 2015). Our study found no significant differences in the ratio of oleic to linoleic acid at any of the kernel developmental stages in either of the inoculation events (data not shown). The level of fatty acids in Inoculation Event 2 remained constant from the dent stage (Inoculation Event 2; S7) to biological maturity (Inoculation Event 2; S52). This is expected as the maize plant defence is no longer as active during the later stages of kernel development. Although no significant correlations were determined between fatty acids and infection indicators, fatty acids were associated with the early stages of kernel maturation, suggesting that fatty acids may be involved in plant defence to fungal infection when infected at the immature stage of kernel development. There also seems to be a similar pattern in the fluctuations of the different fatty acids. Fatty acids show a synchronised increase or decrease over time. This pattern within the fatty acid profile over time could reflect the co-ordination that exists between these molecules in order to facilitate a defence response within the plant to fungal infection.

Of the structural traits evaluated, only silk browning had a significant positive association with infection indicators, suggesting that it could contribute to resistance to *F. verticillioides* infection and/or fumonisin contamination. *Fusarium verticillioides* moves along the silk using free water and, therefore, dry silks prevent this movement into the maize kernels (Reid and Sinha, 1998; Duncan and Howard, 2010). The rate of silk senescence has been described as a resistance factor by Reid *et al*. (2002) who demonstrated reduced susceptibility to infection by *F. verticillioides* as kernels ages and dries out. Silks able to dry out faster will

shorten the period of possible infection. This phenotype was mostly present in susceptible lines (R2565y and I-B) in both seasons. However, it was also observed in resistant line CML 390 in the 2019/20 growing season. This suggests that silk browning may contribute slowing down the rate of infection but it does not represent a formidable barrier to infection and subsequent fumonisin production once the fungus has gained entry.

We conclude that physico-chemical factors such as carbon, nitrogen and C/N could serve as potential indicators of resistance to *F. verticillioides* in maize. Although silk browning has also been associated with infection indicators, it is unlikely that it plays a role in resistance or susceptibility but can still be selected for by breeders to enhance resistance on a structural level. The inbred lines used in this study can now also be classified in terms of their ability to resist the accumulation of not only free fumonisins, but hidden fumonisins as well. These findings will add to the perspective regarding the maize-*Fusarium verticillioides* pathosystem as well as providing more insight into the plant-fungus interaction on a metabolic level with support for additional factors that may influence fungal proliferation and fumonisin biosynthesis. This knowledge will inevitably facilitate the timely selection of resistant maize lines in future and contribute to the future food security status of our country, in time benefitting farmers and consumers alike.

REFERENCES

Achimón, F., Dambolena, J.S., Zygadlo, J.A. and Pizzolitto, R.P. 2019. Carbon sources as factors affecting the secondary metabolism of the maize pathogen *Fusarium verticillioides*. LWT-Food Science and Technology 115: 1–7.

Alberts, J.F., van Zyl, W.H. and Gelderblom, W.C.A. 2016. Biologically based methods for control of fumonisin-producing *Fusarium* species and reduction of the fumonisins. Frontiers in Microbiology 7: 1-33.

ApSimon, J.W. 2001. Structure, synthesis, and biosynthesis of fumonisin B1 and related compounds. Environmental Health Perspectives 109: 245–249.

Bacon, C.W., Glenn, A.E. and Yates, I.E. 2008. *Fusarium verticillioides*: Managing the endophytic association with maize for reduced fumonisins accumulation. Toxin Reviews 27: 411–446.

Bakan, B., Melcion, D., Richard-Molard, D. and 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.

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S. De, Haesaert, G., Karlovsky, P., Oswald, I.P., Seefelder, W., Speijers, G. and Joerg, S. 2013. Masked mycotoxins: A review. Molecular Nutrition and Food Research 57: 165–186.

Berthiller, F., Maragos, C.M. and Dall'Asta, C. 2016. Introduction to masked mycotoxins. Pages 1-13 in: Masked mycotoxins in food: Formation, Occurrence and Toxicological Relevance (Berthiller, F and Dall'Asta, C). Royal Society of Chemistry, London, United Kingdom.

Betancourt, P.D.S., Tolentino, R.G. and Schettino, B. 2017. Proximate Composition, fatty acid profile and mycotoxin contamination in several varieties of mexican maize. Food and Nutrition Sciences 8: 865–872.

Beukes, I. 2015. Pathogenicity and mycotoxin production of the *Fusarium graminearum* species complex in South African grains. MSc Agric thesis, University of Stellenbosch, Stellenbosch, South Africa, 66 pp.

Blandino, M., Reyneri, A. and Vanara, F. 2009. Effect of sowing time on toxigenic fungal infection and mycotoxin contamination of maize kernels. Journal of Phytopathology 157: 7–14.

Bluhm, B.H. and Woloshuk, C.P. 2005. Amylopectin induces fumonisin B1 production by *Fusarium verticillioides* during colonization of maize kernels. Molecular Plant-Microbe Interactions 18: 1333–1339.

Bojja, R.S., Cerny, R.L., Proctor, R.H. and Du, L. 2004. Determining the biosynthetic sequence in the early steps of the fumonisin pathway by use of tree gene-disruption mutants *of Fusarium verticillioides*. Journal of Agricultural and Food Chemistry 52: 2855–2860.

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.

Boutigny, A.L., Scauflaire, J., Ballois, N. and Ioos, R. 2017. *Fusarium temperatum* isolated from maize in France. European Journal of Plant Pathology 148: 997–1001.

Bryla, M., Roszko, M., Szymczyk, K., Renata, J. and Obiedzinski, M.W. 2016. Fumonisins and their masked forms in maize products. Food Control 59: 619–627.

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.

Cao, A., Santiago, R., Ramos, Antonio, J., Marin, S., Reid, L.M. and Butron, A. 2013. Environmental factors related to fungal infection and fumonisin accumulation during the development and drying of white maize kernels. International Journal of Food Microbiology 167: 1–25.

Department of Agriculture, Forestry and Fisheries (DAFF). 2017. A Profile of the South African Maize Market Value Chain. Online publication: https://www.nda.agric.za/docs/AMCP/Citrusmvcp2011-12.pdf (14 June 2019).

Dall'Asta, C., Giorni, P., Cirlini, M., Reverberi, M., Gregori, R., Ludovici, M., Camera, E., Fanelli, C., Batillani, P. and Scala, V. 2015. Maize lipids play a pivotal role in the fumonisin accumulation. World Mycotoxin Journal 8: 87–97.

Dall'Asta, C. and Battilani, P. 2016. Fumonisins and their modified forms, a matter of concern in future scenario? World Mycotoxin Journal 9: 727–739.

Dall'Asta, C., Falavigna, C., Galaverna, G. and Battilani, P. 2012. Role of maize hybrids and their chemical composition in *Fusarium* infection and fumonisin production. Journal of Agricultural and Food Chemistry 60: 3800–3808.

Dall'Erta, A., Cirlini, M., Dall'Asta, M., Del Rio, D., Galaverna, G. and Dall'Asta, C. 2013. Masked mycotoxins are efficiently hydrolyzed by human colonic microbiota releasing their aglycones. Chemical Research in Toxicology 26: 305–312.

Dellafiora, L. and Dall'Asta, C. 2016. Masked mycotoxins: An emerging issue that makes renegotiable what is ordinary. Food Chemistry 213: 534–535.

Desjardins, A.E., Munkvold, G.P., Plattner, R.D. and Proctor, R.H. 2002. *FUM1* - A gene required for fumonisin biosynthesis but not for maize ear rot and ear infection by

Gibberella moniliformis in field tests. Molecular Plant-Microbe Interactions 15: 1157–1164.

Falavigna, C., Lazzaro, I., Battilani, P., Galaverna, G. and Dall'Asta, C. 2013. Cornmeal and starch influence the dynamic of fumonisin B, A and C production and masking in *Fusarium verticillioides* and *F. proliferatum*. International Journal of Food Microbiology 166: 21–27.

Falavigna, C., Lazzaro, I., Galaverna, G., Battilani, P. and Dall'Asta, C. 2013. Fatty acid esters of fumonisins: First Evidence of Their Presence in Maize. Food Additives and Contaminants - Part A 30: 1606–1613.

Flaherty, J.E., Pirttila, A.M., Bluhm, B.H. and Woloshuk, C.P. 2003. *PAC1*, a pH-regulatory gene from *Fusarium verticillioides*. Society 69: 5222–5227.

Foley, D.C. 1962. Systemic Infection of Corn by *Fusarium moniliforme*. Phytopathology 52: 870–872.

Freire, L. and Sant'Ana, A.S. 2018. Modified mycotoxins: An updated review on their formation, detection, occurrence, and toxic effects. Food and Chemical Toxicology 111: 189–205.

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

GrainSA. 2018. New season prospects for maize 2018. Online publication: https://www.grainsa.co.za/new-season-prospects-for-maize (21 January 2019).

Hanway, J.J. 1966. How a corn plant develops. Online publication: https://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1045&context=specialreports (3 February 2019).

Harrison, L.R., Colvin, B.M., Greene, 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.

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.

Humpf, H.U. and Voss, K.A. 2004. Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins. Molecular Nutrition and Food Research 48: 255–269.

Jiménez, M., Mateo, J.J., Hinojo, M.J. and Mateo, R. 2003. Sugars and amino acids as factors affecting the synthesis of fumonisins in liquid cultures by isolates of the *Gibberella fujikuroi* complex. International Journal of Food Microbiology 89: 185–193.

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 and matric potential stress, temperature, growth, and *FUM1* gene expression in two *Fusarium verticillioides* strains from Spain. Applied and Environmental Microbiology 74: 2032–2036.

Juroszek, P. and von Tiedemann, A. 2013. Climatic changes and the potential future importance of maize diseases: A short review. Journal of Plant Diseases and Protection 120: 49–56.

Kachroo, A. and Kachroo, P. 2009. Fatty acid-derived signals in plant defense. Annual Review of Phytopathology 47: 153–76.

Karlovsky, P., Suman, M., Berthiller, F., De Meester, J., Eisenbrand, G., Perrin, I., Oswald, I.P., Speijers, G., Chiodini, A., Recker, T. and Dussort, P. 2016. Impact of food processing and detoxification treatments on mycotoxin contamination. Mycotoxin Research 32: 179–205.

Keller, N.P., Nesbitt, C., Sarr, B., Phillips, T.D. and Burow, G.B. 1997. pH regulation of sterigmatocystin and aflatoxin biosynthesis in *Aspergillus* spp. Phytopathology 87: 643–648.

Keller, S.E., Sullivan, T.M. and Chirtel, S. 1997. Factors affecting the growth of *Fusarium proliferatum* and the production of fumonisin B 1: oxygen and pH. Journal of Industrial Microbiology and Biotechnology 19: 305–309.

Keyser, Z., Vismer, H.F., Klaasen, J.A., Snijman, P.W. and Marasas, W.F. 1999. The antifungal effect of fumonisin B1 on *Fusarium* and other fungal species. South African Journal of Science 95: 455–458.

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.

Kim, E.K., Scott, P.M. and Lau, B.P.Y. 2003. Hidden fumonisin in corn flakes. Food Additives and Contaminants 20: 161–169.

Kim. H. Smith. J.E. Ridenour. J.B. Woloshuk. C.P. and Bluhm. B.H. 2011. *HXK1* regulates carbon catabolism and sporulation. Fumonisin B1 production and pathogenesis in *Fusarium verticillioides*. Microbiology 157: 2658-2669.

Koehler, B. 1942. Natural mode of entrance of fungi into corn ears and some symptoms that indicate infection. Journal of Agricultural Research 64: 421-442.

Lazzaro, I., Falavigna, C., Dall'Asta, C., Proctor, R.H., Galaverna, G. and Battilani, P. 2012. Fumonisins B, A and C profile and masking in *Fusarium verticillioides* strains on

fumonisin-inducing and maize-based media. International Journal of Food Microbiology 159: 93–100.

Li, Y., Lou, L., Cerny, R.L., Butchko, R.A.E., Proctor, R.H., Shen, Y. and Du, L. 2013. Tricarballylic ester formation during biosynthesis of fumonisin mycotoxins in *Fusarium verticillioides*. Mycology 4: 179–186.

Lim, G., Singhal, R., Kachroo, A. and Kachroo, P. 2017. Fatty acid– and lipid-mediated signalling in plant defense. Annual Review of Phytopathology 55: 505–536.

Links, S. 2019. Phenotypic, physico-chemical and genetic responses of maize associated with resistance to *Fusarium Verticillioides*. MSc Agric thesis, University of Stellenbosch, Stellenbosch, South Africa, 76 pp.

Links, S., van Zyl, K., Cassiem, A., Flett, B.C., Viljoen, A. and Rose, L.J. 2020. The association of maize characteristics with resistance to *Fusarium verticillioides* and fumonisin accumulation in commercial maize cultivars. World Mycotoxin Journal 13: 1–14.

Marasas, W.F.O. 1996. Fumonisins: History, world-wide occurrence and impact. Fumonisins in Food 392: 1988–1989.

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

Marasas, W.F.O., Wehner, F.C., Janse van Rensburg, S. and Van Schalkwyk, D.J. 1981. Mycoflora of corn produced in human oesophageal areas in Transkei, Southern Africa. Postharvest Pathology and Mycotoxins Phytopathology 71: 792-796.

Marín, S., Magan, N., Ramos, A.J. and Sanchis, V., 2004. Fumonisin-producing strains of *Fusarium*: A Review of Their Ecophysiology. Journal of Food Protection 67: 1792-1805.

Merrill, A.H., Liotta, D.C. and Riley, R.T. 1996. Fumonisins: Fungal Toxins that Shed Light on Sphingolipid Function. Trends in Cell Biology 6: 218–223.

Miquel, M. and Browse, J. 1992. Arabidopsis mutants deficient in polyunsaturated fatty acid synthesis: Biochemical and Genetic Characterization of a Plant Oleoyl-phosphatidylcholine Desaturase. Journal of Biological Chemistry 267: 1502–1509.

Missmer, S.A., Suarez, L., Felkner, M., Wang, E., Alfred, H.M., Rothman, K.J. and Hendricks, K.A. 2006. Exposure to Fumonisins and the Occurrence of Neural Tube Defects along the Texas – Mexico Border. Environmental Health Perspectives 114: 237–241.

Munkvold, G. P. 2000. Genetically modified, insect resistant maize: Implications for Ear and Stalk Diseases. Plant Health Progress 1: 1-10.

Munkvold, G.P. 2003a. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. European Journal of Plant Pathology 109: 705–713.

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

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

Nayaka, S.C., Udaya Shankar, A.C., Reddy, M.S., Niranjana, S.R., Prakash, H.S., Shetty, H.S. and Mortensen, C.N. 2008. Control of *Fusarium verticillioides*, cause of ear rot of maize, by *Pseudomonas fluorescens*. Pest Management Science 65: 769–775.

Nicolaisen, M., Suproniene, S., Nielsen, L.K., Lazzaro, I., Spliid, H.N. and Justesen, A.F. 2009. Real-time PCR for quantification of eleven individual *Fusarium* species in cereals. Journal of Microbiological Methods 76: 234-240.

Oerke, E.C. and Dehne, H.W. 2004. Safeguarding production-losses in major crops and the role of crop protection. Crop Protection 23: 275–285.

Official Methods of Analysis: AOAC International. 20th edition. 2016. Latimer, L. W. AOAC Official method 925.09, section 32. International publishers, Rockville, Maryland.

Ott, R.L. 1998. An Introduction to statistical methods and data analysis. Duxbury Press, Belmont, California, 837 pp.

Picot, A., Barreau, C., Pinson-Gadais, L., Caron, D., Lannou, C. and Richard-Forget, F. 2010. Factors of the *Fusarium verticillioides*-maize environment modulating fumonisin production. Critical Reviews in Microbiology 36: 221–231.

Picot, A., Barreau, C., Pinson-Gadais, L., Piraux, F., Caron, D., Lannou, C. and Richard-Forget, F. 2011. The dent stage of maize kernels is the most conducive for fumonisin biosynthesis under field conditions. Applied and Environmental Microbiology 77: 8382–8390.

Ranum, P., Pablo Pe˜na-Rosas, J. and Garcia-Casal, M.N. 2014. Global maize production, utilization, and consumption. Annals of the New York Academy of Sciences 1312: 105–112.

Reid, L.M., Nicol, R.W., Quellet, 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.

Reid, L.M., Bolton, A.T., Hamilton, R.I., Woldemariam, T. and Mather, D.E. 1992. Effect of silk age on resistance of maize to *Fusarium graminearum*. Canadian Journal of Plant Pathology 14: 293-298.

Righetti, L., Lucini, L., Giorni, P., Locatelli, S., Dall'Asta, C. and Battilani, P. 2019. Lipids as key markers in maize response to fumonisin accumulation. Journal of Agricultural and Food Chemistry 67: 4064–4070.

Rocha, L.O., Barroso, V.M., Andrade, L.J., Pereira, G.H.A., Ferreira-Castro, F.L., Duarte, A.P., Michelotto, M.D. and Correa, B. 2016. *FUM* gene expression profile and fumonisin production by *Fusarium verticillioides* inoculated in *Bt* and non-*Bt* maize. Frontiers in Microbiology 6: 1–10.

Rose, L.J. 2016. Investigating maize inbred line response following infection by the mycotoxigenic fungus *Fusarium verticillioides*. PhD dissertation, University of Stellenbosch, Stellenbosch, South Africa, 210 pp.

Rychlik, M., Humpf, H.U., Marko, D., Dänicke, S., Mally, A., Berthiller, F., Klaffke, H. and Lorenz, N. 2014. Proposal of a comprehensive definition of modified and other forms of mycotoxins including “masked” mycotoxins. Mycotoxin Research 30: 197–205.

Samapundo, S., Devliehgere, F., Meulenaer, B.D.E. and Debevere, J. 2005. Effect of water activity and temperature on growth and the relationship between fumonisin production and the radial growth of *Fusarium verticillioides* and *Fusarium proliferatum* on Corn 68: 1054–1059.

Sampietro, D.A., Vattuone, M.A., Presello, D.A., Fauguel, C.M. and Catalán, C.A.N. 2009. The pericarp and its surface wax layer in maize kernels as resistance factors to fumonisin accumulation by *Fusarium verticillioides*. Crop Protection 28: 196–200.

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

Scauflaire, J., Gourgue, M. and Munaut, F. 2011. *Fusarium temperatum* sp nov from maize, an emergent species closely related to *Fusarium subglutinans*. Mycologia 103: 586–597.

Shapiro, S.S. and Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). Biometrika 52: 591-611.

Schoeman, A., Flett, B.C., Janse van Rensburg, B., 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.

Seefelder, W., Knecht, A. and Humpf, H.U. 2003. Bound fumonisin B1: Analysis of fumonisin-B1 glyco and amino acid conjugates by liquid chromatography-electrospray ionization-tandem mass spectrometry. Journal of Agricultural and Food Chemistry 51: 5567–5573.

Snedecor, G.W. and Cochran, W.G. 1980. Statistical Methods, 7th Edition. The Iowa State University Press, Ames.

Small, I.M., Flett, B.C., Marasas, W.F.O., Mcleod, A. and Viljoen, A. 2012. Use of resistance elicitors to reduce *Fusarium* ear rot and fumonisin accumulation in maize. Crop Protection 41: 10–16.

Stroshine. R.L. Kirleis. A.W. Tuite. J.F. Bauman. L.F. and Emam. A. 1986. Differences in grain quality among selected corn hybrids. Cereal Foods World 31: 311-316.

Subedi, S. 2016. A review on important maize diseases and their management in Nepal. Journal of Maize Research and Development 1: 28–52.

Thompson, M. and Raizada, M. 2018. Fungal pathogens of maize gaining free passage along the silk road. Pathogens 7: 81.

van Zyl, K. 2015. Resistance in maize to infection and toxin production. MSc Agric thesis, University of Stellenbosch, Stellenbosch, South Africa, 142 pp.

Voss, K.A., Howard, P.C., Riley, R.T., Sharma, R.P., Bucci, T.J. and Lorentzen, R.J. 2001. Carcinogenicity and mechanism of action of fumonisin B1: A Mycotoxin Produced by *Fusarium moniliforme* (= *F. verticillioides*). Cancer Detection and Prevention 26: 1–9.

Waes, J.G., Starr, L., Maddox, J., Aleman, F., Voss, K.A., Wilberding, J. and Riley, R.T. 2005. Maternal fumonisin exposure and risk for neural tube defects: Mechanisms in an *in vivo* Mouse Model. Birth Defects Research- Part A 73: 487–497.

Walley, J.W., Kliebenstein, D.J., Bostock, R.M. and Dehesh, K. 2013. Fatty acids and early detection of pathogens. Current Opinion in Plant Biology 16: 520–526.

Wang, E., Norred, W.P., Bacon, C.W., Riley, R.T. and Merrill, A.H. 1991. Inhibition of sphingolipid biosynthesis by fumonisins-implications for diseases associated with *Fusarium moniliforme* 266: 14486–14490.

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.

Warfield, C.Y. and Gilchrist, D.G. 1999. Influence of kernel age on fumonisin B1 Production in maize by *F. verticillioides*. Applied and Environmental Microbiology 65: 2853–2856.

Weaver, M.A., Abbas, H.K., Brewer, M.J., Pruter, L.S. and Little, N.S. 2017. Integration of biological control and transgenic insect protection for mitigation of mycotoxins in corn. Crop Protection 98: 108–115.

Zhang, Z., Nie, D., Fan, K., Yang, J., Guo, W., Meng, J., Zhao, Z. and Han, Z. 2019. A systematic review of plant-conjugated masked mycotoxins: Occurrence, Toxicology, and Metabolism. Critical Reviews in Food Science and Nutrition 60: 1523–1537.

Table 1. Characteristics of the maize inbred lines selected for structural trait evaluation in the 2018/2019 and 2019/20 growing season.

Inbred Line	Response to *F. verticillioides*[*]	Structural trait evaluated 2018/19	Structural trait evaluated 2019/20	Origin[*]	Colour*	Type*	Adaptation*	Resistance *	Protein content*
R2565y	Susceptible to FER/FUM	✓	✓	ARC-GC South Africa	Yellow	-	Mid-altitude	-	Normal
I-B	Resistant to FER only	✓	✓	ARC-GC South Africa	Yellow	-	-	-	Normal
CML 444	Resistant to FER/FUM	✓	✓	CIMMYT-Zimbabwe	White	Semi-dent	Mid-altitude and drought tolerant	Turcicum leaf blight Grey leaf spot	Normal
CB 222	Resistant to FUM only	✓		ARC-GC South Africa	White	-	Mid-altitude	-	Unknown
CML 390	Resistant to FER/FUM		✓	CIMMYT-Zimbabwe	White	Flint	Mid-altitude	Maize streak virus	Normal

FER- Fusarium ear rot; FUM- Fumonisins; ARC-GC- Agricultural Research Council-Grain Crops; CIMMYT- International Maize and Wheat Improvement Centre;[*]According to Small *et al*. (2012), Rose *et al*. (2016), Okoth *et al*. (2017) and Netshifhefhe *et al*. (2018).

Table 2. Inoculation and sampling times of maize at various kernel developmental stages used for physico-chemical analyses.

Inoculation Event 1: Seven days after pollination (Blister stage)			**Inoculation Event 2: Thirty-five days after pollination (Early dent stage)**		
Sampling time	**Days after inoculation**	**Kernel stage**	**Sampling time**	**Days after inoculation**	**Kernel stage**
S0	0	Blister (R2)	**S0**	0	Early dent (R5)
S7	7	Milk (R3)	**S7**	7	Dent (R5)
S28	28	Early dent (R5)	**S28**	14	Physiological maturity (R6)
S42	42	Late dent (R5)	**S42**	42	Biological maturity (R6)
S52	52	Physiological maturity (R6)	**S52**	52	Biological maturity

S- Sampling time after inoculation; R- Reproductive stage of kernel development.

Table 3. Means of structural traits evaluated in maize inbred lines grown in the 2018/19 and 2019/20 seasons.

Factor	2018/19 Season						2019/20 Season				
	CB 222	CML 444	CML 390	I-B	R2565y		CB 222	CML 444	CML 390	I-B	R2565y
*Mean silk length (cm)	17.8	10.9	-	9.8	12.8		-	13.6	13.6	12.0	9.5
*% Closed husks at anthesis	88	100	-	100	100		-	100	100	100	100
*% Closed husks at harvest	88	100	-	100	100		-	100	100	100	100
*% Ears with silk browning	0	0	-	28	44		-	0	88	16	64
*% Ears with detached	0	0	-	0	16		-	0	0	0	8

Percentage (%) and means represented by the average measurement of 25 randomly selected maize ears for each trait; Dash (-) indicates no data recorded for the specific line.

Table 4. Means of Fusarium ear rot (FER) disease severity, *Fusarium verticillioides* target DNA and total fumonisins in maize inbred lines following artificial inoculation of maize ears with *Fusarium verticillioides* at 7 (Inoculation Event 1) and 35 (Inoculation Event 2) days after pollination (dap).

Factor	INOCULATION EVENT 1 (7 dap)									INOCULATION EVENT 2 (35 dap)								
	Line	CB 222		CML 444		I-B		R2565y			CB 222		CML 444		I-B		R2565y	
	I/C	I	C	I	C	I	C	I	C		I	C	I	C	I	C	I	C
% FER disease severity	S0	-	0 .0 b	-	0.0 b	-	0.0 b	-	10.0 a	S0	-	1.0 a	-	1.0 a	-	1.0 a	-	1.0 a
	S7	0.0 b	3.3 b	3.3 b	0.0 b	0.0 b	0.0 b	6.7 b	48.3 a	S7*	0.0 a	0.0 a	1.0 a	3.3 a	1.7 a	0.0 a	0.0 a	3.3 a
	S28	10.0 c	6.7 cd	6.7 cd	0.0 d	6.7 cd	6.7 cd	76.7 a	50.0 b	S28	3.3 a	6.7 a	6.7 a	10.0 a	6.7 a	0.0 a	10.0 a	6.7 a
	S42*	21.7 c	10.0 cd	6.7 cd	0.0 c	21.7 c	6.7 cd	76.7 a	43.3 b	S42	48.3 a	3.3 c	10.0 bc	10.0 bc	21.7 b	0.0 c	21.7 b	10.0 bc
	S52*	20.0 bc	3.3 e	16.7 cd	6.7 de	30.0 b	3.3 e	70.0 a	60.0 a	S52*	38.3 a	6.7 c	6.7 c	3.3 c	35.0 ab	3.3 c	20.0 bc	3.3 c
Target DNA (ng µL^{-1}) ¥	S0	-	0.001 ab	-	0.002 a	-	0.002 ab	-	0.0 b	S0	-	0.004 b	-	0.047 a	-	0.013 b	-	0.000 c
	S7	0.018 ab	0.0 c	0.002 b	0.010 ab	0.003 ab	0.029 a	0.000 c	0.000 c	S7*	0.003 b-d	0.006 bc	0.160 a	0.121 ab	0.010 bc	0.015 a-c	0.000 d	0.002 cd
	S28*	0.042 ab	0.007 bc	0.009 a-c	0.003 bc	0.042 a	0.003 bc	0.009 bc	0.003 c	S28*	1.500 a	0.128 b	0.179 ab	1.200 ab	1.100 ab	0.302 ab	0.086 ab	0.076 b
	S42	0.044 bc	0.007 de	0.017 cd	0.239 ab	0.390 a	0.006 c-e	0.029 a-c	0.001 e	S42*	2.100 ab	0.323 bc	4.900 a	1.300 bc	2.100 ab	0.228 bc	0.121 c	0.013 d
	S52*	0.493 a	0.004 c	0.656 a	0.013 bc	3.5 a	0.020 bc	0.209 ab	0.029 bc	S52*	2.300 a	3.600 a	0.649 ab	0.066 c	3.400 a	0.474 bc	1.600 a	0.705 ab
Fumonisin (mg kg^{-1}) ¥	S0	-	0.5 ab	-	22.6 a	-	0.3 ab	-	0.1 b	S0	-	6.3 a	-	1.8 a	-	9.3 a	-	3.5 a
	S7*	0.4 bc	0.2 ab	12.8 a	6.5 ab	0.2 bc	0.2 c	0.0 bc	0.0 cd	S7*	0.1 c	0.3 bc	0.2 c	0.2 bc	6.3 a	0.7 bc	4.8 ab	0.1 c
	S28*	11.2 a	10.1 a	0.4 ab	0.1 b	0.5 ab	0.1 bc	0.1 bc	0.0 c	S28*	0.0 de	0.0 e	1.3 a	0.6 ab	0.1 a-c	0.1 b-d	0.1 b-d	0.1 c-e
	S42*	0.2 cd	0.3 bc	0.1 cd	0.2 bc	34.8 a	0.6 bc	1.1 ab	0.0 d	S42*	0.9 a	0.2 a	0.2 a	0.6 a	2.9 a	5.1 a	0.2 a	1.7 a
	S52*	0.4 bc	0.8 bc	0.1 c	0.4 c	76.1 a	8.8 a	52.2 a	2.8 ab	S52*	19.0 ab	1.1 ab	3.3 ab	2.3 b	1.0 ab	28.1 a	4.5 ab	1.2 ab

Means followed by the same alphabetical letter in each row are not significantly different according to Fisher's LSD (*P>0.05*); *****No significant line by treatment interaction; ¥ t-tests provided are according to log transformation of the means; S- Sampling time after inoculation; I- Fungal-inoculated; C- Water-inoculated.

Table 5. Means of physico-chemical traits evaluated in maize flour from inbred lines following artificial inoculation with *Fusarium verticillioides* at 7 (Inoculation Event 1) and 35 (Inoculation Event 2) days after pollination (dap).

Factor		INOCULATION EVENT 1 (7dap)									INOCULATION EVENT 2 (35 dap)							
	Line	CB 222		CML 444		I-B		R2565y			CB 222		CML 444		I-B		R2565y	
	I/C	I	C	I	C	I	C	I	C		I	C	I	C	I	C	I	C
% Moisture¥	S0	-	69.3 a	-	79.8 a	-	70.6 a	-	73.2 a	S0	-	42.4 ab	-	30.8 c	-	44.9 a	-	35.6 b
	S7*	70.3 a	72.9 a	53.4 b	47.1 b	64.7 a	56.0 ab	63.3 a	66.2 a	S7*	33.7 ab	36.3 ab	29.5 b	39.9 ab	36.1 ab	36.7 ab	38.9 a	33.6 ab
	S28*	42.2 ab	39.6 ab	32.2 b	41.3 ab	47.0 a	40.6 ab	44.4 a	52.8 a	S28*	23.7 b	26.9 ab	23.8 ab	25.0 ab	22.7 b	27.0 ab	26.9 ab	33.5 a
	S42*	34.3 a	33.0 ab	35.7 a	32.0 ab	28.3 b	33.0 ab	30.0 ab	35.7 a	S42*	17.0 dc	20.4 bc	10.9 e	15.8 d	21.8 bc	20.4 bc	24.7 ab	30.9 a
	S52	19.7 b	28.2 a	31.8 a	28.6 a	25.3 b	29.1 a	29.3 a	26.7 a	S52	14.4 b	13.4 b	23.2 a	13.6 b	19.7 a	20.7 a	24.6 a	22.0 a
Average pH	S0	-	6.9 a	-	6.8 a	-	6.8 a	-	6.6 a	S0	-	6.2 a	-	6.0 a	-	5.6 a	-	5.9 a
	S7*	6.6 ab	6.8 ab	6.6 ab	6.4 b	6.8 ab	6.7 ab	6.9 ab	7.1 a	S7*	5.8 bc	6.1 a	5.8 b-d	5.7 c	5.8 c	5.7 c	6.0 ab	5.8 bc
	S28*	6.1 ab	6.2 ab	6.2 ab	6.1 ab	5.8 ab	5.5 b	6.3 a	5.5 b	S28	6.2 a	6.2 a	5.7 d	5.9 b-d	6.0 a-c	6.1 ab	5.8 cd	4.8 e
	S42*	6.1 ab	6.2 ab	6.1 ab	6.0 ab	6.1 ab	5.9 b	6.0 ab	6.1 ab	S42*	6.4 a	5.9 ab	5.8 b	5.8 b	6.1 ab	6.2 ab	6.1 ab	6.1 ab
	S52*	6.3 a	6.4 a	6.1 bc	6.1 bc	6.2 ab	6.1 bc	5.9 cd	6.1 d	S52*	6.4 b	6.4 b	5.9 a	6.0 b	6.2 a	6.2 ab	6.1 a	6.2 a
%Carbon¥	S0	-	20.9 a	-	8.7 a	-	17.4 a	-	21.3 a	S0	-	25.5 b	-	31.4 a	-	27.4 b	-	30.9 a
	S7*	16.4 c-e	12.9 e	26.9 ab	33.9 a	19.9 b-d	23.4 a-c	15.4 de	17.1 c-e	S7*	33.1 ab	33.3 ab	33.9 a	33.0 ab	30.4 bc	32.2 a-c	29.6 c	29.4 c
	S28*	33.2 ab	30.0 bc	33.5 a	30.4 a-c	31.2 ab	31.3 ab	27.5 cd	26.5 d	S28*	38.5 b	36.0 c	40.9 a	40.6 ab	34.6 c	34.8 c	34.9 c	31.9 d
	S42*	32.3 a	32.4 a	31.4 a	33.1 a	33.4 a	31.1 a	31.9 a	31.8 a	S42	41.2 ab	39.7 a-c	41.9 a	39.3 a-c	32.8 d	38.3 bc	36.9 c	32.6 d
	S52*	37.4 a	33.9 a	35.6 a	35.0 a	35.2 a	36.2 a	34.7 a	34.6 a	S52*	42.5 a	42.5 a	37.9 bc	41.1 ab	37.2 c	38.5 bc	35.4 c	36.8 c

Factor	INOCULATION EVENT 1 (7dap)									INOCULATION EVENT 2 (35 dap)								
	Line	CB 222		CML 444		I-B		R2565y			CB 222		CML 444		I-B		R2565y	
	I/C	I	C	I	C	I	C	I	C		I	C	I	C	I	C	I	C
% Nitrogen	S0	-	1.1 ab	-	0.6 b	-	1.1 a	-	1.0 ab	S0	-	1.2 a	-	1.4 a	-	1.2 a	-	1.4 a
	S7	1.0 cd	0.657 e	1.3 ab	1.6 a	1.0 cd	1.3 bc	1.0 cd	0.943 de	S7*	1.3 b	1.6 a	1.6 a	1.5 ab	1.2 b	1.5 ab	1.3 b	1.4 ab
	S28*	1.5 a-c	1.5 a-d	1.3 cd	1.5 a-d	1.7 a	1.6 ab	1.4 c-e	1.3 d	S28*	1.8 a-c	2.0 ab	1.9 a-c	2.2 a	1.5 cd	1.7 bc	1.3 d	1.2 d
	S42*	1.5 b	1.4 b	1.5 b	1.5 b	1.8 a	1.5 a	1.5 b	1.4 b	S42*	2.0 a	1.8 ab	1.9 a	1.8 ab	1.4 c	1.6 bc	1.5 c	1.4 c
	S52*	1.7 ab	1.8 a	1.7 ab	1.6 ab	1.5 ab	1.7 ab	1.5 b	1.7 ab	S52*	2.1 a	1.9 ab	1.7 b-d	1.6 b-d	1.5 cd	1.7 a-c	1.4 d	1.6 b-d
Carbon:Nitrogen	S0	-	18.4 a	-	14.5 a	-	15.3 a	-	21.7 a	S0	-	21.8 a	-	22.7 a	-	23.1 a	-	22.1 a
	S7*	15.8 ab	19.7 ab	20.0 ab	21.3 a	20.5 ab	18.5 ab	14.9 ab	18.1 ab	S7*	26.9 a	20.9 b	21.4 ab	22.6 ab	24.6 ab	21.3 b	22.9 ab	20.7 b
	S28	21.5 b	20.5 b	25.1 a	20.3 b	18.7 b	19.9 b	19.6 b	20.1 b	S28*	21.6 bc	18.4 c	21.6 bc	18.9 bc	23.4 ab	20.8 bc	27.2 a	26.9 a
	S42*	22.2 ab	22.8 a	21.6 ab	22.3 ab	19.0 b	21.0 ab	21.6 ab	22.6 a	S42*	21.1 c	22.0 bc	22.0 bc	21.5 c	24.3 ab	24.2 ab	25.0 a	24.3 ab
	S52*	22.6 ab	19.4 b	20.3 b	22.2 ab	23.9 a	21.6 ab	23.9 a	20.3 b	S52*	20.8 b	22.4 ab	23.0 ab	25.7 a	25.6 a	22.3 ab	25.4 a	22.8 ab
% Amylose	S0	-	51.9 b	-	64.5 a	-	55.3 b	-	56.9 b	S0	-	43.3 a	-	47.1 a	-	48.5 a	-	44.7 a
	S7	51.4 cd	62.6 a	45.2 d	44.7 d	53.4 bc	53.2 bc	62.4 a	58.5 ab	S7*	40.3 b	42.8 b	44.7 ab	48.8 a	46.0 a	48.8 a	44.0 ab	44.3 ab
	S28*	41.3 bc	50.4 a	36.7 c	45.0 ab	42.9 ab	47.3 bc	48.2 ab	47.0 ab	S28*	43.9 a	41.1 a	42.2 a	43.7 a	45.4 a	43.8 a	43.5 a	40.4 a
	S42*	42.4 a	45.8 a	43.1 a	41.9 a	44.8 a	39.3 a	46.2 a	39.7 a	S42	46.6 ab	39.4 c	46.6 ab	47.3 a	50.0 a	45.3 a-c	41.0 cd	42.4 b-d
	S52	43.0 b	44.2 b	44.7 ab	41.5 b	42.7 b	48.1 a	43.1 b	43.2 b	S52*	40.5 bc	37.6 c	45.4 ab	48.4 a	41.9 a-c	43.6 a-c	42.7 a-c	49.3 a

Factor	INOCULATION EVENT 1 (7dap)									INOCULATION EVENT 2 (35 dap)								
Line	CB 222		CML 444		I-B		R2565y			CB 222		CML 444		I-B		R2565y		
I/C	I	C	I	C	I	C	I	C		I	C	I	C	I	C	I	C	
% Amylopectin																		
S0	-	48.1 a	-	35.5 b	-	44.7 a	-	43.1 a	S0	-	56.7 a	-	52.9 a	-	51.5 a	-	55.3 a	
S7	48.5 ab	37.4 d	54.8 a	55.3 a	46.6 bc	46.8 bc	37.6 d	41.5 d	S7*	59.7 a	57.2 a	55.3 ab	51.2 b	54.0 ab	51.2 b	56.0 ab	55.7 ab	
S28*	58.7 ab	49.6 c	63.3 a	55.0 bc	57.1 ab	52.7 bc	51.8 bc	53.0 bc	S28*	56.1 a	58.9 a	57.8 a	56.3 a	54.6 a	56.2 a	56.5 a	59.6 a	
S42*	57.6 a	54.2 a	56.9 a	58.1 a	55.2 a	60.7 a	53.8 a	60.3 a	S42	53.4 cd	60.6 a	53.4 cd	52.7 d	50.0 d	54.7 b-d	58.9 ab	57.6 a-c	
S52	57.0 a	55.8 a	55.3 a	58.5 a	57.3 a	51.9 b	56.9 a	56.8 a	S52*	59.5 ab	62.4 a	54.6 bc	51.6 c	58.1 a-c	56.4 a-c	57.3 a-c	50.7 c	

Means followed by the same alphabetical letter in each row are not significantly different according to Fisher's LSD (*P > 0.05*); *****No significant line by treatment interaction;

[¥] t-tests provided according to log transformation of the means; S- Sampling time after inoculation; I- Fungus-inoculated; C- Water-inoculated.

Table 6. Mean fatty acids evaluated in maize flour from maize inbred lines following artificial inoculation with *Fusarium verticillioides* at 7 (Inoculation Event 1) and 35 (Inoculation Event 2) days after pollination (dap).

Factor	INOCULATION EVENT 1 (7dap)									INOCULATION EVENT 2 (35 dap)								
Line	**CB 222**		**CML 444**		**I-B**		**R2565y**			**CB 222**		**CML 444**		**I-B**		**R2565y**	
I/C	I	C	I	C	I	C	I	C		I	C	I	C	I	C	I	C
Palmitic acid (C16:0) (mg g^{-1})¥																	
S0	-	7.1 ab	-	13.9 a	-	4.2 b	-	12.4 ab	**S0**	-	1.4 a	-	0.8 a	-	1.0 a	-	1.5 b
S7*	4.4 b	5.8 ab	12.8 a	6.6 ab	7.5 ab	4.9 b	10.5 a	7.7 ab	**S7***	6.8 a	6.9 a	5.4 a	5.1 a	4.9 a	7.8 a	2.5 b	1.3 c
S28*	2.7 ab	0.7 ab	0.8 ab	1.8 a	1.9 b	3.4 ab	2.6 ab	3.7 a	**S28***	5.1 a	4.5 a	4.8 a	2.2 b	4.5 a	4.2 a	1.5 b	1.6 b
S42*	3.7 bc	2.3 cd	10.1 a	8.0 ab	1.6 d	1.4 d	2.0 cd	3.3 cd	**S42***	4.5 ab	4.2 ab	3.8 ab	4.8 a	5.8 a	4.1 ab	4.7 ab	2.3 b
S52*	0.9 d	1.1 cd	7.5 ab	11.1 a	1.9 cd	4.2 a-c	4.8 b-d	1.1 d	**S52***	5.8 a	5.5 a	5.0 a	6.0 a	4.2 a	6.7 a	6.7 a	4.7 a
Oleic acid (C18:1n9) (mg g^{-1})¥																	
S0	-	2.4 ab	-	1.4 ab	-	0.7 b	-	9.2 a	**S0**	-	0.1 a	-	0.1 a	-	0.1 a	-	0.2 a
S7*	1.3 b-d	1.5 b-c	1.6 cd	0.6 d	5.0 a	2.9 a-c	3.1 ab	2.8 a-c	**S7**	0.8 a-d	0.9 a-c	0.7 b-d	0.4 cd	1.0 ab	1.7 ab	2.0 a	0.4 d
S28*	1.3 bc	1.7 ab	0.4 d	0.5 d	0.9 cd	3.4 c	2.7 ab	4.2 a	**S28***	0.8 a	0.6 a	0.6 a	0.3 b	0.9 a	0.8 a	0.9 a	0.7 a
S42	2.1 a	1.5 a-c	0.5 c	2.2 a	0.9 a-c	0.5 bc	1.2 a-c	1.7 ab	**S42**	0.7 bc	0.6 c	0.6 c	0.8 bc	1.6 ab	1.1 ab	2.0 a	0.6 c
S52*	0.4 b	0.4 b	1.4 ab	2.1 a	1.3 ab	1.4 ab	4.3 a	0.5 b	**S52***	1.0 bc	0.9 bc	0.7 bc	0.9 bc	1.2 a-c	1.1 bc	2.1 a	1.5 ab
Stearic acid (C18:0) (mg g^{-1})¥																	
S0	-	0.2 a	-	0.3 a	-	0.1 a	-	0.3 a	**S0**	-	0.1 ab	-	0.1 ab	-	0.2 a	-	0.1 ab
S7*	0.1 c	0.2 a-c	0.3 bc	0.2 ab	0.3 ab	0.2 a-c	0.2 a-c	0.2 a-c	**S7**	0.2 b	0.2 b	0.1 bc	0.1 bc	0.2 ab	0.3 a	0.2 b	0.1 bc
S28*	0.2 ab	0.2 ab	0.1 b	0.1 b	0.1 b	0.3 a	0.1 b	0.3 a	**S28***	0.1 a	0.1 ab	0.1 a	0.1 a	0.1 a	0.1 a	0.1 a	0.1 a
S42*	0.2 a-c	0.1 bc	0.3 ab	0.4 a	0.2 a-c	0.1 c	0.1 bc	0.2 bc	**S42**	0.1 a-c	0.1 bc	0.1 bc	0.1 a-c	0.2 ab	0.1 a-c	0.2 a	0.1 c
S52*	0.1 b	0.1 b	0.2 ab	0.3 a	0.2 ab	0.2 ab	0.3 ab	0.8 b	**S52***	0.2 bc	0.2 bc	0.1 c	0.1 bc	0.2 bc	0.2 a-c	0.4 a	0.2 ab

Factor	INOCULATION EVENT 1 (7dap)									INOCULATION EVENT 2 (35 dap)								
Line	**CB 222**		**CML 444**		**I-B**		**R2565y**			**CB 222**		**CML 444**		**I-B**		**R2565y**	
I/C	I	C	I	C	I	C	I	C		I	C	I	C	I	C	I	C
Linoleic acid (C18:2n6) (mg g^{-1}) ¥*																	
S0	-	6.7 ab	-	1.8 b	-	3.5 ab	-	17.3 a	**S0**	-	1.4 a	-	0.8 a	-	1.0 a	-	1.5 a
S7*	3.7 bc	4.6 ab	1.9 cd	1.1 d	7.8 ab	5.9 ab	9.3 a	6.0 ab	**S7***	1.5 a	1.4 a	0.8 b	0.9 b	1.5 a	2.1 a	2.9 a	1.5 a
S28*	4.8 ab	3.8 ab	0.7 c	1.0 c	2.5 bc	5.8 ab	5.5 ab	6.0 a	**S28***	1.5 a	1.0 a	1.1 a	0.5 b	1.2 a	1.10 a	1.8 a	1.84 a
S42*	4.2 a	3.0 ab	2.2 ab	2.4 ab	1.4 b	1.3 b	1.6 b	3.9 ab	**S42***	1.6 a	1.3 a	1.2 a	0.7 b	1.9 a	1.2 a	2.3 a	0.7 b
S52*	1.2 a	1.1 a	1.7 a	2.0 a	2.1 a	2.9 a	5.9 a	1.1 a	**S52***	2.1 a	2.0 a	1.4 a	1.9 a	1.4 a	1.9 a	2.1 a	1.5 a
Linolenic acid (C18:3n3) (mg g^{-1}) ¥*																	
S0	-	0.8 a	-	0.3 a	-	3.5 a	-	0.8 a	**S0**	-	0.1 a	-	0.1 a	-	0.1 a	-	0.2 a
S7*	0.4 b	0.5 b	0.2 c	0.1 c	0.5 b	0.7 b	1.9 a	0.6 b	**S7***	0.2 ab	0.1 a-c	0.1 bc	0.1 c	0.1 bc	0.1 a-c	0.2 a	0.2 a-c
S28	0.9 a	0.4 ab	0.1 c	0.1 c	0.2 bc	0.4 ab	0.3 a-c	0.2 a-c	**S28***	0.2 ab	0.1 ab	0.1 b	0.0 c	0.1 ab	0.1 ab	0.2 ab	0.2 a
S42*	0.3 a	0.2 ab	0.1 cd	0.1 cd	0.1 cd	0.1 b-d	0.1 d	0.2 a-c	**S42**	0.2 a	0.2 a	0.1 ab	0.1 a	0.1 ab	0.1 bc	0.2 a	0.1 c
S52*	0.2 a	0.2 a	0.1 a	0.1 a	0.2 ab	0.3 a	0.4 a	0.2 a	**S52***	0.2 a	0.2 a	0.1 ab	0.2 a	0.1 ab	0.2 a	0.1 a	0.1 a

Means followed by the same alphabetical letter in each row are not significantly different according to Fisher's LSD (*P > 0.05*); *****No significant line by treatment interaction; ¥ t-tests provided according to log transformation of the means; S- Sampling time after inoculation; I- Fungus-inoculated; C- Water-inoculated.

Table 7. Pearson's correlation matrix of maize grain physico-chemical properties with Fusarium ear rot (FER) disease severity, *Fusarium verticillioides* target DNA content and total, free fumonisins at 7 (Inoculation Event 1) and 35 (Inoculation Event 2) days after pollination (dap).

INOCULATION EVENT 1 (7 dap)						
Variable	**FER disease severity (I)**	**FER disease severity (C)**	**Target DNA ¥ (I)**	**Target DNA ¥ (C)**	**Free fumonisins ¥ (I)**	**Free Fumonisins ¥ (C)**
Palmitic acid¥	**-0,310**	-0,186	**-0,499**	-0,211	-0,275	-0,176
P-value	0,026	0,150	0,000	0,105	0,058	0,179
Oleic acid¥	0,185	0,087	**-0,308**	-0,218	-0,090	**-0,340**
P-value	0,236	0,486	**0,033**	0,094	0,545	**0,008**
Stearic acid¥	-0,134	-0,150	-0,190	-0,061	-0,027	-0,155
P-value	0,331	0,261	0,197	0,643	0,857	0,236
Linoleic acid¥	0,009	0,077	**-0,393**	**-0,431**	-0,280	-0,216
P-value	0,994	0,542	0,006	0,001	0,054	0,098
linolenic acid¥	-0,224	-0,080	**-0,388**	**-0,407**	**-0,308**	-0,152
P-value	0,120	0,587	0,006	0,001	0,033	0,246
Moisture¥	**-0,373**	-0,131	**-0,709**	**-0,599**	**-0,306**	-0,224
P-value	0,005	0,278	< 0.0001	< 0.0001	0,034	0,085
pH	**-0,334**	-0,179	**-0,516**	**-0,356**	**-0,292**	0,050
P-value	0,018	0,168	0,000	0,005	0,044	0,707
Carbon¥	**0,325**	0,096	**0,658**	**0,455**	**0,393**	0,078
P-value	0,017	0,433	< 0.0001	0,000	0,006	0,553
Nitrogen	0,210	0,033	**0,638**	**0,506**	0,222	0,202
P-value	0,118	0,809	< 0.0001	< 0.0001	0,129	0,122
C/N	0,240	0,097	**0,347**	0,168	**0,358**	-0,044
P-value	0,093	0,394	0,016	0,200	0,013	0,739
Amylose	-0,117	-0,155	**-0,486**	**-0,435**	**-0,374**	0,019
P-value	0,383	0,224	0,000	0,001	0,009	0,884
Amylopectin	0,117	0,155	**0,486**	**0,435**	**0,374**	-0,019
P-value	0,383	0,224	0,000	0,001	0,009	0,884

INOCULATION EVENT 2 (35 dap)						
Variable	**FER disease severity (I)**	**FER disease severity (C)**	**Target DNA ¥ (I)**	**Target DNA ¥ (C)**	**Free fumonisins ¥ (I)**	**Free Fumonisins ¥ (C)**
Palmitic acid¥	0,108	-0,204	0,187	**0,263**	0,204	0,122
P-value	0,457	0,118	0,204	0,042	0,164	0,354
Oleic acid¥	0,118	-0,233	-0,149	0,179	0,273	0,133
P-value	0,319	0,073	0,313	0,172	0,060	0,311
Stearic acid¥	0,079	**-0,268**	-0,156	0,087	0,268	0,133
P-value	0,458	0,039	0,289	0,509	0,066	0,313
Linoleic acid¥	0,194	-0,070	-0,119	-0,148	0,114	-0,041
P-value	0,148	0,594	0,420	0,259	0,439	0,754
linolenic acid¥	0,116	-0,080	-0,179	0,036	-0,188	-0,084
P-value	0,458	0,542	0,224	0,870	0,201	0,358
Moisture¥	**-0,479**	-0,243	**-0,771**	0,022	0,071	-0,121
P-value	0,000	0,061	< 0.0001	< 0.0001	0,632	0,918
pH	**0,545**	-0,079	**0,336**	**-0,695**	0,078	0,014
P-value	< 0.0001	0,551	0,020	0,151	0,599	0,255
Carbon¥	**0,445**	**0,299**	**0,663**	0,187	-0,102	0,149
P-value	0,001	0,020	< 0.0001	< 0.0001	0,434	0,738
Nitrogen	**0,298**	0,248	**0,673**	**0,695**	-0,116	-0,044
P-value	0,038	0,056	0,000	< 0.0001	0,358	0,691
C/N	-0,071	-0,048	**0,526**	**0,547**	-0,136	-0,052
P-value	0,657	0,717	0,131	0,801	0,754	0,935
Amylose	0,020	-0,143	-0,221	-0,033	0,046	-0,011
P-value	0,897	0,275	0,617	0,769	0,985	0,319
Amylopectin	-0,020	0,143	0,074	-0,039	0,003	0,131
P-value	0,897	0,275	0,617	0,769	0,985	0,319

Dap- Days after pollination; I- Fungus-inoculated; C- Water-inoculated; **Bold-** Significant *P*-value with 95% confidence level; ¥Correlations provided according to log transformed mean values; C/N- Ratio of carbon to nitrogen; **Free fumonisins**=FB_1+FB_2+FB_3.

Table 8. Means of total free fumonisins, free hydrolysed fumonisins and hidden fumonisins in four maize inbred lines following inoculation of maize ears with *Fusarium verticillioides* at 7 (Inoculation Event 1) and 35 (Inoculation Event 2) days after pollination (dap).

Factor		INOCULATION EVENT 1 (7 dap)									INOCULATION EVENT 2 (35 dap)							
	Line	CB 222		CML 444		I-B		R2565y			CB 222		CML 444		I-B		R2565y	
	I/C	I	C	I	C	I	C	I	C		I	C	I	C	I	C	I	C
Total free fumonisins (mg kg^{-1}) ¥	S0	-	0.5 ab	-	22.6 a	-	0.3 ab	-	0.1 b	S0	-	6.3 a	-	1.8 a	-	9.3 a	-	3.5 a
	S7*	0.4 bc	0.2 ab	12.8 a	6.5 ab	0.2 bc	0.2 c	0.0 bc	0.0 d	S7*	0.1 c	0.3 bc	0.2 c	0.2 bc	6.3 a	0.7 bc	4.8 ab	0.1 c
	S28*	11.2 a	10.1 a	0.4 ab	0.1 b	0.5 ab	0.1 bc	0.1 bc	0.0 c	S28*	0.0 de	0.0 e	1.3 a	0.6 ab	0.1 a-c	0.1 b-d	0.1 b-d	0.1 c-e
	S42*	0.2 cd	0.3 bc	0.1 cd	0.2 bc	34.8 a	0.6 bc	1.1 ab	0.0 d	S42*	0.9 a	0.2 a	0.2 a	0.6 a	2.9 a	5.1 a	0.2 a	1.7 a
	S52*	0.4 bc	0.8 bc	0.1 c	0.4 c	76.1 a	8.8 a	52.2 a	2.8 ab	S52*	19.0 ab	1.1 ab	3.3 ab	2.3 b	1.0 ab	28.1 a	4.5 ab	1.2 ab
Free hydrolysed fumonisins (mg kg^{-1}) ¥	S0	-	0.0009 a	-	0.0027 a	-	0.0005 a	-	0.0009 a	S0	-	0.000 a	-	0.000 a	-	0.000 a	-	0.0018 a
	S7 *	0.0005 a	0.0203 a	0.0000 a	0.0068 a	0.0012 a	0.0002 a	0.0004 a	0.0006 a	S7 *	0.0000 b	0.000 b	0.0006 b	0.0002 b	0.0009 b	0.0000 b	0.0078 a	0.0015 b
	S28 *	0.0052 a	0.0007 a	0.0002 a	0.0001 a	0.0003 a	0.0002 a	0.0000 a	0.0004 a	S28 *	0.0031 a	0.0000 a	0.0006 a	0.0009 a	0.0074 a	0.0001 a	0.0008 a	0.0000 a
	S42 *	0.0007 ab	0.000 b	0. 000 b	0.0003 ab	0.0080 a	0.0007 ab	0.0003 ab	0.0003 ab	S42 *	0.0113 a	0.0001 c	0.0062 ab	0.0028 a-c	0.0194 a-c	0.0006 a-c	0.0029 a-c	0.0007 bc
	S52 *	0.0008 b	0.0007 b	0.0016 ab	0.0000 ab	0.0425 b	0.0014 ab	0.0231 a	0.0005 b	S52 *	0.0397 a	0.0107 a	0.0129 a	0.0001 c	0.0078 ab	0.0010 c	0.0191 a	0.0019 bc
Hidden fumonisins (mg kg^{-1}) ¥	S0	-	0.0 a	-	0.0 a	-	0.0 a	-	0.0 a	S0	-	0.0 a	-	0.0 a	-	0.0 a	-	0.0 a
	S7*	0.0 a	0.1 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.5 a	S7*	0.0 a	0.0 a	0.2 a	0.0 a	0.0 a	0.0 a	0.4 a	0.2 a
	S28*	0.0 a	0.8 a	0.0 a	0.4 a	0.4 a	1.3 a	0.0 a	0.0 a	S28*	0.3 a	0.0 a	0.2 a	1.5 a	1.1 a	0.0 a	0.3 a	1.7 a
	S42*	1.9 ab	0.0 b	0.5 ab	0.1 ab	16.8 a	0.0 b	0.5 ab	0.0 b	S42*	8.9 a	0.3 a	1.3 a	5.0 a	11.4 a	0.1 a	3.9 a	2.1 a
	S52*	4.0 ab	0.0 b	1.9 b	0.2 b	95.0 ab	0.1 b	63.5 a	3.5 ab	S52*	6.6 bc	5.7 ab	4.3 ab	0.0 c	3.7 bc	0.0 c	25.0 a	3.4 bc

Means followed by the same alphabetical letter in each row are not significantly different according to Fisher's LSD (*P>0.05*);*No significant line by treatment interaction; ¥t-tests provided according to log transformation of the means; S- Sampling time after inoculation; I- Fungal-inoculated; C- Water-inoculated; **Total free fumonisins** = FB_1+FB_2+FB_3; **Free hydrolysed fumonisins** = HFB_1 present before hydrolysis detected using standard fumonisin extraction and hydrolysed fumonisin B_1 analytical standards; **Hidden fumonisins** = Total hydrolysed FB_1-Free FB_1 hydrolysed fumonisin B_1 analytical standards.

Table 9. Pearson's correlation matrix of free hydrolysed and hidden fumonisins with Fusarium ear rot (FER) disease severity, *Fusarium verticillioides* target DNA and total free fumonisins at 7 (Inoculation Event 1) and 35 (Inoculation Event 2) days after pollination (dap).

INOCULATION EVENT 1 (7 dap)				
Variable	**Free, hydrolysed fumonisins¥ (I)**	**Free, hydrolysed fumonisins¥ (C)**	**Hidden fumonisins¥ (I)**	**Hidden fumonisins¥ (C)**
FER disease severity	0,232	**0,390**	0,220	**0,418**
P-value	0,245	0,359	0,390	0,001
Target DNA¥	**0,367**	-0,053	**0,575**	0,254
P-value	0,010	0,687	0,001	0,050
Total free fumonisins¥	**0,464**	0,161	**0,531**	0,000
P-value	0,001	0,220	< 0.0001	0,998

INOCULATION EVENT 2 (35 dap)				
Variable	**Free, hydrolysed fumonisins¥ (I)**	**Free, hydrolysed fumonisins¥ (C)**	**Hidden fumonisins¥ (I)**	**Hidden fumonisins¥ (C)**
FER disease severity	**0,450**	0,200	**0,298**	0,206
P-value	0,481	0,604	0,014	0,113
Target DNA¥	**0,537**	**0,372**	**0,497**	**0,457**
P-value	0,449	0,003	0,000	0,000
Total free fumonisins¥	0,193	**0,460**	0,098	**0,968**
P-value	0,287	0,079	0,505	0,709

Dap- Days after pollination; I- Fungus-inoculated; C- Water-inoculated; **Bold-** Significant *P*-value with 95% confidence level; ¥Correlations provided according to log transformed mean values; **Total free fumonisins** = $FB_1 + FB_2 + FB_3$; **Free hydrolysed fumonisins** = HFB_1 present before hydrolysis detected using standard fumonisin extraction and hydrolysed fumonisin B_1 analytical standards; **Hidden fumonisins** = Total hydrolysed FB_1-Free FB_1 and hydrolysed fumonisin B_1 analytical standards.

Table 10. Pearson's correlation matrix of fatty acids and starch with free hydrolysed and hidden fumonisins at 7 (Inoculation Event 1) and 35 (Inoculation Event 2) days after pollination (dap).

INOCULATION EVENT 1 (7 dap)				
Variable	**Free hydrolysed fumonisins¥ (I)**	**Free hydrolysed fumonisins¥ (C)**	**Hidden fumonisins¥ (I)**	**Hidden fumonisins¥ (C)**
Palmitic acid¥	-0,099	-0,104	-0,266	**-0,299**
P-value	0,502	0,428	0,020	0,960
Oleic acid¥	0,248	-0,231	0,086	-0,175
P-value	0,089	0,076	0,181	0,279
Stearic acid¥	0,138	-0,212	0,038	-0,231
P-value	0,350	0,104	0,076	0,387
Linoleic acid¥	0,134	-0,108	-0,060	-0,246
P-value	0,365	0,413	0,059	0,063
linolenic acid¥	0,105	0,046	-0,121	-0,206
P-value	0,476	0,726	0,115	0,899
Amylose	-0,091	0,194	-0,216	-0,086
P-value	0,539	0,137	0,512	0,035
Amylopectin	0,091	-0,194	0,216	0,086
P-value	0,539	0,137	0,512	0,035

INOCULATION EVENT 2 (35 dap)				
Variable	**Free hydrolysed fumonisins¥ (I)**	**Free hydrolysed fumonisins¥ (C)**	**Hidden fumonisins¥ (I)**	**Hidden fumonisins¥ (C)**
Palmitic acid¥	0,027	-0,011	0,196	-0,007
P-value	0,027	0,529	0,182	0,960
Oleic acid¥	0,165	0,105	**0,351**	0,142
P-value	0,165	0,728	0,015	0,279
Stearic acid¥	0,131	0,070	**0,402**	0,114
P-value	0,131	0,911	0,005	0,387
Linoleic acid¥	0,108	**-0,262**	0,228	-0,242
P-value	0,108	0,227	0,120	0,063
linolenic acid¥	-0,102	-0,052	-0,159	-0,017
P-value	-0,102	0,807	0,279	0,899
Amylose	-0,048	**-0,265**	-0,077	**-0,272**
P-value	-0,048	0,646	0,602	0,035
Amylopectin	0,048	**0,265**	0,077	**0,272**
P-value	-0,048	0,646	0,602	0,035

Dap- Days after pollination; I- Fungus-inoculated; C- Water-inoculated; **Bold-** Significant *P*-value with 95% confidence level; ¥Correlations provided according to log transformed mean values; **Total free fumonisins** = $FB_1 + FB_2 + FB_3$; **Free hydrolysed fumonisins** = HFB_1 present before hydrolysis detected using standard fumonisin extraction and hydrolysed fumonisin B_1 analytical standards; **Hidden fumonisins** = Total hydrolysed FB_1-Free FB_1 and hydrolysed fumonisin B_1 analytical standards.

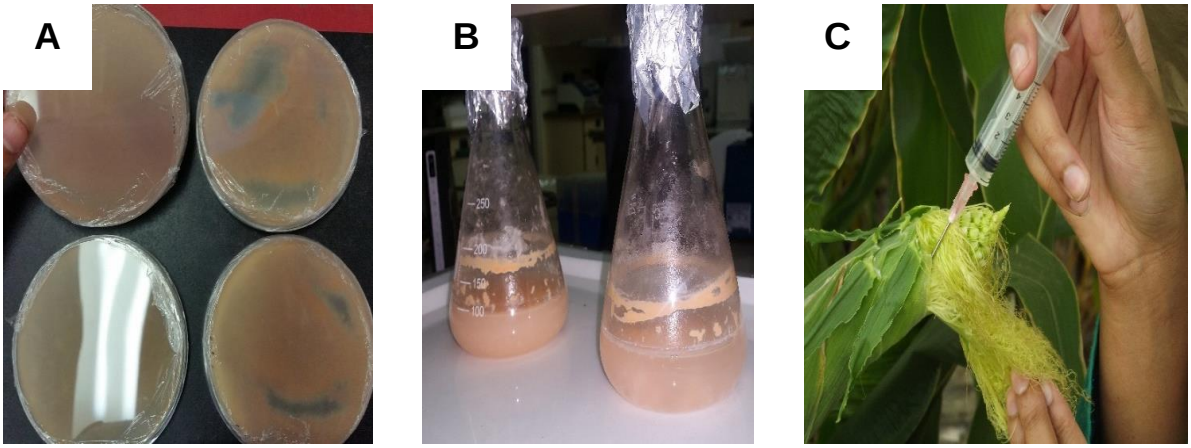

Figure 1. The process leading up to artificial inoculation of maize ears with *Fusarium verticillioides*. **A**: Growth of *F. verticillioides* MRC 826 on potato dextrose agar, **B**: *Fusarium verticillioides* after being transferred to Armstrong media and continuous shaking at room temperature, **C**: Artificial inoculation of the primary maize ear with the adjusted spore suspension using a needle and syringe.

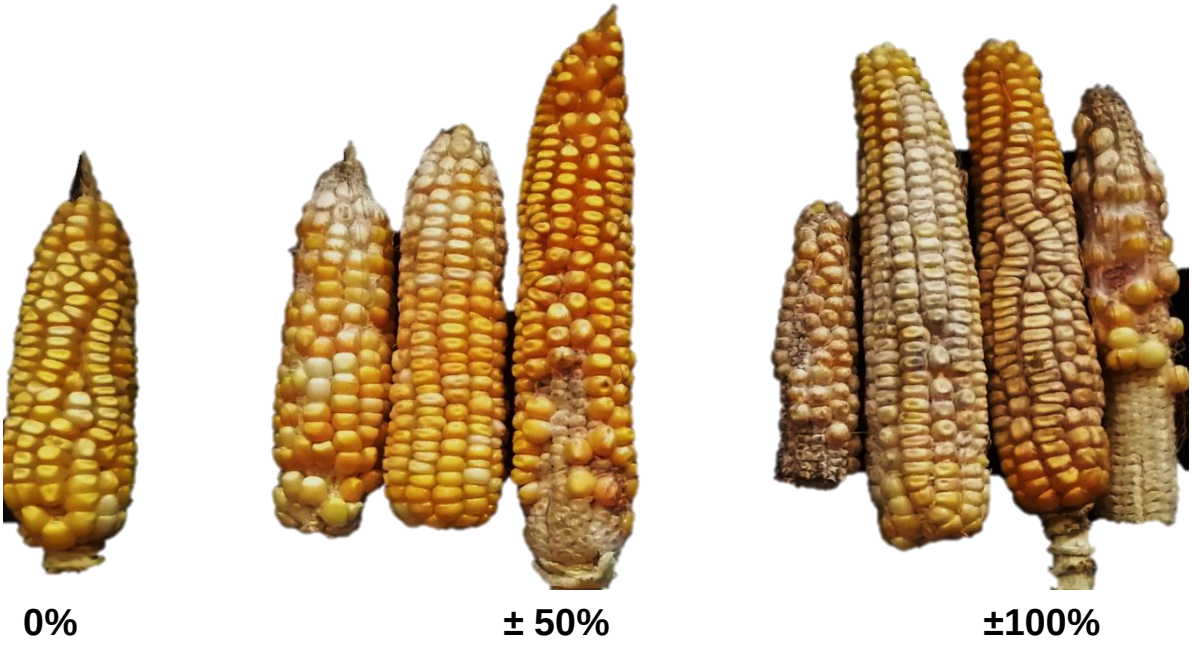

Figure 2. Scale used for the visual rating of Fusarium ear rot severity of the maize ears at harvest.

Figure 3. Types of insect-feeding damage recorded in 2018/2019 tunnel trial.

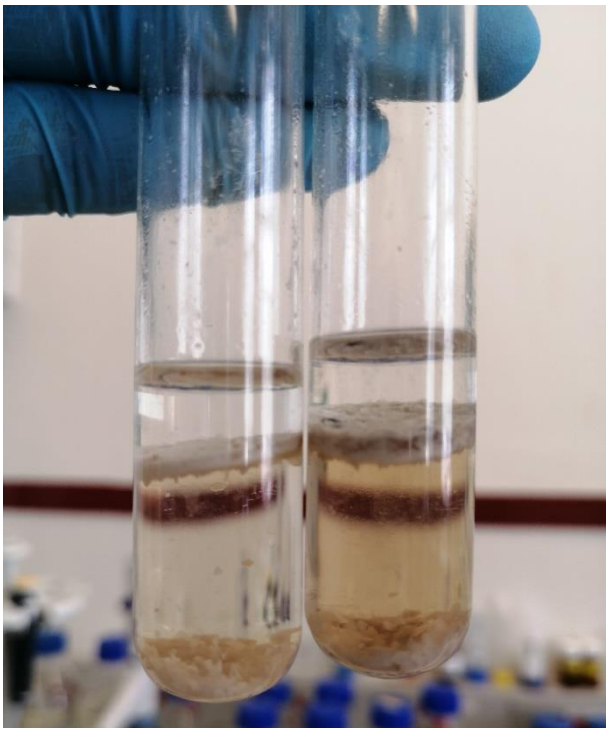

Figure 4. Fatty acid separation layer resulting in an upper-hexane phase used for analysis by gas-chromatography.

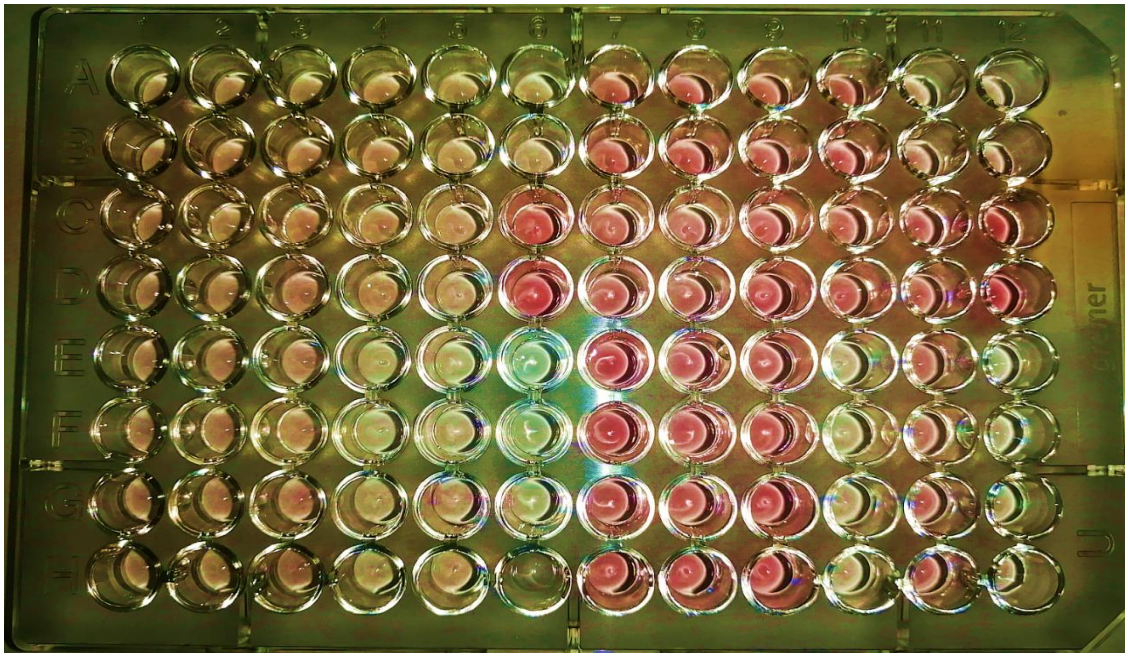

Figure 5. Quantification of amylose and total starch by spectrophotometry. Amylopectin is calculated as the difference between the total starch percentage and the percentage amylose.

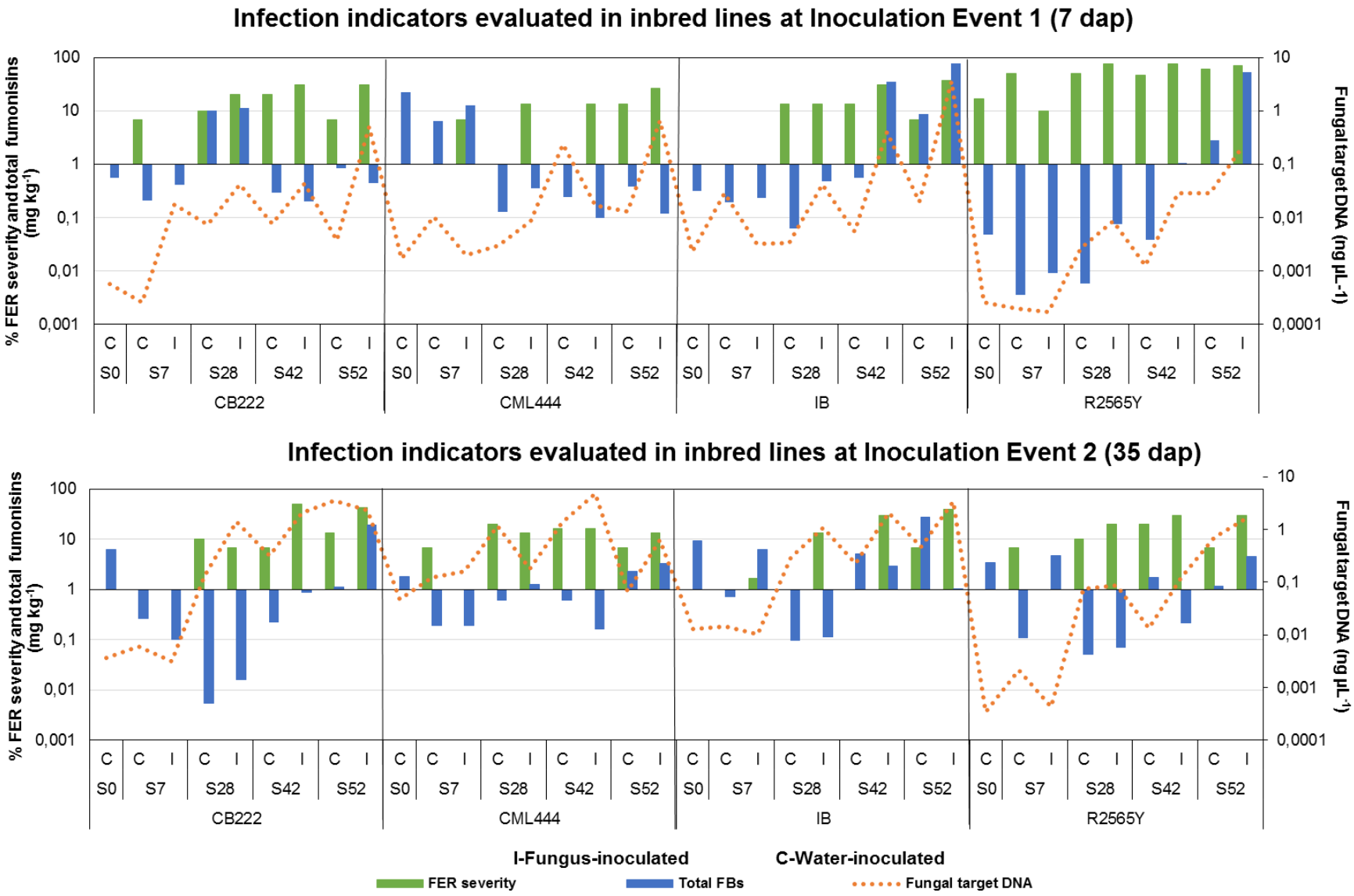

Figure 6. Mean Fusarium ear rot (FER) severity, total free fumonisins (FBs) (FBs=FB_1+FB_2+FB_3) and *Fusarium verticillioides* target DNA in maize inbred lines CML 222, CML 444, I-B and R2565y inoculated 7 and 35 days after pollination (dap) and sampled at 0, 7, 28, 42 and 52 days after inoculation (dai).

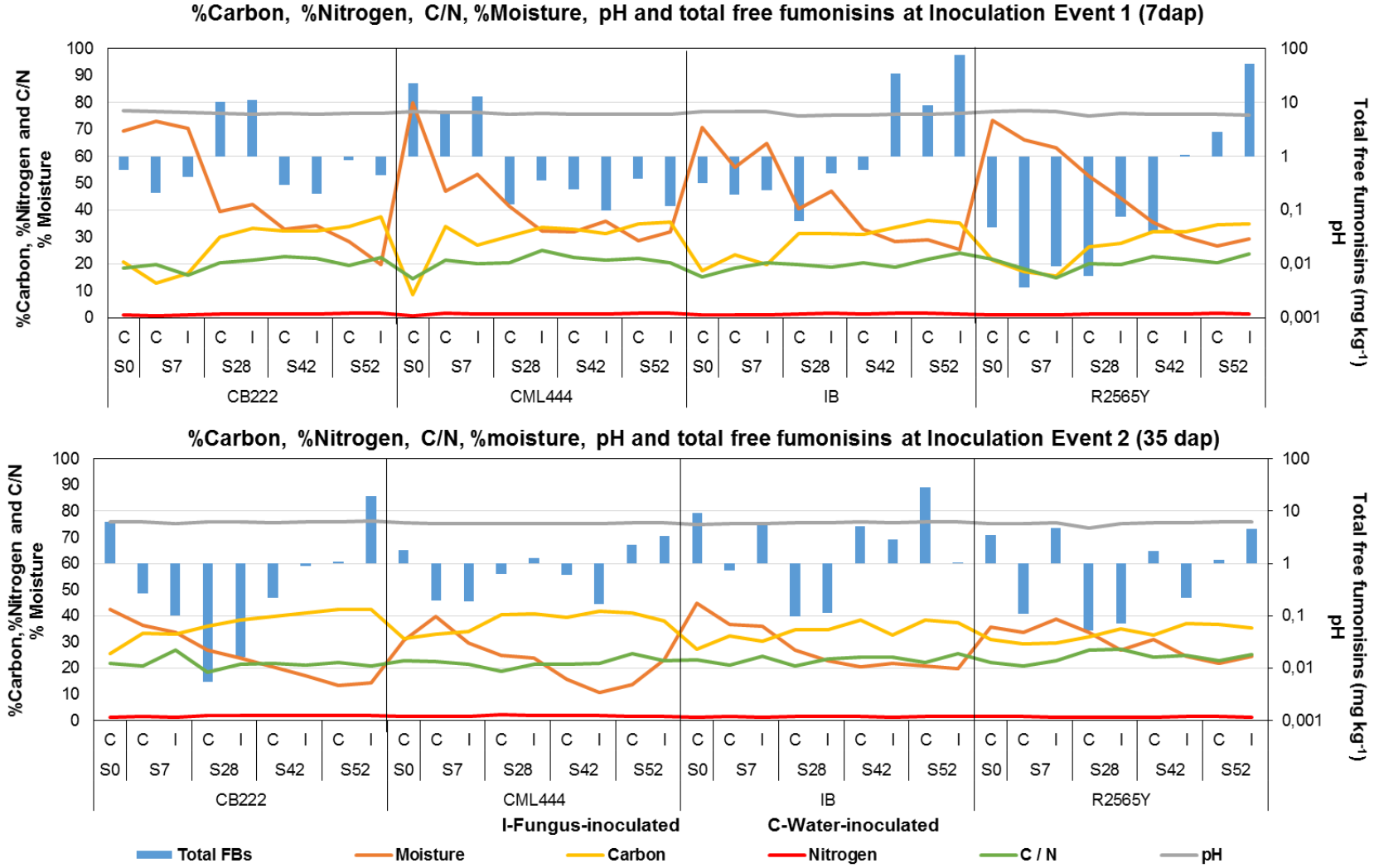

Figure 7. Carbon percentage (%), nitrogen %, the ratio of carbon to nitrogen (C/N), kernel pH, % moisture and total free fumonisins (FBs) (FBs=FB_1+FB_2+FB_3) in maize inbred lines CB 222, CML 444, I-B and R2565y inoculated 7 and 35 days after pollination (dap) and sampled at 0, 7, 28, 42 and 52 days after inoculation (dai).

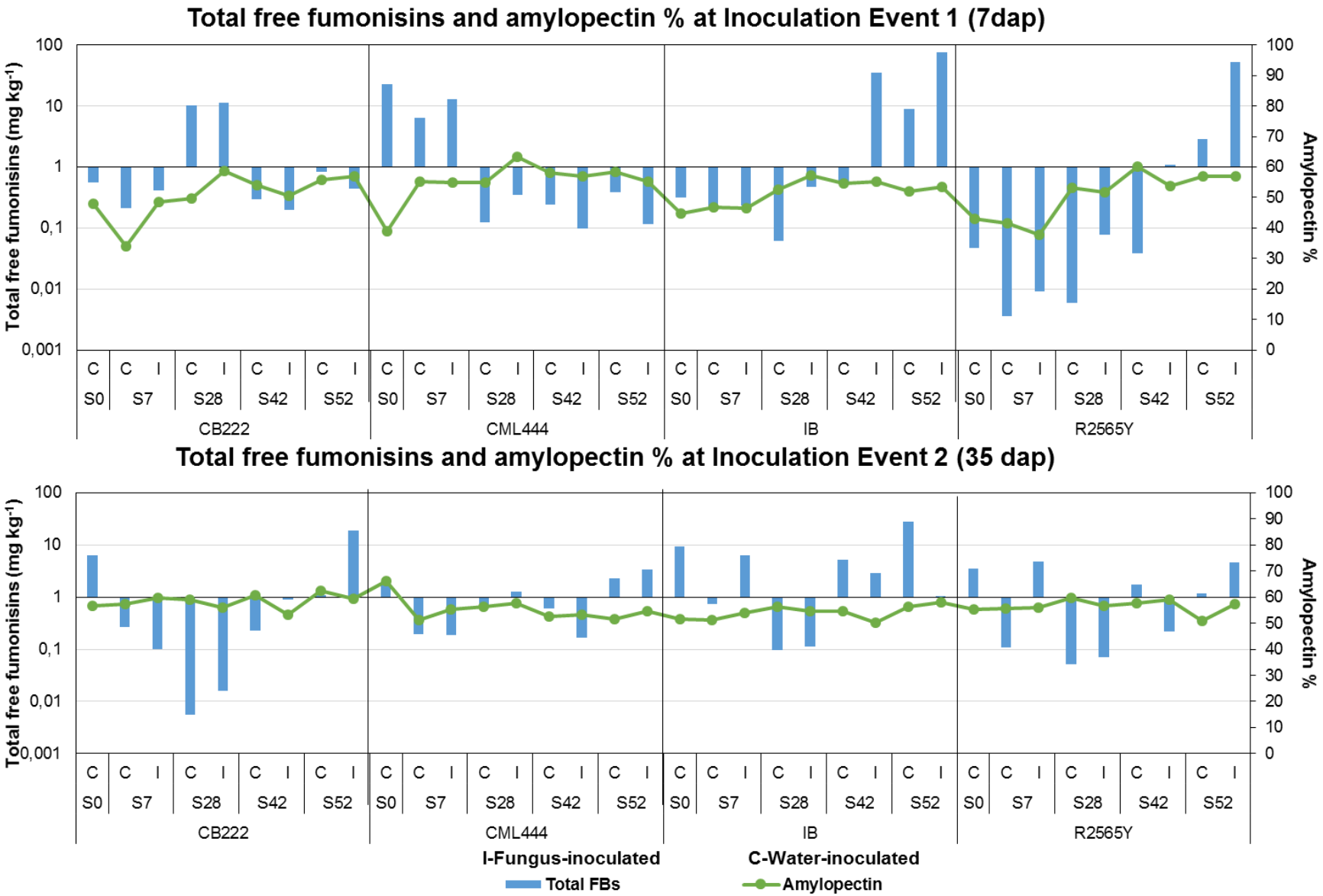

Figure 8. Total free fumonisins (FBs)(FBs=FB_1+FB_2+FB_3) and amylopectin content in four maize inbred lines CB 222, CML 444, I-B and R2565y inoculated 7 and 35 days after pollination (dap) and sampled at 0, 7, 28, 42 and 52 days after inoculation (dai).

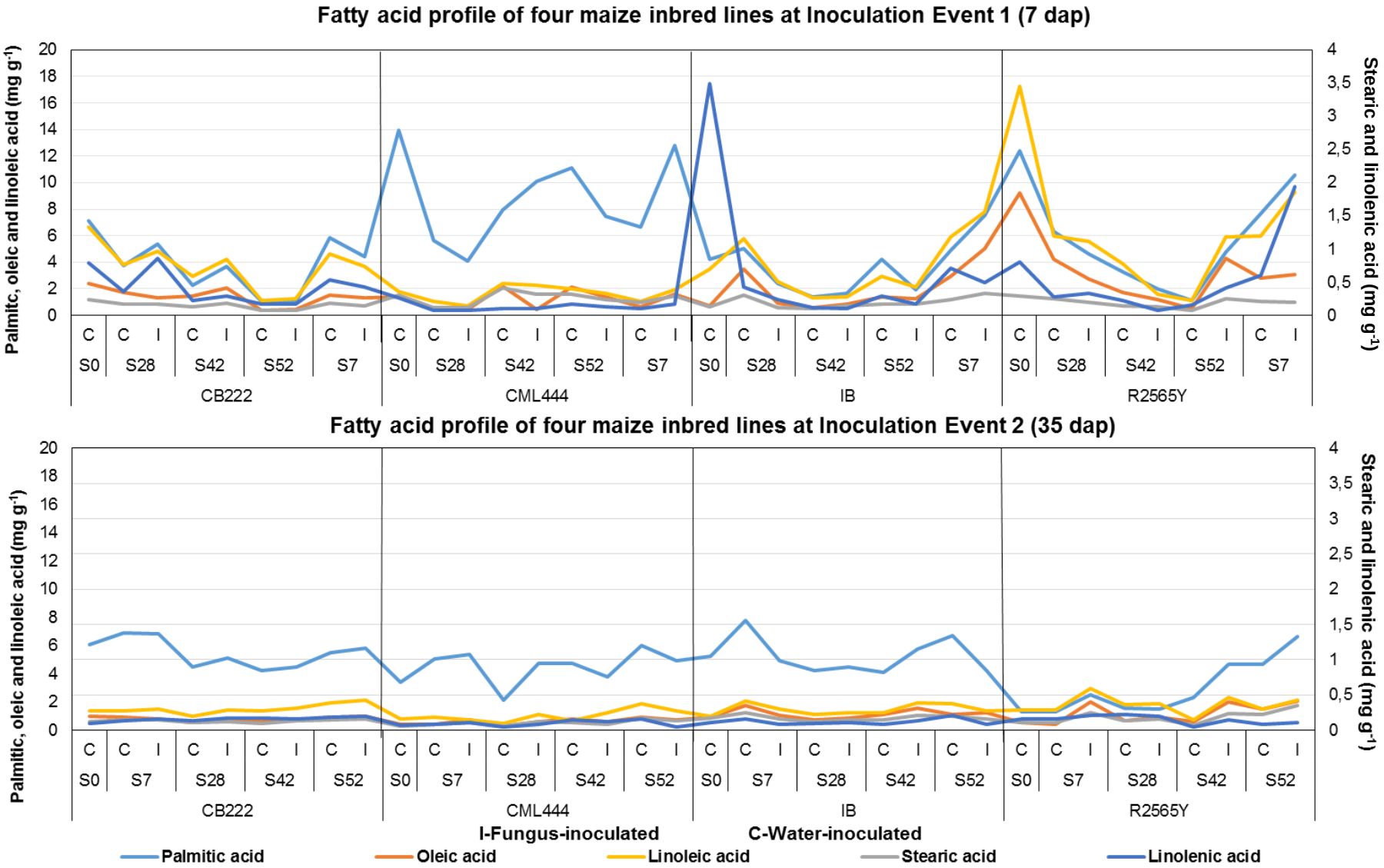

Figure 9. Fatty acid content in four maize inbred lines inoculated with *Fusarium verticillioides* MRC 826 at 7 and 35 days after pollination in maize inbred lines CB 222, CML 444, I-B and R2565y sampled at 0, 7, 28, 42 and 52 days after inoculation.

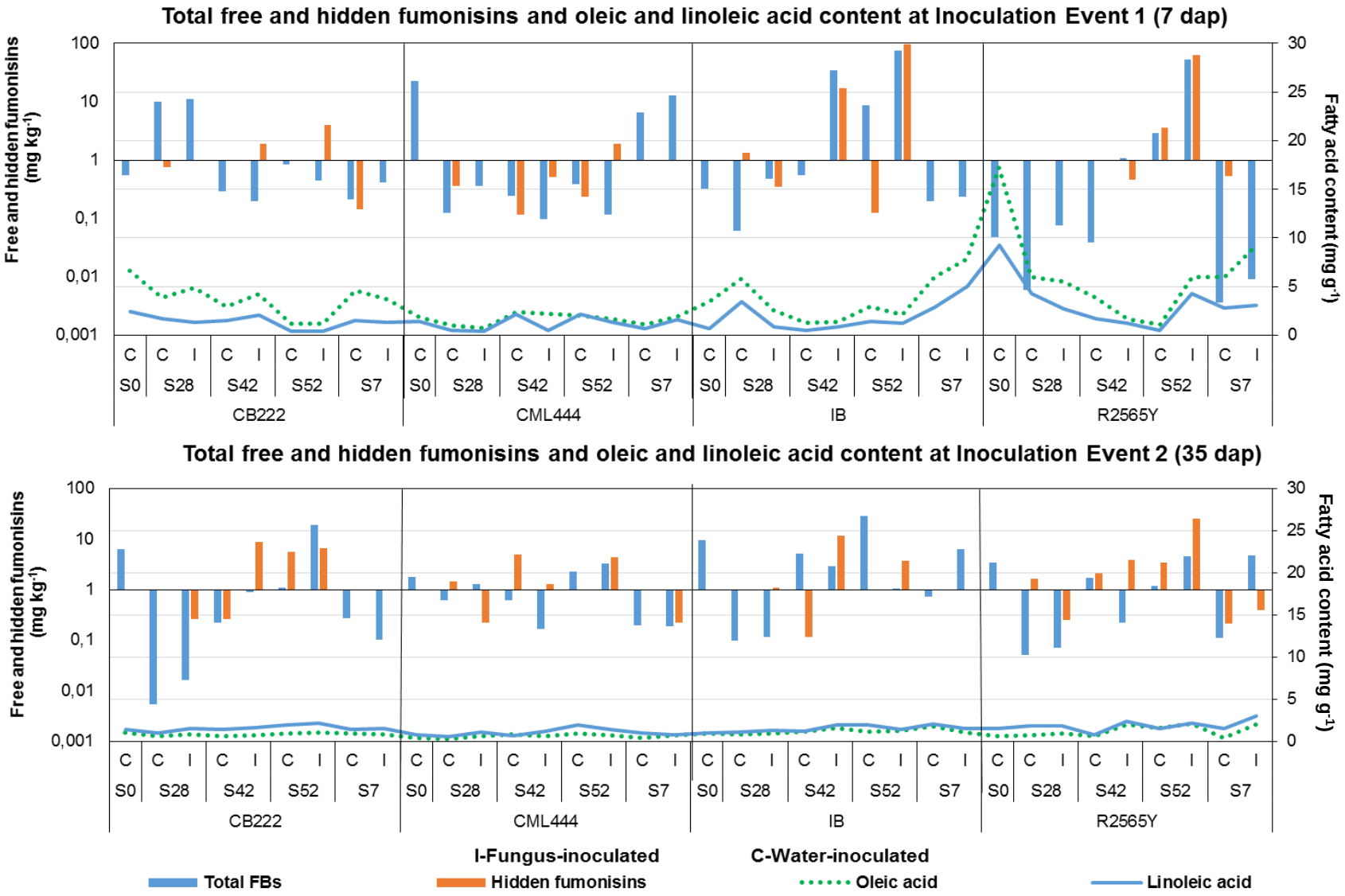

Figure 10. Total free fumonisins (FBs) (FBs=FB_1+FB_2+FB_3), hidden fumonisins (hidden fumonisins=Total hydrolysed fumonisins-FB_1) and oleic and linoleic fatty acid content in four maize inbred lines inoculated 7 and 35 days after pollination (dap) and sampled at 0, 7, 28, 42 and 52 days after inoculation (dai).

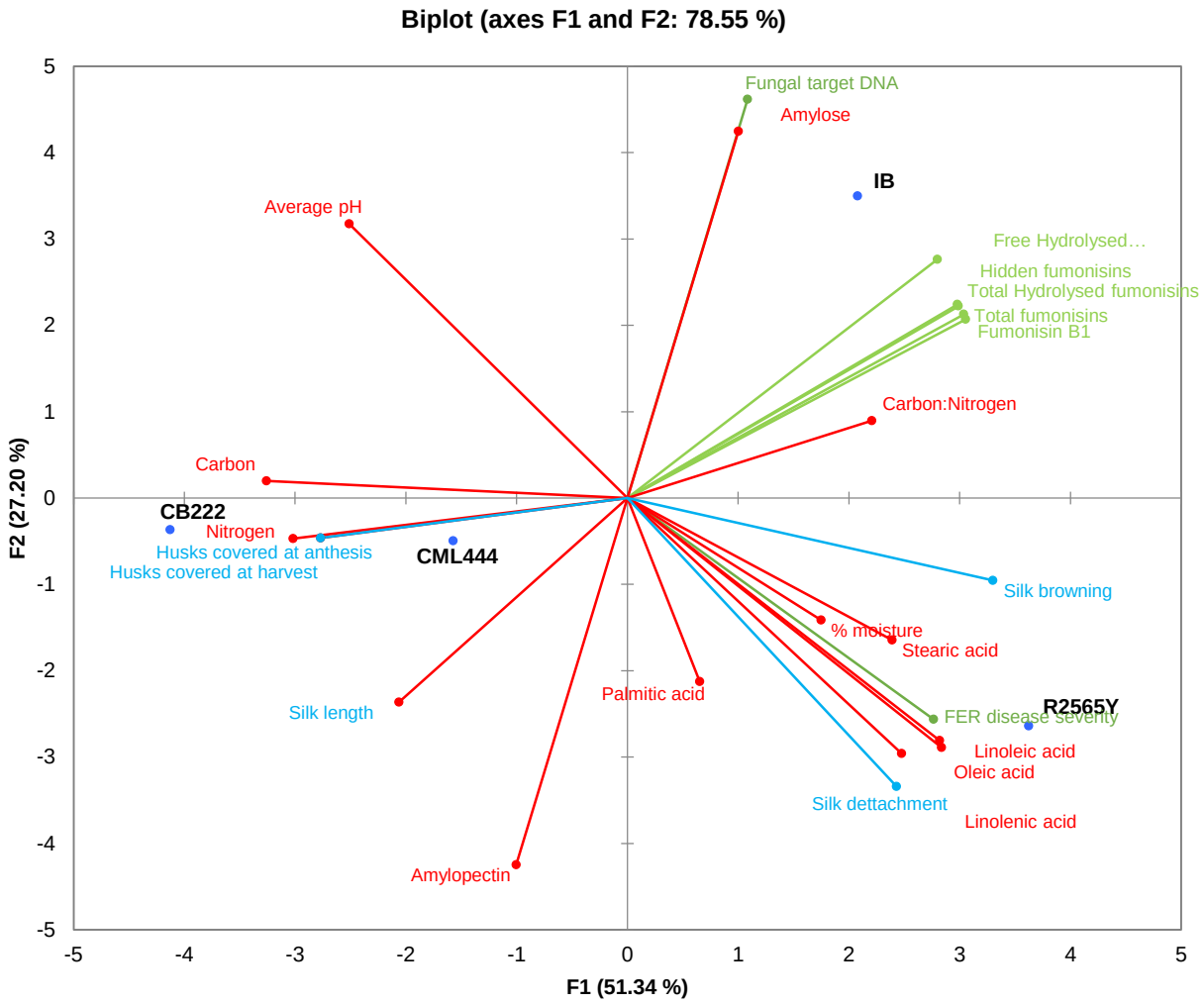

Figure 11. Principle component biplot demonstrating the relationship between structural traits (husk coverage at anthesis and harvest, silk length, silk browning and silk detachment) (blue), infection indicators (Fusarium ear rot (FER) disease severity, *Fusarium verticillioides* target DNA and total free fumonisins (FB_1+FB_2+FB_3)) as well as fumonisin derivatives (free fumonisin B_1 (FB_1), free hydrolysed fumonisins, total fumonisins and hidden fumonisins) (green) and physico-chemical properties (Moisture, pH, carbon, nitrogen, carbon: nitrogen, oleic, linoleic, linolenic, palmitic and amylose and amylopectin) (red) for maize inbred lines CML 444, CB 222, R2565y and I-B inoculated at 7 and 35 days after pollination and sampled at 0, 7, 28, 42 and 52 days after inoculation during the 2018/19 season.

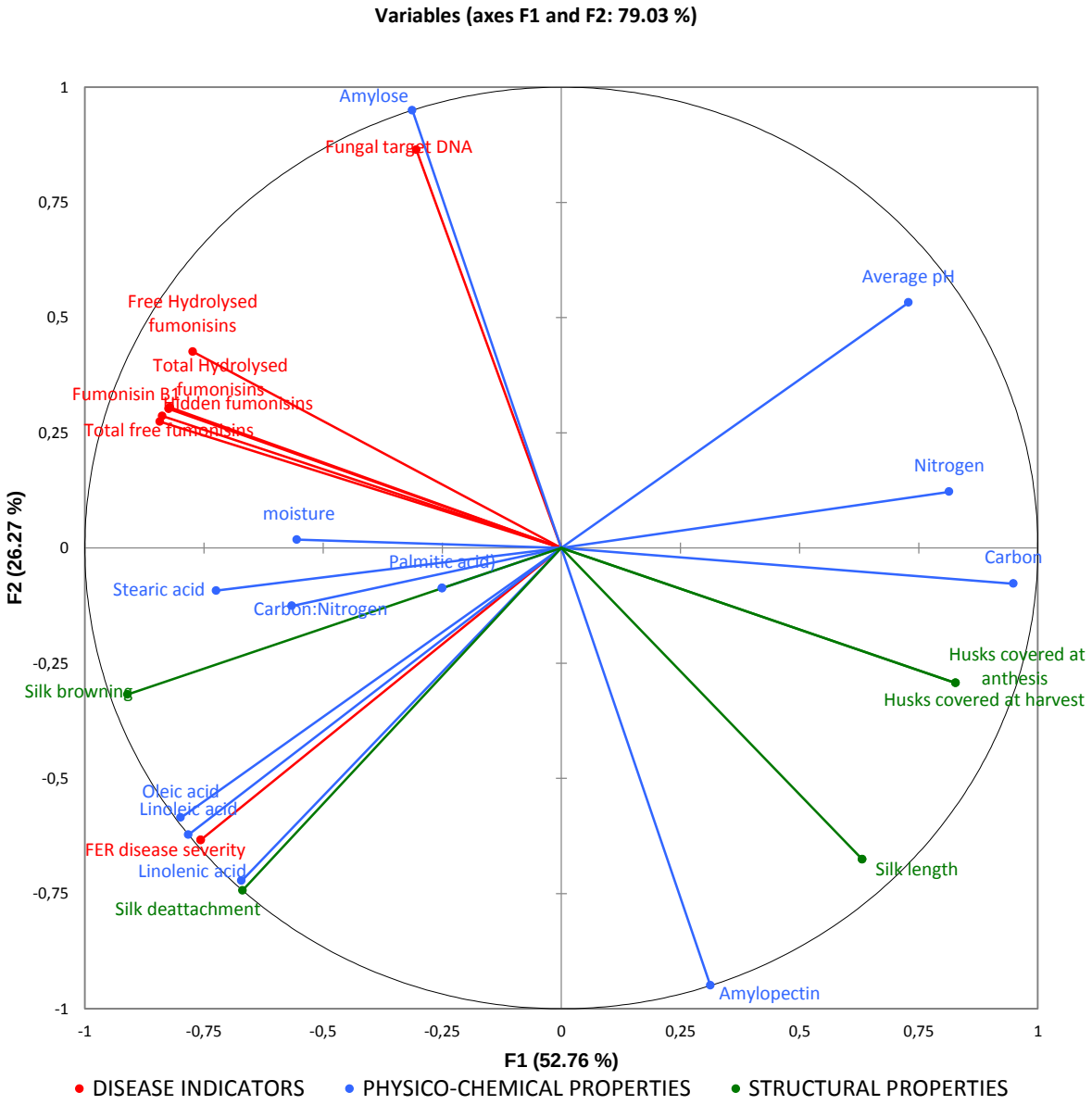

Figure 12. Multifactor analysis of structural traits (husk coverage at anthesis and harvest, silk length, silk browning and silk detachment) (green), infection indicators (Fusarium ear rot (FER) disease severity, *Fusarium verticillioides* target DNA, total free fumonisins (FB_1+FB_2+FB_3)) as well as fumonisin derivatives (free fumonisin B_1 (FB_1), free hydrolysed, total fumonisins and hidden fumonisins) (red) and physico-chemical properties (Moisture, pH, carbon, nitrogen, carbon: nitrogen, oleic, linoleic, linolenic, palmitic and amylose and amylopectin) (blue) for maize inbred lines CML 444, CB 222, R2565y and I-B inoculated at 7 and 35 days after pollination and sampled at 0, 7, 28, 42 and 52 days after inoculation during the 2018/19 season.

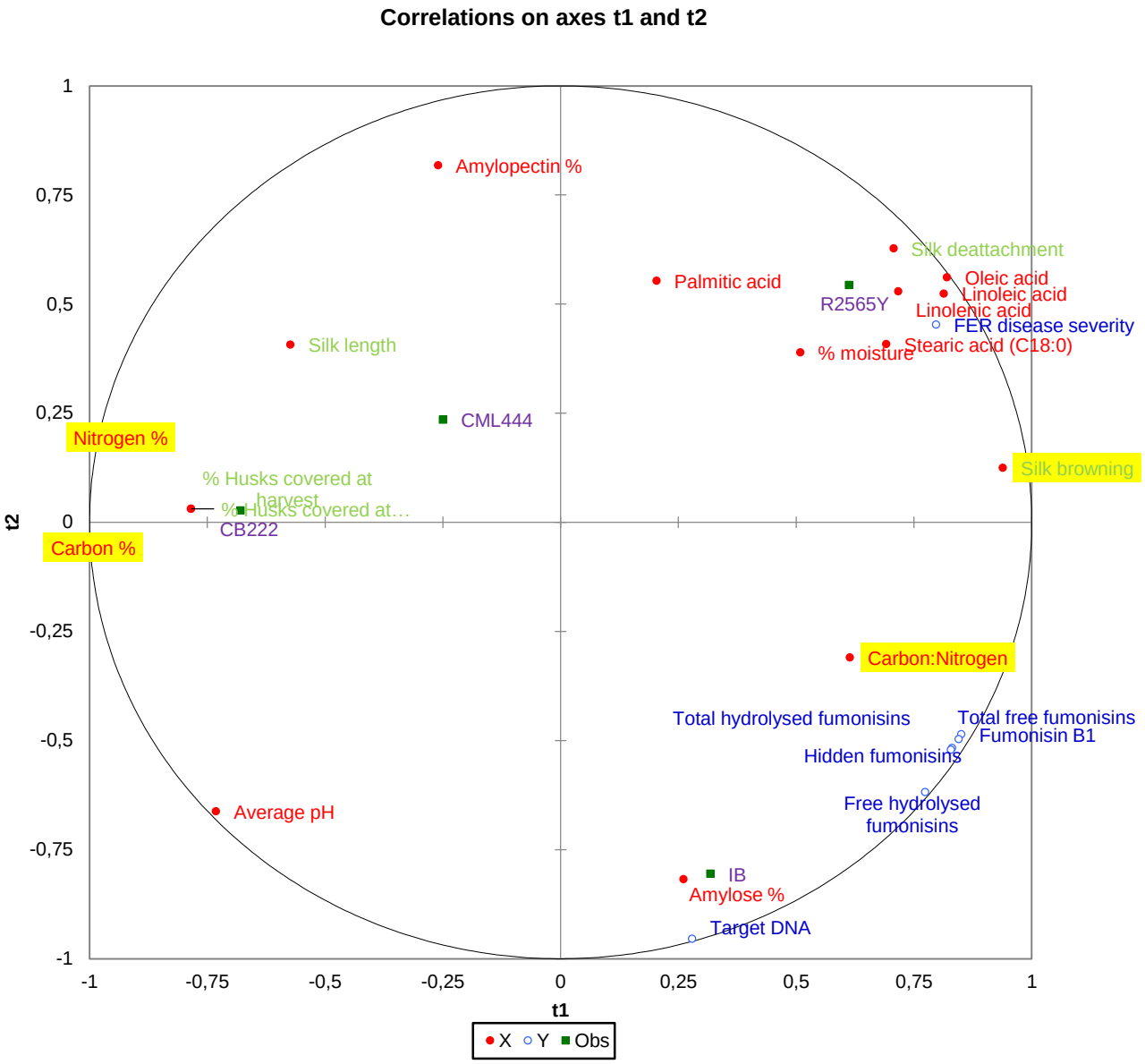

Figure 13. Partial least squares regression analysis of structural traits (husk coverage at anthesis and harvest, silk length, silk browning and silk detachment) (green), infection indicators (Fusarium ear rot (FER) disease severity, *Fusarium verticillioides* target DNA and total free fumonisins (FB_1+FB_2+FB_3)) as well as fumonisin derivatives (free fumonisin B_1 (FB_1), free hydrolysed, total fumonisins and hidden fumonisins) (blue) and physico-chemical properties (Moisture, pH, carbon, nitrogen, carbon: nitrogen, oleic, linoleic, linolenic, palmitic and amylose and amylopectin) (red) evaluated in maize inbred lines CML 444, CB 222, R2565y and I-B inoculated at 7 and 35 days after pollination (dap) and sampled at 0, 7, 28, 42 and 52 days after inoculation (dai) during the 2018/19 season. Highlighted factors are positively correlated with infection indicators.

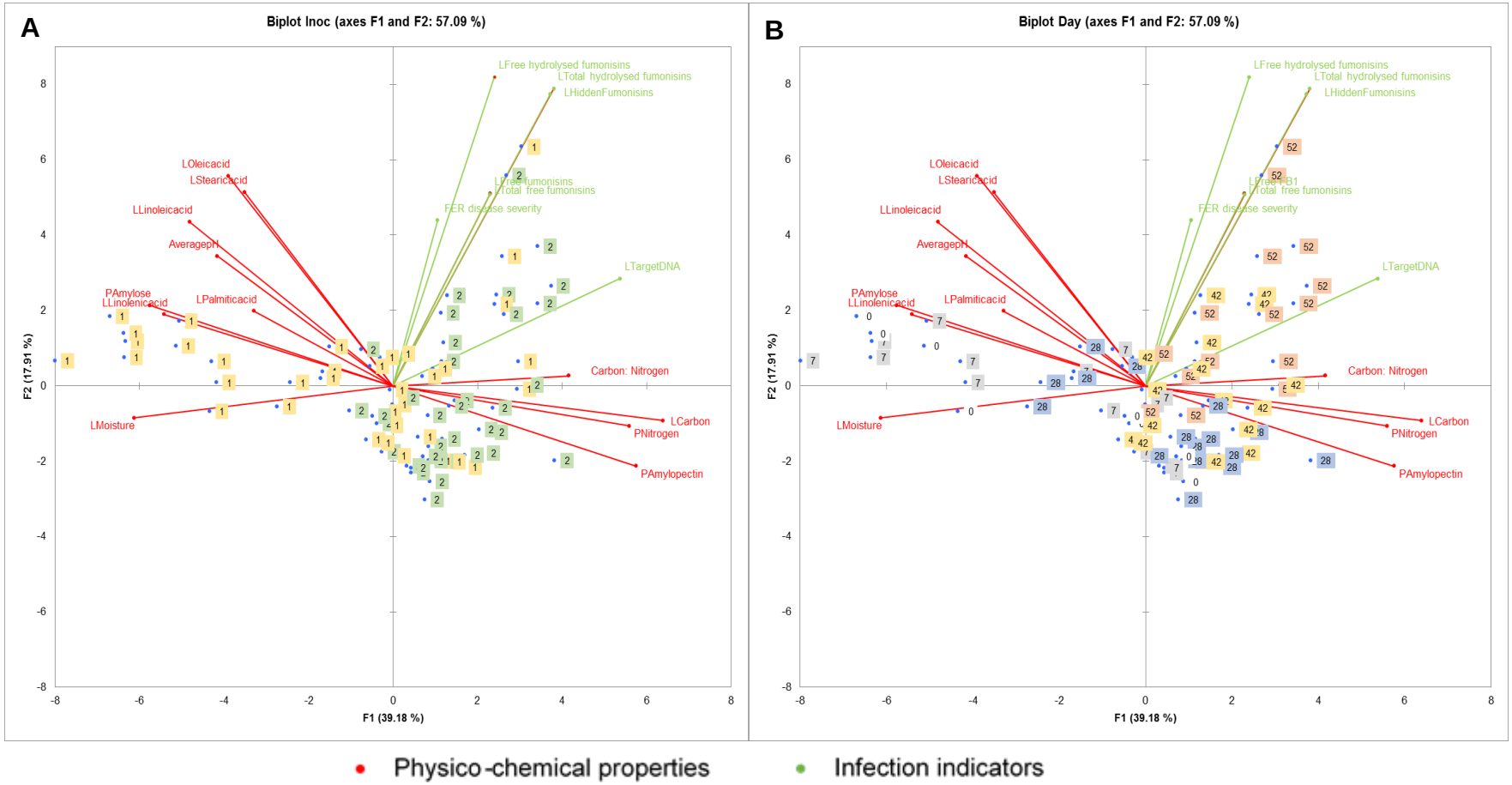

Figure 14. Principle component biplots demonstrating the relationship between infection indicators (Fusarium ear rot (FER) disease severity, *Fusarium verticillioides* target DNA and total free fumonisins (FB_1+FB_2+FB_3)) as well as fumonisin derivatives (free fumonisin B_1 (FB_1), free hydrolysed fumonisins, total fumonisins and hidden fumonisins) (green) and physico-chemical properties (Moisture, pH, carbon, nitrogen, carbon: nitrogen, oleic, linoleic, linolenic, palmitic and amylose and amylopectin) (red) in maize inbred lines CML 444, CB 222, R2565y and I-B inoculated at 7 (Inoculation Event 1) and 35 (Inoculation Event 2) days after pollination during the 2018/19 season and sampled at 0, 7, 28, 42 and 52 days after inoculation. **A**: Relationship between infection indicators, physico-chemical properties and Inoculation Events 1 and 2, **B**: Relationship between infection indicators, physico-chemical properties and sampling time after inoculation (0, 7, 28, 42 and 52 dai).