

**Assessing the effect of decortication on sorghum grain mold fungi and
concomitant mycotoxins**

A thesis submitted in accordance with the requirements for the degree of
Magister Scientiae Agriculturae

by

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DECLARATION

I, Masisi Thabiso Vincent, declare that the *MSc. Agric.* dissertation that I submit for the *MSc. Agric.* degree qualification at the University of the Free State is my independent work, and I have not previously submitted it for a qualification at another institution of higher education.

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Masisi Thabiso Vincent

A handwritten signature in black ink, appearing to read 'Masisi Thabiso Vincent', written over a horizontal line.

--- June 2021---

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PREFACE

Chapter 1. An overview of fungal pathogen complex associated with sorghum grain mold

The first chapter gives an overview on the sorghum (*Sorghum bicolor*) production, uses of the crop as well as abiotic and biotic constraints to the production and uses of sorghum. Additionally, dehulling in the sorghum industry and the potential benefits thereof in plant pathology are also discussed. The chapter briefly reviews the fungal causal organisms that contribute to the sorghum grain mold, followed by a detailed discussion about *Fusarium graminearum* species complex and concomitant mycotoxins, their effect on humans and livestock and their management strategies.

Chapter 2. Evaluating the effect of decortication on sorghum grain mold fungi and concomitant mycotoxins

In order to determine effect of decortication, three commercial cultivars with contrasting grain characteristics were used from the National Cultivar Trial. The effect of decortication was tested on this grain to see its influence on fungal and mycotoxins reduction. The decorticated grain was plated on artificial media for different fungi to be isolated. Fungi were morphologically and molecularly identified, through phylogenetics to determine the evolutionary relationships between identified fungi.

Chapter 3. Evaluating and comparing on-ground (*in situ*) with gridded weather data

The statistical agreement between the weather data obtained from ARC-ISCW (on-ground weather stations) and NASA POWER (simulated gridded data) was investigated to determine the accuracy of gridded data compared to the on-ground weather stations data. Weather stations associated with sorghum field trials were used and parameters such as minimum and maximum temperature, minimum and maximum relative humidity and rainfall were used. Statistical agreement between the

two datasets was determined. Number of missing values of each station and parameter were calculated. Additionally, coefficient of agreement (d), mean bias error (MBE), Nash-Sutcliffe efficiency coefficient (NSE), Pearson's correlation coefficient (R) and root mean square error (RMSE) statistical agreement tools were utilised to determine the accuracy of NASA POWER data against on-ground weather data. Additionally, plots were generated to visualise these relationships.

Chapter 4. Assessing the effect of weather on colonization of grain by mold fungi and pre-harvest mycotoxin accumulation

Historic data from previous field trials were utilised. This consisted of total fungal biomass, *Fusarium graminearum* species complex (FgSC) DNA biomass and mycotoxin concentrations. The mentioned disease parameters were correlated with weather parameters to investigate their relationship with the same weather parameters as mentioned in Chapter 3. The weather parameters strongly associated with disease parameters and conducive days during anthesis were determined through correlation analyses and non-linear regression.

CHAPTER 1: AN OVERVIEW OF FUNGAL PATHOGEN COMPLEX ASSOCIATED WITH SORGHUM GRAIN MOLD

INTRODUCTION

The sorghum grain mold (SGM) pathosystem includes the interaction between the host (sorghum, *Sorghum bicolor* (L.) Moench.), the pathogen (fungal complex) and the environment (abiotic and biotic factors). Grain mold can be described as the deterioration of sorghum grain as the result of fungal complexes that infect and colonise sorghum spikelets between flowering and harvest (Bandyopadhyay *et al.*, 2000; Thakur *et al.*, 2006). Although over 40 genera of fungi are associated with SGM, the *Fusarium graminearum* Schwabe species complex (*FgSC*) is the most widely distributed pathogenic fungal group associated with the disease (Tesfaendrias *et al.*, 2011). The *FgSC* produces economically important mycotoxins such as deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) which have detrimental health effects on livestock and humans when consumed (Marroquín-Cardona *et al.*, 2014).

Prior to food product processing and consumption, sorghum needs to go through the process of decortication, also referred to as debranning. This process refers to the physical removal of the outer layers of the kernel which make up the seed coat (Van der Merwe *et al.*, 2005). The purpose of this process is to remove polyphenolic compounds, which are undigestible to humans when consumed, due to binding with dietary proteins. These compounds are associated with the outer layers of the kernel and are removed to increase the palatability of sorghum flour (Munck *et al.*, 1982; Mwasaru *et al.*, 1988; Awika *et al.*, 2003). Coetzee (2015) demonstrated that decortication could remove *FgSC* that colonises different layers of the kernel and associated mycotoxins. However, decortication was limited in removing deep-seated

fungi and potentially mycotoxins from the endosperm of the kernel. Therefore, the first research aim of this study is to investigate the establishment of selected fungi within the sorghum kernel, through decortication, to deepen the understanding of where the fungi are in the grain and how much decortication is required to remove these organisms in a manner which is economically viable.

Sorghum grain mold develops under moist and warm conditions (Thakur *et al.*, 2006). Cognisance of these factors will contribute to our understanding of the pathosystem and the development of good crop management strategies. Weather data has been used in multiple studies to generate disease risk models. However, the source of the data may vary, and local ground station data or gridded assimilated weather data can be utilised for these purposes. Furthermore, using historic SGM data and weather data, the second aim is to evaluate the accuracy and reliability of gridded weather data from National Aeronautics and Space Administration/ Prediction of Worldwide Energy Resources (NASA POWER, USA) in comparison to the weather data obtained from the Agricultural Research Council (ARC, South Africa) and finally to measure the impact of weather parameters on SGM. To provide context and a clearer understanding of the research objectives a literature review is presented below.

The centre of origin for sorghum

Sorghum is a member of the Poaceae family, commonly known as the grasses. It is mostly accepted as being a crop native to Africa (Kimber, 2003). Although, the specific country of origin is still under speculation, Vinall *et al.* (1936) reported that sorghum is indigenous to Egypt. Partly supporting this report, Dahlberg and Wasylikowa (1996) stated that the border between Egypt and Sudan is the centre of origin for sorghum and where early domestication occurred. Doggett (1970) holds a contrary view and

suggests that West Africa is sorghum's origin. This view is partially based on reports of ancient trade between West Africa and the United States of America (USA), although this does not confirm that sorghum originated in West Africa (Sorghum Trust, 2016). The enormous diversity of sorghum types found in Sudan suggests this region may be the true centre of origin (Tarr, 1962). Similarly, the Sorghum Trust (2016) also reports on a great variety of wild and domesticated sorghum types in Sudan as supported by archaeological reports. According to Wendorf *et al.* (1992) and corroborated by Sorghum Checkoff (2016) sorghum was first domesticated in North-East Africa, where Sudan is located, it could be assumed that Wendorf *et al.* (1992) might have been referring to Sudan. In contrast, Vavilov *et al.* (1992) classifies India as a secondary source of origin due to the wide variation of sorghum plant species found in India. Although, Zohary and Hopf (2000) stated that sorghum is not a crop native to India, and that it migrated from East Africa into India, as the wild types of the crop are limited to Africa only. Therefore, it is more commonly accepted that because of trade between countries, sorghum spread to Egypt and India, perhaps explaining the diversity of theories regarding the origin of the crop (Doggett, 1970). Identifying the origin of a crop is important as this information can be used to collect germplasm from wild types that display favourable characteristics to be used in breeding programs to promote plant health (Bandyopadhyay *et al.*, 2000).

Plant structure

Sorghum growth stages are classified into two major phases, vegetative (stages 0-5) and reproductive (stages 6-9; Figure 1). At emergence, the coleoptile is the first of the leaves to appear (stage 0). After coleoptile leaf maturity, the leaf collar becomes visible, which serves as the intersection between the lamina and the leaf sheath at the node. The final leaf to emerge is the flag leaf, which is reduced in size compared to

former leaves. When the collar of this leaf appears, the plant is in boot stage (stage 5). The leaves are completely extended and are at the greatest light reception phase. The head is almost completely developed and is embedded in the sheath of the flag leaf and the elongation of the peduncle is initiated which later will support the panicle. Flowering occurs at stage 6, i.e., the developed head sheath of the flag leaf in boot stage is now extended by the rapidly growing peduncle, commencing at the apex and continues sequentially downwards. Stage 7 is the soft dough stage and is associated with ~ 50% of dry matter accumulation. Grain at the soft dough stage can easily be pressed between two fingers, usually resulting in the appearance of a milky fluid. When the grain can no longer be easily squeezed and ~ 75% of dry matter has been accumulated, the hard dough stage (stage 8) is distinguished. The final stage (stage 9) is physiological maturity, and the formation of the black layer can be seen near the base of grain at the intersection between the grain and the florets (Gerik *et al.*, 2003). The genotype-environment interaction influences the time required for a sorghum plant to reach each developmental stage (Vanderlip, 1993).

The value of understanding host growth stages is critical to making crop management decisions and gathering information valuable to plant disease epidemiology. Monitoring the host plant contributes to ensuring that the pathosystem is fully understood, as well as deciphering the relationship between each growth stage and weather parameters to unravel host-pathogen-environment interactions. Furthermore, identifying growth stages enables the timely application of agrochemicals for the control of diseases, insects and weeds (Bock and Nutter, 2012). For yields and crop protection to be optimized, production requirements of the crop need to go beyond that of only fulfilling the weather demands of the crop and need to integrate good management practices at critical physiological growth stages. An

understanding of the development and the growth of sorghum can be advanced with the knowledge of crop anatomical structures (Figure 2).

The morphological characteristic of sorghum enables it to tolerate drought conditions more readily than other crops such as maize (*Zea mays L*) (Mahmood, 2012). Leaf xerophytic qualities enable a more water efficient transpiration rate and as a result limit water loss. Sorghum has half the leaf area of maize and when subjected to above average temperatures ($\geq 33\text{ }^{\circ}\text{C}$) leaves roll up. Additionally, a waxy cuticle is present and prevents drying under these extreme conditions. Sorghum has a well branched, deep root system (~ 2 meters) that allows it to draw significantly more water than maize. Lastly, sorghum can undergo a dormant phase during drought periods and continue growing when conditions improve (Mahmood, 2012; Ramatoulaye *et al.*, 2016; Phuong, 2018). Sorghum can compensate for yield loss if growth is compromised by producing ear-bearing tillers during physiological maturity, resulting in an additional grain source (Gerik *et al.*, 2003; Awika and Rooney, 2004). Sorghum can out-perform other crops under various adverse environmental conditions such as those experienced in dry (≤ 400 mm) and hot ($31\text{-}32\text{ }^{\circ}\text{C}$), or waterlogged areas (Awika and Rooney, 2004; Hadebe *et al.*, 2017; Tack *et al.*, 2017; Phuong, 2018).

The inflorescence of sorghum, once grain-bearing is referred to as a panicle (head; Figure 2), an individual panicle, dependent on the variety, has the potential to carry 800 to 3000 kernels (Department of Agriculture, Forestry and Fisheries; DAFF, 2010). The genotype and the environment in which sorghum is grown influences the panicle colour (Dykes *et al.*, 2005; Figure 3). The panicle can be red in colour as determined by R-Y- genes, white with genes R-yy/rryy or lemon yellow with rrY- genes (Dykes *et al.*, 2005). Abiotic factors may also influence the panicle colour, for example

lemon yellow varieties may turn brownish yellow prior to drying because of bleaching by the sun (Rooney and Miller, 1981; Figure 3).

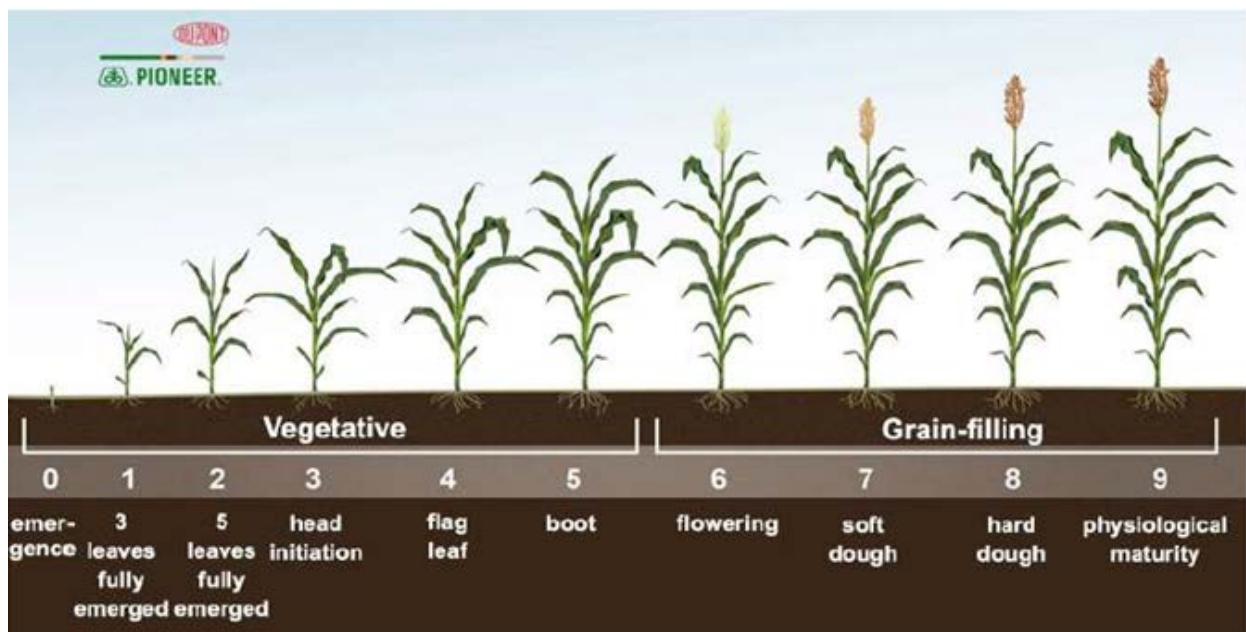


Figure 1: Sorghum plant growth stages, annotating the stage number in light brown section and the stage name in the dark brown section of the image (Pioneer, 2014).

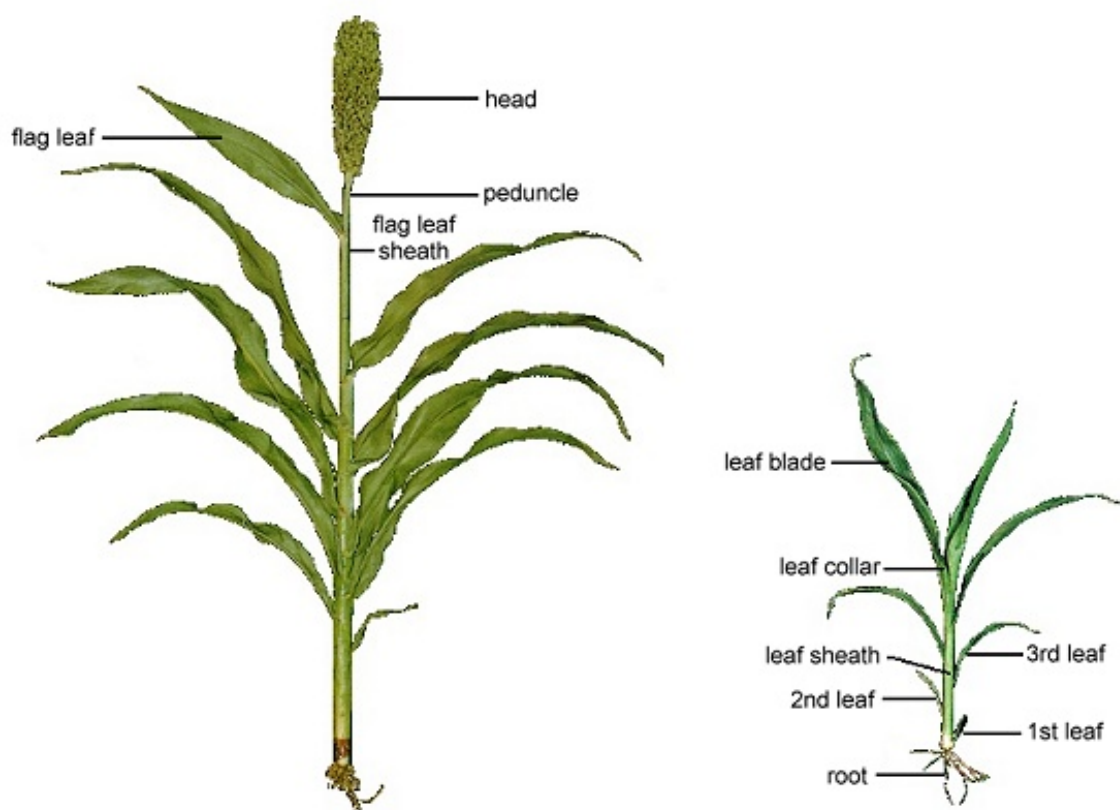


Figure 2: Botanical structure of grain sorghum plant (Warrick, 2000).



Figure 3: Sorghum grain colours (Republic Ranches, 2015).

Sorghum grain consists of multiple layers, primarily the embryo (germ), endosperm and seed coat, with the latter further subdivided into the pericarp and testa (Figure 4; Taylor, 2003). Sorghum grain is made up of ~ 8% pericarp, ~ 10% embryo and ~ 82% endosperm. The pericarp comprises three layers, namely epicarp, mesocarp and endocarp (Figure 4). The epicarp is the outer layer consisting of pigmented rectangular cells that are thick walled. The mesocarp, the middle layer, is comprised of several layers of cells, these being the determining factor of pericarp thickness, while the endocarp is the innermost layer responsible for systemic transport of moisture in the grain and is comprised of cross and tube cells (Rooney and Miller, 1981).

The Z-gene controls the mesocarp thickness, where a thinner mesocarp is controlled by dominant genes, whereas thicker structure is moderated by recessive genes (Rooney and Miller, 1981). A thinner pericarp is attached tightly to the endosperm making the grain suitable for decortication through mechanical milling. Mechanical decortication can be achieved with devices such as Tangential Abrasive Dehulling Device (TADD) and Pedal Operated Sorghum Dehuller (Van der Merwe *et*

al., 2005; Lazaro *et al.*, 2013). In contrast, a thicker pericarp is loosely attached to the endosperm and thus grains are more suitable for decortication by hand (Bassey and Schmidt, 1989; Beta *et al.*, 2001).

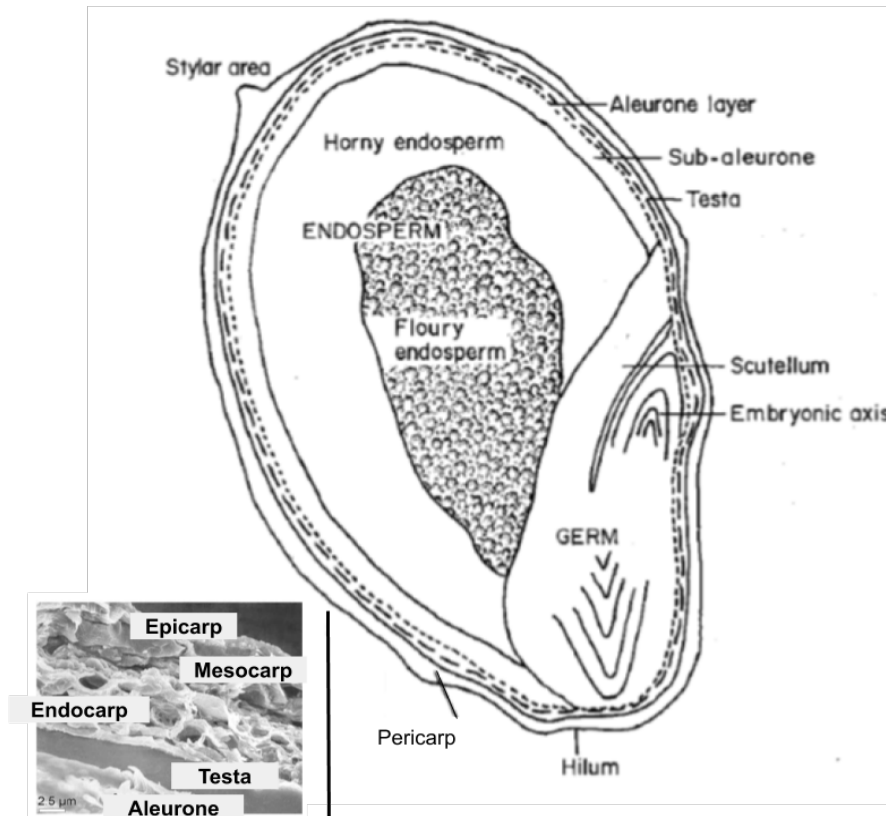


Figure 4: Cross section of the structural components of sorghum grain and scanning electron photomicrograph of sorghum grain layers consisting of: epicarp; mesocarp; endocarp; testa; aleurone (Taylor, 2003; Earp *et al.*, 2004).

Sorghum grain uses are determined by grain characteristics, primarily kernel colour, which is driven by phytochemical components. Phytochemicals are biological active chemical compounds, produced by plants for basic functions or as defence mechanisms against pests and diseases, that are found in the pericarp and testa of the grain (Taylor, 2003; Awika and Rooney, 2004; Mendoza and Silva, 2018). White sorghum varieties have little to no detectable levels of phytochemicals, i.e., phenols or anthocyanins, while red varieties carry a significant amount of phenols but do not have tannins. In contrast, brown varieties contain higher levels of tannins in the testa

(Awika and Rooney, 2004). Thus generally, dark coloured sorghum grains are known to have a high phytochemical content, i.e., tannins, phenolic acids and anthocyanins compared to light coloured varieties (Mwasaru *et al.*, 1988; Reichert *et al.*, 1988; Rao *et al.*, 2012). The phenolic compounds found in the grain are associated with different flavours that produce sour or bitter tastes (Purseglove, 1972; Phuong, 2018). In South Africa, sorghum is classified based on phytochemical content and thus the appearance of the grain. The grading of grain sorghum was created to separate feed, fodder and malting uses. Four classes namely, GM (malt and does not have a dark testa), GL (low tannins and does not have a dark testa), GH (high tannins and have a dark testa) and class other (if it does not comply with the standards for either GM, GL, or GH; Government Gazette, 2016). Sorghum grain is high in starch (73.8%), protein (12%) and vitamin B complex (B₁, B₂ and B₃; Adegbola *et al.*, 2013). Resistance to diseases, including grain molds, has been associated with grain phenolic compound concentration which limits fungal growth and development (Lo *et al.*, 1999). These phenolic compounds are usually associated with darker pericarp colours (Menkir *et al.*, 1996; Awika *et al.*, 2003; Dicko *et al.*, 2005; Dykes *et al.*, 2005). There is a relationship between resistance of sorghum to grain mold and to the production of phenolic compounds such as flavan-4-ol phenolics (Jambunathan *et al.*, 1991; Rao *et al.*, 2012), caffeic acid, cinnamic acid, ferulic acid, gallic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, protocatechuic acid and vanillic acid (Hahn *et al.*, 1983). The production of anthocyanins, apigeninidin and luteolinidin near infection sites of sorghum seedlings infected by *F. proliferatum* (Matsushima) Nirenburg and *F. thapsinum* Klittich, Leslie, Nelson and Marasas, have been observed (Huang and Backhouse, 2004). Moreover, Rao *et al.* (2012) found a high concentration of flavan-4-ol in red variety compared to white variety. As a result, red variety were resistant to

mold while white variety were not. Knowledge of the host crop physiology and associated organs contribute to ensuring host plant protection is appropriately deployed for disease management. Further understanding of the production systems associated with host crops can assist in ensuring stresses are mitigated or detected early enough to be corrected.

Agronomy

The minimum soil temperature for germination of sorghum seed is 15 °C, at a 10 cm depth beneath the soil surface, resulting in approximately 80% germination of planted seed. Low temperatures (5-7 °C) may predispose sorghum to infection by a complex of pathogens especially at emergence (stage 0; McLaren and Rijkenberg, 1991). The base temperature for sorghum is 10 °C, i.e., temperatures below may be detrimental to seed germination and seedling development (Du Plessis, 2008). If these conditions at planting cannot be avoided, seed should be sown at a shallower depth and seed treatments are recommended (Gerik *et al.*, 2003; Du Plessis, 2008).

Temperatures between 27-30 °C are vital for optimal development of sorghum during the entire growing season. Sub-optimum temperatures of 21 °C can occur during development of sorghum. However, no severe damage or yield losses have been reported (DAFF, 2010). A study conducted in the USA indicated that temperatures that exceed the optimum range during early-vegetative (stage 2-4), pre-, and post-flowering phases (stage 5-8), may significantly reduce sorghum yield (Tack *et al.*, 2017). Sorghum is cultivated in rainfall areas with a range of 400-800 mm per annum but successful production in areas where a range of 300-700 mm rainfall have been documented (Du Plessis, 2008; DAFF, 2010).

Weed control is important at and during leaf emergence and development (stage 1-2), as weed competition reduces the sun, water and nutrient uptake by young

sorghum plants (Vanderlip,1993). Nutrient application in the form of top dressing may become essential prior to flowering (stage 6) as the plant would have utilised 70%, 60% and 80% of nitrogen, phosphorous and potassium requirements, respectively. It should be noted that the soil types affect recommended dosages. Higher nutrient content in the soil may ensure optimum grain fill and thus the amount of fertilizer that needs to be applied depends only on the yield target (Vanderlip, 1993; Gerik *et al.*, 2003; Pannar seeds, n.d).

Sorghum production

Sorghum is the fifth most cultivated cereal crop globally after maize, wheat (*Triticum aestivum* L), rice (*Oryza sativa* L), and barley (*Hordeum vulgare* L), and has remained relevant during the 21st century (Sorghum Checkoff, 2016; Taylor, 2018). Sorghum is Africa's second most important crop as approximately 20 million tonnes per annum are harvested, which equates to a third of sorghum produced globally (Mwadalu and Mwangi, 2013). In South Africa, sorghum is considered the third most important grain crop after wheat and maize (Du Plessis, 2008). In the 2008/2009 season, the Free State province was the largest producer of sorghum responsible for 62% of production followed by Mpumalanga and Limpopo, with 24% and 8%, respectively (Crop Estimate Committee, 2009). However, after a decade, in 2018, a significant shift was observed and Mpumalanga became the main sorghum production area with an increase in the area planted and the tonnage harvested, 7 500 ha and 40 125 tons, respectively. The North West province became the second largest production area followed by the Free State (Crop Estimate Committee, 2009; 2018; Figure 5).

The 2015/2016 drought in South Africa, resulted in a decrease in land area used for planting and disrupted the yield for many crops including sorghum (Sihlobo, 2018; Figure 6). Locally, the sorghum area planted has declined annually compared

to the increase in oilseed crops (Crop Estimate Committee, 2018; Sihlobo, 2018). Despite this, in 2019 an increase by 19 200 ha of sorghum area planted was reported compared to the 28 800 ha planted during the previous year (Crop Estimate Committee, 2019). According to Sihlobo (2019) this increase is explained by drought tolerance of sorghum and that it has become the more suitable substitute when maize cannot be planted.

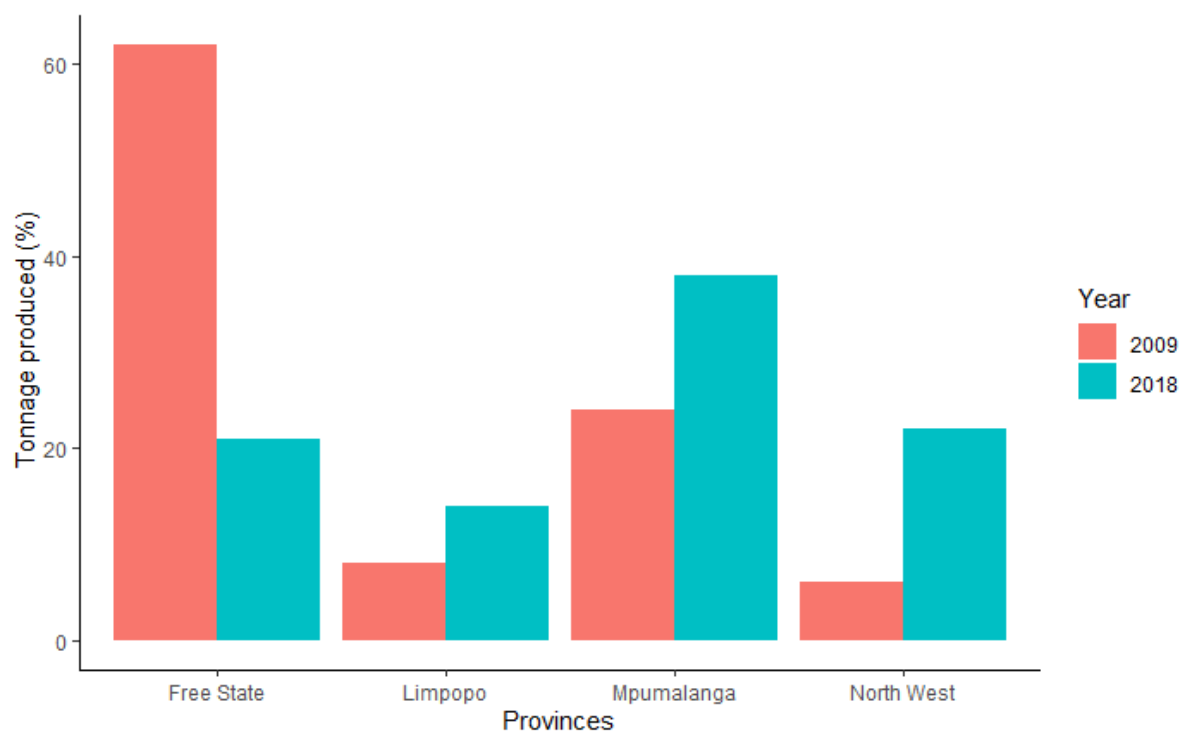


Figure 5: Comparison of 2009 and 2018 sorghum production statistics in South Africa (Crop Estimate Committee, 2009; 2018).

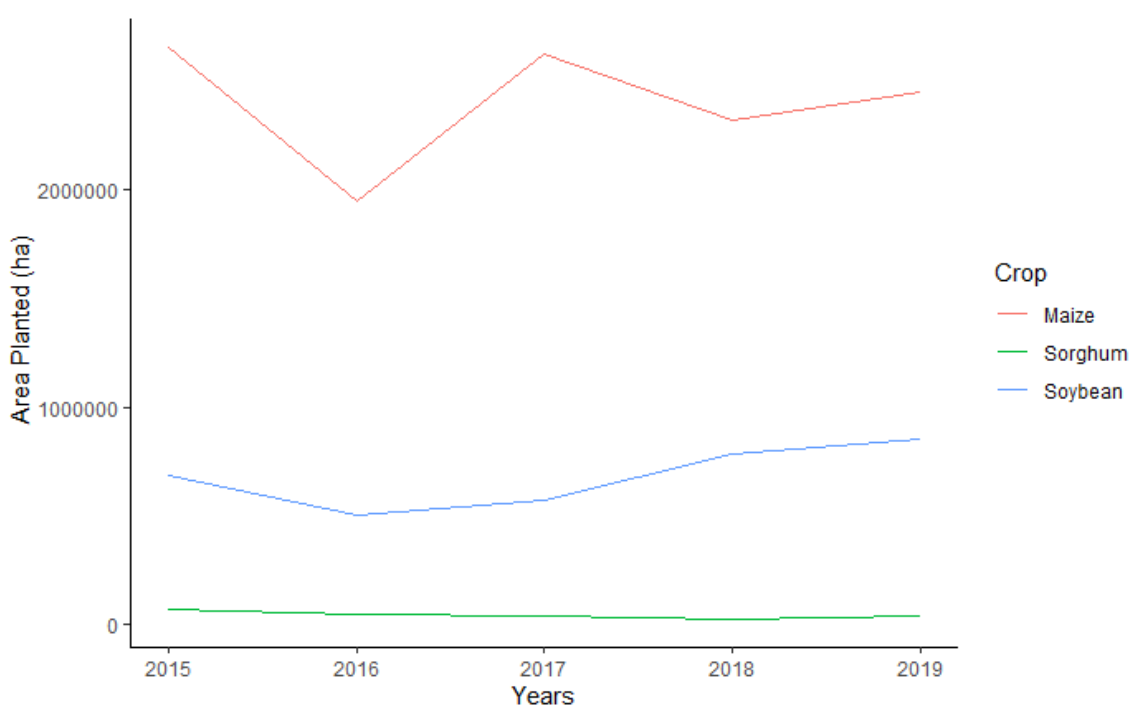


Figure 6: Comparison of area planted (hectares) of maize, sorghum and soybean in South Africa from 2015 to 2019 (Crop Estimate Committee, 2016; 2018;2019).

Uses of sorghum

Sorghum plays an important role in health, food security and the economy of many countries (Adegbola *et al.*, 2013). In developing countries sorghum is used as a food source in the form of bread, pasta, traditional beer, porridge (Mabele), and sorghum meal (Matalbella). Sorghum is considered a staple food, as it is consumed frequently, in large quantities and is a dominant part of the diet in a community, serving as their primary source of calorie intake (Duodu *et al.*, 2003). Sorghum is considered a staple food in more than 30 countries in the semi-arid regions and at least 500 million people rely on it as their daily meal (Reddy *et al.*, 2010). In contrast, developed countries such as in Australia, Europe, South America and North America, make use of sorghum as a source for animal feed (Bhagavatula *et al.*, 2013; Ramatoulaye *et al.*, 2016).

Sorghum grain is gluten free and therefore safe to eat for people who are gluten sensitive, although sorghum bread is often flatter and denser due to the lack of gluten.

Alternate gluten free products, such as almond flour or coconut flour, are very costly and not readily available compared to products with gluten (Lee *et al.*, 2007; Medeiros *et al.*, 2011). As a result, sorghum may be appealing to the gluten free market and is cheaper than maize and wheat (Phuong, 2018). Sorghum grain contains high antioxidant content, making it attractive to customers looking for healthier diets (Anglami, 1998; Phuong, 2018). Malted sorghum (GM class) can be used in the brewing industry, as it can provide malt extract at a lower cost than barley (Middleman, 1986; Ogbeide, 2011). Adegbola (2013) stated that a one-to-one ratio of sorghum and barley malt may be used to brew beer, reducing the input costs but partially retaining the quality of a barley malt. Darker coloured sorghum varieties are used for this purpose since they produce a bitter taste which is preferred in brewing (Doggett, 1970).

Additionally, sorghum presents a promising opportunity for the biofuel industry (Rigdon *et al.*, 2013). In 2014, the increased yield of approximately 3.4 ton ha⁻¹ made the crop more appealing in South Africa, with a potential use in the biofuel industry development. Bloomberg News (2013) stated that an approximate 771 000 tonnes per annum was allocated to be utilised for this purpose although Sihlobo (2018) estimated 500 000 tonnes per annum would be utilised. However, due to the decrease in area planted and annual yield, the government reconsidered sorghum as a biofuel crop (Sihlobo, 2018) and the use of sorghum as a biofuel crop is not currently a local priority. However, in Nigeria and the USA it is utilised by the biofuel industry (Agbro and Ogie, 2012; Phuong, 2018).

Constraints in sorghum production

Major constraints in sorghum production may be associated with abiotic and biotic factors including drought, insects, birds, pathogens and weeds (Thakur *et al.*, 2006;

Amelework *et al.*, 2016). The importance of each of these constraints differs from area to area as well as within and between countries. In countries such as Ethiopia, South Africa and Uganda shoot fly (*Atherigona soccata* Rondani) is a reported problem while in Mozambique it has a lesser impact (Wortmann *et al.*, 2006). Shoot fly attacks sorghum plants in the first to sixth leaf stage and hatched larvae feed on the growth points. Infestations of up to 43% were reported in late plantings in South Africa (Van Rensburg and Van den Berg, 1992). Systemic insecticides can provide efficient control against shoot fly, while cultural practices such as early planting can reduce the risk of infestation (Van Den Berg *et al.*, 2005). Resistance breeding against shoot fly may be a solution to this biotic stress and the discovery of 29 resistant quantitative trait loci by Satish *et al.* (2009) may aid in this quest.

The white-eyed parakeet (*Aratinga leucophthalma* Muller), ruddy ground dove (*Columbina talpacoti* Temminck), house sparrow (*Passer domesticus* L), picazuro pigeon (*Patagioenas picazuro* Temminck) and blue-black grassquit (*Volatinia jacarina* L) are the bird species documented to feed on sorghum (Mofokeng and Shargie, 2016). However, in South Africa the red billed quelea (*Quelea quelea* L) are the most destructive birds reported on sorghum as they feed prolifically on grain. Quelea birds are a problem in the Eastern Cape, Karoo, KwaZulu-Natal midlands, and Western Cape (Faansie, 2016). Controlling birds necessitates scaring the birds daily which is labour intensive and therefore increases production costs (Mwadalu and Mwangi, 2013). Spraying the active ingredient, Fenthion, commonly known as Quelea-tox, in infested areas is often applied (The New Humanitarian, 2009). The chemical is registered for control of birds and is used in veterinary facilities. The potential negative effect of this chemical is to the environment, especially the poisoning of wild birds (INCHEM, n.d). In extreme cases, blowing up bird flocks with dynamite or firebombs

in places where they are concentrated is practiced (The New Humanitarian, 2009). Many small-scale producers tend to avoid the use of white sorghum varieties and select varieties with tannins since they are unpalatable to birds (Mwadalu and Mwangi, 2013).

Striga spp. (*Striga asiatica* (L) Kuntze and *Striga hermonthica* (Delile) Bentham) commonly known as witchweed are parasitic weeds that reduce cereal production including sorghum (DAFF, 2010; Kenyi *et al.*, 2017; Belay, 2018). These species damage the crop before emergence and parasitize the host roots, using their water and nutrients, leading to leaf wilting, chlorosis and rolling (DAFF, 2010; Williams and Boehm, 2017). Damage caused by *Striga* spp. vary according to cultivar and infestation levels (Kenyi *et al.*, 2017). Globally it is estimated that 20 to 80% yield loss in sorghum is attributable to this weed. In Africa, losses of 40% are estimated, while in South Africa 50 to 100% yield loss may occur (Lagoke *et al.*, 1991 cited by Belay, 2018; Hearne, 2009; Joubert, 2013). Once the weed has established itself in the field it can produce approximately 500 000 seeds per plant which can survive in the soil for up to 20 years (DAFF, 2010; Koichi *et al.*, 2010). Control of witchweed can be achieved by the application of the herbicide 2,4-D (Korwar and Friesen, 1984). The cost of this chemical makes it less affordable to small scale farmers (Mrema *et al.*, 2017). Use of resistant cultivars offers an affordable and environmentally friendly approach against *Striga* spp., as these can reduce seed production and thus the reservoir in the soil (Belay, 2018). Cultural practices such crop rotation with cotton and peanuts also help in the management of *Striga* spp. (DAFF, 2010).

Abiotic factors, such as periods of dry weather during the production which may occur during any crop growth stage, pose a threat to sorghum production (Rao, 2004). However, research has shown that certain growth stages are more vulnerable to water

deficiencies. Water stress at the beginning of the growing season leads to poor crop stands and delays the flower primordia (stage 6). A reduction in dry mass accumulation occurs if water stress coincides with grain filling (stage 7-8). Cultivars that have drought escape characteristics, such as early maturity, shallow soil surface root systems (utilising water after short rainfall events) and decreased leaf area (reduced transpiration) may reduce the risk (Simane *et al.*, 1998; Tumwesigye and Musiitwa, 2002; Rao, 2004). Understanding risks associated with sorghum production will also afford producers the opportunity to optimise their cropping systems.

WHAT IS GRAIN MOLD?

SGM is a biotic plant disease, is the fungal deterioration of sorghum grain as the result of a complex of fungi that infect and colonise sorghum spikelets between flowering (stage 6) and harvest (stage 9; Bandyopadhyay *et al.*, 2000). More than 40 genera of fungi are associated with grain mold of sorghum (Forbes *et al.*, 1992). Parasitic fungi commonly isolated from grains include *Curvularia* spp. and *Fusarium* spp. which can infect immature grain and may penetrate the pericarp (Figure 4), given favourable environmental conditions. Saprophytic species which are commonly isolated include *Alternaria* spp., *Cladosporium* spp., and *Phoma* spp. (also known as *Epicoccum* spp.). These are not true pathogens but superficial grain colonisers that occur at later grain development stages and cause minor damage (Williams and Rao, 1981; Forbes *et al.*, 1992). However, *Cladosporium* spp. have been isolated from immature sorghum grain seven days post-flowering which suggest they may also be true grain mold pathogens (Melake-Berhan, 1996). Grain mold is often confused with grain weathering, which is colonisation of grain after physiological maturity under high moisture conditions (Thakur *et al.*, 2006). The relationship between grain mold and weathering in relation

to sorghum growth stages, including the time difference between infection, colonisation and sporulation are illustrated in Figure 7 (Thakur *et al.*, 2006).

Sorghum grain mold is a concern in areas where warm air temperatures (25-35 °C) and high humidity (85-90%) coincide with the flowering (stage 6) to harvest stages (Stage 9; Bandyopadhyay *et al.*, 2000; Little *et al.*, 2012). It is important to note that variations in weather at various developmental stages will support various species of the fungal complex and result in different fungi prevailing at any one time (Menkir *et al.*, 1996).

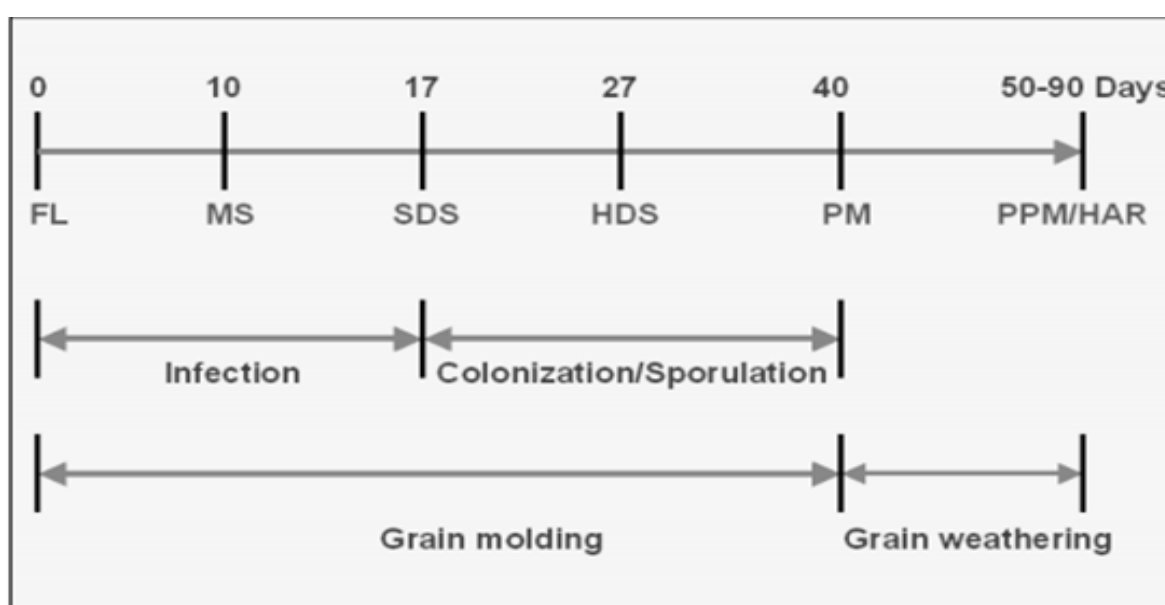


Figure 7: Relationship of grain mold and weathering with sorghum host crop during the physiological growth stages measured in days. FL = Flowering; MS = Milk stage; SDS = Soft dough stage; HDS = Hard dough stage; PM = Physiological maturity; PPM = Post physiological maturity; HAR = Harvesting (Thakur *et al.*, 2006).

***Fusarium graminearum* species complex**

Fusarium graminearum is a homothallic fungus, capable of sexual reproduction from a single organism, as opposed to heterothallic species which has sexes that reside in different individuals and require two compatible partners to produce sexual spores. *MAT1-1* and *MAT1-2* are two idiomorphs (mating types) that allow *Fusarium* species,

including *F. graminearum* to reproduce sexually (Miedaner *et al.*, 2008). Sexual reproduction in this fungus has been observed in the laboratory suggesting that members of this group may cross in the field under natural conditions (Goswami and Kistler, 2004). Sexual outcrossing of the mating types may aid the pathogen to adapt to a changing environment for it to survive and to bring changes to the population structure that may overcome plant host resistance genes (McDonald, 1997; Xu, 2012).

Fusarium graminearum has been classified under *Fusarium* section Discolor. This section differentiates *Fusarium* species from others of the same genus by producing chlamydospores, having thick-walled, septate macroconidia and an absence of microconidia (Leslie and Summerell, 2006). The morphology of *F. graminearum* is characterized by the production of macroconidia, that are relatively slender, thick-walled and can be straight to slightly curved. The macroconidia can be 5 to 6 septate with distinct septa. Unlike other *Fusarium* spp., the presence of macroconidia is rare. Chlamydospores may form, but this happens over an extended period, and thus the absence of chlamydospores is not a diagnostic character (Figure 8; Leslie and Summerell, 2006).

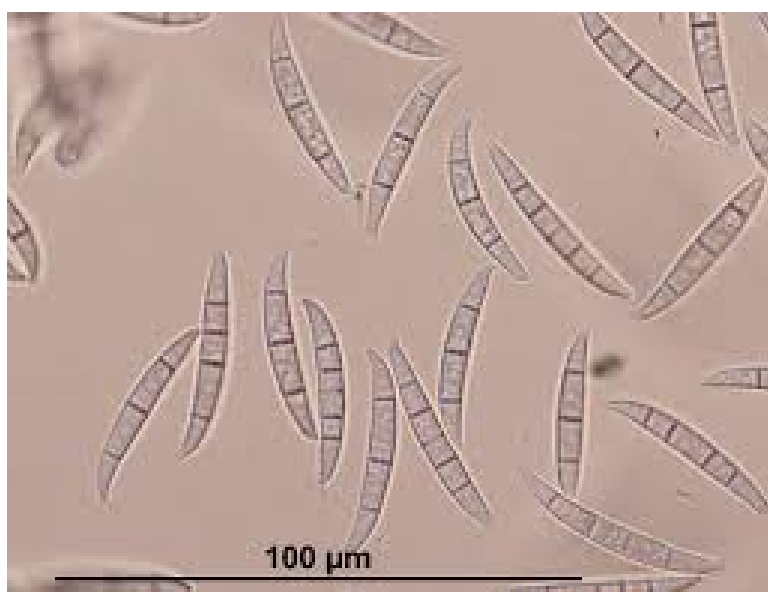


Figure 8: Micrograph of *Fusarium graminearum* macroconidia (Integrated Crop Protection, 2018).

The recognition that members within *F. graminearum* have geographic and host preferences led to phylogenetically varying cryptic species that form what is now known as the *FgSC*. Multi-locus genotyping coupled with genealogical concordance of phylogenetic species recognition was used to designate the phylogeny of the species status, identifying 16 cryptic species (O'Donnell *et al.*, 2000, Taylor *et al.*, 2000; O'Donnell *et al.*, 2004; Starkey *et al.*, 2007; O'Donnell *et al.*, 2008; Yli-Mattila *et al.*, 2009; Sarver *et al.*, 2011; Aoki *et al.*, 2012; Table 1). Three of the species within the *F. graminearum* complex (*F. meridionale*, *F. acaciae-mearnsii* and *F. cortaderiae*) are associated with sorghum grain in South Africa, the principal crop host of this study (Mavhunga, 2013). Further classification of the *FgSC* members is based on trichothecene chemotypes.

Trichothecenes are secondary metabolites or mycotoxins and arbitrarily divided into four groups namely type A, B, C and D. Groups specific to *Fusarium* are type A and B while both type C and D are not produced by this genus. The difference between type A and B trichothecenes is the carbonyl structure connected to the C-8, which is absent in type A (Desjardins, 2006; Köppen *et al.*, 2010). Type A *Fusarium* trichothecenes include trichodermol, trichodermin, 4,15-diacetoxyscirpenol, neosolaniol, T-2 toxin, isotrichodermol, calonectrin and 7,8-dihydroxy calonectrin which are regarded as being of great importance compared to type B trichothecenes because of their greater level of toxicity (McCormick *et al.*, 2011). Type A trichothecenes, such as T-2 and HT-2 mycotoxins are produced by *F. oxysporum* and *F. sporotrichioides* and *F. poae*. Type B trichothecenes, including DON and NIV are produced by *FgSC* and *F. culmorum*. Type B trichothecenes produced by the *FgSC*

are classified into three chemotypes namely 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON) which produce the mycotoxin deoxynivalenol and the NIV chemotype which produces nivalenol (Boutigny *et al.*, 2011). Zearalenone is also a primary mycotoxin associated with the *FgSC*, commonly detected in different cereal crops including sorghum (Richard, 2007).

Some members of the *FgSC* display variability in terms of host specificity and chemotype production but reasons for this are still unclear (Beukes *et al.*, 2016; Hao *et al.*, 2017). *F. boothii* is mostly associated with maize but it has been detected on barley and wheat, producing only 15-ADON (Boutigny *et al.*, 2011; Aoki *et al.*, 2012; Mavhunga, 2013). *F. brasiliicum* is primarily associated with wheat and produces 3-ADON and NIV (Boutigny *et al.*, 2011; Aoki *et al.*, 2012). Whereas *F. acaciae-mearnsii*, *F. cortaderiae*, and *F. meridionale* are associated with sorghum and primarily produce NIV, they have been detected on other cereal crops such as maize and wheat where they, except for *F. meridionale*, produce 3-ADON (Boutigny *et al.*, 2011; Lamprecht *et al.*, 2011; Aoki *et al.*, 2012; Mavhunga, 2013). *F. graminearum sensu stricto* has been detected on maize roots, wheat and barley (Boutigny *et al.*, 2011; Lamprecht *et al.*, 2011) and produces 3-ADON, 15-ADON and NIV (Aoki *et al.*, 2012).

Table 1: *Fusarium graminearum* species complex lineages and associated trichothecenes.

Species	Lineage	Mycotoxin	Reference
<i>F. austroamericanum</i>	1	3-AcDON and NIV	O'Donnell <i>et al.</i> , 2004
<i>F. meridionale</i>	2	NIV	O'Donnell <i>et al.</i> , 2004
<i>F. boothii</i>	3	15-AcDON	O'Donnell <i>et al.</i> , 2004
<i>F. mesoamericanum</i>	4	3-AcDON and NIV	O'Donnell <i>et al.</i> , 2004
<i>F. acaciae-mearnsii</i>	5	3-AcDON and NIV	Roux <i>et al.</i> , 2001
<i>F. asiaticum</i>	6	3-AcDON, 15-AcDON and NIV	O'Donnell <i>et al.</i> , 2004
<i>F. graminearum sensu stricto</i>	7	3-AcDON, 15-AcDON and NIV	Akinsanmi <i>et al.</i> , 2004
<i>F. cortaderiae</i>	8	3-AcDON and NIV	O'Donnell <i>et al.</i> , 2004
<i>F. brasilicum</i>	9	3-AcDON and NIV	O'Donnell <i>et al.</i> , 2004
<i>F. vorosii</i>	Not assigned lineages	15-AcDON	Starkey <i>et al.</i> , 2007
<i>F. gerlachii</i>		NIV	Starkey <i>et al.</i> , 2007
<i>F. aethiopicum</i>		15-AcDON	O'Donnell <i>et al.</i> , 2008
<i>F. ussurianum</i>		3-AcDON	Yli-Mattila <i>et al.</i> , 2009
<i>F. nepalense</i>		15-AcDON	Sarver <i>et al.</i> , 2011
<i>F. louisianense</i>		NIV	Sarver <i>et al.</i> , 2011
<i>Fusarium</i> spp. NRRL 34461		3-AcDON	Starkey <i>et al.</i> , 2007; Aoki <i>et al.</i> , 2012

Inoculum, dissemination, disease cycle and infection process

F. graminearum produces sexual and asexual spores namely ascospores and macroconidia, respectively (Figure 9; Wagacha and Mothomi, 2007). The sexual stage of the fungus is critical for the initiation of disease (Goswami and Kistler, 2004; Beyer *et al.*, 2005) although recent research suggests that initial infection may also be

caused by macroconidia (Gale *et al.*, 2002). Infected crop residues such as maize, sorghum and wheat from previous seasons serve as shelter for the fungus to overwinter, as a result helping with spore dissemination. Furthermore, weeds are known to be principal sources of inoculum in the field (Gale *et al.*, 2002; Goswami and Kistler, 2004). The mature sexual fruiting body (perithecium) that develops on crop residues, releases ascospores into the air and these are subsequently spread by air currents, insects, or rain to the host crop (Xu, 2003). Macroconidia are spread by rain, insects or to a lesser extent air currents and primarily cause secondary infection on the flowering spikelets (Miedaner *et al.*, 2008). Flowering (Stage 6) is the vulnerable growth stage of the sorghum crop for subsequent grain infection by *FgSC* (Figure 7; Thakur *et al.*, 2006). Research has shown that the lodicules are a source of energy for the fungi (Figure 10; Das *et al.*, 2012).

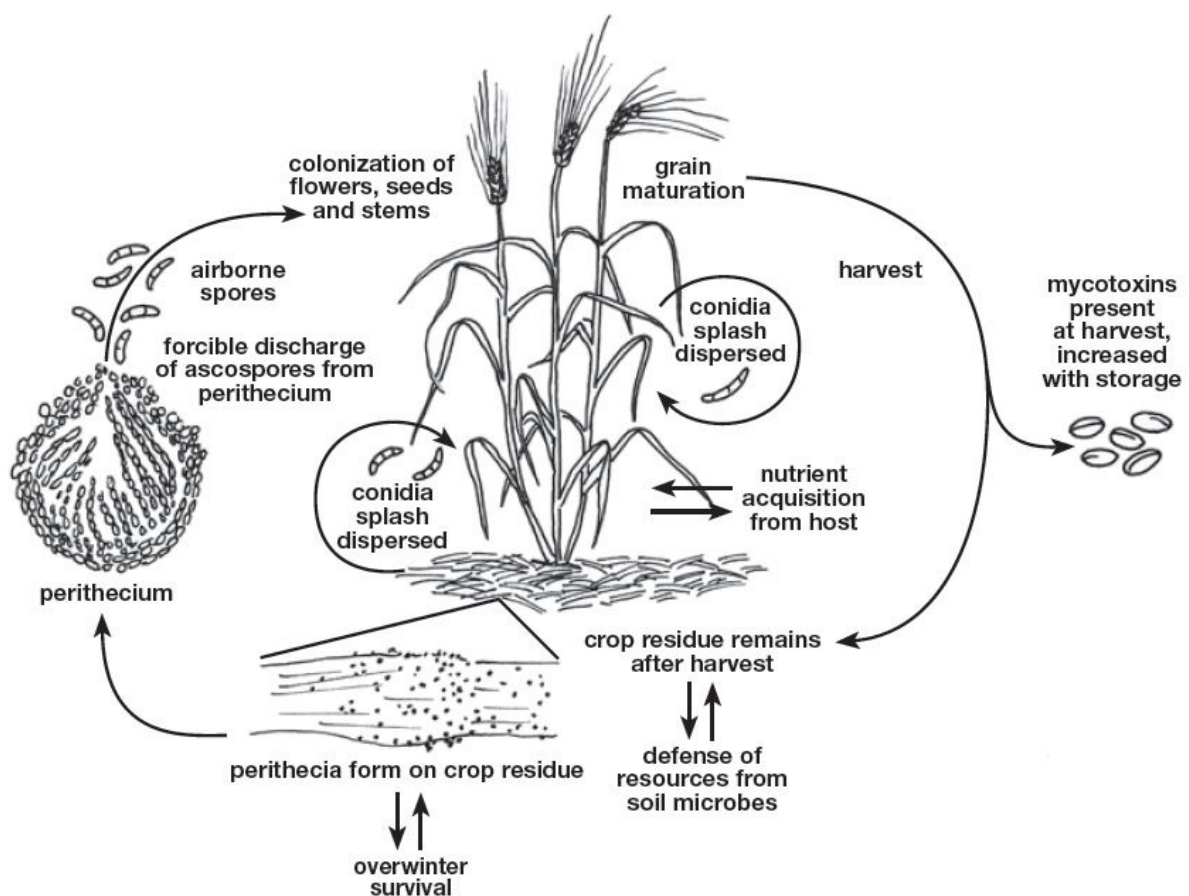


Figure 9: A disease cycle of *F. graminearum* on cereal crops (Trail, 2009).

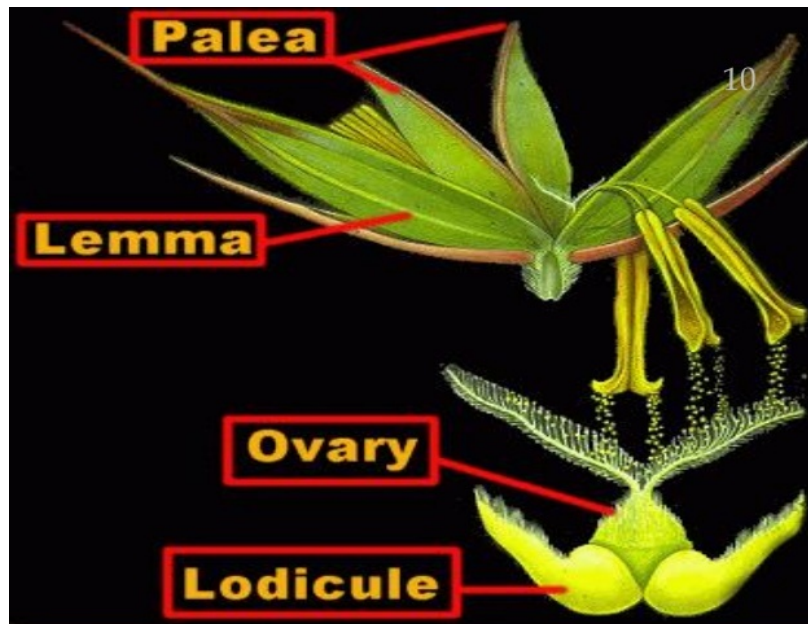


Figure 10: Floral anatomy of the sorghum plant (Gayatonde, 2016). Mycelium growth first occurs on the hilar end of the grain (Figure 3) where it transfers to the pericarp surface (Das *et al.*, 2012). The fungus enters the pericarp where it can cause internal infection of the embryo and endosperm (Thakur *et al.*, 2006). Infection is not only limited to the grain but may extend to the spikelet tissues such as lemma, lodicules, glumes and palea and subsequently progresses to the base of the flower (Figure 10; Bandyopadhyay *et al.*, 2000). The internal surfaces of these tissues are thin walled and as such increase the likelihood of penetration by *FgSC*, thus providing multiple opportunities for infection (Tekle *et al.*, 2012).

Symptoms and signs

A pinkish-white powdery mycelium is produced on the grain by *FgSC* in which over time the mycelium becomes pink and fluffy (Thakur *et al.*, 2006). A change in environmental conditions soon after flowering may cause the grain to appear symptomless, allowing the fungus to remain within grains without the manifestation of external symptoms (Navi *et al.*, 2005). It is only when grain is surface sterilized and plated onto artificial media that mold fungi become evident.

Economic importance

Infected grain

Grain mold is a constraint in sorghum growth, where a 100% loss of yield in susceptible cultivars has been recorded, thus affecting the marketing of the grain (Williams and Rao, 1981). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) estimated losses in Africa and Asia to be around US\$ 130 million due to this disease complex (ICRISAT, 1992). Over two decades later monetary losses due to grain mold seems to have increased by 19%, as WHO/FAO (2012) records losses of up to US\$ 670 million. Audilakshmi *et al.* (2007) reported a 10-30% reduction in the market price of sorghum associated with mold score ratings of 3 and 4, where 1 no mold and a rating of 5 indicated 50% mold on grain. Thus, the physical appearance of produce has a direct influence on the pricing, even though discoloured grain does not imply internal infection (Das *et al.*, 2012). Grain value is further reduced by compromised seed mass, germination, nutritional value, seed viability and presence of mycotoxins (Williams and McDonald, 1983; Thakur *et al.*, 2006). Grain mold fungi can induce early development of the black layer, a condition linked to physiological maturity of sorghum (Figure 1). When grain is subjected to humidity levels above 95% pre-harvest sprouting of grain in the panicle may occur approximately 15 days post-fertilisation (Bandyopadhyay *et al.*, 2000; Das *et al.*, 2012).

Impact of mycotoxins

When grain is infected with mold, it is under significant threat of concomitant mycotoxin accumulation. Mycotoxins are secondary metabolites which are produced by specific toxin producing fungi before harvest when they invade the crop host during the season or post-harvest in storage (Miller, 1995; Placinta *et al.*, 1999). They pose a serious risk to world food security as infected grain cannot be consumed by humans or livestock.

Additionally, safety issues are raised when the contaminated grain is ingested as they can induce health problems in humans and livestock (Navi *et al.*, 2005; De Ruyck *et al.*, 2015; Misihairabgwi *et al.*, 2019). As SGM is associated with mycotoxins, DON, NIV and ZEA, sorghum grain products are at risk of mycotoxin accumulation (Misihairabgwi *et al.*, 2019). Mycotoxicosis occurs because of ingesting mycotoxin contaminated grain (Bhat *et al.*, 2010). The effects of mycotoxins differ according to the toxin involved, the quantity ingested, as well as the immune system and the age of the victim (WHO/FAO, 2012).

DON is the trichothecene most widespread globally and is synthesised by *F. graminearum* and *F. culmorum* (W.G.Smith) Saccardo (Sarlin *et al.*, 2006; Misihairabgwi *et al.*, 2019). Trichothecenes have a passive movement through human membranes where they are absorbed. Bhat *et al.*, (2010) reported that, if trichothecene contaminated feed is consumed, the mycotoxicosis symptoms can be seen within hours. A European Commission study between 2002 and 2003 found that 57% of grain samples were contaminated with DON with maize being the most contaminated, followed by wheat, barley, oats and rye (Schothorst and Van Egmond, 2004). When chickens feed on DON contaminated grain, the toxin can be transferred embryonically as low amounts have been detected in eggs (Bhat *et al.*, 2010). The toxin causes food refusal in farm animals, nausea and vomiting in humans and is referred to as vomitoxin (Rotter *et al.*, 1996).

NIV is produced by *FgSC*, *F. culmorum*, *F. cerealis* and *F. poae*. Compared to DON, it is produced at low concentrations but is more toxic to humans and livestock (Ryu *et al.*, 1988; Desjardins, 2006; Pestka, 2007). Once the toxin is in the system of the individual (humans or livestock) it may inhibit protein synthesis, deoxyribonucleic

acid (DNA) and ribonucleic acid (RNA) replication and, as a result, lead to cell death and poisoning of lymphatic and intestinal systems (De Lucca, 2007).

ZEA is a phytoestrogenic mycotoxin affecting the reproductive system of humans and animals (Turcotte *et al.*, 2005). It is commonly associated with *F. graminearum*, *F. cerealis*, *F. culmorum*, *F. equiseti* and *F. sporotrichioides* (Kumar *et al.*, 2008; Misihairabgwia *et al.*, 2019). Although it is commonly associated with maize, it is often observed in other crops such as barley, millet, sorghum and wheat (Matumba *et al.*, 2014; De Ruyck *et al.*, 2015).

DISEASE QUANTIFICATION

Accurate measurement of disease is important as it quantifies the prevalence and extent of damage caused, which is essential to establish the necessity and effectiveness of appropriate management strategies (Agrios, 2005). The accuracy and precision obtained during disease or pathogen assessment will offer a measure of the relationship between disease mitigation theory and its application (Shokes *et al.*, 1987 cited by Nutter *et al.*, 1991; Nutter and Schultz, 1995; Nutter *et al.*, 2006). This is supported by Kranz (1988) stating that disease measurement is crucial to epidemiological research, crop loss assessments, and plant disease surveys, in order for applications to be viable.

Disease and pathogen assessments refer to two different concepts that cannot be used interchangeably. Disease assessment refers to the measurement of disease symptoms (i.e., host response to injury visible to the human eye using visual estimates or electronic sensors) while pathogen assessment refers to the direct measurement of the pathogen population for example, using enzyme-linked immunosorbent assay (ELISA), ergosterol or quantitative polymerase chain reaction (qPCR) (Nutter *et al.*, 2006).

Measurement of grain mold

Visual estimates

Visual estimates of SGM have been proven to be biased and unreliable in terms of the true value of the disease or pathogen colonisation. However, due to the development of the new techniques the problem has been alleviated and, as a result, accurate and precise methods of measurement can be applied (Nutter *et al.*, 2006; Mpofu and McLaren, 2014). Visual estimation of grain mold severity based on a global common scale indicates the degree of surface colonisation of the grain. There are well-defined rating scales, including 1-5 grain mold (1 = no mold and 5 $\geq$ 50% grain mold) (Bandyopadhyay and Mughogho, 1988) and a similar 1-9 scale (1 = no mold and 9 $\geq$ 75% grain mold (Rao *et al.*, 2012). These scales are easy to apply and are very convenient since screening of large samples can be done in a relatively short time (Bandyopadhyay and Mughogho, 1988; Rao *et al.*, 2012). Although grain mold infection starts from flowering growth stage (Forbes *et al.*, 1992; Thakur *et al.*, 2006) both the scales are designed for use in-field at physiological maturity (Thakur *et al.*, 2006; Das *et al.*, 2012) and do not consider progressive colonisation. Visual assessments do not always reflect the extent of tissue colonisation by pathogens, particularly in grain molds of sorghum caused by a pathogen complex that colonises the grain both superficially and internally (Coetzee, 2015). This may sometimes lead to misinterpretations of the data, leading to incorrect conclusions about overall disease in a crop and the application of inappropriate disease management practices. Screening for resistance in plant breeding programs can also be jeopardised when using visual scoring of SGM, potentially placing incorrect sorghum cultivars as susceptible when in fact the fungus is growing on the peripheral layers of the kernel causing little internal damage. Conversely, susceptible cultivars can be categorized as

resistant when the internal structure is infected with a fungus despite the cleaner outward appearance (Audilakshmi *et al.* 1999; Mpofu and McLaren, 2014).

Ergosterol analysis

Ergosterol, a sterol in the cell membranes of fungi, functions as a regulator of membrane fluidity and permeability (Yang *et al.*, 2015). Quantification of ergosterol offers a means of measuring total fungal colonisation within host tissues (Doohan, 1999). Seitz *et al.* (1977) introduced total fungal biomass quantification using ergosterol, measured by ultraviolet spectrometry to both detect the sterol presence and to compare its concentration in sorghum grain fractions. However, both Seitz *et al.* (1977) and Jambunathan *et al.* (1991) used high performance liquid chromatography (HPLC) to quantitatively measure ergosterol content. Jambunathan *et al.* (1991) reports on a technique which makes use of an alcohol-base for ergosterol extraction and the subsequent use of hexane (non-polar solvent) for separation. Following extraction there is an evaporation phase that leaves behind a precipitate that is reconstituted in HPLC-grade methanol. The final step of the technique is separation using a C-18 reverse column chromatographic column under a high pressure and optical detection at 285 nm (Gessner and Schmitt, 1996). Furthermore, ergosterol concentration provides an indication of the extent of internal mold colonisation which is not externally visible (Mpofu, 2009; Mpofu and McLaren, 2014).

Audilakshmi *et al.* (1999) indicated that bias with visual ratings may arise due to the visibility of superficial exterior symptoms on white grain cultivars compared to red and brown cultivars. These findings were corroborated in a grain mold study conducted at the University of the Free State, including 11 sorghum genotypes, where no correlation was recorded between visual assessment and ergosterol accumulated in the grain inoculated with four fungal pathogens. This question's the accuracy of

categorizing genotypes as either resistant or susceptible in resistance screening programs based solely on visual ratings (Mpofu and McLaren, 2014; Coetzee, 2015). In contrast, two independent studies provide the antithesis, Seitz *et al.* (1983) and Rao *et al.* (2012), where a significant positive correlation between visual estimates of grain mold and the measurement of ergosterol implies that either one of the methods can be used. Although, ergosterol provides a measure of total fungal biomass it is unable to quantify specific species present. This limitation can be overcome with the use of molecular techniques such as qPCR (Nicholson *et al.*, 2003).

Mycotoxin measurement

Mycotoxins are stable in nature and can thus survive processing and remain in food products. They are persistent and measurement is therefore of great importance to mitigate losses in the food or feed industry and ensure food safety (Shephard *et al.*, 1996; Lee and Ryu, 2017). Many countries have introduced laws that enforce mycotoxin measurements to determine whether products have levels of mycotoxins above regulatory levels (Rahmani *et al.*, 2009). In South Africa only a few mycotoxins are regulated including aflatoxins, DON, fumonisins and patulin (Viljoen, 2003; Katerere *et al.*, 2007; Misihairabgwi *et al.*, 2019). Depending on the product produced South African legislation restricts the maximum amount of DON to 1000-2000 µg/kg. The U.S-Food and Drug Administration (FDA) has set a maximum limit for DON at 1000 µg/kg for all wheat-derived products, whereas, the European Union has set a maximum limit of 1250 µg/kg for DON on all raw cereals other than durum wheat, oats (*Avena sativa*) and maize (Lancova *et al.*, 2008; Bhat *et al.*, 2010). Despite the effects of NIV, it is not internationally regulated (Pasquali *et al.*, 2010). The Food and Agriculture Organization of the United Nations has set a total tolerable level of ZEA in foods within the range of 50 µg/kg-1000 µg/kg, while a tolerable daily intake of 0.5

µg/kg body weight for ZEA has been established by the European food safety authority (Marroquín-Cardona *et al.*, 2014).

The non-homogeneous nature of mycotoxins in seed lots or consignments requires accurate sampling of the grain that will serve as a representative sample of the consignments. The preparation of samples before assessments is important in determining a quantitative measure of the toxins present (Turner *et al.*, 2009). Furthermore, Janse van Rensburg *et al.* (2011) suggest many factors contribute to the efficient quantification of mycotoxins, including, sample size, replication, available laboratories and technique applied. Janse van Rensburg *et al.* (2011) compared sub-samples of 25 and 250 g, replicated 25 times each and 5 g aliquots were analysed. The results indicated an increasing sample size and replication significantly improved the accuracy of the quantified mycotoxin.

Mycotoxins can be qualitatively (presence or absence) and quantitatively (specific measure or count) assessed using several techniques (Zheng *et al.*, 2006). This includes techniques such as the use of Thin-Layer Chromatography (TLC), HPLC, liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS; Zheng *et al.*, 2006; Bhat *et al.*, 2010) and ELISA (Rahmani *et al.*, 2009).

TLC is among the most common and simplest qualitative techniques, able to detect multiple mycotoxins simultaneously. The technique uses what is referred to as “matrix coated plates”. The compounds are mixed with specific solvents in the plates, which propel the compounds to separate and move along the plate. The distance displaced by these compounds is monitored and used to determine the identity of the mycotoxins. Just like any other form of detection, accuracy is ensured with a parallel run of a positive control (Bhat *et al.*, 2010). HPLC is the quantitative technique used to measure mycotoxins such as aflatoxin, fumonisins, ochratoxin, patulin,

trichothecenes (including DON and NIV) and ZEA in feed and food. This technique is dependent of fluorescent detection. Some mycotoxins, however, (e.g. fumonisins) lack chromophores, atoms responsible for giving a compound its colour, and therefore it is not accurately assessed by HPLC quantification. However, derivatization seems to aid in this obstacle by making compounds to be analysed the fluorescent derivatives. Coupling the HPLC technology with mass spectrometry (MS) eliminates such processes since it uses atmospheric pressure chemical ionisation to achieve accurate, efficient, precise and sensitive detection of mycotoxins, regardless of their fluorescence (Rahmani *et al.*, 2009; Santini *et al.*, 2009; Silva *et al.*, 2009).

HPLC-MS has gained favour among commercial laboratories for mycotoxin quantification due to its ability to measure multiple mycotoxins from different matrices with a single extraction procedure (Zheng *et al.*, 2006; Silva *et al.*, 2009; Bhat *et al.*, 2010). The technique reduces laborious steps needed in mycotoxins extraction, such as a clean-up process, eliminating substances in the test extract which may interfere with analyte detection (Rahmani *et al.*, 2009).

ELISA test kits are available on the market which may be used to detect mycotoxins in samples, qualitatively and quantitatively. Specifically developed antibodies capture the required analyte and a colour indicates whether the mycotoxin is present or not. The intensity of the colour reaction, based on photospectrometry, can indicate mycotoxin concentration (Woloshuk, n.d; Herrman, 2002). Test kits are easy to use and take less time than more advance technologies, however separate kits are required to detect each mycotoxin (Turner *et al.*, 2009).

It is worth noting that the technique used and location where mycotoxins are measured also significantly affect the outcome. A strong correlation ($R^2 = 0.996$) was obtained between the results of an ELISA and HPLC tests, although ELISA results

suggested higher concentrations than those with HPLC. A non-significant correlation between the results of two independent laboratories about the quantified mycotoxin content was found (Janse van Rensburg *et al.*, 2011). Therefore, it is important to note that time and expense in the approach to disease quantification may not be suitable for all pathosystems.

DECORTICATION: TANGENTIAL ABRASIVE DEHULLING DEVICE

Decortication, also known as debranning or dehulling, is a dry milling process that refers to the removal of the outer layers of the kernel which make up the seed coat (Figure 4). Sorghum grain needs to be dehulled and milled into flour before it can be sold to consumers and different levels of decortication are associated with various products (Van der Merwe *et al.*, 2005). Sorghum with red pericarps that are resistant to grain mold can be dehulled and milled into white flour (Thakur *et al.*, 2006).

The need for highly efficient milling by the industry necessitates the use of TADD devices (Reichert *et al.*, 1986), which can be applied in the laboratory for small scale sampling of grains. The machine consists of bottomless sample cups placed above an abrasive material (i.e., eight or twelve cups). The rotation of the abrasive material progressively scours the grain due to the close contact between the sample and the abrasive material (Oomah *et al.*, 1981; Reichert *et al.*, 1986). An air flow generated by a fan extracts the bran/hulls into collection bags to ensure sample separation (Oomah *et al.*, 1981; Reichert *et al.*, 1986; Chen *et al.*, 2013).

TADD was originally developed to measure the hardness of the grain, which is expressed as the difference in weight of the grain before and after decortication, determined from the percentage weight loss or as the time spent to remove peripheral layers of the kernel (Reichert *et al.*, 1986). Hogan *et al.* (1964) first introduced the principle of tangential abrasion where rice outer kernel layers were removed

successively without causing damage to the kernel, which is ideal as the kernels preserve their value. Despite the advantage of multi-sampling, a few limitations such as the abrasive material, generally sandpaper, wearing down with use, can produce inconsistent results over time. This can cause kernel breakage which results in excessive endosperm loss with the bran fraction (Mwasaru *et al.*, 1988; Chiremba, 2012).

Since grain mold discolours the grain, TADD could be used to improve the cosmetic value of the grain by removing the discoloured layers and subsequently affecting the market price positively (Audilakshmi *et al.*, 2007). This machine could also be a means to track fungal deposition and concomitant mycotoxins in the grain. Since the fungal complex that colonises and produces the mycotoxin in the grain can be found in the peripheral layers or be deep-seated in the endosperm of the grain (Bandyopadhyay *et al.*, 2000), dehulling can be used to identify where unwanted fungi and mycotoxins occur in grains and facilitate their removal. Coetzee (2015) conducted a model study whereby, sorghum grain was decorticated, to provide 1, 2, 4- and 6-minute fractions. After which colonisation by *FgSC* and mycotoxin levels were measured to determine the potential of decortication in reducing colonisation as well as mycotoxin accumulation. Based on the results of the study it was reported that decortication at three to four minutes which is the equivalent of 30% mass loss, was necessary to reduce the infection or *FgSC* fungal biomass. It was also recorded that DON and ZEA occur in the peripheral layers as decortication can easily remove these toxins. However, NIV was shown to be in the endosperm and 22% grain mass removal was needed to decrease its levels.

MANAGEMENT STRATEGIES FOR GRAIN MOLD

Chemical control

The use of agricultural chemicals such as fungicides against grain mold causing pathogens may provide a degree of protection. This disease control strategy is deemed to be not economical feasible especially for subsistence producers since they are costly or may not be able to be applied efficiently due to the lack of available infrastructure (Scheffer, 1997; Bandyopadhyay *et al.*, 2000). Bhat *et al.* (2010) reports that even if chemicals are effective in controlling fungi, they may also induce stress that may favour the production of mycotoxins. An *in vitro* fungicide study using azoxystrobin (Amistar[®]), epoxiconazole (Opus[®]), propiconazole (Tilt[®]), prochloraz (Sportak[®]) and tebuconazole (Folicur[®]) investigated the effects on the growth of *F. graminearum* mycelium inoculated on petri dishes and on wheat grains. Although the chemicals significantly reduced mycelial growth, inoculated wheat grains indicated that DON levels had increased significantly over the incubation period (Ramirez *et al.*, 2004).

Marley and Ajayi (1999) reported that timing of chemical application has a significant effect on the reduction of grain mold. Cromei *et al.* (2001) proposed the application of azoxystrobin, carbendazim and tebuconazole at mid-flowering of maize, as this was seen to have not only increased 1000 kernel weight but to have reduced ear rot incidence by 33% for both azoxystrobin and carbendazim and 50% for tebuconazole. Audilakshmi *et al.* (2007) reported that application of propiconazole at flowering of sorghum with repeated applications at ten-day intervals significantly decreased grain mold severity.

Biological control

Biological control refers to the use of a natural enemy to control or suppress a pathogen (Handelsman and Stabb, 1996). This method is often used to lower the impact that the disease control strategy has on the environment (Musyimi *et al.*, 2012). Furthermore, the application of biological control has received much attention due to its potential to decrease the production of mycotoxins (Matarese *et al.*, 2012; Nguyen *et al.*, 2017). Bacterial species, such as *Brevibacillus* spp. and *Streptomyces* spp., were chosen as potential biological control agents in Argentina against Fusarium head blight and production of DON in wheat (Palazzini *et al.*, 2007). Palazzini *et al.* (2016) recorded a significant reduction of disease severity through applying *Bacillus subtilis* (62-76%) and *Brevibacillus* spp (42-58%). In addition, it was observed that even though small dosages of these bacteria were applied, DON did develop although at low levels which were almost undetectable.

Cultural practises

Monoculture, where the same, or related plant species are continuously grown in succession, leads to an increased accumulation of pathogen inoculum (Janvier *et al.*, 2007). Crop rotation is the practise of cultivating a series of various unrelated crops across growing seasons in the same land and can be used to reduce pathogen inoculum build-up (Janvier *et al.*, 2007; Reza, 2016). Removal of related crops such as maize, oats, and wheat or planting of alternative non-host crops such as soybeans (*Glycine max*) in a rotation system may reduce the inoculum of *F. graminearum* (Lori *et al.*, 2009). In a rotation system where maize was followed by wheat or any related crops, an increase in the risk of mycotoxin accumulation was observed (Mansfield *et al.*, 2005). The preceding crop seems to make available the inoculum for the subsequent susceptible crop, planted in the following year. A rotation system of

maize/soybean/wheat was shown to be sufficient to reduce DON levels in wheat (Schaafsma *et al.*, 2001).

Crop residues left on the field aid in the saprophytic survival of *F. graminearum*. Owing to the enzymatic nature of the fungus it can degrade the residues and use them as nutrient source to sustain continued active growth compared with residues that are buried through tillage practices. Hence, burying the residues in the soil, within 10-20 cm, through tillage may help in reducing the inoculum build up (Edwards, 2004; Leplat *et al.*, 2013). However, the limitation of this practice is the ability of *FgSC* to survive (although not necessarily grow actively) for more than 4 years on and in buried residues.

Different types of nitrogen fertilizers have an influence on *F. graminearum* infestations (Bernhoft *et al.*, 2012). An increase in inoculum or disease symptoms was seen with the application of ammonium nitrate and calcium ammonium nitrate (Edwards, 2004; Leplat *et al.*, 2013). In contrast, urea has been shown to reduce disease symptoms, halt the reproduction of fungi, restrict chlamydospores formation and delay ascospores maturation (Edwards, 2004; Leplat *et al.*, 2013). Incorrect application of nitrogen lead to plants being vulnerable to molding since it interferes with cell wall and chemical makeup of the host plant (Van Arendonk *et al.*, 1997). In contrast, soils with insufficient or excessive amounts of nitrogen also render plants vulnerable to infection by *F. graminearum*. Thus, different nitrogen types and quantities need to be balanced to control infections in an effective manner (Lori *et al.*, 2009).

CONCLUSION

Sorghum grain mold not only causes economic losses for the producers but also poses a significant health risk for humans and livestock when contaminated grain products

are consumed. It is therefore necessary that strategies are in place to mitigate and manage grain mold. Moreover, it is worth noting that even though decortication may remove a portion of the fungal biomass and mycotoxins, this may not be sufficient to successfully reduce the risk where concentrations are too high. Understanding the SGM pathosystem through epidemiological knowledge of the fungal complex and mycotoxin production is essential if disease control strategies are to be optimized. Information concerning the host crop is also important to fully comprehend the importance of this disease. The correct management practices that target both in-field and harvested grain can only be implemented through the complete understanding of the pathosystem and ensure the successful use of processing techniques where economically feasible and the production of healthy products for consumption. Ultimately, integrating plant protection into all spheres of crop production and the food system will provide sustenance and sustainability to our society.

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CHAPTER 2: EVALUATING THE EFFECT OF DECORTICATION ON SORGHUM GRAIN MOLD FUNGI AND CONCOMITANT MYCOTOXINS

ABSTRACT

Sorghum production is expected to increase in the future, and with Africa's growing population, this crop might assist in meeting the rising need for grain foods. However, a biotic constraint such as sorghum grain mold (SGM) may place strain on the production and quality thereof. The fungal deterioration of sorghum grain, SGM, results due to a complex of fungi infecting and colonising sorghum spikelets, between flowering and harvest. Associated SGM fungi are responsible for the production of mycotoxins, potentially rendering grain unsuitable for animal and human consumption. The current study was proposed to evaluate the presence of fungi associated with superficial and deep-seated colonisation of sorghum grain through applying a processing tool, Tangential Abrasive Dehulling Device (TADD), to identify which fungi, colonise grain layers. Furthermore, concomitant mycotoxins of aflatoxin (AF; B₁, B₂, G₁ and G₂), deoxynivalenol (DON), fumonisins (FB; B₁, B₂ and B₃), zearalenone (ZEA) and 15-acetyldeoxynivalenol (15-ADON) were investigated on a portion of the grain samples (N = 24). Grain utilised were obtained from previously evaluated field trials for the National Cultivar Trial. Three commercial cultivars, PAN8816, PAN8706W and NS5511 were planted across two localities, Cedara and Greytown, with seven planting dates in total. The TADD was applied to dehull each cultivar-environment replicate, at 1, 2, 4 and 6 minutes (T₀ = whole grain; decorticated fractions = T₁, T₂, T₄, T₆). After identifying surface sterilisation times appropriate for each decorticated fraction, macroscopic and morphological observations were conducted of fungal growth observed. Fungi present were counted and expressed as percentage fungal load (isolation frequency). Selected genera were grouped, and sequencing was conducted to confirm fungi present. Primers *ITS* 1 and *ITS* 4 and *EF* 1 and *EF* 2 were utilised to identify fungi to a genus level and *Fusarium* specific identification, respectively. Decortication proved to have significant effect on reducing grain percentage, consequently exposing different grain fractions for fungal frequency determination. *Alternaria* spp., *Epicoccum* spp. and *Phoma* spp. did not only colonise superficial layers of the grain but could also be found deep-seated, in the endosperm, associated with T₆. *Colletotrichum* spp. and *Exserohilum* spp. were found in T₁, while *Curvularia* spp. were found at T₁ and T₆, suggesting that these fungi were grain layer specific and could be removed easily at the recommended decortication rates, with the exception of deep-seated *Curvularia* spp. *Fusarium* spp. was found to colonise the superficial layers and to be deep-seated as expected. Only four isolates of *Fusarium* spp. could be identified to specie level and shown to colonise specific layers of the grain. The isolates were identified as *F. graminearum*, this fungus was associated with T₀, T₁ and T₂, although, specific lineages were not determined in this study. This indicates that the fungus was both found in the superficial and deep layers of sorghum grain. Mycotoxins were detected in only nine samples, and only one sample exhibited ZEA mycotoxin concentration (1250 µg/kg) higher than the maximum legislative limit. All the recorded DON concentrations were lower than the maximum legislative level. The results emphasise the need to not only to focus on decortication since it is not solely effective for removal of fungi and mycotoxins but to also to focus on cultural practises and resistance breeding to combat the colonisation of grain by fungi and concomitant mycotoxin accumulation.

Keywords: Tangential Abrasive Dehulling Device, cultivars, superficial fungi, deep-seated fungi.

INTRODUCTION

Globally sorghum (*Sorghum bicolor* (L.) Moench) is relied on as an important cereal crop, for food, feed and fibre, fourth most relied on after barley (*Hordeum vulgare*), maize (*Zea mays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aestivum* L.; Reddy *et al.*, 2010). Sorghum is composed of starch, protein and vitamin B which are vital health components for both human and livestock nutrition (Adegbola *et al.*, 2013). Thus, making this crop important in food insecure countries especially in Africa, as eight of the world's most food insecure countries are found in Africa (Concern Worldwide US, 2020). It should be noted that the current exponential rate of population growth, the change in climate and the increasing demand for food will result in an estimated 60% requirement for a rise in food production by 2050 (Alexandratos and Bruinsma, 2012). This potentially poses a problem, particularly in Africa, since the majority of its countries have semi-arid areas, where conditions are not only harsh, i.e., heat, insufficient rain, soils with poor nutrients, as a result not ideal for crop production, but are also populated with billions of citizens, relying on home-grown crops (Silva *et al.*, 2004). However, grain crops such as sorghum may help in the growing demand of grain food (De Wet, 1986; Rao *et al.*, 2012).

Sorghum is associated with drought tolerance, the ability to grow under waterlogged soils (Awika and Rooney, 2004; Phuong, 2018) and for resilience in the above-mentioned environmental conditions (Tarr, 1962; Du Plessis, 2008). In the semi-arid tropics, sorghum products provide staple meals for 500 million people in 30 countries (Sorghum Checkoff, 2010). According to Reddy *et al.* (2010) ~ 55% of sorghum produced is for food consumption and ~ 33% is utilised for livestock feed. Globally, sorghum production is projected to rise by ~ 9% (Reddy *et al.*, 2010). Although, biotic constraints to sorghum production may hamper this growth, fungal

pathogens associated with field and post-harvest stages may pose a threat to quantity and quality of this production increase.

Sorghum grain mold affects sorghum production influencing yield, marketability, and crop usage (Das *et al.*, 2012). The fungal deterioration of sorghum grain as the result of a complex of fungi that infect and colonise sorghum spikelets between flowering and harvest is associated with SGM (Bandyopadhyay *et al.*, 2000). Studies indicated that sustained periods of high relative humidity (85-95%) and warm temperatures (21-35 °C), coinciding with the period between flowering and grain maturation are more conducive to increasing the severity of SGM (Bandyopadhyay *et al.*, 2000; Tarekegn, 2002; Thakur *et al.*, 2006; Das *et al.*, 2012; Little *et al.*, 2012). Variations in weather at the different developmental stages will however, support various species of the fungal complex, involving more than 40 genera, and result in different fungi prevailing at any one time (Forbes *et al.*, 1992). These include, but are not limited to, *Alternaria* spp., *Bipolaris* spp., *Cladosporium* spp., *Colletotrichum* spp., *Curvularia* spp., *Exserohilum* spp., *Fusarium* spp. and *Phoma* spp. (Forbes *et al.*, 1992; Bandyopadhyay *et al.*, 2000).

Losses associated with SGM include decreases in yield, grain mass, density, size, seed germination and associated grain discolouration, i.e. moldy grain and mycotoxin production (Williams and Rao, 1981; Thakur *et al.*, 2006). Williams and Rao (1981) recorded that a 100% loss can be achieved in susceptible cultivars. Whereas Singh and Bandyopadhyay (2000) documented 30-100% yield loss, although this is dependent on the prevailing environmental conditions at critical growth stages and the cultivar used. The economic losses, observed in Africa and Asia have recorded losses annually reaching an approximate total loss of US\$ 130 million (Thakur *et al.*, 2006).

The above-mentioned genera not only infect and colonise grain sorghum but are also responsible to produce mycotoxins, toxic secondary metabolites (Balota, 2012), potentially rendering grain unsuitable for animal and human consumption (Navi *et al.*, 2005). *Alternaria* spp., *Aspergillus* spp., *Epicoccum* spp. and *Fusarium* spp. produce mycotoxins which are of considerable concern. Tenuazonic acid is often associated with *Alternaria* spp. and *Epicoccum* spp. (Astoreca *et al.*, 2019), whereas aflatoxins are produced by *Aspergillus* spp. (Okoth *et al.*, 2017). *Fusarium* spp. are responsible for many mycotoxins including fumonisins, moniliformin, T-2 toxin, zearalenone (ZEA), nivalenol (NIV) and deoxynivalenol (DON). The latter three mycotoxins are closely associated with the *Fusarium graminearum* Schwabe species complex (FgSC). *F. meridionale*, *F. acaciae-mearnsii* and *F. cortaderiae*, are three species associated with the FgSC, which are commonly associated with sorghum grain in South Africa (Köppen *et al.*, 2010; Mavhunga, 2013). Mycotoxins can accumulate in the field, as well as during post-harvest storage. However, FgSC associated colonisation and concomitant mycotoxin accumulation is because of field related infections (Bhat *et al.*, 2010).

Generally, fungi associated with SGM colonise the grain superficially, resulting in the moldy appearance. However, in the case where fungi penetrate and deeper infection occurs, concomitant mycotoxins are produced, posing a threat to food safety (Thakur *et al.*, 2006). Therefore, sorghum primarily aimed for human consumption needs to have the outer layers of the kernel removed, i.e., decorticated, prior to milling, as the seed coat is considered less nutritious and contaminative to the flour during milling (Munck *et al.*, 1982; Awika *et al.*, 2003; Van der Merwe *et al.*, 2005). Additionally, infected grain deteriorates and as a result renders the grain useless in

processing as it can no longer be effectively decorticated (Jambunathan *et al.*, 1991; Forbes *et al.*, 1992; Bandyopadhyay *et al.*, 2000).

There is a considerable amount of research conducted on the decortication of sorghum grain in the field of food science, especially with regards to the influence on grain colour and hardness associated with product development and nutrition (Mwasaru *et al.*, 1988; Van der Merwe *et al.*, 2005; Bean *et al.*, 2006). However, limited research exists on the identification of the fungi and mycotoxins present on the grain fractions which remain after decortication. To determine if fungi associated with SGM are superficial or deep-seated, decortication affords plant pathologists the opportunity to initiate answering questions associated with fungal infections and their prevalence within grain fractions. The current study aims to identify fungi obtained from different fractions of sorghum grain to potentially identify which fungi are located within the structure of the grain. Additionally, the presence of mycotoxins was assessed and quantified on a limited sample size of the grain, to identify the need for future studies. We hypothesise that superficial fungi will be identified in grain decorticated at one and two minutes, where deep-seated fungi will be identified at grain decorticated at four and six minutes. In understanding these components, great value will be added to the processing of sorghum grain by knowing which fungi colonise the layers of the grain and at what rate of decortication process is needed to remove them, as well as if this process also affects concomitant mycotoxin.

MATERIALS AND METHODS

Field trials

Grains utilised in this study were collected from previously evaluated field trials, 2016-2018, conducted by the University of the Free State for the National Cultivar Trial (NCT). For the purpose of this study, three commercial cultivars were selected in order

to ensure varying plant and grain characteristics, these include PAN8816 (red), PAN8706W (white) and NS5511 (brown) genotypes. Trials were planted across two localities, Cedara (29.5477°S, 30.2667°E), and Greytown (29.0549°S, 30.6085°E; KwaZulu-Natal) for the above-mentioned seasons. Five planting dates were performed at Greytown; 1 and 23 November 2016, 12 and 28 December 2016 and 5 January 2017 (PN1, PN2, PN3, PN4 and PN5, respectively). Two planting dates at Cedara were included, planted on 5 December 2017 (PN6) and 1 November 2018 (PN7). Multiple planting dates were included to ensure flowering at various periods and to subject the developing grains to various weather conditions. The seven planting dates were viewed as independent environments and thus providing varying disease potentials. At soft dough panicles were bagged with aerated bags to minimise bird damage, particularly by the red billed quelea (*Quelea quelea*), in the field. The development of grain mold relied on natural inoculum and standard agronomic practices were followed to maintain each locality. Grains were harvested at 14.5% moisture and stored at room temperature (~ 25 °C) at the University of the Free State. The moisture content mentioned above is a recommended level for safe, and long-term storage and to also minimize any quality deterioration (Sadaka *et al.*, 2015). Additionally, a mite solution (NuPro Aerosol) is applied in storage to protect against the insect damage. Weather data including minimum and maximum temperature and relative humidity were collected for each season, from World Weather Online (2021; a and b), data are shown in the supplementary material (Table 1).

Decortication

The tangential abrasive dehulling device (TADD) consisted of eight sample cups with dimensions of 5 cm diameter by 5 cm depth, and a capacity to hold 50 g of seed per cup. The TADD was used to dehull each cultivar-environment replicate, a total of 210 samples (3 cultivars, 5 times, 7 planting numbers and 2 replicates) were prepared and weighed prior to decortication. The width between the abrasive surface, a horizontal rotating disc of sandpaper, and the cups was adjusted to 1 mm. During the dehulling process, the cups were closed with a fibre-glass lid so that the grain could move freely around without escaping. The grain was dehulled as they reached the abrasive surface, this process was maintained at 1725 revolutions per minute. An exhaust port was connected between the TADD and a compressor machine to collect bran. The samples, a maximum of 50 g in duplicates, (however some samples had a minimum weight of 30 g, as minimal grain was harvested), were dehulled at 1, 2, 4 and 6 minutes (T1, 2, 4 and 6, respectively). At each time interval, the abraded grain was removed from the cups by a vacuum suction system and weighed prior to storage. A control for each cultivar, recorded as zero minutes (T0), was reserved from the sample and not dehulled. The decorticated grain mass was measured, and the percentage weight loss reported for each time interval (Oomah *et al.*, 1981). A regression between the percentage grain removed and decortication time was plotted. The Abrasive Dehulling Index (AHI) was calculated by multiplying the inverse of the slope of the regression line by 60 to evaluate grain hardness. The AHI assesses the time in seconds taken by the abrasion to remove one percent of the grain mass, where a higher AHI value is associated with harder grain (Reichert *et al.*, 1988).

Surface sterilisation

To determine the fungal genera present and the extent of their colonisation, surface sterilisation was required, however, as decorticated samples varied from whole undisturbed grain to a 15% grain kernel, surface sterilisation times needed to be adjusted accordingly. Cultivar PAN8816 was used to investigate which sterilisation time would be appropriate for each decortication time. Five sterilisation times were selected, 180 (as the standard practice; Tesfaendrias, 2006), 90, 60, 30 and 15 seconds. The dehulled grain from each TADD time was sterilized with 1% Sodium Hypochlorite (NaOCl) and plated on the 90 mm Petri dishes filled with potato dextrose agar (PDA; BioLab) (three grains per plate) amended with 0.1 g/L streptomycin sulphate salt antibiotic (Glentham Life Sciences Ltd; Muvea *et al.*, 2018),. Petri dishes were transferred to the incubator for 7-days at 25 °C, observations were conducted after 24 hours and recording were taken daily. Once complete the fungal growth per plated grain was recorded using a binary method where 1 represented the presence of fungi and 0 presented the absence of fungi. The percentage colonisation was calculated per plate for each treatment and analysis of variance (ANOVA) was applied to determine the appropriate sterilisation time for each TADD time. A total of 125 Petri dishes were plated out.

Fungal incidence and isolation frequency

Once the surface sterilisation times were identified for each TADD time, the grain mold fungal incidence was determined on seeds obtained from the above decorticated samples. Grain was randomly selected from each cultivar-environment treatment and disinfected with 1% NaOCl. A 180 seconds surface sterilisation was applied to the unhulled control grain. Whereas 30 seconds were applied to the T1 and T2 dehulled grain and 15 seconds to the T4 and T6 dehulled grain, respectively. After each surface

sterilisation the samples were rinsed in sterile water three times. The samples were subsequently dried on a blotting paper under the laminar flow.

Sterile techniques were used to aseptically plate out the grain onto PDA amended with 0.1 g/L streptomycin sulphate salt antibiotic. Three grain samples were plated onto each Petri dish, yielding a total of 210 plates. The Petri dishes were incubated at 25 °C for 7-days, observations were conducted after 24 hours and recordings were taken daily. Once complete the fungal growth per plated grain was recorded where the presence of fungi on a grain was given a number i.e. 1. Where 1 represented the presence of fungi and 0 presented the absence of fungi. In the case where one grain had more than one fungus, all the present colony were given allocated a number (1) as well. The percentage colonisation was calculated per plate for each treatment. Colonies per plate were counted and expressed as percentage fungal load (isolation frequency).

Morphological examination of isolated fungi

The morphology of the fungi from cultures was macroscopically characterized by evaluating the colony characteristics (colour and texture) and further separated and grouped at genus level according to these features. Furthermore, the representative fungi sampled from each of the grouped fungi were subsequently studied using wet mount slides under a light microscope, to confirm fungal genera. A small amount of mycelium was placed on a glass slide and stained with lactophenol cotton-blue to further distinguish the fungi according to the arrangement and spore type or shape (Gaddeyya *et al.*, 2012; Kalman *et al.*, 2020).

A selection of 36 isolates were made according to various genera and were sub-cultured to generate pure cultures. After sufficient colony development samples

were further purified by placing a portion of mycelia on water agar (WA) to allow for a single hyphal tip or spore isolation. Single hyphal tip or spore were placed on streptomycin sulphate salt amended PDA plates for seven days to allow fungal growth. Once fully grown the mycelium was carefully scraped off and placed in a 2 mL Eppendorf tube and stored in the freezer at ~ -80 °C. The mycelium served as a source of pure genetic material. The above described methods are illustrated in Figure 1.

Molecular identification

Genomic DNA extraction

The total genomic DNA of the aforementioned fungal mycelium was isolated using Zymo Research Quick-DNA fungal/bacterial microprep kit (Zymo Research, n.d) following the manufacturer's instructions. The quality and quantity of the extracted genomic DNA were determined by using a NanoDrop 2000 spectrophotometer (Thermo Scientific). The purity of the DNA was measured at 260/280 nm.

Polymerase Chain Reaction (PCR) protocol

The amplification of the *Internal Transcribed Spacer (ITS)* gene region was achieved by using forward primer *ITS 1* (5'-TCC GTA GGT GAA CCT GCG G-3' and reverse primer *ITS 4* (5'-TCC TCC GCT TAT TGA TAT GC-3') respectively (Rex *et al.*, 2019). Each reaction consisted of 2x Kapa3G Plant PCR buffer, 10 µM for each primer, 1U of KAPA3G Plant DNA polymerase and 10 ng of each DNA sample, respectively. The conditions used for PCR (Bio-Rad T100™ thermal cycler) included a pre-incubation period of 95 °C for 2 minutes, followed by 30 cycles of 95 °C for 20 seconds, 53 °C for 15 seconds, 72 °C for 30 seconds and a final extension step at 72 °C for 1 minute. As this study focuses on the *FgSC*, once samples were identified as *Fusarium* spp. by means of the *ITS* fragments the *Elongation Factor (EF)* partial gene region for these

DNA samples were amplified as described above by using *EF 1* (5'- AAG GCT GTT GCT TTC GTC C -3') and *EF 2* (5'- GCA AGC AAT GTG GGC AGT G -3') primers as forward and reverse primers, respectively (O'Donnell *et al.*, 2000). The reaction conditions were similar as described above, but the annealing temperature was modified to 56 °C.

Gel electrophoresis

In order to evaluate the purity and quality of the PCR fragments a 1% (w/v) agarose gel was prepared containing 0.5 µg/mL ethidium bromide in 0.5x TAE buffer [20 mM Tris-HCl pH 8.0, 0.28% (v/v) acetic acid and 0.5 mM EDTA pH 8.0]. Before separation, each DNA sample was mixed with loading buffer [0.015% (w/v) bromophenol blue and 2.5% (w/v) ficoll]. The gel was separated for 40 min at 80 V.cm⁻¹. Ultraviolet (UV) light illumination was used to visualize the DNA fragments and it was photographed with the Gel Doc TM EZ Imager (BIO-RAD).

The fragments that were successfully amplified were excised from the gel with a clean sharp scalpel and respectively placed in a 1.5 mL microcentrifuge tube. DNA fragments were purified from the gel by using the FavorPrep™ GEL/PCR Purification Kit according to the manufacturer's instruction (Favorgen®, n.d). The quantity and quality of the purified fragments were confirmed and visualized on a 1% (w/v) agarose gel as described above. The DNA samples were stored in the (-20 °C) freezer until required for further use.

DNA sequencing

The purified PCR products were sequenced using the BigDye™ Terminator v 3.1 Cycle sequencing kit (Applied Biosystems) following manufacturer's specifications. Each sequencing reaction was made up of 3 µL of the purified PCR fragment, 3.2 pmol

primer, 1 µL of premix and 2 µL of the sequencing buffer. The sequence PCR reaction was performed using the forward primer of *ITS* and *EF* regions, respectively. The amplification conditions were: one cycle of 96 °C for 1 minute, followed by 25 cycles of 96 °C for 1 minute, 50 °C for 5 seconds and 60 °C for 4 minutes. This same amplification regime was applied for both *ITS* and *EF* fragments.

The sequencing reactions were purified by adjusting the volume to 20 µL and adding 125 mM EDTA and 2 volumes of 100% ethanol to each reaction. The tubes were vortexed briefly and left to incubate at room temperature for 15 minutes, then centrifuged at 12 000 *g* for 15 minutes at 4 °C. The supernatant was carefully removed using a pipette and the pellet washed with 70% (v/v) ethanol and recentrifuged at 12 000 *g* for 5 minutes at 4°C and the pellet dried. The composition of the nucleotides was determined on an ABI3500 Genetic analyser, this was conducted at the Department of Genetics, University of the Free State.

DNA sequence editing and alignment

Sequences were first analysed, and the identity determined by using Basic Local Alignment Search Tool (BLAST). Chromas Lite v.2.01 (Technelysium Pty. Ltd) and Bio-Edit version 2.6.4 were used to determine sequence quality and to edit the sequences where necessary. Sequences that had few base pairs (80 to 300 base pairs) were re-sequenced with the reverse primers (*ITS* 4 and *EF* 2) using the same method described above. MAFFT (Multiple alignment program for amino acid or nucleotide sequences) online service was used for sequence alignment.

Phylogenetic tree construction

Bayesian Inference analysis

A Bayesian Inference (BI) analysis was performed on the *ITS* and *EF* datasets respectively by using MrBayes v.3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The nucleotide substitution model for the BI analysis was determined using the Akaike Information Criterion (AIC) in jModeltest v. 2.1.1 (Guindon and Gascuel, 2003; Darriba *et al.*, 2012). GTR+G+I was the most suited for both the *ITS* and *EF* datasets. The MrBayes analyses were initiated from a random tree and four Markov Chain Monte Carlo (MCMC) chains were run at 5 000 000 generations, where every 500th tree was sampled. The analysis was run until the average standard deviation of split frequencies was below 0.01. The first 500 sampled trees were discarded as burnin.

Colony frequency

The colony frequency (CF) was determined by counting number isolates of each species on grain samples, divided by the total number of isolates of all species multiplied by 100 (Rodrigues and Menezes, 2005).

Mycotoxin analysis

Representative samples from each cultivar, planting number except, PN4 and PN5 (excluded due to low fungal frequency) and each TADD time were selected for mycotoxin extraction and analysis (N = 24). Planting dates were chosen based on samples with the highest amount fungi present at mycological studies. Sorghum grain were sub-sampled to 10 g portions which were ground into fine powder placed into 50 mL falcon tubes. The samples were sent to the Southern African Grain Laboratory (SAGL) for extraction and quantification of aflatoxin (AF; B₁, B₂, G₁ and G₂), DON, fumonisins (FB; B₁, B₂ and B₃), ZEA and 15-Acetyldeoxynivalenol (15-ADON). SAGL

deployed a LC-MS/MS approach for quantification. It is an ISO 17025 accredited testing laboratory and as part of ongoing quality assurance procedures it participates in several proficiency testings. Mycotoxin extraction and analyses were not performed at the University of the Free State due to restrictions imposed on campus access due to COVID-19. Additionally, only limited samples were set for analysis due to the costs associated with the mycotoxin analysis at an accredited laboratory, however the benefit is the data are accredited.

Data analysis

Data were imported from Google Sheets using the 'gsheet' package (Conway, 2020). A ANOVA was conducted to measure the extent of variation between the groups in the data collected from the various sterilisation and TADD times. The *aov()* function was utilised for this analysis including data exploration, wrangling and visualisation using the 'tidyverse' package (Wickham *et al.*, 2019). The 'agricolae' package (De Mendiburu, 2020) was used to obtain Fisher's Protected least significant difference (LSD) calculated at $p < 0.001$, so to separate the means of effects measured, the *LSD.test()* function was used for this analysis. Analyses were performed using R (version 4.0.3; R Core Team, 2020) within RStudio (version: 1.3.15093; RStudio Team, 2020).

RESULTS

Elucidating fraction surface sterilisation

The CF on sorghum grain was significantly ($p < 0.001$) influenced by TADD fraction, sterilisation time and the interactions between these factors (Table 1). The interaction between TADD fraction and sterilisation time explained 13.95% of the total variation observed, while TADD fractions and sterilisation time explained 42.40% and 10.29%

of the variation observed, respectively according to partitioning of the sum of sum of squares (Table 1). The lowest CF was observed at T6 (mean CF = 39.76%) and T4 (mean CF = 49.12%), which did not differ significantly from one another (Table 2). In contrast, the highest CF was observed at T0 (mean CF = 100%) and T1 (mean CF = 89.12%), also grouping together. According to Fisher's Protected LSD ($p < 0.001$) T2 did not group with either the highest or the lowest CF therefore forming three extreme clusters. The highest CF was recorded for 15 second surface sterilisation (mean CF = 87.8%) and 30 second sterilisation time (mean CF = 77.1%) which did not differ significantly from one another, however, 30 second was not significantly different from 60 second sterilisation time (mean CF = 70.53%). No significant difference was recorded among 180 second (mean CF = 57%) and 90 second surface sterilisation (mean CF = 59.8%) as both had the lowest colony frequency. Consequently, surface sterilisation at 90 seconds and 60 seconds also had no significant difference among them. These results were applied to the experiment to determine which sterilisation time is suitable for different TADD fractions to limit complete sterilisation grain and significant reduction of true fungal presence.

Effect of Decortication by tangential abrasive dehulling device

Decortication rates

As illustrated in Figure 2 the decortication by TADD proved to be successful, showing a positive relationship between the time taken to decorticate the grain and the loss obtained as a result thereof. The regression plot indicates a positive relationship between the time taken to decorticate grain samples and grain mass loss. Determining decortication rates was done to obtain different grain fractions to be studied for fungal colonisation and detection thereof by sequencing. Extra notes and figures are provided in the supplementary material for readers interested in the effects of interaction

between cultivars, planting numbers and TADD time, but are not discussed in detail as the results are not pertinent to the research question.

Abrasive Dehulling Index

ANOVA (Table 3) indicated that the time taken to remove one percentage of grain, as a characteristic of grain hardness and the potential ability of fungi to colonise grain, differed significantly ($p < 0.001$) due to cultivar and planting number (PN). Cultivar and PN explained 27.30% and 38.65% of the variation observed according to the partitioning of the sum of squares, respectively. PAN8706W (mean Abrasive Dehulling Index (AHI; mean AHI = 5.75, Table 4) recorded the lowest decortication time in seconds compared to NS5511 (mean AHI = 6.35) and PAN8816 (mean AHI = 7.16). According to Fisher's Protected LSD all three cultivars were significantly different from one another. PN7 has the highest mean value of time in seconds to abraded grain (mean AHI = 7.34), although this did not differ with the rate recorded for PN4. The latter also did not differ significantly with PN1. Furthermore, PN5 and PN6 grouped together as well as PN3 and PN2, respectively.

Phylogenetic tree analysis

Identification of fungal species based on morphological characteristics is insufficient to properly differentiate the isolates to species-specific levels. Therefore, species identification based on molecular markers presented a rapid approach. The phylogenetic tree constructed from *ITS* data indicated a close relationship between isolates and voucher specimens. A total of 119 sequences from 87 voucher specimens (Table 5) and 32 isolates from this investigation were used in the phylogenetic analysis. The *ITS* gene region displayed a degree of variation within the isolates of this study. Consequently, the phylogenetic tree (Figure 3) was divided into eight

clades, i.e., A to H, where clade A to F formed a sister group to clades G and H. The isolates in clade A clustered with *Epicoccum* spp. voucher specimens with a good Bayesian posterior probability (PP) value (PP = 0.9296) where all isolates in Clade B clustered with *Phoma* spp. voucher specimens (PP = 0.9973). However, within the same clade (B) voucher specimens of *Epicoccum* spp. formed sister groups with *Phoma* spp. voucher specimens (PP = 0.9973). Isolates in Clade C were identified as *Curvularia* spp. (PP = 1). Isolates with homology to voucher specimens of genera *Bipolaris* spp. (Clade D), *Exserohilum* spp. (Clade E), *Alternaria* spp. (Clade F) grouped in respective clades. *Fusarium* isolates clustered with their respective voucher specimens (Clade G) where all *Colletotrichum* isolates grouped with voucher specimens on clade H. Both clades G and H had a strong PP value of 1 indicating a match for the true identity of these isolates.

Ten isolates were identified as *Fusarium* after *ITS* sequencing, subsequently the *EF* gene region was used in an attempt to identify *Fusarium* isolates to species level. Only four of the ten sequenced isolates clustered with *F. graminearum* (PP = 0.9819; Figure 4). Four isolates did not cluster with any of the voucher specimens on the tree and could not with certainty be identified by molecular data in this study. The remaining two isolates were unsuccessfully amplified.

Colony frequency

Seventy-three percent of the 210 decortication samples tested positive for the presence of fungi. According to macroscopic groupings confirmed through sequencing, ANOVA (Table 6) on CF revealed a significant difference due to fungi, TADD and the interaction between the two variables ($p < 0.001$). Planting date and cultivar did not have significant effect on the CF, as such they were removed from analysis due to the lack of variation presented. The interaction TADD and Fungi (Table

6) explained 3.39% of the variation, while TADD and Fungi explained 0.75% and 16.71% of the variation observed according to the partitioning of the sum of squares, respectively. There was no significant difference between T6 (6.55%) and T4 (7.44%), recording the lowest CF (Table 7), however, these did not differ significantly with T2 (8.33%). T0 (11.90%) recorded the highest CF and was not significantly different to T1 (11.31%).

Alternaria spp. (31.12%) recorded the highest CF compared to all the identified species (Table 7). This was followed by *Phoma* spp. (16.48%) and *Bipolaris* spp. (12.22%) which were significantly different from each other based on the means separation. However, *Fusarium* spp., (8.34%) were not significantly different from *Bipolaris* spp. *Colletotrichum* spp. recorded the lowest CF although this was not significantly different from *Curvularia* spp. (0.71%), *Epicoccum* spp. (3.33%) and *Exserohilum* spp. (0.48%). The two-way interaction between TADD and fungi had a significant effect on the colony frequency. *Alternaria* spp., *Bipolaris* spp., *Epicoccum* spp., *Fusarium* spp. and *Phoma* spp. were identified in all TADD fractions. In contrast, *Colletotrichum* spp. was associated with T1, *Curvularia* spp. with T0 and T6 while *Exserohilum* spp. associated with T1 only. *F. graminearum* were associated with T0, T1 and T2.

Mycotoxin analysis

Twenty-four representative samples were sent for mycotoxin analysis and no AF (B₁, B₂, G₁ and G₂) and FB (B₁, B₂ and B₃) were detected in any of the samples. Only nine of the twenty-four samples tested had mycotoxins (15-ADON, DON and ZEA) present, these samples were from PN2, PN3 and PN7. Three mycotoxins, 15-ADON, DON and ZEA, were detected in concentrations of 272, 106 to 461 and 28 to 1249 µg/kg,

respectively. ZEA was only detected in PAN8706W and PAN8816, while 15-ADON was the only mycotoxin detected in NS5511 (Table 8).

DISCUSSION

Decortication proved to have significant effect on SGM, showing that certain fungi only colonise specific layers of the grain. Furthermore, it was also indicated that different sterilisation time were associated with different TADD fractions, thus allowing for significant fungal growth on each fraction. The use of 180 seconds as most accepted time for surface sterilisation (Tarekegn, 2002; Tesfaendrias, 2006; Mpofu, 2009) would 'wash away' a significant amount of fungi at the decorticated fractions. Therefore, adjusting the surface sterilisation time for the respective TADD times was justified in this study. Similar findings were observed by Girton (1936) and Alemu (2014), stating that longer sterilise durations of grain with NaOCl, resulted in less fungus present. The solution seems to disinfect the grain from the inside since it penetrated the grain because of easier penetration in dehulled grain. A shorter time is accordingly required to surface sterilise such grain. However, the grain that was used as the control sterilized at 180 seconds yielded 100% fungal growth, it can be assumed that the grain was protected by the seed coat.

Decortication of sorghum could be used to improve the cosmetic value of the grain by removing the discoloured layers and subsequently affect the market price positively with only 5% of grain mass loss (Audilakshmi *et al.*, 2007). However, Van der Merwe *et al.* (2005) recommends at least 12% of the grain to be decorticated if it is to be used for human consumption. Furthermore, decortication is used to remove outer layers of sorghum which are known to be less nutritious and could be contaminative in the flour during milling of the grain since they are naturally crumbly

(Munck *et al.*, 1982; Awika *et al.*, 2003). However, decortication by TADD is a method used in the lab to measure the hardness of the grain (Reichert *et al.*, 1986). According to the results of this study, close to 10% of the grain was removed for every minute of decortication, suggesting food processing only recommends approximately one minute of decortication.

Abrasive dehulling index is a quantitative technique or tool used to measure for grain hardness, where a higher AHI value is associated with harder grain (Reichert *et al.*, 1988). A distinction is made between hard and soft grain, where they are associated with a mean AHI value of 15.7 and 7.7, respectively (Mwasaru *et al.*, 1988; Reichert *et al.*, 1988). In this study, PAN8816, a red genotype, recorded a high AHI compared to the remaining two cultivars, this was followed by NS5511, a brown genotype and PAN8706W, a white genotype. It should be noted that all the cultivars had a mean AHI < 15.7 indicating that they have soft endosperm. Additionally, all three cultivars exhibited AHIs that were partially similar to those of soft grain. Implying grains could break easily under abrasive material as they had a lower AHI. Contrasting to our study, Reichert *et al.* (1988) found that the coloured grains were softer compared to non-coloured grains. The discrepancy could perhaps be explained by weather variables, the level of grain mold in each sample, as well as variable weights used during dehulling. Hard grain resists the fungal invasion to the internal tissues of the grain, therefore prohibiting its deterioration (Waniska, 2000; Katile *et al.*, 2010). In terms of fungal colonisation, the cultivars were identical; however, due to excessive infection, the interior tissues degraded, making them vulnerable to breaking during decortication (Bandyopadhyay *et al.*, 2000).

The current study served as an initial approach to identify fungi to species level on decorticated grain fractions. The time taken to dehull the grain indicated

decortication had a significant effect on the reduction of fungi present in the grain. As LSD values grouped T4 and T6 together, and T0 and T1 together, the assumption can be made that latter fraction are associated with superficial colonisation, whereas T4 and T6 are associated with deep-seated colonisation. However, T2 did not group with either time interval meaning it could be associated with layers between the superficial and deep layers (mesocarp layers; intermediate). When Coetzee (2015) examined the fungal biomass of *F. graminearum* on decorticated samples, they discovered that the fungal biomass was considerably decreased at high decortication rates (T4 and T6). Furthermore, the results of the current study confirm this, where the un-hulled grain showed 100% fungal growth compared to the rest of the dehulled grain showing a progressive reduction in the amount of fungal biomass.

The study's main goal was to identify fungi isolated from decorticated sorghum grain using molecular phylogenetics. In this study only eight fungi were identified to genus level with strong posterior probability support values (Schoch *et al.*, 2012). Figure 3 indicated the grouping of *Epicoccum* spp. and *Phoma* spp. into one clade (B), indicating homology due to high PP values. The short branches observed between these species in the clade could suggest that both entities represent the same biological species (Ministry of Public Health, 2020). The clade can therefore represent *Phoma* spp. since most of the species in the clade are *Phoma* spp. Four isolates were successfully identified as *F. graminearum* by using the *EF* gene region (Figure 4). Sequence data can be used to construct a phylogenetic tree, thus providing a clear insight into specific species' evolutionary ties. However, as seen in Figure 4 there is a non-grouping of four isolates with any of the voucher specimens. An assumption could be made that the isolates sequence data (base pairs) were insufficient for an accurate phylogenetic analysis (Bogusz and Whelan, 2017; Balaban *et al.*, 2019). In order to

further identify the remaining four isolates of *F. graminearum*, the future use of DNA-directed RNA polymerase second largest subunit (RPB2; O' Donnell *et al.*, 2010) can be utilised.

Tarekegn (2002) and Tesfaendrias (2006) molecularly identified and measured the isolation frequency of fungal complexes on whole grains but not on the decorticated grain, potentially suggesting the depth at which fungi penetrate and colonise grain. Both researchers identified *Alternaria* spp., *Curvularia* spp., *Epicoccum* spp., and *Fusarium* spp. across environments, and reported *Alternaria* spp. as the highest isolation frequency. These results are consistent with the findings of the current study, where *Alternaria* spp. had the highest fungal frequency followed by *Phoma* spp. and *Bipolaris* spp. and *Fusarium* spp. Fungi such as *Colletotrichum* spp., *Curvularia* spp., *Exserohilum* spp. and *Epicoccum* spp. were isolated in low frequencies. As there was a significant relationship between fungal genera and TADD fractions, some fungi were only present in specific TADD fractions. In this study, *Colletotrichum* spp. was identified in T1, *Curvularia* spp. at T0 and T6 only, and *Exserohilum* spp. was only found in the T1 fraction. These results corroborate that *Colletotrichum* spp., *Curvularia* spp. and *Exserohilum* spp. are superficial contaminants of sorghum grain (Singh and Bandyopadhyay, 2000; Thakur *et al.*, 2006; Chandrashekar and Satyanarayana, 2007). *Exserohilum* spp. and *Colletotrichum* spp. are foliar pathogens of sorghum and hence their presence may be found in the grain (McLaren and Rothmann, 2019). However, *F. graminearum* were associated with T0 and T1 (superficial layers; Coetzee, 2015) and T2 (mesocarp).

Saprophytic species commonly isolated from sorghum grain include *Alternaria* spp., *Epicoccum* spp. and *Phoma* spp, and are not considered true pathogens but superficial grain colonisers that occur at later grain development stages and cause

minor damage (Williams and Rao, 1981; Forbes *et al.*, 1992). *Curvularia* spp. and *Fusarium* spp. are able to penetrate the grain from the pericarp into the endosperm (Chandrashekar and Satyanarayana, 2007) as was evident in this study. However, *Alternaria* spp. and *Phoma* spp. were observed to follow the same trend despite being known as saprophytic species. Forbes *et al.* (1992) reported that these species could be weather dependent, implying that they may colonise deeper in the grain if environmental conditions are favourable. This implies that the environmental conditions during the sorghum growth stages (supplementary Table S1) were conducive for the effective colonisation of the grain by these fungi. *Alternaria* spp. is favoured by relative humidity and temperature $\geq 70\%$ and $25\text{ }^{\circ}\text{C}$, respectively (Sharma and Ahir, 2018; Meena *et al.*, 2021). While *Phoma* spp. requires relative humidity of 70% and temperatures between 22 and $26\text{ }^{\circ}\text{C}$ for effective colonisation (Do Amaral *et al.*, 2004; Oliveira *et al.*, 2017; Oliveira *et al.*, 2018), all these conditions are in alignment with the weather conditions indicated in supplementary Table S1.

The study's estimate of 10% decortication suggests that only the most superficial fungi may be eliminated. Internal infection caused by fungi that create mycotoxins, on the other hand, may constitute a concern to food safety (Thakur *et al.*, 2006). As a result, no amount of decortication would be effective in removing the fungi if penetrated to the deeper layers of the grain; accessing the diseased area would necessitate up to 30% decortication (Coetzee, 2015). However, this percentage is higher than the recommended decortication rate of 12% in processing (Van der Merwe *et al.*, 2005) making this method un-economical.

Due to the limited samples which were analysed for mycotoxins, the effects of cultivar, planting date and TADD could not be quantified. Rose *et al.* (2016) indicated that fungal frequency as measured by visual or quantitative molecular techniques is

not always proportional to mycotoxins accumulation, therefore careful consideration must be taken when grading produce on fungal frequency. As a result, this could bias researchers into thinking that high fungal frequency is equal to having high mycotoxin concentrations (Bhat *et al.*, 2010). In a study conducted by Coetzee (2015) 180 samples were analysed for the effect of cultivar, environment and TADD fraction on mycotoxin variation, where significant difference among each factor was reported. However, this study only 24 samples were selected for the analysis and therefore inhibited successful further analyses to confirm main effects driving variation.

Mycotoxin legislation varies across countries and depends on the grain, the product and product use, i.e. livestock or human consumption. Depending on the product produced, South African legislation restricts the maximum amount of DON of 1000-2000 µg/kg. The U.S-Food and Drug Administration (FDA) has set a maximum limit for DON at 1000 µg/kg for all wheat-derived products (CAST, 2003). Whereas, the European Union has set a maximum limit of 1250 µg/kg for DON on all raw cereals other than durum wheat, oats and maize (Lancova *et al.*, 2008; Bhat *et al.*, 2010). It is worth noting that the mycotoxins concentration quantified for DON in this study were lower than the maximum legislative limit required by all jurisdictions listed above. However, the Food and Agriculture Organization of the United Nations has set a total tolerable level of ZEA in foods within the range of 50 µg/kg-1000 µg/kg (Marroquín-Cardona *et al.*, 2014), in this study only one sample exceeded the maximum legislative limit (1250 µg/kg). It is probable to assume that since these mycotoxins are below legislative limit, sorghum consumers are not at risk. However, it is difficult to confirm this assumption based on the sample numbers used in the study.

CONCLUSION

Decortication aims to remove the bran and germ from the endosperm as these outer layers are known to be less nutritious and may be a contaminant in the flour during milling of the grain since they are naturally crumbly. However, it can be used in plant pathology to answer questions associated with fungal infections and their prevalence within grain fractions. Great value is added to the processing of sorghum grain by knowing which fungi colonise the layers of the grain and at what rate of decortication is needed to remove them, as well as understanding if this process also affects concomitant mycotoxin accumulation. As sorghum production is estimated to grow, understanding the impacts of fungal penetration and internal colonisation may help in protecting the growing demand for sorghum grain food. However, concomitant mycotoxin contamination threatens food safety and as such contributing to food insecurity. Toxigenic superficial fungi, such as *Alternaria* spp., *Epicoccum* spp. and *Phoma* spp. were detected, as well as fungi associated with deep-seated colonisation, i.e. *Fusarium* spp. Furthermore, the former mentioned fungi were also identified in the deep layers of grain, posing a threat through potential mycotoxin contamination. It should be noted that mycotoxins are stable in nature and can thus survive processing and remain in food products. Since decortication is not an economically viable option to reduce fungi other alternative methods should be explored to prevent or reduce fungal colonisation of sorghum grain and consequent mycotoxin contamination. Cultural practices and resistance breeding could be the answer to limit grain mold at different environments. The use of mineral adsorbents in produce contaminated with mycotoxins could help in reducing mycotoxins concentrations. Additionally, techniques such as metabarcoding could assist in identifying fungi quickly, potentially indicating the risk for mycotoxin presence and the need to assess the presence of toxigenic fungi. Future studies could quantify the presence of the fungi which are deep-seated

through real-time PCR to determine the severity of colonisation, and potential mycotoxin accumulation.

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TABLES AND FIGURES

Table 1: Analysis of variance on the effect of surface sterilisation time to reduce superficial fungal contamination on decorticated grain.

Source of variation	DF	SS	MS	F value	p-value
TADD	4	65840	16460	31.63	0.00
Sterilisation time	4	15975	3994	7.68	0.00
TADD x sterilisation time	16	21667	1354	2.60	0.00
Replication	1	289	289	0.57	0.46
Residual	99	51508	520		

DF = Degrees of freedom; SS = sum of squares; MS = mean squares ($p = 0.001$); TADD, Tangential Abrasive Dehulling Device

Table 2: Efficiency of interaction between surface sterilisation time and minutes of decortication by Tangential Abrasive Dehulling Device (TADD) on reduction of fungal contamination (colony frequency; %) on dehulled grain.

TADD decortication fraction (minutes)	Sterilisation time (seconds)					Mean
	15	30	60	90	180	
0	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a
1	93.2 ^{ab}	93.2 ^{ab}	93.2 ^{ab}	93.2 ^{ab}	72.8 ^{abcd}	89.12 ^a
2	86.4 ^{abc}	86.4 ^{abc}	86.4 ^{abc}	59.6 ^{cde}	52.8 ^{def}	74.32 ^b
4	73 ^{abcd}	59.8 ^{cde}	66.6 ^{bcd}	13.2 ^g	33 ^{efg}	49.12 ^c
6	86.4 ^{abc}	46.2 ^{def}	6.6 ^g	33.2 ^{efg}	26.4 ^{fg}	39.76 ^c
Mean	87.80 ^a	77.12 ^{ab}	70.53 ^{bc}	59.84 ^{cd}	57.00 ^d	70.46

Treatments with the same letter are not significantly different according to Fisher's Protected LSD ($p < 0.001$). LSD: TADD (12.80), LSD: Sterilisation time (12.80) and LSD: TADD X Sterilisation time (28.62).

Table 3: Analysis of variance on grain hardness tested from three commercial cultivars tested in seven environments in South Africa using abrasive dehulling index (AHI).

Sources of variation	DF	SS	MS	F value	p-value
Cultivar	2	69.83	34.91	80.60	0.001
Planting number	6	98.85	16.47	38.03	0.001
Residual	201	87.07	0.43		

DF = Degrees of freedom; SS = sum of squares; MS = mean squares, ($p < 0.001$).

Table 4: The effect of planting number and cultivar on the dehulling efficiency as indicated by time taken in seconds for decortication (Abrasive dehulling Index; AHI) on three commercial cultivars tested in seven environments in South Africa.

Main effects			
Planting Number	Mean AHI	Cultivar	Mean AHI
1	6.88 ^b	NS5511	6.35 ^b
2	5.42 ^d	PAN8706W	5.75 ^c
3	5.56 ^d	PAN8816	7.16 ^a
4	7.11 ^{ab}		
5	6.38 ^c		
6	6.21 ^c		
7	7.34 ^a		

Treatments with the same letter are not significantly different according to Fisher's Protected LSD ($p < 0.001$). LSD: Cultivar = 0.21; LSD: Planting number = 0.34. Planting number: 1 = 1 Nov 2016, 2 = 23 Nov 2016, 3 = 12 Dec 2016, 4 = 28 Dec 2016, 5 = 5 Jan 2017, 6 = 5 Dec 2017 and 7 = 1 Nov 2018

Table 5: Voucher specimens obtained from the GenBank used in the identification of fungi isolated from sorghum grain.

Accession number	Fungi
KR912313	<i>Epicoccum</i> spp.
JF449836	<i>Epicoccum</i> spp.
KT355005	<i>Epicoccum</i> spp.
LC543647	<i>Epicoccum</i> spp.
LC543646	<i>Epicoccum</i> spp.
KF986447	<i>Epicoccum</i> spp.
MT079314	<i>Epicoccum</i> spp.
KJ171035	<i>Epicoccum</i> spp.
MK680810	<i>Epicoccum</i> spp.
MN089646	<i>Epicoccum</i> spp.
MN202770	<i>Epicoccum</i> spp.
MN202771	<i>Epicoccum</i> spp.
MK841458	<i>Epicoccum</i> spp.
KJ170968	<i>Epicoccum</i> spp.
MK370650	<i>Epicoccum</i> spp.
HQ914873	<i>Epicoccum</i> spp.
LT592927	<i>Epicoccum</i> spp.
MN944541	<i>Epicoccum</i> spp.
MN215621	<i>Epicoccum</i> spp.
MN215622	<i>Epicoccum</i> spp.
MH497563	<i>Epicoccum</i> spp.
KU715154	<i>Phoma</i> spp.
KU715125	<i>Phoma</i> spp.
MT557514	<i>Phoma</i> spp.
MT557442	<i>Phoma</i> spp.
MT557416	<i>Phoma</i> spp.
MT557213	<i>Phoma</i> spp.
MN452486	<i>Phoma</i> spp.
MT420621	<i>Phoma</i> spp.
HE861845	<i>Curvularia</i> spp.
MK804384	<i>Curvularia</i> spp.
MG664783	<i>Curvularia</i> spp.
MH414897	<i>Curvularia</i> spp.
NR158444	<i>Curvularia</i> spp.
MT341911	<i>Curvularia</i> spp.
KU253770	<i>Curvularia</i> spp.
KF171515	<i>Bipolaris</i> spp.
MH864478	<i>Bipolaris</i> spp.
MH864763	<i>Bipolaris</i> spp.
KY905677	<i>Bipolaris</i> spp.
MG727655	<i>Exserohilum</i> spp.
MG727641	<i>Exserohilum</i> spp.
MG727593	<i>Exserohilum</i> spp.
MG727575	<i>Exserohilum</i> spp.
MG727616	<i>Exserohilum</i> spp.

Table 5 continued: Voucher specimens obtained from the GenBank used in the identification of fungi isolated from sorghum grain.

Accession number	Fungi
KX426692	<i>Alternaria</i> spp.
MT487771	<i>Alternaria</i> spp.
MW009030	<i>Alternaria</i> spp.
MW008969	<i>Alternaria</i> spp.
MW032199	<i>Alternaria</i> spp.
MT557479	<i>Alternaria</i> spp.
MT453271	<i>Alternaria</i> spp.
MT557563	<i>Alternaria</i> spp.
MW081304	<i>Alternaria</i> spp.
MN822550	<i>Alternaria</i> spp.
KR822131	<i>Alternaria</i> spp.
MN206894	<i>Alternaria</i> spp.
MK6449964	<i>Alternaria</i> spp.
MK466369	<i>Alternaria</i> spp.
MK649969	<i>Alternaria</i> spp.
MK78022	<i>Fusarium</i> spp.
MT603307	<i>Fusarium</i> spp.
MT558855	<i>Fusarium</i> spp.
MN452637	<i>Fusarium</i> spp.
MN452419	<i>Fusarium</i> spp.
MN452134	<i>Fusarium</i> spp.
MT557534	<i>Fusarium</i> spp.
MN154167	<i>Fusarium</i> spp.
KY426422	<i>Fusarium</i> spp.
MT446136	<i>Fusarium</i> spp.
MT446106	<i>Fusarium</i> spp.
MT4466075	<i>Fusarium</i> spp.
MN428026	<i>Fusarium</i> spp.
MK990154	<i>Fusarium</i> spp.
MK841429	<i>Fusarium</i> spp.
MH582472	<i>Fusarium</i> spp.
MG250447	<i>Fusarium</i> spp.
MN634682	<i>Fusarium</i> spp.
MH582471	<i>Fusarium</i> spp.
MN882831	<i>Fusarium</i> spp.
JQ517492	<i>Fusarium</i> spp.
KX421422	<i>Fusarium</i> spp.
MK087641	<i>Fusarium</i> spp.
MF754105	<i>Fusarium</i> spp.
MF405438	<i>Colletotrichum</i> spp.
NR163522	<i>Colletotrichum</i> spp.
MN889462	<i>Colletotrichum</i> spp.
KX645872	<i>Colletotrichum</i> spp.

Table 6: Analysis of variance for fungal colony frequency as influenced by decortication by Tangential Abrasive Dehulling Device (TADD) on three commercial cultivars tested in seven environments in South Africa.

Source of variation	Df	SS	MS	F value	p-value
TADD	4	7595	1899	3.87	0.00
Fungi	7	169589	24227	49.46	0.00
Fungi X TADD	28	34443	1230	2.51	0.00
Residuals	1640	803261	490		

DF = Degrees of freedom; SS = sum of squares; MS = mean squares; TADD, Tangential Abrasive Dehulling Device; ($p < 0.001$).

Table 7: Two-way interaction between fungal genera and Tangential Abrasive Dehulling Device time taken in minutes for decortication on fungal frequency (%)

Fungal genera	TADD fraction					Mean colony frequency
	0	1	2	4	6	
<i>Alternaria</i> spp.	48.61 ^a	37.1 ^b	31.19 ^{bc}	20.43 ^{de}	18.25 ^{def}	31.12 ^a
<i>Bipolaris</i> spp.	16.07 ^{efg}	4.37 ^{ijklm}	11.5 ^{efghij}	17.26 ^{ef}	11.9 ^{efghi}	12.22 ^c
<i>Colletotrichum</i> spp.	0 ^m	0.79 ^m	0 ^m	0 ^m	0 ^m	0.16 ^d
<i>Curvularia</i> spp.	1.19 ^{lm}	0 ^m	0 ^m	0 ^m	2.38 ^{ijklm}	0.71 ^d
<i>Exserohilum</i> spp.	0 ^m	2.38 ^{ijklm}	0 ^m	0 ^m	0 ^m	0.46 ^d
<i>Epicoccum</i> spp.	2.38 ^{ijklm}	4.76 ^{hijklm}	1.98 ^{klm}	6.34 ^{hijklm}	1.19 ^{lm}	3.33 ^d
<i>Fusarium</i> spp.	7.02 ^{ghijklm}	14.08 ^{efgh}	10.31 ^{fghijkl}	4.36 ^{ijklm}	5.95 ^{hijklm}	8.34 ^c
<i>Phoma</i> spp.	19.96 ^{de}	26.98 ^{cd}	11.67 ^{efghij}	11.11 ^{efghijk}	12.69 ^{efghi}	16.48 ^b
Mean colony frequency	11.9 ^a	11.31 ^{ab}	8.33 ^{bc}	7.44 ^c	6.55 ^c	9.11

Treatments with the same letter are not significantly different according to Fisher's Protected LSD ($p < 0.001$). LSD: Fungi (4.23), LSD: TADD (3.35) and LSD: Fungi x TADD (9.47).

Table 8: Mycotoxin concentrations detected over varying planting dates (planting number), cultivars and Tangential Abrasive Dehulling Device (TADD) fractions.

Planting number	Cultivar	TADD	Aflatoxin (ug/kg)	Fumonisin (ug/kg)	DON (ug/kg)	15-ADON (ug/kg)	ZEA (ug/kg)
1	NS5511	6	-	-	-	-	-
3	NS5511	2	-	-	-	-	-
2	NS5511	6	-	-	-	-	-
6	NS5511	0	-	-	-	-	-
1	NS5511	2	-	-	-	-	-
1	NS5511	0	-	-	-	-	-
1	NS5511	1	-	-	-	-	-
2	NS5511	4	-	-	-	-	-
7	NS5511	4	-	-	-	-	-
7	NS5511	1	-	-	-	272	-
2	PAN8706W	0	-	-	461	-	28
3	PAN8706W	6	-	-	106	-	-
7	PAN8706W	2	-	-	129	-	285
7	PAN8706W	0	-	-	442	-	1249
3	PAN8706W	2	-	-	-	-	-
2	PAN8706W	2	-	-	-	-	-
1	PAN8706W	1	-	-	-	-	-
3	PAN8816	1	-	-	-	-	-
2	PAN8816	1	-	-	-	-	-
2	PAN8816	0	-	-	-	-	-
7	PAN8816	2	-	-	-	-	-
7	PAN8816	6	-	-	-	-	-
7	PAN8816	4	-	-	-	-	-
3	PAN8816	0	-	-	-	-	295

Multi mycotoxin: Aflatoxin total = Sum of Aflatoxin B₁ + B₂ + G₁ + G₂; Fumonisin total = Sum of Fumonisin B₁ + B₂ + B₃; Planting number: 1 = 1 Nov 2016, 2 = 23 Nov 2016, 3 = 12 Dec 2016, 4 = 28 Dec 2016, 5 = 5 Jan 2017, 6 = 5 Dec 2017 and 7 = 1 Nov 2018.

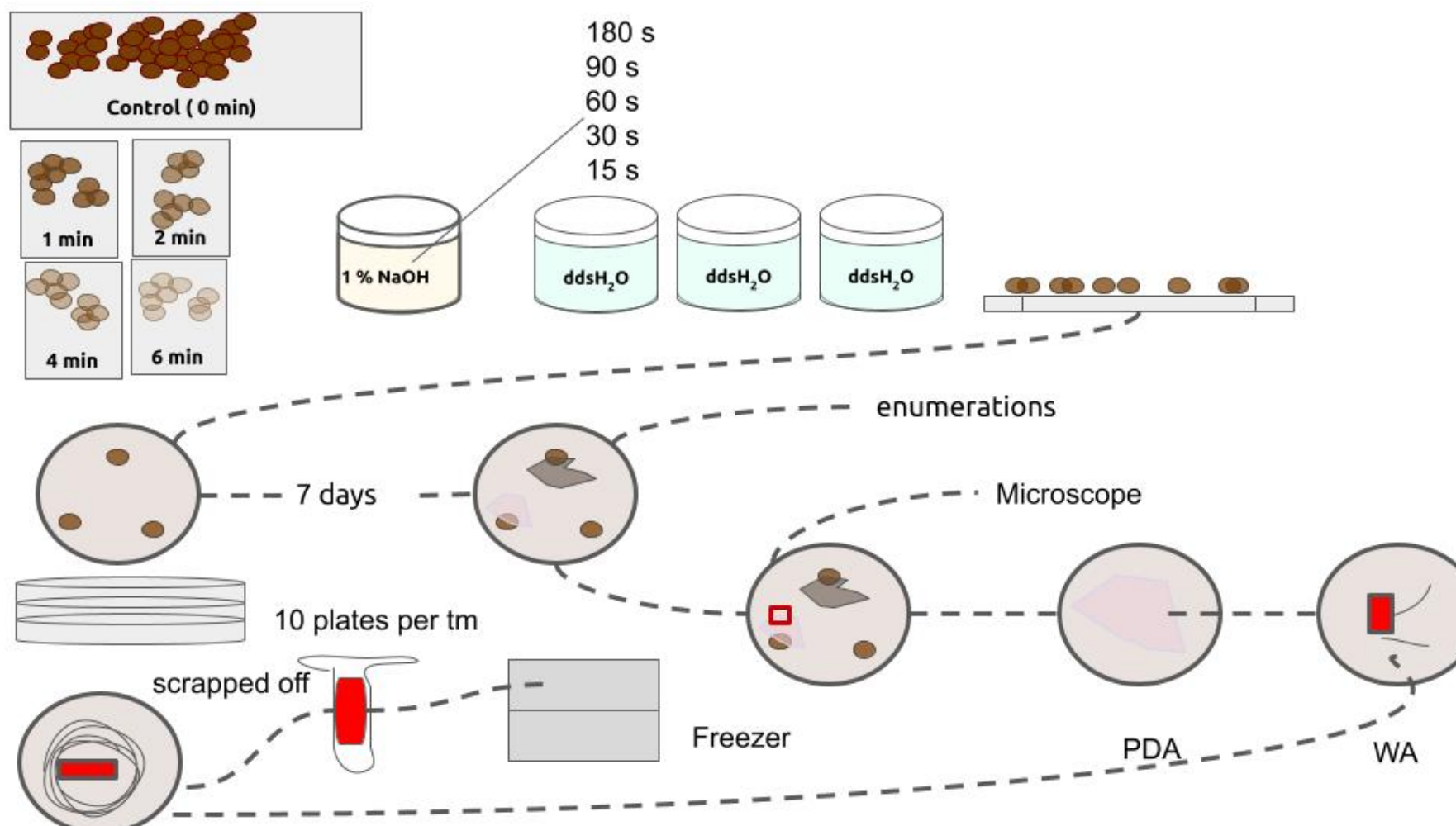


Figure 1: An illustration depicting all the stages from grain surface sterilisation till pure culture collection.

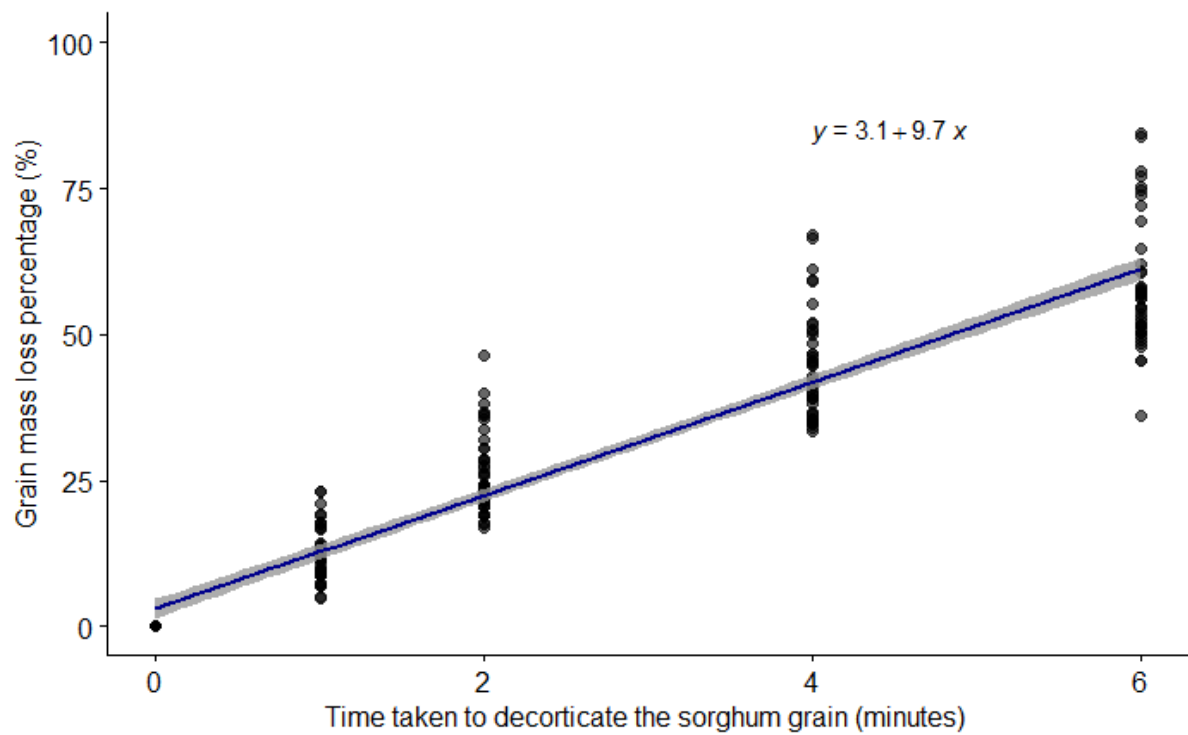


Figure 2: The relationship between grain mass loss percentage obtained and time taken to decorticate sorghum grain.

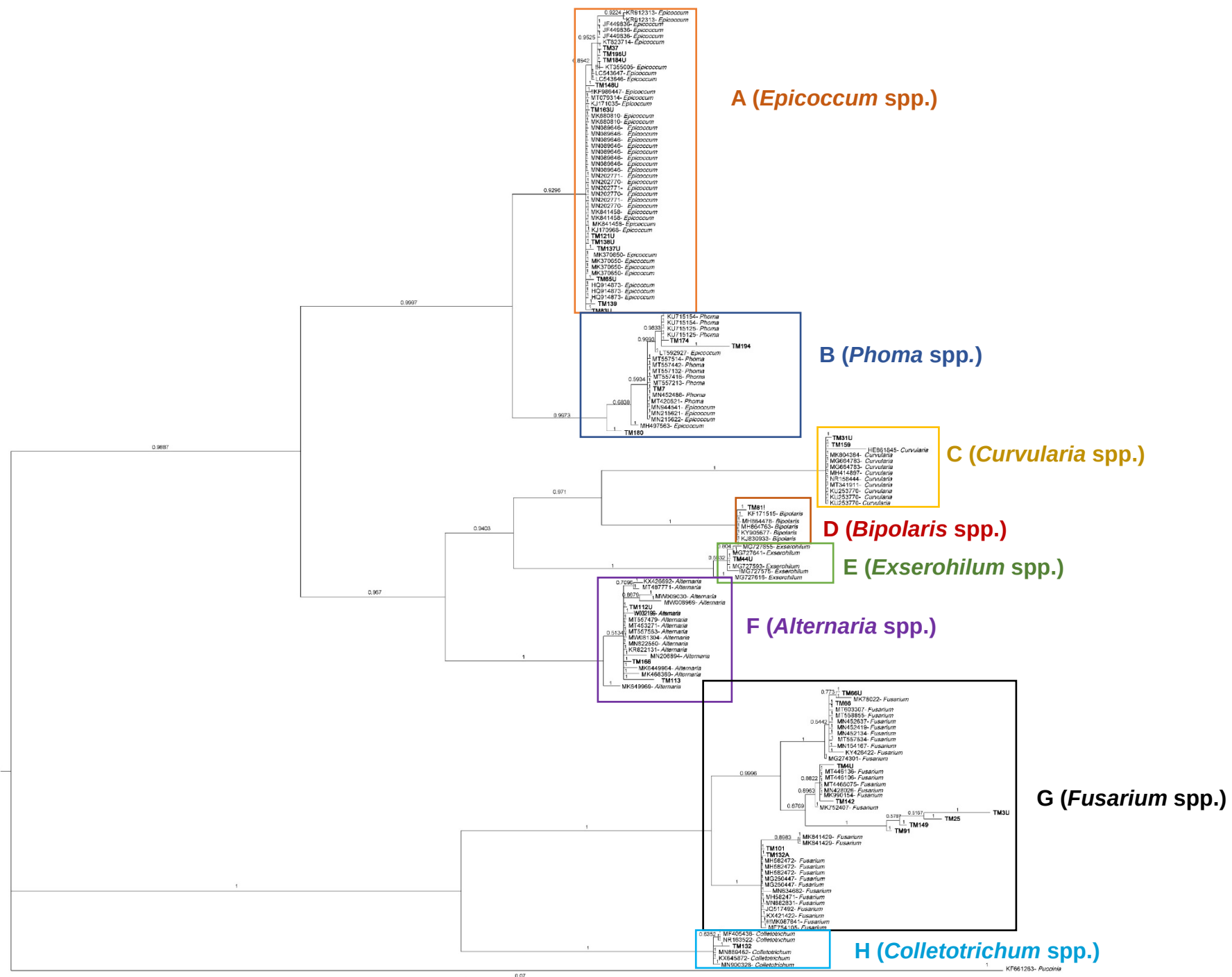


Figure 3: Phylogenetic tree based on the ITS gene sequence data set. Bayesian posterior probability values indicted on the nodes. The tree was rooted with *Puccinia* spp.

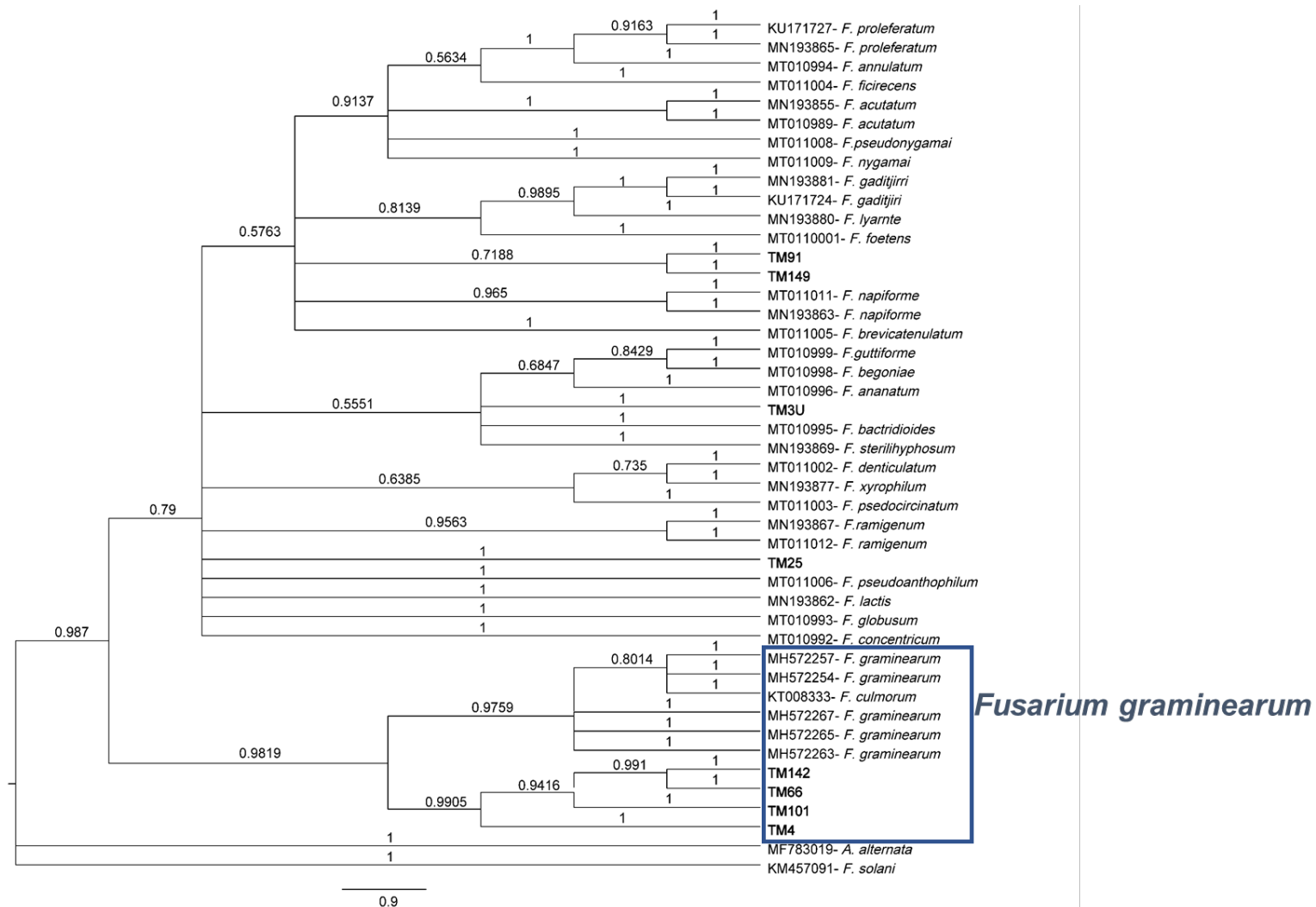


Figure 4: Phylogenetic tree based on the *ITS* gene sequence data set. Bayesian posterior probability values indicated on the nodes. The tree was rooted with *Alternaria alternata* and *Fusarium solani*.

CHAPTER 3: EVALUATING AND COMPARING ON-GROUND (*IN SITU*) WITH GRIDDED WEATHER DATA

ABSTRACT

Weather elements are important abiotic factors that drive plant disease epidemics where pathogen populations are favoured in the presence of susceptible host populations. The application of weather data in epidemiology largely depends on obtaining the data from on ground stations (OGS). However, in some cases OGS data are incomplete or are not present in areas of interest. These issues may have confounding effects on dependent variables measured against independent weather variables. Consequently, skewing the quantification of the stimuli and response interactions between the host, pathogen and environment. To compensate for these complications, assimilated gridded data (GD) are used since they offer complete daily long-term data. Additionally, GD can be utilised in areas where OGS are absent. The objectives of this study were to compare the weather data obtained from 11 OGS with NASA POWER data and to evaluate the degree of statistical agreement between variables. Daily minimum (TMIN) and maximum (TMAX) temperature (°C), rainfall (RAINFALL; mm) and mean relative humidity (RH; %) were selected as variables. A significant statistical agreement was reported between OGS and NASA POWER data for both temperature parameters, but not moisture parameters. The coefficient of agreement (d), Mean Bias Error (MBE), Nash-Sutcliffe efficiency coefficient (NSE) and Root Mean Square Error (RMSE) were 0.93, -0.07 °C, 0.73 and 1.85 °C, as well as 0.92, -1.11 °C, 0.75 and 2.32 °C, for TMAX and TMIN, respectively. However, NASA POWER underestimated TMIN at all stations, while TMAX was overestimated in 55% of the stations. Nonetheless, results from our study confirm those of previous research which indicated that GD are sufficient to be used in lieu of OGS or as weather data alternatives for temperature parameters. In contrast, RAINFALL and RH, performed unsatisfactorily. RAINFALL was associated with d , MBE, NSE and RMSE values of 0.58, 0.14 mm, 0.18 and 8.30 mm, while 0.78, 3.38%, -0.44 and 9.50%, were associated with RH, respectively. Based on the results of temperature parameters, NASA POWER database presents an opportunity to be used as substitutes for temperature parameters, however caution should be taken when utilising moisture parameters. The additional benefit is that NASA POWER data are open source, which affords researchers with no or limited capital to pursue epidemiological research.

Keywords: NASA POWER, statistical agreement, heat islands, weather variability.

INTRODUCTION

The concept of the disease tetrahedron in plant pathology emphasises the interaction of the environment, pathogen and host plant as influenced by man. The initial step in understanding the interactions between these components is to monitor and accurately measure the components (Franci, 2001). The environment is defined as the conditions or surroundings in which an organism lives. Subsequently, the development of plant disease epidemics is driven by environmental factors stimulating host and pathogen population responses (Velásquez *et al.*, 2018). Elements of weather such as air temperature, and moisture parameters, rainfall and relative humidity, trigger plant disease epidemics, as the pathogen is favoured in the presence of a susceptible host plant (Velásquez *et al.*, 2018; Shah *et al.*, 2019). The temporal and spatial components of the environment need to be considered when assessing weather parameters. A distinction between weather and climate is necessary in the field of epidemiology, as epidemics can span between the two. The short-term atmospheric conditions that prevail at a given location and time are referred to as weather. In contrast, climate refers to the average weather data at a given location over a longer period, i.e., 30-years (National Oceanic and Atmospheric Administration, 2021).

On-ground stations (OGS), measuring weather parameters through the use of approved instruments, placed either on land or in oceans are able to collect a variety of parameters, whereas weather data from the atmosphere are collected by use of radar, satellites and weather balloons. Prior to data storage in databases, the incoming data go through observational quality checks where outliers, spikes and excessive ranges are detected through patterns by computers (Actively Learn, 2019; Joint Research Center, 2021). In scenarios where no weather stations are located, spatial

interpolation can be implemented, through calculating values at uncertain points by utilising known values from nearby points. Subsequently, local weather data are mathematically manipulated for another station with no instruments or sufficient data, hence spatial interpolation could be used as an alternative for obtaining OGS data (Magarey *et al.*, 2001).

Gridded weather data assimilated by millions of meteorological data are available from a wide range of sources and databases, i.e., land and ocean observations, meteorological balloons, radar imagery and remote sensing (Monteiro, 2015; Monteiro *et al.*, 2018; Duarte and Sentelhas, 2020). Gridded data (GD) are geospatial datasets that provide weather observation over extended time spans and are created by interpolation of meteorological observations or model-based forecasts (Parker, 2016; Speedwell Weather, 2021).

Few organisations in South Africa provide on-ground weather data and include the Agricultural Research Council - Institute of Soil, Water and Climate (ARC-ISWC; 2014), Hortec Analytical Services and Weather Stations Services (2020), as well as South African Weather Service (2021). These data are usually the intellectual property of the OGS proprietor, although, in some cases these data can be purchased from the proprietor with cost related to the number of weather variables required (Valeriano *et al.*, 2019). Parameters that are commonly captured include rainfall, relative humidity, temperature, solar radiation, wind speed and direction. The resolution and density of OGS are also dependent on the source.

In contrast, various institutes generate and make use of GD to provide long-term and daily weather data through online platforms, which are usually freely available. Examples include the Climate Forecast System Reanalysis, European Centre for Medium-Range Weather Forecasts, National Centre for Environmental

Prediction, National Centre for Atmospheric Research, Japanese Meteorological Agency Climate Data Assimilation System and National Aeronautics and Space Administration/Prediction of Worldwide Energy Resources (NASA POWER; Aboelkhair *et al.*, 2019). Parameters often collected here include cloud cover, rainfall, relative humidity, temperature, solar radiation, sunshine hours and wind speed. The spatial and temporal variation of the databases are driven by elevation, topography, vegetation and weather seasons (Duarte and Sentelhas, 2020).

On-ground station weather data referred to as the “gold standard”, imply the weather data collection is occurring under optimal deployment and calibration and offer accurate observational data. In addition, OGS can offer data in real time as some instruments are versatile and have dual purposes, for example radar can detect both storms and gather rainfall data (Colston *et al.*, 2018; VAISALA, 2019; THERMOPRO, 2019). Additionally, weather data derived from spatial interpolation is relatively cheap since simulated data are used instead of paying for specific or missing locations (Magarey *et al.*, 2001). However, instrument calibration is required where data quality may not be sufficient, or data are not available, due to a lack of maintenance or software malfunction. In the latter situation, neighbouring stations with small distance between them may be used, however sometimes the distances may be too great or even so still have incomplete data in order to successfully deploy interpolation (Moeletsi and Walker, 2012; Monteiro *et al.*, 2018). The inability to routinely maintain and update weather data software may be the reason behind missing data. Additionally, the cost of OGS maintenance may be to the extent that stations need to be shut down, and data collection can no longer be continued (Pandey *et al.*, 2016; Colston *et al.*, 2018).

Daly (2006) and Tozer *et al.* (2012) used spatial interpolation to estimate the amount of daily rainfall for an area of interest where no stations were available. However, they observed that in areas where stations are further apart from the area of interest the interpolated rainfall fields only provided rough estimates of the rainfall events. This method always introduces error in the interpolated dataset making it difficult to test their validity especially in areas with low numbers of weather stations. This makes their use unreliable according to Chen *et al.* (2004) and Mizukami and Smith (2012). Gridded data can be used in areas where there are no OGS or in areas where required data are incomplete. Some GD can be extracted online for free from any location, with known geographical coordinates, and offer reliable, continuous (i.e. complete), long-term and daily data over space and time (Pinker *et al.*, 1995; Lakshmi and Susskind, 2000; Stahl *et al.*, 2006; Fang *et al.*, 2009).

In order to alleviate pressures associated with OGS it would normally be expected of the government or research institutions to invest in the establishment of new OGS since they cause interferences in investigations outcomes (El Afandi, 2014). Additionally, weather stations are located in developed regions for weather monitoring in cities or airports, a greater volume of stations could cover multiple locations, particularly in remote areas (Moeletsi and Walker, 2012; Colston *et al.*, 2018; Valeriano *et al.*, 2019). The procurement and construction of these stations will require a financial injection, as well as increased access to electricity and equipment maintenance. However, electricity power shortages due to “load shedding”, the increasing threat of coronavirus cases and unemployment crisis add compounded pressures on OGS weather data collection (Business Insider SA, 2020; Mathe, 2020).

To overcome obstacles associated with spatial interpolation, alternative reliable weather data sources, such as GD, need to be explored and evaluated. El Afandi

(2014) compared the Climate Forecast System Reanalysis data base and the OGS observations, concluding that this GD source has compatibility with all OGS observations, except for relative humidity and dew point temperature. Subsequently, El Afandi (2014) noted GD can be used to compensate for missing data from OGS. Duarte and Sentelhas (2020), in Brazil, compared two GDs, DailyGridded and NASA POWER against OGS. Their study showed that DailyGridded had a superior performance compared to NASA POWER and should also be considered to compensate for missing data associated with OGS. DailyGridded is only available in Brazil, whereas NASA POWER is a global database.

The NASA POWER gridded system has a long history of providing meteorological data for research purposes (Stackhouse *et al.*, 2004). The information from this online archive has been available since 1983. The meteorological data available include, but are not limited to, rainfall, relative humidity, solar radiation, temperature (mean, minimum and maximum) and wind speed (Stackhouse, 2020). There are numerous recent successful studies that compare and evaluate the use of the NASA POWER and the ground truth data from OGS. These include the simulation modelling of water requirements for maize in South Africa (Moeletsi and Walker, 2012), estimation of maize yield in Brazil (Duarte and Sentelhas, 2020), and a rotavirus epidemiology study in Bangladesh, Brazil, India, Nepal, Pakistan, Peru, South Africa and Tanzania (Colston *et al.*, 2018).

NASA POWER uses a set of models and algorithms to obtain their data. The Goddard Earth Observing System (GEOS) model is used to estimate air temperature, estimating every three hours. Air temperature is re-interpolated from other sources such as ground stations, meteorological balloons, and radar imagery. NASA POWER rainfall data is obtained from Global Precipitation Climate Project, additionally, other

sources such as microwave imagery and atmospheric infrared sources are used to enhance estimations. Relative humidity is estimated using air temperature derived from GEOS system, atmospheric pressure and specific humidity parameters from the NASA's Modern-Era Retrospective analysis for Research and Applications assimilation model (Monteiro *et al.*, 2018). The daily data collected from NASA POWER is at global grid scale of 0.5°x 0.5° (50 km X 50 km) latitude and longitude (Srivastava *et al.*, 2020). The focus of this study was to test the reliability of NASA POWER in supplying continuous weather data against the OGS weather data from the ARC-ISCW in order to evaluate a potential alternative in plant pathological and epidemiological research. The aim of this study is to compare the weather data obtained from 11 OGS with NASA POWER data through evaluating the statistical agreement between the two datasets. We hypothesise that there is statistical agreement between the weather parameters obtained from OGS and those from the simulated gridded data.

MATERIALS AND METHODS

Selection of sites

Weather data collection sites for this study were selected based on their proximity to sorghum field trial locations, of which two are used in Chapter 4 of this dissertation, as well as other known sorghum grain mold (SGM) prone areas (Figure 1). These stations are maintained by the ARC-ISCW. Table 1 provides the weather station, location, elevation, coordinates of the selected stations, field trial location, and the distance between the weather station and the field trial site.

Weather databases

ARC-ISCW data

The ARC-ISCW has 530 automated stations around South Africa which are tracked and calibrated. Daily observations of the 11 selected on ground-based weather stations were purchased from the ARC-ISCW for the period of 2007 to 2014, starting from the 1st of December until 31st of May of each year, the period associated with sorghum grain panicle development. The chosen weather parameters for the study were maximum (TMAX, °C) and minimum (TMIN, °C) temperature, rainfall (RAINFALL, mm) and minimum and maximum relative humidity (RH, %).

NASA POWER data

Maximum and minimum temperature were collected at 2 m, as this would be the most appropriate height for the sorghum crop (Sorghum Checkoff, 2016). Additionally, the above corresponding daily synthetic data for the same periods with equivalent weather parameters were obtained from NASA POWER. The minimum and maximum RH percentage from ARC-ISCW was averaged in order to be consistent with average RH percentage daily GD from NASA POWER (Stackhouse, 2020). The 'nasapower' package (Sparks, 2018) was used to extract the corresponding data from the NASA POWER database.

Data analysis

Analytic workflow

The performance of the daily NASA POWER weather data was evaluated and compared to the observed OGS data, through investigating accuracy and goodness-of-fit compared to the observed data. Data processing and analyses, as well as visualisations, were performed with R (version 4.0.3; R Core Team, 2020) within RStudio (version: 1.3.15093; RStudio Team, 2020). The observational data from

weather stations was imported from Google Sheets using the 'gsheet' package (Conway, 2020). The 'tidyverse' package (Wickham *et al.*, 2019) was used to read, tidy and save the data.

Statistical metrics

The following statistical metrics were used to evaluate each weather parameter at each location; coefficient of agreement (d), mean bias error (MBE), Nash-Sutcliffe efficiency coefficient (NSE), Pearson's correlation coefficient (R) and root mean square error (RMSE). Each of these statistics are explained briefly below.

The coefficient of agreement (d), also known as the Willmott index of concordance (Willmott, 1981), is the standardised estimate of the rate of error of the prediction. Values between 0-1 are recorded, whereby, 1 indicates a good match between the estimate and the observed value, values ≥ 0.8 are satisfactory, 0.5-0.79 are not satisfactory and 0 indicates no match or a lack of agreement (Duarte and Sentelhas, 2020).

Mean bias error (MBE) measures the systemic or average bias between the estimated and the actual values. This parameter is interpreted by values ranging between any positive to negative values, where positive values indicate overestimation and negative values estimate an underestimation. A zero value indicates the absence of MBE (Singh *et al.*, 2013).

The Nash-Sutcliffe efficiency coefficient (NSE) measures the statistical agreement between simulated or estimated values relative to the actual or observed values. Values from negative infinity to positive 1 are recorded, whereby, the value of one indicates a perfect match between the two values (estimated and observed) and values below zero indicate the observed data are superior or a better predictor

(Colston *et al.*, 2018). Values of NSE < 0.5 are regarded as less satisfactory and NSE > 0.5 are considered a good fit (Moriassi *et al.*, 2007).

Root mean square error (RMSE) provides an indication of the difference between estimated values and actual or observed values, as well as to provide a calculation of the total error in the predictions compared to the observed values. Values from 0 to infinity are assigned to RMSE, where zero suggests a lack of error (Moeletsi and Walker, 2012).

Data visualisation

Scatter plots were generated to ascertain the potential presence of correlations between ARC-ISCW station-based and NASA POWER values. To determine the strength of the linear relationship between variables Pearson's correlation coefficient, also known as the accuracy index (R), was applied. Where values from - 1 to +1 are reported. A value of 1 indicates a perfect strong positive relationship and moderate correlations are indicated by values between 0.5 and 0.7 (Fernando, 2021). Weak associations are regarded as $R < 0.5$ (Clark, 2018). The 'ggscatter' package (Kassambara, 2020) was utilised to plot the correlations.

The coordinates of selected sorghum field sites (red points) and nearest ARC-ISCW weather stations (blue points) were used for NASA POWER data extraction and were mapped using the 'rnatualearth' package (South, 2017). Additionally, 'ggspatial' (Dunnington, 2020) and 'ggrepel' (Slowikowski, 2020) were used to add arrows and annotate the map, as well as to ensure labels did not overlap. Both OGS and sorghum localities were plotted in order to illustrate the nearest OGS available for each sorghum test locality.

RESULTS

The shortest and greatest distance between the OGS and sorghum test site were 1.19 km and 47.80 km, indicating a wide spatial variation between sites. The ARC-ISCW data showed a high amount of missing data where all the stations had more than 20% missing data for the period of the study (Table 2-5). Rainfall was the parameter with the greatest amount of missing data. The locality with the greatest frequency of discontinuous data was Viljoenslaagte.

Evaluation of maximum temperature

The statistical metrics and errors of TMAX comparisons from NASA POWER and ARC-ISCW data are provided in Table 2. The maximum values of NSE (0.86) and d (0.97) are recorded at Viljoenslaagte, indicating statistical agreement between the estimated and the observed values. The lowest values of NSE and d (0.10 and 0.81, respectively) are observed at Sekhukhune, suggesting that the estimation of the data by NASA power is less satisfactory ($NSE < 0.5$), however $d > 0.8$ indicates a strong goodness of fit. The lowest RMSE value of 1.52 °C was reported at Viljoenslaagte and Potchefstroom, while Sekhukhune had the highest RMSE value of 3.22 °C, indicating that the degree of error for NASA POWER was greater at Sekhukhune compared to the other sites. Furthermore, ten of the 11 stations have RMSE values less than 3°C. Additionally, MBE values ranged from -2.88 °C at the Sekhukhune to 1.30 °C at Cedara, indicating that NASA POWER underestimated TMAX at Sekhukhune and overestimated TMAX at Cedara. Six of the 11 stations showed positive MBE indicating that the TMAX was higher than the actual values at these stations. All the stations, except for Doornkop (0.88), showed a strong Pearson's correlation coefficient, greater than 0.89 (Figure 2), suggesting a large similarity between the two datasets.

Evaluation of minimum temperature

The accuracy and goodness of fit of TMIN from NASA POWER compared to ARC-ISCW are presented in Table 3. Eight of the 11 stations had a $d > 0.9$, the highest values were 0.98 at Sekhukhune, contrasting with Doornkop where $d \sim 0.83$. Despite the former recording the lowest value, agreement between the two datasets at all stations were recorded. MBE ranged from $-2.53\text{ }^{\circ}\text{C}$ to $-0.17\text{ }^{\circ}\text{C}$ at Doornkop and Potchefstroom, indicating the greatest underestimation and the lowest underestimation, at Doornkop and Potchefstroom, respectively. All of the TMIN data from NASA POWER had a negative MBE, indicating a systemic underestimation compared to the observed ARC-ISCW data. All NSE values with the exception of Doornkop (0.54) were above 0.6. The highest recorded NSE was 0.9 in Potchefstroom, indicating NASA POWER as a good fit for TMIN. Eight of the 11 stations had $\text{RMSE} < 3\text{ }^{\circ}\text{C}$, where RMSE ranged from $1.15\text{ }^{\circ}\text{C}$ to $3.71\text{ }^{\circ}\text{C}$ in Sekhukhune and Doornkop, respectively. The maximum and minimum values of R , 0.95 and 0.89, (Figure 3) were observed at Potchefstroom and Bothaville stations, respectively, indicating a strong association between NASA POWER and ARC-ISCW.

Evaluation of rainfall

All stations (Table 4) have unsatisfactory d values (0.06-0.72). The lowest recorded d parameter was associated with Bothaville (0.06). These results indicated an absence of goodness of fit between the two datasets. The MBE values range from -2.13 mm in Cedara to 1.05 mm in Bothaville, indicating both underestimation and overestimation at some stations. All stations had $\text{NSE} < 0.5$ indicating poor statistical agreement between NASA POWER and ARC-ISCW for rainfall, with a negative value for this metric at Cedara. This suggests that ARC-ISCW provided data which holds true to actual rainfall events compared to the gridded NASA POWER data. Bothaville

recorded the highest RMSE (27.38 mm) while the lowest RMSE (4.88 mm) was recorded in Viljoenslaagte. Subsequently, the highest degree of error in NASA POWER data estimated rainfall events unsatisfactorily at Bothaville. Five sites, Morgenzon, Potchefstroom, Bothaville, Cedara and Doornkop had $R < 0.5$ indicating weak relationships between the two weather databases for rainfall. The six remaining sites had moderate correlation coefficients between 0.5 and 0.68 (Figure 4).

Evaluation of relative humidity

Average RH indicated variable goodness of fit among sites (Table 5). The d values range from 0.70-0.90 at Doornkop and Bothaville as well as Sekhukhune, respectively, indicating a poor to satisfactory fit. Furthermore, MBE ranged from -7.54-11.63% at Cedara and Bothaville, respectively. Two sites, Cedara and Viljoenslaagte had negative MBE values suggesting an underestimation of RH. The remaining nine sites overestimate RH, according to the MBE parameter. The RH from NASA POWER performed poorly relative to the observed data at the ARC-ISCW indicated by NSE values less than 0.5, except for Sekhukhune site, $NSE > 0.5$ (Table 5). This indicated that ten of the stations have less statistical agreement with the observed values. In addition, the RMSE ranged from 6.55-13.91% in Sekhukhune and Bothaville, respectively, suggesting Sekhukhune as the site with best fit and Bothaville the worst fit according to RMSE. All the stations have $R > 0.7$, with the minimum and maximum values ranging from 0.76-0.87 at Cedara as well as Viljoenslaagte and Sekhukhune, respectively (Figure 5).

DISCUSSION

As technology has advanced, sources of weather data have increased in their availability, number of measurements available and the quality thereof, ranging from

manual and mechanical OGS to assimilated GD. This study indicated the agreement and disagreement among weather parameters measured and estimated by OGS and GD, providing an indication for the application of simulated weather data in plant pathology and plant disease epidemiology. On-ground stations usually only have the capacity to record a small, or limited number of weather parameters at a particular locality, whereas GD such as NASA POWER, can be used as a potential alternative source of data which provides a greater selection of weather parameters. However, in this study only most commonly collected OGS parameters were evaluated against NASA POWER data. These included TMAX, TMIN, RAINFALL and RH. These parameters are also the most commonly applied variables in plant disease epidemiological studies. Schaafsma and Hooker (2007) found positive correlation between daily rainfall, relative humidity and temperature in the development of *Fusarium* head blight and accumulation of deoxynivalenol in wheat. This suggests that weather variables are significant abiotic factors that drive plant disease epidemics where the pathogen is favoured on susceptible plants. Therefore, understanding the effects of these variables on disease will be vital in developing suitable disease risk management systems.

The performance evaluation of the NASA POWER data, compared to the OGS (ARC-ISCW) data, relied on the statistical metric applied, the site and the weather variable. The use of the NASA POWER database may serve as an alternative source in instances where i) weather stations have missing data, ii) do not have data for specific sites or iii) choosing of certain weather variables over others based on the fact that some may have high degrees of accuracy when compared to the observed data. Temperature parameters, TMAX and TMIN, were the only variables with superior

performance compared to moisture parameters, RAINFALL and RH, as suggested by average values of the calculated statistical metrics, i.e., d , MBE, NSE, RMSE and R.

Aboelkhair *et al.* (2019), Egyptian researchers, recorded average d , R and RMSE values of 0.8, 0.75 and 5.23 °C for TMAX respectively and 0.87, 0.86 and 3.9 °C for TMIN, respectively. However, they recorded higher MBE values for TMAX and TMIN compared to this study (0.49 °C and 1.29 °C, respectively). Nonetheless it was concluded that the results showed a sufficient goodness of fit and precision relative to the actual values. Colston *et al.* (2018), from the United States of America (USA), recorded an average of NSE value of 0.29. However, this varied according to the different regions with NSE > 0.5 in some instances. Consequently, the results of the current study are superior compared to the findings of their study and as a result, suggest that our results for TMAX and TMIN are sufficient to fill in missing data for OGS or to be used as OGS data alternatives.

A study conducted, in Italy, by Negm *et al.* (2018) stated that inland weather stations with high elevations (> 300 m above sea level), tend to have inaccurate estimations for air temperature. This was further supported by Aboelkhair *et al.* (2019) stating that this was evident in the Egyptian weather stations with elevations greater than 100 m. The results of this study contradict those of Negm *et al.* (2018) as TMAX and TMIN had sufficient agreement between observed and simulated data. Furthermore, locations evaluated in Valeriano *et al.* (2019) were 800 m above sea level and had strong correlations ($R = 0.8$) for air temperature, similar to those observed in our study. Srivastava *et al.* (2020), from Northern India, reported a relationship between the overestimation of air temperature and rainfall. During the days when rainfall occurs the OGS measures the amount of rainfall accurately while the NASA POWER algorithms or models for temperature may not detect the change

in air temperature. This may be as a result of the immediate cooling of the area with a consequent overestimation of the temperature by NASA POWER, reported in this study.

A consistent systemic underestimation of the TMIN (-1.12 °C) was observed in the current study as indicated by negative MBE values, showing that dataset from NASA POWER underestimated this variable. Similarly, in China, Bai *et al.* (2010) found comparable results with the average systemic underestimation of -1.4 °C. In contrast, in the USA, White *et al.* (2008) found that TMIN was overestimated by NASA POWER with an average of 1.1 °C. Furthermore, in South Africa, Moeletsi and Walker (2012) found an overestimation of TMIN by NASA POWER with an average of 2.83 °C. According to Bai *et al.* (2010) and Che *et al.* (2016), both reporting from China, this type of discrepancy needs further research because it is not due to location-specific or seasonal variation. Although, TMIN was underestimated the application in plant disease epidemiology may still be useful, as a range of optimum temperatures may be used to calculate conducive days for disease development. The observed underestimation may therefore be counteracted by the awareness of its presence and making a locality specific adjustment accordingly.

Zhou *et al.* (2004) and Ren *et al.* (2007) highlight the effects of heat islands, where stations located closer to urban areas have higher temperatures than the surrounding or outlying areas, such as forests or water bodies due to urban infrastructure, i.e., roads or buildings absorbing and re-emitting the heat of the sun. The United States Environmental Protection Agency (US EPA, 2014) reported that heat islands are produced regardless of the size of the city or town. However, it was also noted that heat islands have an direct proportionate relationship with the size of the city or the town. As a result, TMIN could be underestimated by the NASA POWER

models because the effect of heat islands is not accounted for in model development. Conversely, GD models will not detect the rise in the air temperature where heat islands occur. A similar assumption could be made with regard to the underestimation of TMIN in this study. For the effect of heat islands to not have an influence on the surrounding atmosphere, US EPA (2014) recommend 1.5 km distance from a potential landscape. For instance, there is 1.2 km distance between ARC-ISCW OGS and the nearest populated town in the city of Potchefstroom, a distance below the recommendations of the US EPA. Contrary to the distance, the city recorded high R. As mentioned above the effect of heat islands decreases with size of the city, e.g., Potchefstroom is a smaller city (164.2 km²) compared to Johannesburg (1645 km²) where the effect of heat islands would be more apparent as the urban area is larger. Thus, cognisance must be taken at the town/city area as well as population, which could drive heat island variation and a thorough detailed investigation should be made to check the proximity of the stations used in the study with cities and the sizes of those cities, if disease risk associations or predictions are to be made.

RAINFALL and RH were the only weather variables that performed unsatisfactorily. When daily NASA POWER RAINFALL and RH was compared with the data from the weather stations in Eastern Asia, Europe, Sub-Saharan Africa, North America and South America, the R for RAINFALL was between 0.32 and 0.44 and between 0.32 and 0.77 for RH (Van Wart *et al.*, 2015). Duarte and Sentelhas (2020) recorded R and *d* values for RAINFALL and RH of 0.39 and 0.55 and 0.35 and 0.77, respectively. Our study recorded stronger R and higher *d* values for RAINFALL of 0.48 and 0.58, and 0.81, (R) and 0.78 (*d*) for RH, respectively, which are similar to the above previous findings. Monteiro *et al.* (2018) found that RH was slightly overestimated, by 3.5%, which is close to the results of this study, 3.38%. Additionally,

RH also had a moderate R (0.69), strongly agreeing with results of Van Wart *et al.* (2015), with the exception of the current results outperforming their results with regard to R. Despite the strong correlation NSE values were less than 0.5 showing statistical disagreement and high error (RMSE; 9.51%) values compared to other variables. This indicates that the observations from the OGS are sufficient or should be preferred to those of NASA POWER, where available. The data from ARC-ISCW for RH (minimum and maximum RH) was averaged in order to correspond with NASA POWER, this could also have contributed to the confounded results.

According to Colston *et al.* (2018) the collection of rainfall data can be a challenge to measure at OGS and therefore GD for rainfall may perform weakly due to modelling occurring with collected data. Rainfall is linked with cloud systems presenting complexities with regards to cloud structures or shapes being organised differently across spatial scales. Due to their high variability this makes the measurement of atmospheric variables across land difficult (Rahman and Sengupta, 2007). Rubel and Rudolf (2001) found that NASA POWER could not realistically estimate rainfall. Additionally, Contractor *et al.* (2015) stated that there is no gridded system that consistently estimates rainfall closer to the actual values. Monteiro *et al.* (2018) reported that NASA POWER naturally shows poor performance relative to the observed data ($R = 0.42$ and $d = 0.62$) which were the smallest values among variables tested, as reported in this study.

The high volume of missing data from the OGS, resulting in discontinuous data are significant in this study. Grassini *et al.* (2015) stated that, in order to have quality results OGS need to have $< 20\%$ missing data. White *et al.* (2011) reported that the mismatches with regard to OGS data and NASA POWER could be due to missing data. Therefore, sources of data must be improved so that GD results can be improved

to be closer to the OGS data obtained. In order to enhance the quality of the results, in case of missing data from the OGS, researchers could delete or remove the equivalent dataset from NASA POWER to ensure compatibility with the OGS data. Additionally, the use of other gridded systems may be investigated compared to the NASA POWER data, if available. Lastly, using aggregated data from both databases to compare them against each other, making aggregates of 7-, 10-, 14-day and monthly means could be investigated to determine if they would not produce better quality results. Aboelkhair *et al.* (2019) reported that monthly means for temperature offered a better fit compared to both daily maximum and minimum temperature. Furthermore, Van Wart *et al.* (2015) also found that the 14-day mean of rainfall data showed better performance compared to daily values. This suggestion was also made by Monteiro *et al.* (2018).

CONCLUSION

The results obtained in the study conclude that the NASA POWER database can produce satisfactorily results for both TMAX and TMIN, indicating that the NASA POWER database presents an opportunity to be used as substitutes in areas where there may be no on-ground weather stations, missing data are present, or capital is not available for data purchasing. In contrast, RAINFALL and RH performed poorly, although better than some other countries previously reported, suggesting OGS data offers superior data. The potential use of the NASA POWER gridded data in the area of plant pathology and other natural and agricultural disciplines, provides South African researchers with leverage regarding available and continuous weather data, combatting missing data. The 'usability' of NASA POWER in agronomic research in countries, such as Brazil (Duarte and Sentelhas, 2020), China (Bai *et al.*, 2010), South Africa (Moeletsi and Walker, 2012) and United States of America (White *et al.*, 2011)

has been reported. The use of this database in plant pathology is not yet common in South Africa, however greater use internationally has been noticed. The decision to make use of GD is highly dependent on the research question being posed and the application thereof within the discipline, as well as the available budget and quality of OGS data. However, we trust that this study has indicated some potential uses for TMAX and TMIN, with applications in epidemiology in the field of plant pathology.

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TABLES AND FIGURES

Table 1: Environmental description of 11 selected weather stations used as test localities to compare on-ground stations and National Aeronautics and Space Administration/ Prediction of Worldwide Energy Resources

ARC longitude	ARC latitude	Elevation (m above sea level)	Sorghum location	Weather station locality	Location number	Distance to the field trial (Km)
27.41	-27.27	1401	Goedgedacht	Viljoenslaagte	8	47.80
28.35	-25.60	1168	Klipdrift	Pretoria	11	26.71
29.72	-26.87	1612	Platrand	Morgenzon	5	37.72
29.72	-26.87	1612	Amersfoort	Morgenzon	10	18.73
29.72	-26.87	1612	Perdekop	Morgenzon	13	33.12
28.95	-26.99	1512	Holmdene	Gladdedrift	2	20.10
28.95	-26.99	1512	Val	Gladdedrift	9	22.23
27.07	-26.73	1349	Potchefstroom	Potchefstroom	14	4.01
28.29	-28.16	1721	Bethlehem	Bethlehem	6	8.89
26.58	-27.15	1266	Klerksdorp	Bothaville	3	33.44
29.83	-24.91	1446	Leeuwkraal	Sekhukhune	12	1.94
27.33	-26.94	1415	Dover	Moray	1	28.81
27.33	-26.94	1415	Parys	Moray	4	15.60
30.26	-29.54	1068	Cedara	Cedara	7	1.19
28.00	-24.49	1000	Alma	Doornkop	15	7.12

Table 2: Statistical agreement summary between on-ground stations (OGS) and National Aeronautics and Space Administration/ Prediction of Worldwide Energy Resources for maximum temperature (°C; TMax).

Weather stations localities	<i>d</i>	MBE (°C)	NSE	RMSE (°C)	Missing values (%)
Viljoenslaagte	0.97	-0.29	0.86	1.52	75.37
Pretoria	0.94	0.78	0.76	1.73	53.37
Morgenzon	0.94	1.07	0.76	1.78	40.99
Gladdedrift	0.95	-0.62	0.82	1.57	38.79
Potchefstroom	0.96	-0.33	0.83	1.52	53.66
Bethlehem	0.96	-0.46	0.85	1.53	50.00
Bothaville	0.95	0.32	0.78	1.87	50.06
Sekhukhune	0.81	-2.88	0.10	3.22	50.00
Moray	0.95	0.22	0.83	1.54	48.31
Cedara	0.91	1.30	0.71	2.39	54.25
Doornkop	0.93	0.11	0.75	1.73	35.23
Mean	0.93	-0.07	0.73	1.85	50.00

d = Coefficient of agreement, MBE = Mean Bias Error, NSE = Nash-Sutcliffe efficiency coefficient, RMSE = Root Mean Square Error.

Table 3: Statistical agreement summary between on-ground stations and NASA POWER for minimum temperature (°C, TMIN).

Weather stations localities	<i>d</i>	MBE (°C)	NSE	RMSE (°C)	Missing values (%)
Viljoenslaagte	0.95	-1.13	0.82	2.07	75.31
Pretoria	0.96	-0.26	0.86	1.76	53.37
Morgenzon	0.92	-1.00	0.77	2.37	40.89
Gladdedrift	0.91	-1.18	0.75	2.50	38.79
Potchefstroom	0.97	-0.17	0.90	1.52	53.66
Bethlehem	0.94	-0.32	0.82	1.99	50.00
Bothaville	0.89	-1.96	0.65	3.14	51.17
Sekhukhune	0.98	-0.36	0.87	1.15	50.00
Moray	0.88	-2.39	0.61	3.25	48.32
Cedara	0.92	-0.98	0.74	1.98	54.32
Doornkop	0.83	-2.53	0.54	3.71	35.23
Mean	0.92	-1.12	0.76	2.31	50.1

d = Coefficient of agreement, MBE = Mean Bias Error, NSE = Nash-Sutcliffe efficiency coefficient, RMSE = Root Mean Square Error.

Table 4: Statistical agreement summary between on-ground stations and NASA POWER for rainfall (mm, RAINFALL).

Weather stations localities	<i>d</i>	MBE (mm)	NSE	RMSE (mm)	Missing values (%)
Viljoenslaagte	0.70	-0.20	0.35	4.88	75.44
Pretoria	0.53	0.96	0.24	9.18	53.99
Morgenzon	0.65	-0.09	0.21	6.37	49.51
Gladdedrift	0.72	0.33	0.43	6.88	43.20
Potchefstroom	0.55	0.93	0.21	7.22	56.41
Bethlehem	0.66	-0.25	0.22	5.67	50.54
Bothaville	0.06	1.05	0.00	27.38	49.93
Sekhukhune	0.62	-0.01	0.25	5.67	51.58
Moray	0.62	0.21	0.33	4.98	48.31
Cedara	0.68	-2.13	-0.57	6.44	54.32
Doornkop	0.55	0.8	0.16	6.89	35.32
Mean	0.58	0.15	0.17	8.32	51.69

d = Coefficient of agreement, MBE = Mean Bias Error, NSE = Nash-Sutcliffe efficiency coefficient, RMSE = Root Mean Square Error.

Table 5: Statistical agreement summary between on-ground stations and NASA POWER for relative humidity (% RH).

Weather stations localities	<i>d</i>	MBE (%)	NSE	RMSE (%)	Missing values (%)
Viljoenslaagte	0.76	-6.18	-0.57	9.78	75.37
Pretoria	0.80	4.25	-0.45	8.98	53.37
Morgenzon	0.86	1.56	0.29	6.85	40.98
Gladdedrift	0.83	4.53	0.08	8.43	38.78
Potchefstroom	0.80	6.15	-0.29	9.55	53.66
Bethlehem	0.83	3.06	-0.03	7.87	50.00
Bothaville	0.70	11.63	-1.76	13.91	50.06
Sekhukhune	0.90	3.13	0.59	6.55	50.00
Moray	0.76	8.19	-0.99	11.19	48.31
Cedara	0.74	-7.54	-0.08	9.75	54.25
Doornkop	0.70	8.45	-1.73	11.71	35.23
Mean	0.79	3.38	-0.29	9.51	50.00

d = Coefficient of agreement, MBE = Mean Bias Error, NSE = Nash-Sutcliffe efficiency coefficient, RMSE = Root Mean Square Error.

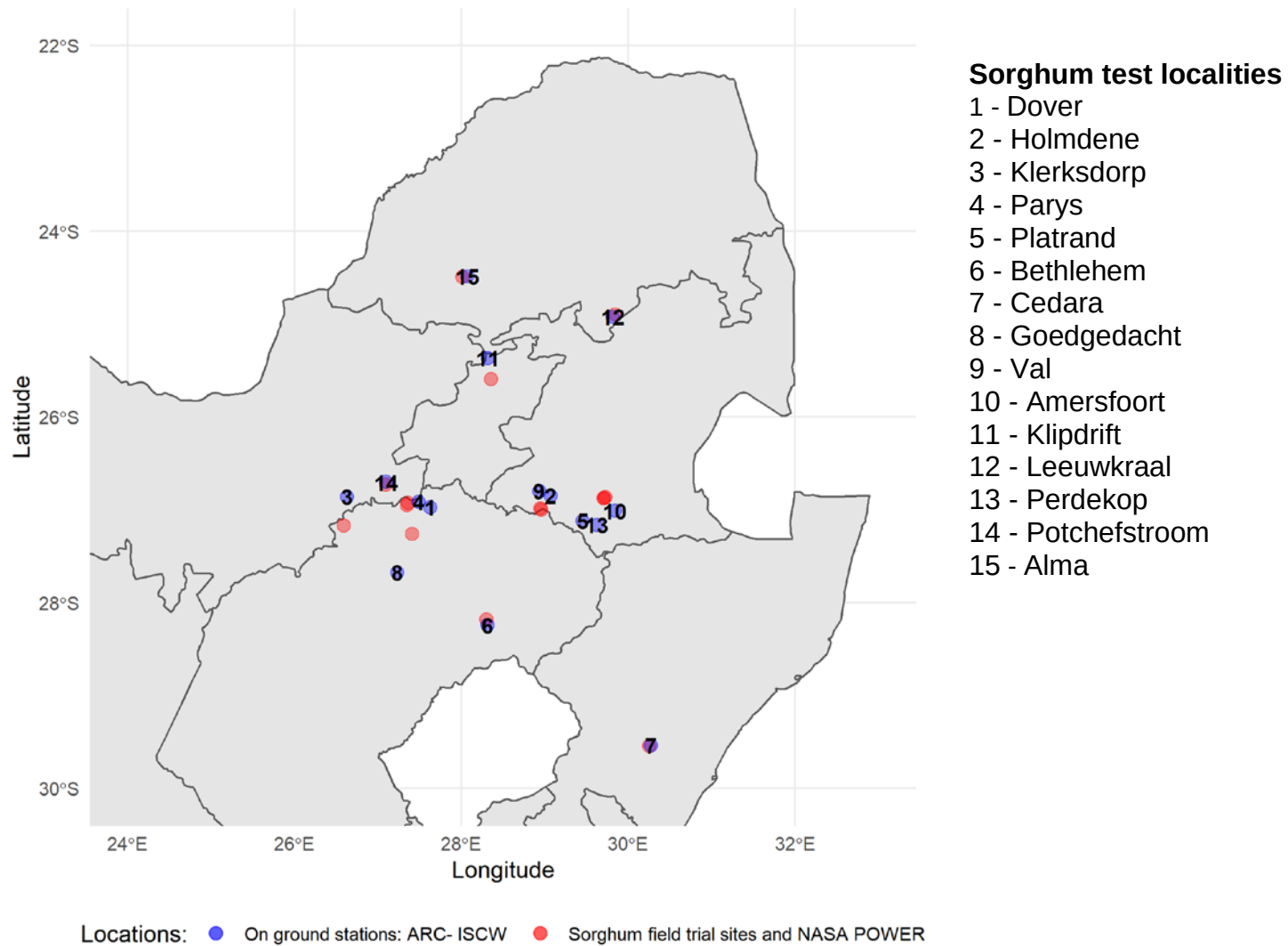


Figure 1: Spatial distribution of the ARC-ISCW and NASA POWER localities based on sorghum test locations.

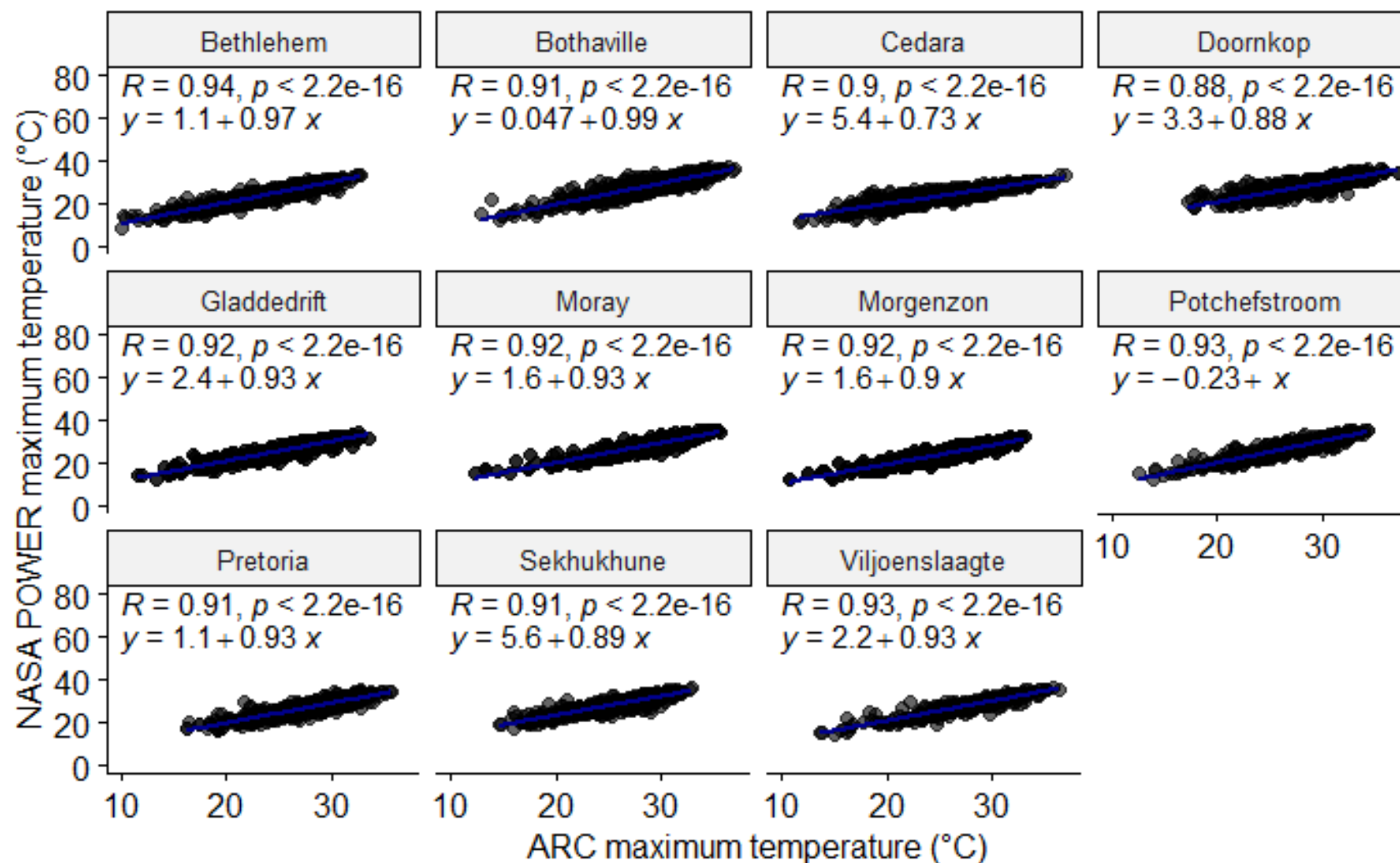


Figure 2: Relationship between daily maximum temperature (°C; TMAX) obtained from ARC and NASA POWER gridded data at 11 selected weather stations.

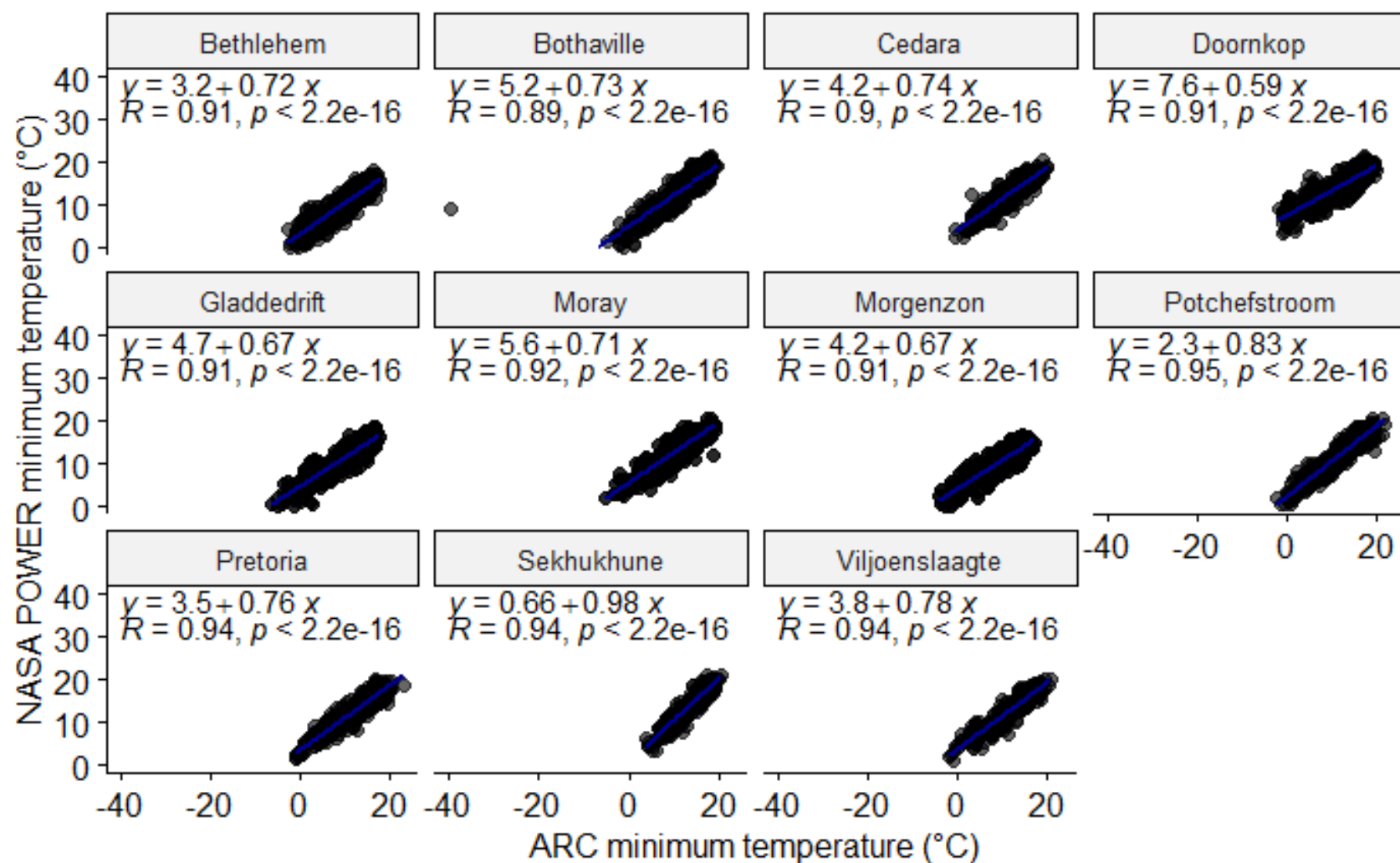


Figure 3: Relationship between daily minimum temperature (°C; TMIN) obtained from ARC and NASA POWER gridded data at 11 selected weather station

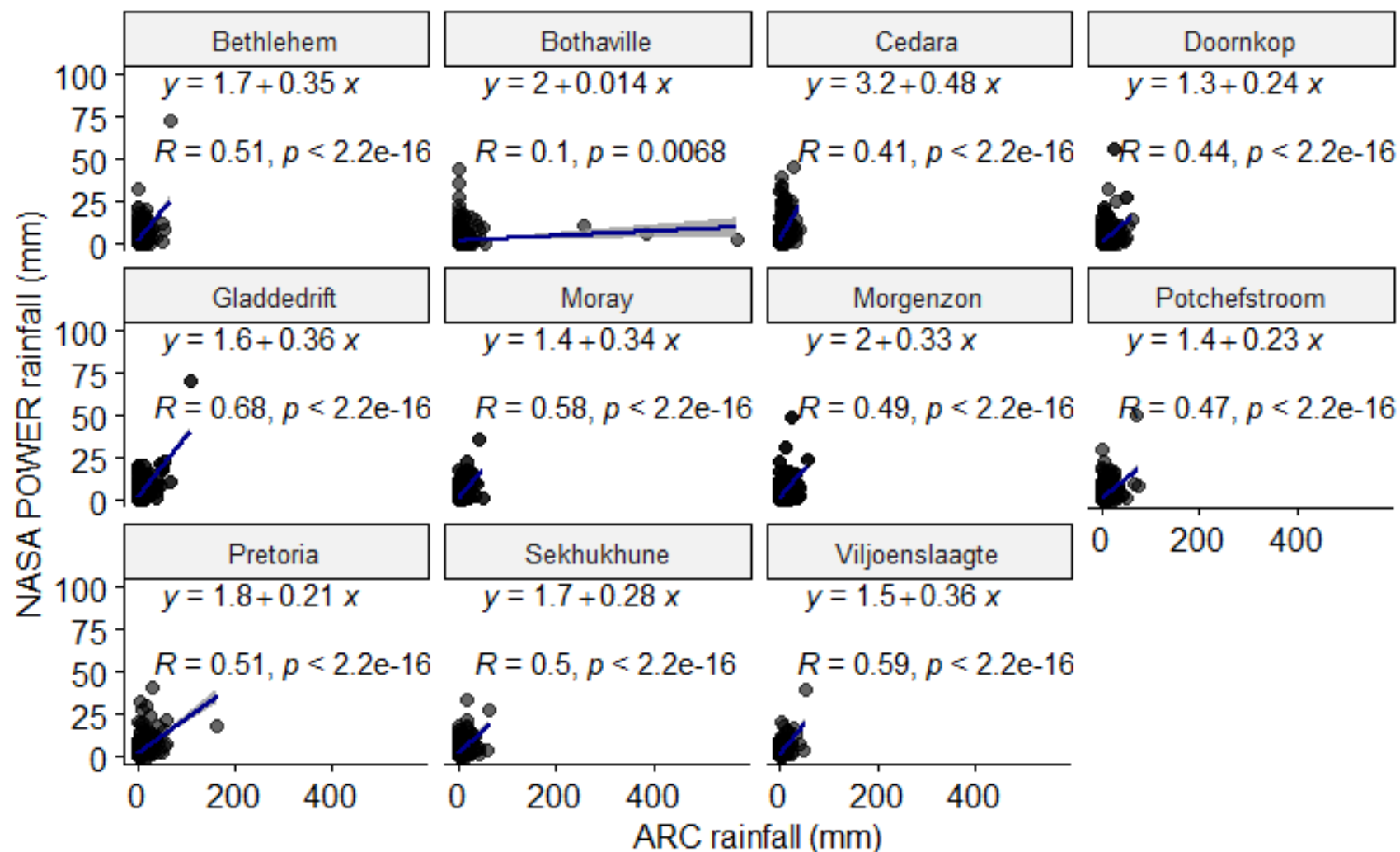


Figure 4: Relationship between daily rainfall (mm, RAINFALL) obtained from ARC and NASA POWER gridded data at 11 selected weather stations.

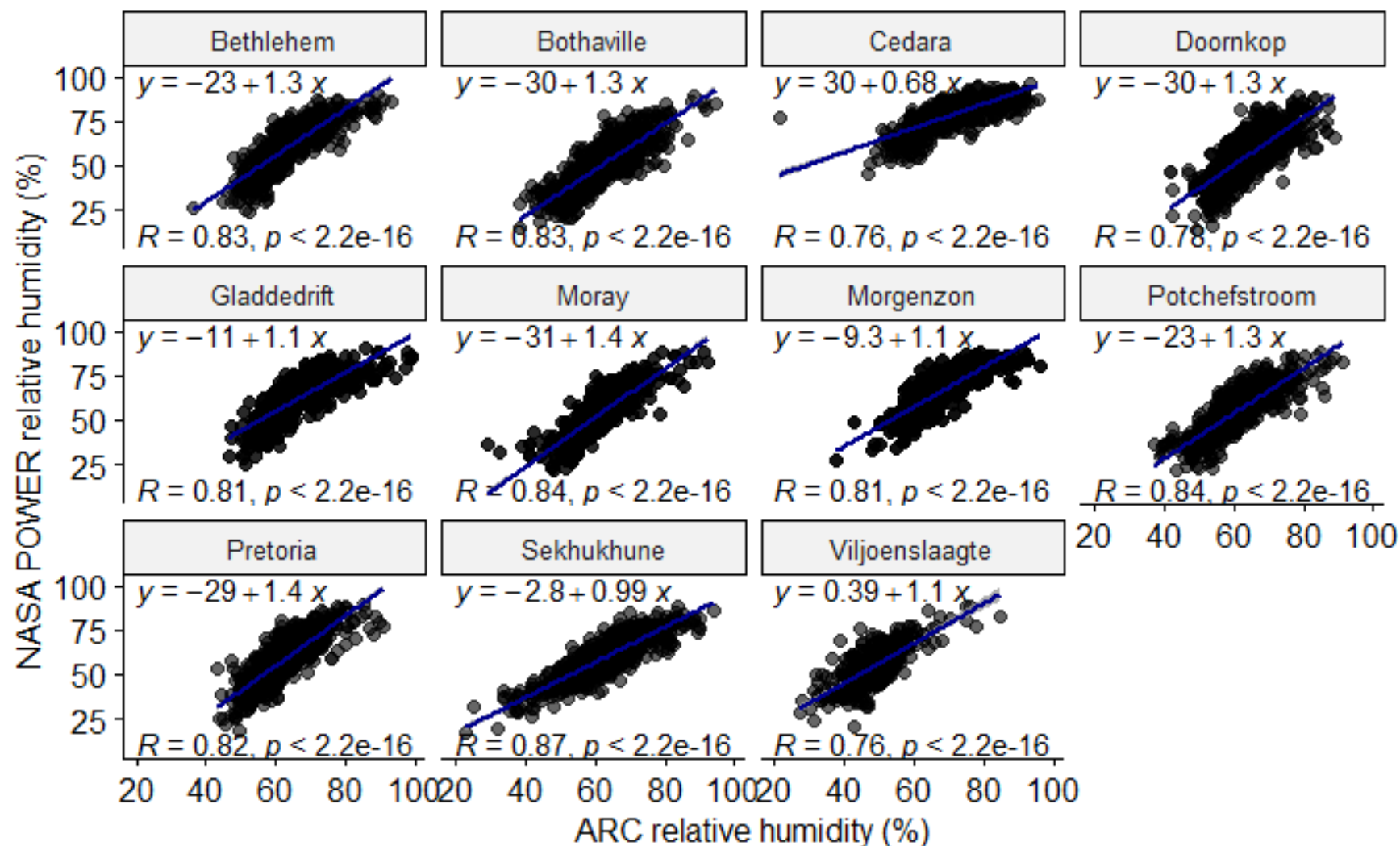


Figure 5: Relationship between daily relative humidity (% RH) obtained from ARC and NASA POWER gridded data at 11 selected weather stations.

CHAPTER 4: ASSESSING THE EFFECT OF WEATHER ON COLONIZATION OF GRAIN BY MOLD FUNGI AND PRE-HARVEST MYCOTOXIN ACCUMULATION

ABSTRACT

In South Africa, sorghum is considered the third most important grain crop after wheat and maize. However, sorghum grain mold (SGM), a pre-harvest biotic constraint, negatively affects the yield and quality of the crop across the globe. Additionally, fungi associated with SGM produce mycotoxins in the grain, potentially rendering grain unsuitable for animal and human consumption. The *Fusarium graminearum* species complex (*FgSC*) is the most widely distributed and pathogenic fungal complex associated with SGM. The aim of this study was to identify specific weather variables associated with SGM development (total fungal biomass and *FgSC* specific), mycotoxin accumulation (deoxynivalenol, DON; nivalenol, NIV; and zearalenone, ZEA) and to quantify conducive periods during anthesis required to initiate infection and grain colonisation. Historic field trial data from sorghum planted between 2009 and 2013 at two localities, Alma and Cedara were used. The relationships between meteorological parameters and total fungal biomass, *FgSC* biomass and mycotoxin accumulation (DON, NIV and ZEA) were analysed using i) specific days after flowering (DAF) and ii) conducive weather periods. Alma had the highest total fungal biomass (4978 µg/g) compared to Cedara (3041.6 µg/g). In contrast, Cedara had the greatest *FgSC* DNA (1216 µg/g) while the lowest value (0 µg/g) was detected from Alma. The maximum levels of DON, NIV and ZEA were detected in grain from Cedara, i.e. 864, 1380 and 2490 µg/kg, respectively. Significant positive correlations between ergosterol concentration and rainfall ($R = 0.63$) at 44 DAF and relative humidity (RH) ($R = 0.64$) at 46 DAF were recorded. Furthermore, a significant positive correlation was recorded between rainfall ($R = 0.63$) at 57 days after flowering and *FgSC* concentrations. No significant relationships were recorded between meteorological parameters and mycotoxins. Non-linear regression analyses through an exponential model best explained the relationship between RH at 44 DAF and mean ergosterol ($p < 0.05$), with an adjusted R^2 accounted for 97% of the variation. Rainfall parameters both for ergosterol and *FgSC* DNA were not significant ($p > 0.05$). Although, the number of conducive maximum temperature days accounted for 81% of the variation in grain colonisation as reflected by *FgSC* DNA concentrations. A sharp increase in *FgSC* DNA was associated with five conducive days of maximum temperature between 20 and 28 °C. A relationship between mean *FgSC* DNA concentration and conducive rainfall (ppt > 1 mm) days was identified and the adjusted R^2 accounted for 62% of the variation. An increase in *FgSC* DNA was associated with three to eight days of conducive ppt > 1 mm, after which a negative relationship was observed. Excessive wetness may be limiting inoculum transmission. A positive relationship between mean *FgSC* DNA and conducive RH > 70% was recorded, a pseudo R^2 of 0.98 was reported. Six conducive days with RH > 70% yielded an increase in the mean *FgSC* DNA concentration. The identification of critical periods for infection and their relationship with weather parameters could assist in the development of disease management strategies, e.g., the selection of planting dates in order to escape infection windows. Furthermore, identifying conducive periods during anthesis could contribute to modelling and the timely use of fungicide applications, ultimately reducing repeated applications, ensuring cost-effective and ecologically friendly control methods. Future data collection aimed at model refinements could consider combinations of meteorological parameters or interactive models with agronomic factors aiding in improving the prediction of the likelihood of infection.

Keywords: Conducive days, flowering, epidemiology, real time, ergosterol.

INTRODUCTION

Sorghum is considered the third most important grain crop after wheat and maize in South Africa (Du Plessis, 2008). According to the 2019/2020 average tonnage, Limpopo had the highest sorghum grain production (44 037 tons), while KwaZulu-Natal produced a significantly lower tonnage (2 850 tons, Crop Estimate Committee, 2019; Crop Estimate Committee, 2020). However, sorghum grain mold (SGM), a pre-harvest biotic constraint, influences the yield and quality of the crop across these provinces (Das *et al.*, 2012). Subsequently, compromised seed mass, reduced germination ability, seed viability and nutritional value, as well as the presence of mycotoxins affect the value and marketability of sorghum grain (Williams and McDonald, 1983; Thakur *et al.*, 2006). The mycotoxins present in the grain potentially renders it unsuitable for animal and human consumption (Navi *et al.*, 2005). The unpredictability of weather interactions with the host and pathogen results in disease which complicates SGM pre-harvest control. The sporadic appearance of SGM and reliance on environmental conditions initiating infection and mycotoxin accumulation have necessitated a deeper understanding of the meteorological requirements (Brustolin *et al.*, 2013).

SGM is the fungal deterioration of sorghum grain as the result of a complex of fungi that infect and colonise sorghum spikelets between flowering and harvest (Bandyopadhyay *et al.*, 2000). The severity of SGM in the field is exacerbated by the prolonged periods of warm and moist weather that coincides with the time between flowering and grain maturation (Forbes *et al.*, 1992; Thakur *et al.*, 2006; Das *et al.*, 2012). Pathogenic fungi, such as *Curvularia* spp. and *Fusarium* spp. can penetrate the endosperm of grain, resulting in deep-seated infections (Forbes *et al.*, 1992; Tarekegn, 2002; Chandrashekar and Satyanarayana, 2007). Saprophytic fungi associated with

SGM include, *Alternaria* spp. or *Phoma* spp., that only colonise mature grain and as a result are restricted to the peripheral grain layers. However, exposure to extended periods of high moisture may provide a window of infection for the saprophytic fungi to penetrate and partially degrade the endosperm (Glueck and Rooney, 1980).

The *F. graminearum* species complex (*FgSC*) is the most widely distributed and pathogenic fungal complex associated with SGM. Three of the species within the *F. graminearum* complex (*F. meridionale*, *F. acaciae-mearnsii* and *F. cortaderiae*) are associated with sorghum grain in South Africa, the principal crop host of this study (Mavhunga, 2013). Economically important mycotoxins such as, deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) are produced by *FgSC* and are detrimental to the health of livestock and humans when consumed (Rao *et al.*, 2012; Marroquin-Cardona *et al.*, 2014). When food products infested with DON are ingested, food refusal in livestock is caused, as well as nausea and vomiting in humans, hence the name vomitoxin (Rotter *et al.*, 1996). The uptake of NIV-contaminated products may inhibit protein synthesis, replication of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and, as a result, lead to cell death and poisoning of lymphatic and intestinal systems (De Lucca, 2007). ZEA is a phytoestrogenic mycotoxin that affects the reproductive system of humans and animals, consequently animal breeding challenges are observed and association with cervical cancer have been made (Bandyopadhyay *et al.*, 2000; Turcotte *et al.*, 2005; Reddy *et al.*, 2010).

Temperatures between 24 and 28 °C (Doohan *et al.*, 2003) and a relative humidity (RH) greater than 90% are required during anthesis (De Wolf *et al.*, 2003) for initiation of infection by *FgSC*. A minimum of 5 mm daily rainfall per day at four to seven days before ear formation triggers the production of *F. graminearum* and DON on wheat. In contrast a minimum temperature < 10 °C halt DON production

(Schaafsma and Hooker, 2007). Furthermore, Wegulo (2012) reported a significant positive correlation between *F. graminearum* biomass and DON production, indicating a possible direct proportional relationship between the weather conditions favouring fungal biomass build-up and conditions that favour mycotoxin accumulation.

Field trials conducted in Canada by Sutton *et al.* (1980) indicated that rainfall $\leq$ 60 mm for $\sim$ eight days during maize silking stage may favour both the incidence of *F. graminearum* and ZEA production. Temperatures of 21-29 °C, and relative humidity between 75-100%, were favourable for the development of SGM in Indiana, USA (Melake-Berhan *et al.*, 1996). Gray *et al.* (1971) reported that average rainfall of 100 mm per month and a temperature of 24.4 °C were ideal for *Fusarium* spp. development on sorghum panicles in Kentucky (USA). However, these reports were made more than two decades ago, and the growing awareness of the variation within the *FgSC* (from 9 cryptic species in 2004, to 16 lineages in 2012; O'Donnell *et al.*, 2004, 2008; Starkey *et al.*, 2007; Yli-Mattila *et al.*, 2009; Sarver *et al.*, 2011; Aoki *et al.*, 2012), adaptation to the environment and host specificity calls for new research to better understand the mechanism and the impact of meteorological variables on pre-harvest infection of sorghum grain by these pathogens (Miller, 2001; Suga *et al.*, 2016). Given the relationship between meteorological variables, *F. graminearum* and concomitant mycotoxin production, a shift in focus is needed for pre-harvest management strategies that would avoid or minimise the growth of *F. graminearum* in the field and also lower the risk of DON, NIV and ZEA contamination.

The identification of critical periods for infection and their relationship with weather parameters could assist in the development of disease management strategies. For instance, adjustment of the planting date may also be utilized to avoid time of year with high levels of disease pressure, consequently escaping windows of

infection. Furthermore, this information could contribute to modelling and the timely use of fungicide applications. As a result, the number of applications required could be reduced and the efficiency maximised, ensuring cost-effective and ecologically friendly control methods (Tarekegn, 2002; Brustolin *et al.*, 2013).

The aim of this study was to identify specific weather variables associated with SGM development, mycotoxin accumulation and to quantify conducive periods during anthesis required to initiate infection and grain colonization. The study focussed on assessing relationships with total fungal biomass, *FgSC* DNA levels and mycotoxin content (DON, NIV and ZEA) using data collected from diverse environments (Van Rooyen, 2019).

MATERIALS AND METHODS

Historic data (Van Rooyen, 2019) from the Collaborative Research Programme: Sorghum Production and End-Use Product Development for Increased Food Security supported by the Howard G. Buffett Foundation through the Norman E. Borlaug Institute for International Agriculture of Texas A&M AgriLife Research were used. Field trials were planted during the 2009 to 2013 seasons at two localities, Alma (24.4841°S, 28.0705°E; Limpopo) and Cedara (29.5477°S, 30.2667°E; KwaZulu-Natal). Five planting dates were used at Cedara; 26 November 2009, 24 November and 13 December 2010, 21 November and 19 December 2011. Three planting dates were included for Alma, 20 November and 11 December 2012, and 29 October 2013. Sorghum lines and grain properties were evaluated in field experiments, and disease occurrences were dependant on natural inoculum. No chemical treatments were applied to manage disease.

Sorghum grain mold parameters measured in harvested grain were total fungal biomass, the level of *FgSC* colonization and mycotoxins. A brief outline is provided for context, as details on the parameter extraction and analysis are provided in Van Rooyen (2019).

In the Van Rooyen (2019) study, ergosterol analysis, was used to determine total fungal biomass, it was extracted according to Jambunathan *et al.* (1991) and quantified using High Performance Liquid Chromatography, and detection at 280nm. Total biomass was calculated from a standard curve (Ergosterol, Sigma). Furthermore, quantitative Polymerase Chain Reaction was used to determine *FgSC* biomass by applying the protocol described by Nicolaisen *et al.* (2009). FgramB379 and Fgram411, forward and reverse primers were applied with matrix matched standards, yielding a $\mu\text{g/g}$ *FgSC* DNA concentration. Additionally, mycotoxin extraction and analyses of DON, NIV and ZEA were conducted using the Liquid Chromatography Mass Spectrometry protocol described by Small *et al.* (2012).

In the current study, weather data including minimum (TMIN) and maximum (TMAX) temperature ($^{\circ}\text{C}$), rainfall (ppt; mm) and RH (%) were extracted from NASA Prediction of Worldwide Energy Resources (NASA POWER). This meteorological database was selected due to the volume of missing values associated with local weather data collected, as presented in Chapter 3.

DATA ANALYSIS

Summary statistics for the historic data taken from Van Rooyen (2019) were generated, indicating the mean, standard deviation, minimum and maximum observations of total fungal biomass, *FgSC* biomass and mycotoxins. The relationships between meteorological parameters and total fungal biomass, *FgSC*

biomass and mycotoxin accumulation (DON, NIV and ZEA) were analysed using i) specific days after flowering (DAF) and ii) conducive weather periods.

Statistical workflow of days after flowering

Planting dates were used to estimate flowering period using mean cultivar flowering from the past season observations, at ~70 days after planting (dap). Pearson's correlation (R) was conducted to determine relationships between the disease parameters and weather parameters associated with each day, subsequent from flowering to hard dough period, known as days after flowering. The correlation interval was set at $-0.5 \geq R \leq 0.5$ ($p = 0.05$) and any correlation coefficient outside this range was excluded from further analyses. Only parameters with $R \geq 0.6$ were selected for regression analyses (Figure 1 and Figure 2). Subsequent re-analysis was done with meteorological parameters identified to be significant and relationships between them and the disease parameters were determined using non-linear regression.

Non-linear analysis of conducive days

As no significant relationships were found between weather parameters associated with DAF and colonization of sorghum grain by *FgSC* and mycotoxins, an alternate protocol was attempted to identify periods of disease-favourable conditions and their relationship with disease development. It could be assumed that the reason to this was the use of estimated flowering dates as opposed to actual flowering dates. Sorghum flowers emerge and open sequentially over a six to fifteen-day period with most genotypes flowering over six to nine days (Dogget, 1970). As a result, counts of days with conducive weather in a ten-day period subsequent to predicted flowering dates in each season were generated. The definition of conducive days was based on literature and selection via an iteration of best-fit models (Grains Research and Development Corporation, n.d). The TMAX and TMIN ranges associated with

favourable infection periods, were daily $20^{\circ}\text{C} \geq \text{TMAX} \leq 28^{\circ}\text{C}$ and $\text{TMIN} \geq 15^{\circ}\text{C}$. Selected moisture parameters were mean daily $\text{RH} > 70\%$ and $\text{ppt} \geq 1 \text{ mm}$. The count of the conducive days within the ten-day period was conducted to create a rolling window of conducive periods subsequent to flowering for the duration of the anthesis. The conducive days were grouped (i.e. zero-ten days) and the mean *FgSC* DNA ($\mu\text{g/kg}$) and DON, NIV and ZEA ($\mu\text{g/kg}$) concentrations associated with each conducive count in the post-flowering periods were generated.

Non-linear regression analyses were used to determine the relationship between variables. The goodness of fit measured for each model was determined using the R^2 for the Gompertz and exponential type 1 regressions, and the pseudo R^2 for the logistic model (Schabenberger and Pierce, 2001). The Gompertz model was used to determine the relationship between mean *FgSC* DNA and the conducive days of maximum temperature (TMAX). The exponential type 1 model was applied to describe the relationship between mean *FgSC* DNA concentration and conducive rainfall days, and a logistic model was fitted to the relationship between mean *FgSC* DNA concentrations and number of conducive RH days. A linear model was used to describe the relationship between *FgSC* DNA concentrations and mycotoxins, DON and NIV, no significant relationship was associated with ZEA.

Analytical workflow

Data processing and analyses, as well as visualisations, were performed with R (version 4.0.3; R Core Team, 2020) within RStudio (version: 1.3.15093; RStudio Team, 2020). The 'tidyverse' package (Wickham *et al.*, 2019) was used to read, wrangle, tidy and save the data. The `st()` function from the 'vtable' package was used to generate the summary statistics (Huntington-Klein, 2021). The easynls package

was used to conduct and fit non-linear regressions (Arnhold, 2017). The 'nasapower' (Sparks, 2018) package was to retrieve meteorological data from NASA POWER.

RESULTS

Sorghum grain mold data summary

According to Van Rooyen (2019) the greatest and the lowest concentrations of ergosterol were detected in grain samples collected from Alma (7.2-4978 µg/g). Total fungal biomass in grain collected from Cedara ranged from 13.3-3041.6 µg/g. The greatest *FgSC* DNA was detected in grain collected from Cedara, 1216 µg/g and the lowest value, 0 µg/g was detected in samples from Alma. At both Alma and Cedara samples were collected with no mycotoxins, but the maximum levels of DON, NIV and ZEA were detected in grain from Cedara, i.e. 864, 1380 and 2490 µg/kg, respectively (Table 1). For a detailed report and discussion of these results associated with grain characteristics please refer to Van Rooyen (2019).

Summary of weather parameters at trial sites

In the current study the weather data was generated from NASA POWER, temperatures at Alma during the experimental period ranged from -1.89-38.46 °C with average minimum and maximum temperatures of, 13.14 and 27.88°C, respectively. Relative humidity ranged from 10.86-91.72%, with a mean RH of 49.17%. Daily rainfall observed at Alma ranged from 0-55.78 mm, with an average of 1.48 mm. In contrast, Cedara experienced milder conditions than Alma, where temperatures ranged from 0.23-35.77 °C, with mean minimum and maximum temperatures of 11.37 and 23.12 °C, respectively. Moisture parameters were higher at Cedara with a RH range of 22.95-97.46%, with an average of 72.63%. Rainfall ranged from 0 to 130.66 mm with a mean of 3.91 mm during the experimental period (supplementary Table S8).

Specific parameters associated with infection

From the current study significant positive correlations between ergosterol concentrations within grains and rainfall ($R = 0.63$) at 44 DAF and RH ($R = 0.64$) at 46 DAF were recorded (Figure 1). Furthermore, a significant positive correlation was recorded between rainfall ($R = 0.63$) at 57 DAF and *FgSC* concentrations (Figure 2). However, no significant relationships were recorded between meteorological parameters and the mycotoxins, DON, NIV and ZEA and since all R were < 0.5 , these were excluded from further analysis. Non-linear regression analyses through an exponential model best explained the relationship between RH at 44 DAF and mean ergosterol ($p < 0.05$; Table 2 and Figure 3), with an adjusted R^2 that accounted for 97% of the variation. Rainfall parameters both for ergosterol and *FgSC* DNA were not significant ($p > 0.05$; Table 2).

Conductive periods during anthesis

In the study, the number of conducive TMAX days accounted for 81% of the variation (Figure 4A) in grain colonisation as reflected by *FgSC* DNA concentrations. A sharp increase in *FgSC* DNA was associated with five conducive days of TMAX between 20 and 28 °C which reflects a distinct threshold for colonization of grain by *FgSC*. The sharp decline at 10 days between 20-28 °C was observed this may indicate a saturation of conducive days between 20-28 °C.

The exponential type 1 model best described the relationship between mean *FgSC* DNA concentration and conducive rainfall (ppt > 1 mm) days and the adjusted R^2 accounted for 62% of the variation (Figure 4B). An increase in *FgSC* DNA was associated with three to eight days of conducive ppt > 1 mm, after which a negative relationship was observed which could be related to restricted movement of inoculum by excessive moisture. A logistic regression model best described the relationship

between mean *FgSC* DNA production and conducive RH > 70%, with a pseudo $R^2 = 0.98$ (Figure 4C). Six conducive days with RH > 70% yielded an increase in the mean *FgSC* DNA concentration, and a terminal phase in the model, i.e., at which no subsequent increase in disease severity was evident, was recorded from eight conducive days of RH > 70%. Additionally, although no conducive weather periods could be associated with mycotoxins as measured in the current study, according to the re-analysis of Van Rooyen (2019) data, we deduce that an increase in *FgSC* DNA concentrations can provide an indication of increased DON and NIV under similar weather conditions conducive for infection (Figure 5A and Figure 5B).

DISCUSSION

To properly anticipate the infection of sorghum panicles by grain mold fungi in the field, a thorough knowledge of the causal relationship between meteorological conditions and pathogens causing SGM is required. Identification of plant pathological causal relationships can create new knowledge about the intricate relationships that drive plant disease epidemics and the management thereof (David *et al.*, 2016). As a result, the relationship between meteorological parameters, the pathogen and resultant disease, were investigated. The moderate positive correlation recorded between mean ergosterol and RH at 46 DAF, suggests that RH $\geq 70\%$ may be regarded as a threshold at which an increase in ergosterol content may be expected. Bandyopadhyay *et al.* (2000), Thakur *et al.* (2006), Das *et al.* (2012) and Little *et al.* (2012) reported associations between high RH, from 85 to 95%, and visual grain mold scorings. However, this method of scoring disease only targets the superficial, visual colonisation of sorghum grain and not the total, superficial and deep-seated, fungal biomass as indicated in the present study (Mpofu and McLaren, 2014). Furthermore, according to Prom *et al.* (2003) and Perkowski *et al.* (2008) extended periods of

humidity led to higher colonisation of superficial fungi. To our knowledge this is the first field study in South Africa on SGM quantifying the RH conducive for total fungal biomass accumulation.

Alma yielded an ergosterol content, calculated from summary statistics, of which is considerably higher than that recorded at Cedara. According to mean RH, Alma is a dry environment relative to Cedara, and the high levels of ergosterol in this environment could be due to other SGM pathogens that are adapted to dry conditions (Prom *et al.*, 2003). In addition, the maximum RH for Alma was ~ 91.72%, which is greater than the 70% threshold suggested in this study, potentially creating a window of infection for different fungal pathogens that thrive in humid conditions (Prom *et al.*, 2003; Navi *et al.*, 2005). In contrast, *FgSC* maximum levels in Cedara (1216 µg/g) were higher compared to Alma (37.2 µg/g), which may indicate that Cedara provides a more suitable environment for *FgSC* colonisation. Additionally, Cedara is an experimental station and as a result the crops are rotated per season. Furthermore, other SGM pathogens might have infected sorghum before the *FgSC* window of infection, as result *FgSC* may have been out-competed in Alma compared to Cedara (Reid *et al.*, 1999). This indicates the importance of adding more environments to account for spatial and environmental variation with regard to modelling of conducive parameters for infection.

Mycotoxin (DON, NIV and ZEA) correlations with meteorological data were not significant ($R < 0.05$) and as such were removed from further analyses. Vogelgsang *et al.* (2017) stated that mycotoxin concentrations were only partially impacted by meteorological parameters and that other factors most likely played a more significant role in mycotoxin contamination. Forecasting of sorghum infection by *FgSC* and mycotoxin contamination could be improved by a combination of meteorological data

with agronomical and genetic parameters. Müller *et al.* (2010) demonstrated this in a two-year field study in Germany that studied the effects of environment, topography, and agronomic management factors in over 300 wheat specimens on DON and ZEA contamination of grain. In addition, Vogelgsang *et al.* (2017) deployed a Fusaprog prediction model developed in the early 2000's that accounted for crop developmental stages and local meteorological data on DON levels in over 298 wheat specimens. This indicates that a similar approach may have to be taken with the sorghum crop and DON, NIV and ZEA contamination.

A system that predicts the onset of infection and disease incidence could help improve fungicide efficacy (Rossi *et al.*, 2001), through forecasting timely recommendations and as a result mitigate severe yield and quality losses, thereby increasing food safety. The findings of this study contribute to defining conducive periods of rainfall, relative humidity and temperature associated with *FgSC* infection of grain. A sharp increase in *FgSC* DNA was associated with five conducive days of TMAX between 20-28 °C, and these results are corroborated by literature which indicated that *FgSC* causes infections at temperatures ≥ 20 °C on wheat (Osborne and Stein, 2007; Xu *et al.*, 2007). However, unlike sorghum, *FgSC* in wheat requires a shorter period of only three days of conducive temperatures to cause infection (Rossi *et al.*, 2001) which may be related to the flowering patterns of the respective crops. Sorghum producers can use this to their advantage, as they would have to apply fungicides at a later stage and residual activity of the fungicides could last longer compared to wheat. In addition, De Wolf *et al.* (2003) reported a combination of three conducive days of RH > 90% and temperatures between 15-30 °C favourable for wheat infection, whereas this study indicated sorghum required six days of RH > 70%. Although the relative humidity reported in this study was lower than in De Wolf *et al.*

(2003), we speculate that the prolonged period, of six days compared to three, humidity may have improved infection by *FgSC* (Schöneberg *et al.*, 2018). In this study, an increase in *FgSC* DNA was associated with three to eight days of conducive rainfall > 1 mm, after which a negative relationship was observed. This decline may be related to the restricted movement of inoculum by excessive moisture after eight days (inoculum wash) (Paulitz, 1996). Moschini and Fortugno (1996) reported that two conducive days of rainfall ≥ 0.2 mm were required for *FgSC* infection of wheat. In contrast, Brustolin *et al.* (2013) stated that the volume of rainfall was insignificant but rather that the duration of moisture played a greater role to induce infection. De Wolf *et al.* (2003) stated that discontinuous wetness could still result in infection, but the pathogen's efficiency would be reduced. Therefore, it can be inferred that infection by the *FgSC* is moisture dependent, and that the duration of the event and the amount of rainfall may be patho-system dependent. Additionally, the differences in conducive days required for infection and the favourable meteorological conditions could also be due to the different species being cultivated, sorghum and wheat, and this would require further epidemiological research to confirm.

No conducive weather could be associated with mycotoxins, however, using the historic data (Van Rooyen, 2019) significant correlations were reported between *FgSC* DNA and DON and NIV concentrations, respectively. A deduction is that an increase in *FgSC* DNA concentrations can provide an indication of a risk of increased DON and NIV levels under weather conditions conducive for *FgSC* infection. The results recorded by Coetzee (2015), indicated that NIV concentrations were significantly higher than DON in the sorghum crop. This was because three of the cryptic species of *F. graminearum* associated with sorghum infection are NIV producers (Mavhunga, 2013). Vogelgsang *et al.* (2017) reported that meteorological

conditions during flowering favorable to *FgSC* can be critical for infection and, eventually mycotoxin production. It may be assumed that measures taken to mitigate the infection of sorghum *FgSC* would also target the reduction in mycotoxin production.

According to Van Rooyen (2019) Cedara compared to Alma appeared to be more conducive for mycotoxin production. South Africa has legislation that sets the maximum permitted number of different mycotoxins in grain e.g., DON concentrations of ~ 1000-2000 µg/kg depending on the envisaged products. The United States-Food and Drug Administration (FDA) has set a maximum limit for DON at 1000 µg/kg for all wheat-derived products (CAST, 2003). Additionally, the European Union has set a maximum limit of 1250 µg/kg for DON on all raw cereals other than durum wheat, oats and maize (Lancova *et al.*, 2008; Bhat *et al.*, 2010). DON mycotoxin concentration quantified in the Van Rooyen (2019) study were lower compared to the maximum legislative limit required by all federations listed above, indicating that DON levels locally are low despite disease-favourable weather conditions. The converse was recorded for NIV and ZEA, the Scientific Committee on Food of European Commission set maximum legislative limit of 0.7 µg/kg for dietary intake for NIV (Schothorst and Van Egmond, 2004). It is noticeable that even though Alma recorded the lowest concentrations of NIV, they are still higher than maximum tolerable amount. The Food and Agriculture Organization of the United Nations has set a total tolerable level of ZEA in foods to be within the range of 50-1000 µg/kg (Marroquín-Cardona *et al.*, 2014). The maximum amount of ZEA in both Alma and Cedara exceeded the maximum legislative limit (50-1250 µg/kg). Although, no weather correlations were significant, cognisance should be taken for the presence and accumulation of mycotoxins and further study is essential.

CONCLUSION

The results highlighted meteorological parameters during anthesis that drive SGM infection caused by *FgSC*, although, the inclusion and elucidation of additional grain mold fungi, such as *Alternaria* spp. and *Phoma* spp. could account for some of the variation observed in this study. Future data collection aimed at model refinements could consider combinations of meteorological parameters or interactive models with agronomic factors aiding in improving the prediction of the likelihood of infection. The utilization of estimated flowering dates as opposed to actual flowering dates in modelling, would require further research that includes collection of actual flowering dates in order to solidify the findings of the dissertation. The conducive days required for infection and meteorological conditions differ between sorghum and other crops, hence new models specifically for the sorghum would have to be developed to increase the accuracy and reliability in understanding these specific associations. Thus, the current study provides the basis on which to build future research aimed at *FgSC* infection and concomitant mycotoxin risk models under contrasting climatic conditions and a range of genotypes.

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a newly discovered cereal head blight pathogen from the Russian Far East.
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TABLES AND FIGURES

Table 1: Historic sorghum grain mold data summary statistics for Alma (Limpopo) and Cedara (KwaZulu-Natal), across eight planting dates and 22 sorghum lines.

	Alma (n = 66)				Cedara (n = 110)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Ergosterol ($\mu\text{g/g}$)	728.21	871.55	7.2	4978	499.27	555.85	13.3	3041.6
<i>FgSC</i> DNA ($\mu\text{g/g}$)	5.91	5.44	0	37.2	126.67	180.6	1.2	1216
DON ($\mu\text{g/kg}$)	5.72	10.46	0	54.5	69.51	134.81	0	864
NIV ($\mu\text{g/kg}$)	8.54	8.97	0	31.9	97.53	195.42	0	1380
ZEA ($\mu\text{g/kg}$)	5.55	15.55	0	76.7	108.02	273.97	0	2490

n = number of samples; SD = standard deviation; Min = minimum; Max = maximum

Table 2: Parameter estimates for nonlinear regression used to describe the relationship between average ergosterol content ($\mu\text{g/g}$) and *FgSC* DNA ($\mu\text{g/g}$) recorded in 22 sorghum lines at two localities, Cedara (KwaZulu-Natal) and Alma (Limpopo), and average relative humidity and rainfall during anthesis.

Dependent variable	Term	Co-efficient estimate	Pr ($> Z $)
Mean Ergosterol	Intercept	1.113	0.255
	^a RH ₄₆	0.083	0.001*
Mean Ergosterol	Intercept	5.914	0.028
	^b PPT ₄₄	0.233	0.159
Mean <i>FgSC</i>	Intercept	2.529	0.034
	^a PPT ₅₇	0.323	0.066

^aRH₄₆ = Average relative humidity at 46 days after flowering; PPT₄₄ = Average rainfall at 44 days after flowering, PPT₅₇ = Average rainfall at 57 days after flowering, * = significance at p = 0.05.

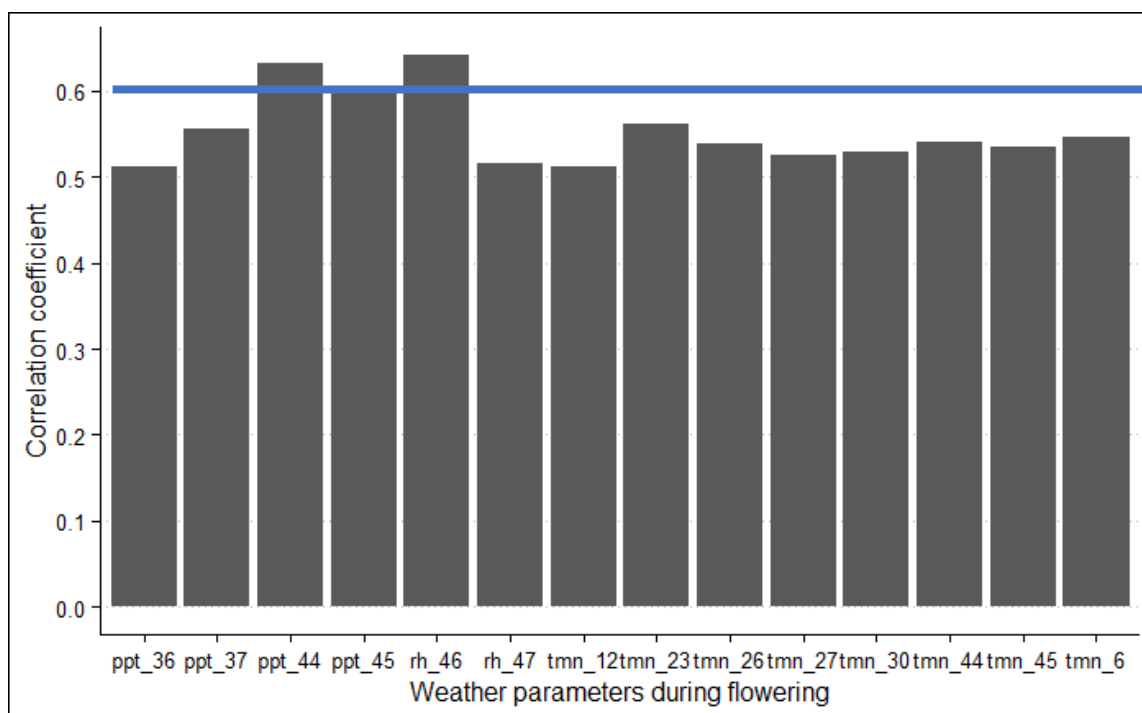


Figure 1: Correlation analysis to identify weather parameters (ppt = rainfall, mm; rh = relative humidity, % and tmn = minimum temperature, °C) associated with specific days after flowering and ergosterol concentration ($\mu\text{g/g}$). Only relationship $R > 0.5$ are indicated.

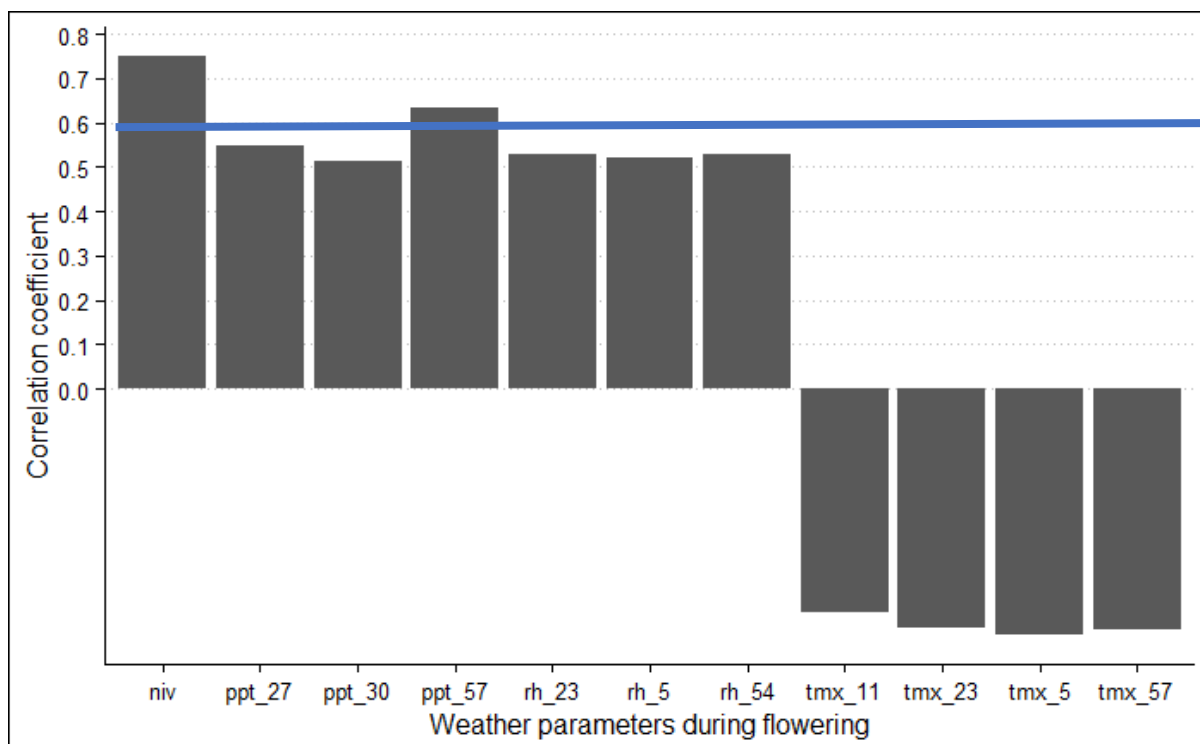


Figure 2: Correlation analysis to identify weather parameters (ppt = rainfall, mm; rh = relative humidity, % and tmx = maximum temperature, °C) associated with specific days after flowering and *Fusarium graminearum* DNA concentration (µg/g). Only relationship $R > 0.5$ are indicated.

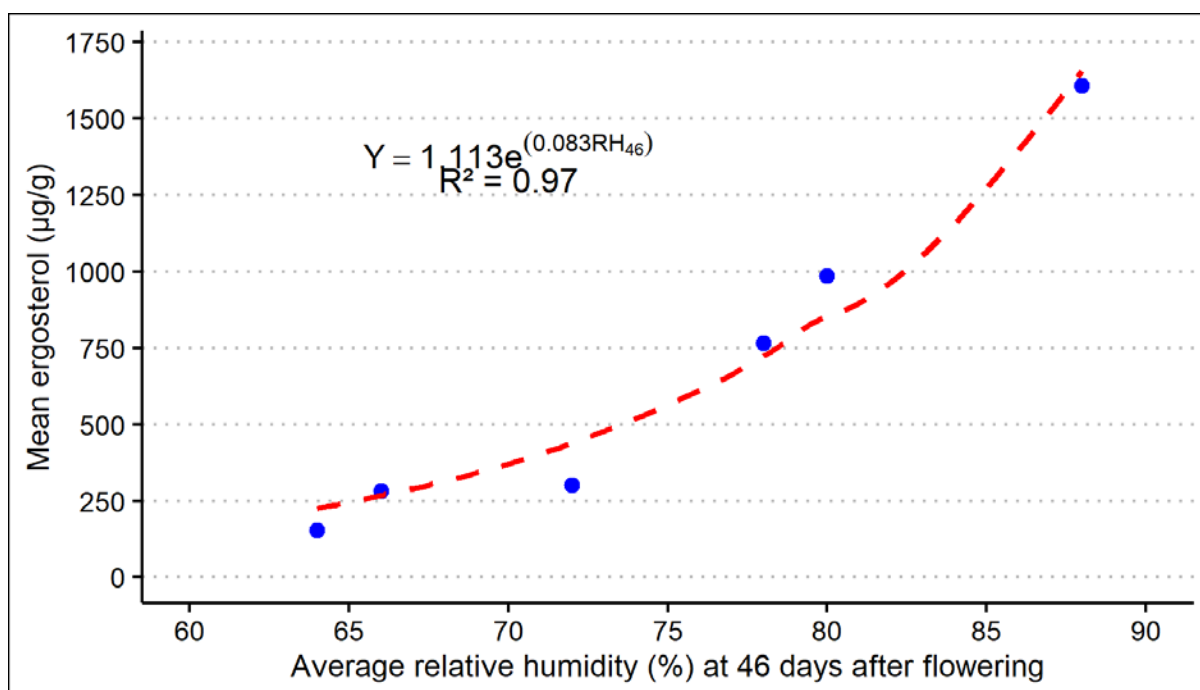


Figure 3: Relationship between ergosterol concentrations (µg/g, •) and relative humidity (%) 46 days after flowering measured at two localities, Cedara (KwaZulu-Natal) and Alma (Limpopo) in 22 sorghum lines, and predicted regression line (- - -).

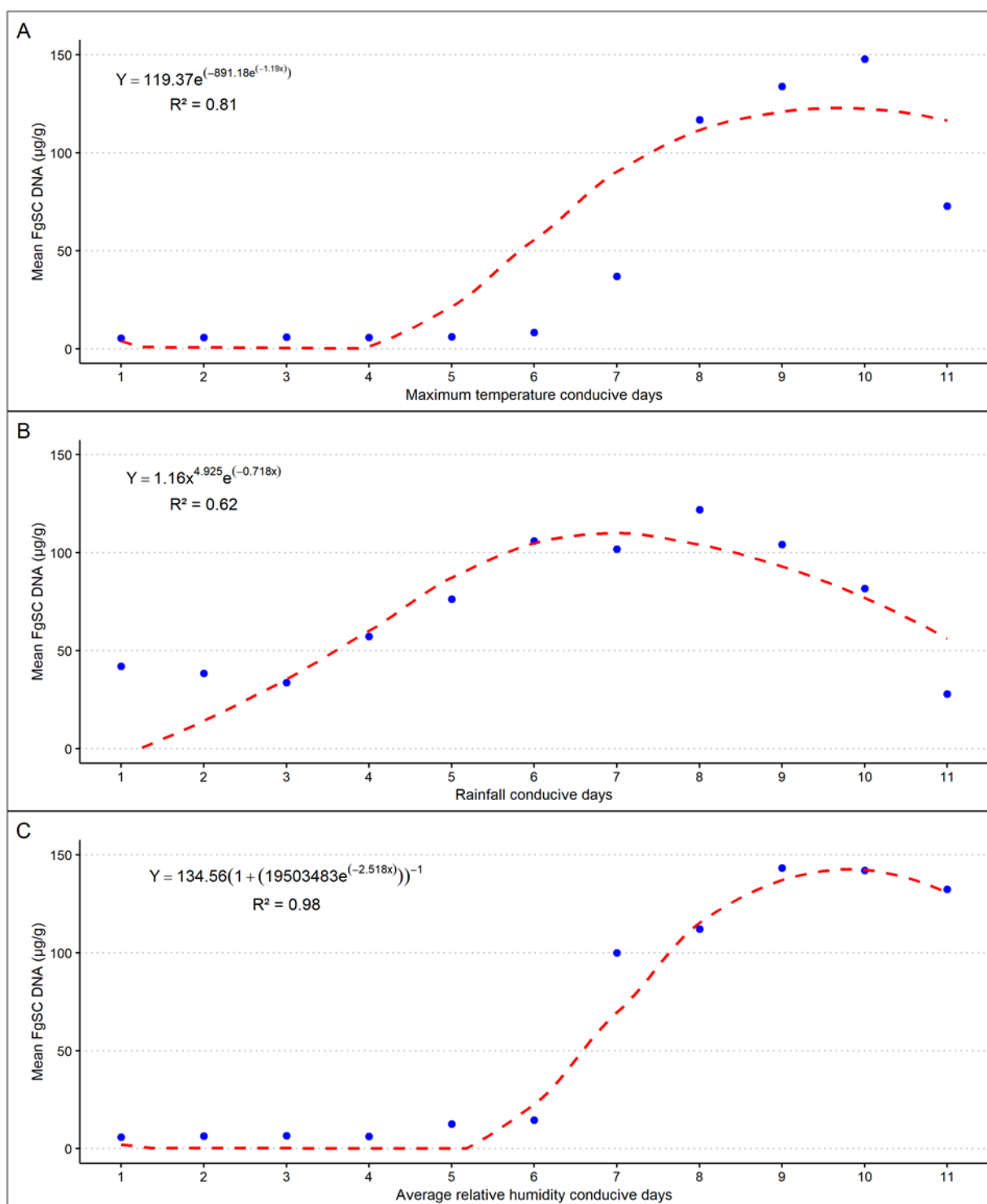


Figure 4: Relationships between observed mean *FgSC* DNA concentrations (µg/g; ●) and conductive days of maximum temperature between 20 and 28 °C (A) using the Gompertz model; rainfall > 1 mm (B) using exponential type 1 model and average relative humidity > 70% (C) using a logistic model indicating predicted values (- - -) in each model.

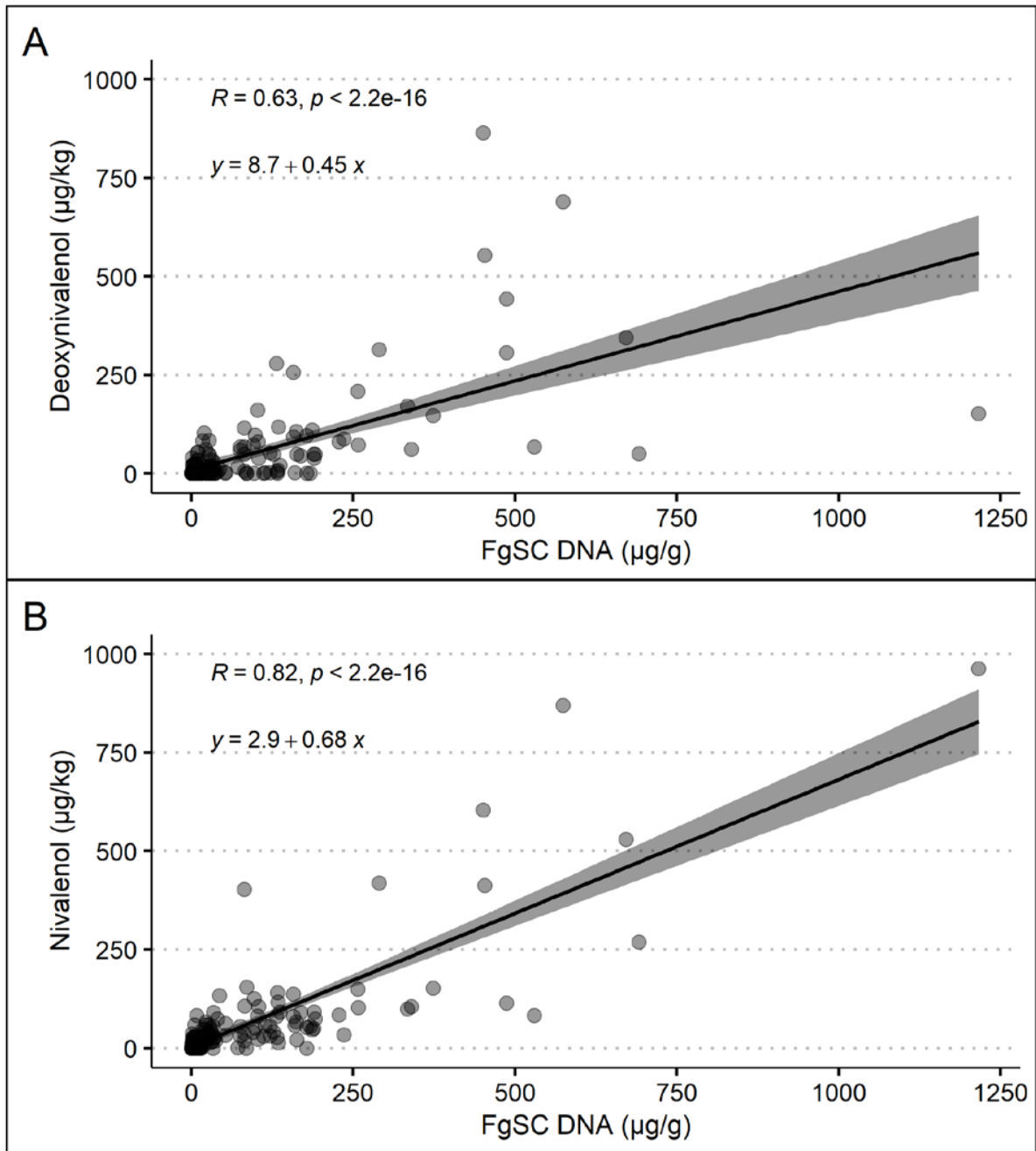


Figure 5: Relationship between *FgSC* DNA concentrations (µg/kg) and mycotoxins deoxynivalenol (A) and nivalenol (B) concentrations measured at two localities in 22 sorghum lines.

SUPPLEMENTARY MATERIALS

Chapter 2

Supplementary Table S1: Mean monthly weather data for the 2016/17 season in Greytown and 2017/18 and 2018/19 for Cedara in South Africa.

Greytown 2016/17			
Months/year	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)
December	28	13	74
January	26	13	79
February	25	15	82
March	26	13	77
April	24	11	76
May	23	10	71
June	22	15	65
Cedara 2017/18			
January	28	14	72
February	27	15	76
March	26	15	77
April	24	14	74
May	23	18	66
June	22	11	58
Cedara 2018/19			
December	28	15	75
January	27	14	76
February	27	15	78
March	26	16	78
April	24	14	78
May	25	14	61
June	23	10	53

Effect of Decortication by tangential abrasive dehulling device

Decortication rates

ANOVA (Table S2) revealed that the total percentage grain mass loss achieved with decortication by TADD differed significantly due to cultivar, decortication time (TADD) and planting number. Two-way interactions between cultivar x planting number, cultivar x TADD and planting number x TADD were recorded. Additionally, a significant three-way interaction between cultivar x planting number x TADD was also recorded.

Significant differences in grain hardness ($P < 0.001$) varied among the sorghum cultivars as seen with varying amounts of grain mass loss percentage (GMLP). PAN8706W had the highest GMLP (mean GMLP 31.53%, Table S3) compared to NS5511 and PAN8616 which had mean GMLPs of 26.20 and 27.19%, respectively. According to means separations these cultivars were significantly different from each other. GMLPs at different planting numbers (PN) ranged from a mean value of 23.34% at PN7 to 34.14% at PN2 (Table S3) with no significant differences between the former, PN4 and PN5. Means separations indicated that PN2 and PN3 (not significantly different from one another) distinctly had more GMLP followed by PN1 and PN6 (not significantly different from one another). Decortication by TADD over time (from zero to six minutes) expressed by GMLP differed significantly with each time fraction forming single clusters as seen in Table S3.

A genotype X environment interaction (planting number and cultivar; Table S4) recorded significant differences ($P < 0.001$). The performance of PAN8706W in PN3 under decortication is significantly similar to that of NS5511 in PN 2. Followed by PAN8706W in PN1 and NS5511 in PN3. These cultivars under these environments had more grain loss compared to the remaining cultivars X planting date conditions. Although PAN8706W in PN6 did not differ significantly from NS5511 in PN3. It is also noticeable that PAN8816 in PN1, 2, 3, 5 and 6 did not differ significantly to PAN8706W in PN4 and 5. Furthermore, NS5511 in PN4, 5 and 7 and PAN88816 in PN4 and 7 performed similar to PAN8706W in PN7.

The two-way interaction between TADD and cultivar (Table S5) was significantly different ($P < 0.001$). There is a consistent pattern observed in the results from Figure 1 and Table 5, a positive relationship between the time taken to decorticate the grain and the loss obtained as a result thereof. The greater the decortication time the higher

the grain loss as depicted in Figure S1. PAN8706W at T6 decortication, had the highest percentage grain loss compared to the remaining cultivars under all TADD treatments. All the cultivars at their respective treatments clustered separately except for NS5511 and PAN8816 at the T1 decortication time. The former cultivar was significantly different to PAN8706W under the same treatment.

The interaction between planting number and TADD (Table S6) was significantly different ($P < 0.001$), with PN2 at T6 decortication time having the highest decortication loss compared to all planting dates. This was followed by PN 3 under the under same decortication time. PN4, 5, 6 and 7 at T1 decortication yielded the lowest losses. The results from the significant three-way interactions ($P < 0.001$) corresponded with those above indicating that PN2 and 3 has the most GMLP compared to other PNs especially under six and four minutes. In addition, that PAN8706W has the highest losses compared to the remaining cultivars. Fisher's Protected LSD indicated that NS5511 sowed at PN2 dehulled at T6 had more percentage loss compared to the remaining cultivar X TADD X planting number conditions (Table S7). Furthermore, the performance of PAN8607W in PN1, 2 and 3 under T6 grouped together. As expected, T1 decortication yielded low grain losses as compared to the other TADD times. PAN8816 in PN5 had the lowest losses even though this did not differ significantly with NS5511 in PN1, 5, 6 and 7 and PAN8706W in PN6 and 7 and PAN8816 in PN4, respectively.

Table S2: Analysis of variance for Tangential Abrasive Dehulling Device (TADD).

Sources of variation	DF	Sum of squares	Mean squares	F value	Prob level
Cultivar	2	1128	564	85.39	0.00
TADD	4	93348	23337	3531.93	0.00
Planting number	6	3206	534	80.67	0.00
Replication	1	3	3	0.38	0.54
Cultivar X Planting number	12	1418	118	17.88	0.00
Cultivar X TADD	8	890	111	16.84	0.00
Planting number X TADD	24	1932	80	12.18	0.00
Cultivar X Planting number X TADD	48	1804	38	5.67	0.00
Residual	104	687	7		

Table S3: Mean grain loss percentages for cultivar, planting date and Tangential Abrasive Dehulling Device (TADD).

Main effects					
TADD	Loss %	Cultivar	Loss %	Planting number	Loss %
0	0.00 ^e	PAN8706W	31.53 ^a	1	29.08 ^b
1	12.39 ^d	NS5511	26.20 ^b	2	34.14 ^a
2	26.34 ^c	PAN8816	27.19 ^c	3	33.31 ^a
4	44.40 ^b			4	24.38 ^{cd}
6	58.40 ^a			5	25.62 ^c
				6	28.25 ^b
				7	23.34 ^d

Treatments with the same letter are not significantly different according to Fisher's Protected LSD ($P < 0.001$). TADD LSD (1.11), Planting number LSD (1.31) and Cultivar LSD (0.86)

Planting number: 1 = 1 Nov 2016, 2 = 23 Nov 2016, 3 = 12 Dec 2016, 4 = 28 Dec 2016, 5 = 5 Jan 2017, 6 = 5 Dec 2017 and 7 = 1 Nov 2018

Table S4: Two-way interactions between planting number and cultivar for grain loss percentage.

Planting number	PAN8706W	PAN8816	NS5511
1	34.33 ^c	27.08 ^{ef}	25.82 ^{gh}
2	37.12 ^b	26.28 ^{efg}	39.01 ^{ab}
3	40.10 ^a	27.52 ^{ef}	32.33 ^{cd}
4	27.04 ^{ef}	23.91 ^{hi}	22.19 ⁱ
5	27.42 ^{ef}	26.57 ^{ef}	22.87 ⁱ
6	30.66 ^d	27.51 ^e	25.80 ^{gh}
7	24.06 ^{ghi}	23.71 ^{hi}	22.28 ⁱ

Treatments with the same letter are not significantly different according to Fisher's Protected LSD ($P < 0.001$). Cultivar X Planting number LSD (2.27)

Planting number: 1 = 1 Nov 2016, 2 = 23 Nov 2016, 3 = 12 Dec 2016, 4 = 28 Dec 2016, 5 = 5 Jan 2017, 6 = 5 Dec 2017 and 7 = 1 Nov 2018

Table S5: Two-way interactions between Tangential Abrasive Dehulling Device (TADD) and cultivar for grain loss percentage.

TADD	PAN8706W	PAN8816	NS5511
0	0.00 ^l	0.00 ^l	0.00 ^l
1	13.97 ^j	12.14 ^{jk}	11.08 ^k
2	31.15 ^g	25.29 ^h	22.59 ⁱ
4	47.55 ^d	41.40 ^f	44.23 ^e
6	65.00 ^a	52.16 ^c	58.04 ^b

Treatments with the same letter are not significantly different according to Fisher's Protected LSD ($P < 0.001$). TADD X Cultivar LSD (1.92)

Table S6: Two-way interactions between planting number and Tangential Abrasive Dehulling Device for grain loss percentage.

Planting number	TADD				
	0	1	2	4	6
1	0.00 ^o	16.76 ^l	29.62 ^l	40.17 ^g	58.83 ^c
2	0.00 ^o	14.27 ^{lm}	32.67 ^h	53.79 ^{de}	69.95 ^a
3	0.00 ^o	15.09 ^l	31.12 ^{hi}	53.02 ^{de}	66.53 ^b
4	0.00 ^o	11.42 ^{mn}	21.53 ^k	37.33 ^g	51.63 ^e
5	0.00 ^o	8.51 ⁿ	23.55 ^{jk}	40.13 ^g	55.92 ^{cd}
6	0.00 ^o	10.30 ⁿ	25.23 ^j	48.09 ^f	57.64 ^c
7	0.00 ^o	9.59 ⁿ	20.66 ^k	38.21 ^g	48.28 ^f

Treatments with the same letter are not significantly different according to Fisher's Protected LSD ($P < 0.001$). Planting number X TADD LSD (2.94).

Planting number: 1 = 1 Nov 2016, 2 = 23 Nov 2016, 3 = 12 Dec 2016, 4 = 28 Dec 2016, 5 = 5 Jan 2017, 6 = 5 Dec 2017 and 7 = 1 Nov 2018

Table S7: Three-way interactions between planting number, cultivar and Tangential Abrasive Dehulling Device for grain loss percentage.

Planting number	Cultivar	TADD				
		0	1	2	4	6
1	PAN8706W	0.00 ^O	16.90 ^{HIJK}	35.13 ^{vwX}	46.08 ^{mnop}	73.58 ^b
	PANN8816	0.00 ^O	23.10 ^{DEFG}	29.00 ^{yzAB}	36.30 ^{uvw}	47.00 ^{klmnop}
	NS5511	0.00 ^O	10.29 ^{LMN}	24.74 ^{ABCDE}	38.18 ^{stuv}	55.92 ^{efgh}
2	PAN8706W	0.00 ^O	18.30 ^{GHI}	37.50 ^{uv}	55.40 ^{efghi}	74.40 ^b
	PANN8816	0.00 ^O	12.40 ^{JKLM}	28.50 ^{yzABC}	39.20 ^{rstuv}	51.30 ^{hijkl}
	NS5511	0.00 ^O	12.12 ^{KLM}	32.00 ^{wxy}	66.77 ^c	84.16 ^a
3	PAN8706W	0.00 ^O	19.40 ^{FGH}	43.20 ^{pqrs}	60.30 ^{de}	77.60 ^b
	PANN8816	0.00 ^O	11.10 ^{LM}	24.40 ^{ABCDEFG}	45.21 ^{opq}	56.88 ^{efg}
	NS5511	0.00 ^O	17.23 ^{HIJ}	25.79 ^{zABCD}	53.55 ^{ghij}	65.10 ^{cd}
4	PAN8706W	0.00 ^O	14.00 ^{IJKL}	24.90 ^{ABCDE}	39.20 ^{rstuv}	57.10 ^{ef}
	PANN8816	0.00 ^O	9.04 ^{LMN}	21.39 ^{DEFGH}	38.05 ^{tuv}	51.11 ^{hijklm}
	NS5511	0.00 ^O	11.23 ^{LM}	18.30 ^{GHI}	34.75 ^{vwX}	46.67 ^{lmnop}
5	PAN8706W	0.00 ^O	11.80 ^{LM}	23.00 ^{DEFG}	44.80 ^{opq}	57.50 ^{ef}
	PANN8816	0.00 ^O	5.88 ^N	29.45 ^{yzA}	40.32 ^{qrstu}	57.23 ^{ef}
	NS5511	0.00 ^O	7.86 ^{MN}	18.21 ^{GHI}	35.28 ^{uvwX}	53.03 ^{ghij}
6	PAN8706W	0.00 ^O	8.90 ^{MN}	30.40 ^{xyz}	50.70 ^{ijklmn}	63.30 ^{cd}
	PANN8816	0.00 ^O	11.77 ^{LM}	24.30 ^{BCDEF}	47.70 ^{klmnop}	57.70 ^{ef}
	NS5511	0.00 ^O	10.23 ^{LMN}	20.97 ^{DEFGH}	45.88 ^{nop}	51.92 ^{ghijk}
7	PAN8706W	0.00 ^O	8.50 ^{MN}	23.90 ^{CDEF}	36.40 ^{uvw}	51.50 ^{hijkl}
	PANN8816	0.00 ^O	11.67 ^{LM}	20.00 ^{EFGH}	43.00 ^{pqrst}	43.88 ^{pqr}
	NS5511	0.00 ^O	8.61 ^{MN}	18.09 ^{GHI}	35.23 ^{uvwX}	49.46 ^{ijklmno}

Treatments with the same letter are not significantly different according to Fisher's Protected LSD ($P < 0.001$). Cultivar X Planting number X TADD LSD (5.09).

Planting number: 1 = 1 Nov 2016, 2 = 23 Nov 2016, 3 = 12 Dec 2016, 4 = 28 Dec 2016, 5 = 5 Jan 2017, 6 = 5 Dec 2017 and 7 = 1 Nov 2018

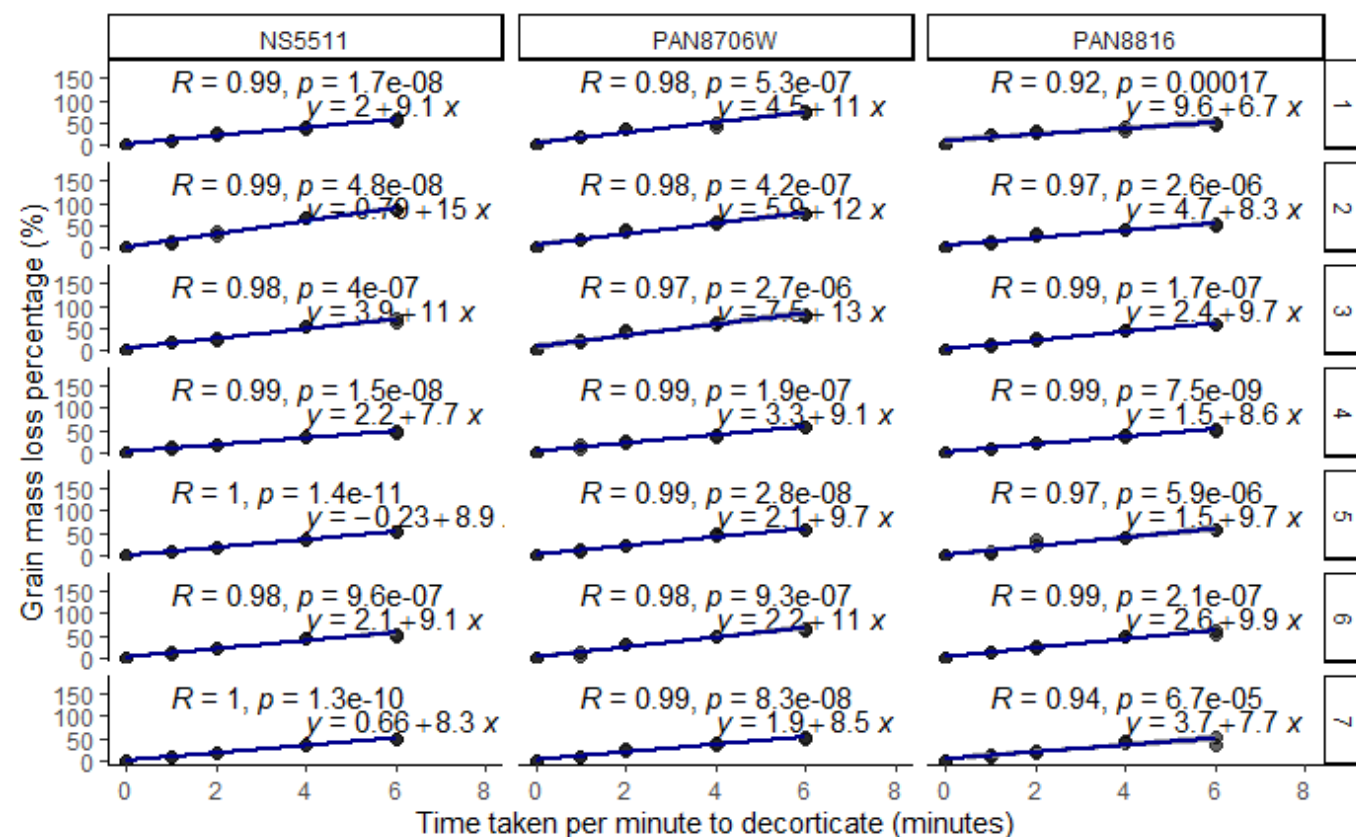


Figure S1: The relationship between grain mass loss percentage obtained at different *planting numbers, cultivars and minutes taken to decorticate each cultivar.

*1 = 1 November 2016, 2 = 23 Nov 2016, 3 = 12 Dec 2016, 4 = 28 Dec 2016, 5 = 5 Jan 2017, 6 = 5 Dec 2017 and 7 = 1 Nov 20

Chapter 4

Supplementary Table S8. Meteorological summary statistics for Alma (Limpopo) and Cedara (KwaZulu-Natal) for the duration of the experimental period.

Variable	Alma				Cedara			
	Mean	SD	Min	Max	Mean	SD	Min	Max
TMIN (°C)	13.14	4.82	-1.89	22.89	11.37	4.12	0.23	19.68
TMAX (°C)	27.88	4.55	11.44	38.46	23.12	4.61	6.93	35.77
RH (%)	49.17	14.94	10.86	91.72	72.63	13.55	22.95	97.46
Rainfall (mm)	1.48	3.33	0	55.78	3.91	7.35	0	130.66

SD = Standard deviation, Min = minimum, Max = Maximum, TMIN = minimum temperature, TMAX= maximum temperature.