RELATIONSHIP BETWEEN SORGHUM PLANT AND GRAIN CHARACTERISTICS, COLONISATION BY MYCOTOXIGENIC *FUSARIUM* SPP. AND MYCOTOXIN LEVELS

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Submitted in fulfilment of the requirements for the degree

Philosophiae Doctor

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JANUARY 2019

Contents

DECLARATION I

ACKNOWLEDGEMENTS II

DEDICATION III

PREFACE IV

CHAPTER 1 1

A REVIEW OF GRAIN MOLD OF SORGHUM 1

1.1. INTRODUCTION 1

1.2. SORGHUM GRAIN MORPHOLOGY 4

1.3. SORGHUM GRAIN MOLD ETIOLOGY 5

1.3.1. Grain mold causal organisms 6

1.3.2. Infection pathway and symptoms associated with grain mold 7

1.4. MYCOTOXIN PRODUCTION 9

1.4.1. Fumonisins 10

1.4.2. Trichothecenes 12

1.4.3. Zearalenone 14

1.4.4. Moniliformin 14

1.5. ECONOMIC IMPORTANCE 15

1.6. FACTORS INFLUENCING SORGHUM GRAIN MOLD AND THEIR ROLE IN DISEASE MANAGEMENT 16

1.6.1. Weather 16

1.6.2. Chemical environment 18

1.6.2.1. Pesticides 18

1.6.2.2. Fungicides 18

1.6.3. Biotic environment 20

1.6.3.1. Biological control of sorghum grain mold 20

1.6.4. Cultural practices 21

1.6.4.1. Avoidance 21

1.6.4.2. Rotation/tillage 22

1.6.4.3. Harvesting 23

1.6.4.4. Dehulling (Pearling) 23

1.6.4.5. Post-harvest storage 24

1.7. GRAIN MOLD RESISTANCE IN SORGHUM 24

1.7.1. Physical properties 25

1.7.1.1. Panicle compactness 25

1.7.1.2. Grain hardness 25

1.7.1.3. Glumes 26

1.7.1.4. Waxy layer 27

1.7.1.5. Dormancy 27

1.7.2. Chemical properties ... 27
1.7.2.1. Phenolic compounds ... 27
1.7.2.2. Antifungal proteins ... 29

1.8. CONCLUSION ... 30

1.9. REFERENCES ... 31

CHAPTER 2 ... 48

PLANT AND GRAIN CHARACTERISTICS THAT INFLUENCE GRAIN MOLD SEVERITY IN SORGHUM ... 48

2.1. Abstract ... 48

2.2. INTRODUCTION ... 49

2.3. MATERIALS AND METHODS ... 50
2.3.1. Field trials ... 50
2.3.2. Grain mold assessment ... 51
2.3.2.1. Grain visual ratings ... 51
2.3.2.2. Evaluation of ergosterol content to determine total fungal biomass in sorghum grain ... 51
2.3.2.3. qPCR to quantify FGSC in sorghum grain ... 52
2.3.2.3.1. DNA extraction ... 52
2.3.2.3.2. qPCR ... 53
2.3.3. Mycotoxin accumulation in sorghum grain associated with FGSC ... 54
2.3.4. Data analysis ... 55

2.4. RESULTS ... 55
2.4.1. Grain mold assessment ... 55
2.4.1.1. Grain visual ratings ... 55
2.4.1.2. Evaluation of ergosterol content to determine fungal biomass in sorghum grain ... 56
2.4.1.3. qPCR to quantify FGSC in sorghum grain lines ... 57
2.4.2. Mycotoxin accumulation in sorghum grain lines associated with FGSC ... 58

2.5. DISCUSSION ... 59

2.6. CONCLUSION ... 64

2.7. REFERENCES ... 64

CHAPTER 3 ... 83

SORGHUM GRAIN METABOLITES IN DEFENSE AGAINST SORGHUM GRAIN MOLD ... 83

3.1. Abstract ... 83

3.2. INTRODUCTION ... 84

3.3. MATERIALS AND METHODS ... 85
3.3.1. Total phenolic content of sorghum florets at anthesis and grain at harvest ... 85
3.3.2. HPLC analysis of phenolic compounds ... 86
3.3.3. Protein extraction ... 87
3.3.4. Total protein analysis in sorghum grain ... 87

3.3.5. Chitinase activity in sorghum grain 88
3.3.6. Glucanase activity in sorghum grain 88
3.3.7. Data analysis 88

3.4. RESULTS 89
3.4.1. Total phenolic content of sorghum florets during anthesis and grain at harvest 89
3.4.2. HPLC analysis of phenolic compounds 90
3.4.3. Total protein analysis in sorghum grain 91
3.4.4. Chitinase activity in sorghum grain 91
3.4.5. Glucanase activity in sorghum grain 91

3.5. DISCUSSION 92

3.6. CONCLUSION 95

3.7. REFERENCES 95

CHAPTER 4 126

THE EFFECT OF ROTATION SYSTEMS ON GRAIN MOLD AND ROOT ROT OF SORGHUM 126

4.1. Abstract 126

4.2. INTRODUCTION 127

4.3. MATERIALS AND METHODS 128
4.3.1. Field trials 128
4.3.2. Soil properties 129
4.3.2.1. Nutrient levels and pH 129
4.3.2.2. Microbial activity 129
4.3.3. Root rot assessments 130
4.3.3.1. Root visual rating and root mass 130
4.3.3.2. Ergosterol content as a measure of fungal biomass in roots 130
4.3.3.3. qPCR to quantify FGSC in roots 131
4.3.3.3.1. DNA extraction 131
4.3.3.3.2. qPCR 131
4.3.4. Mycotoxins associated with FGSC in roots 132
4.3.5. Grain mold assessments 133
4.3.5.1. Grain visual rating and grain yield 133
4.3.5.2. Evaluation of ergosterol content to determine fungal biomass in grain 133
4.3.5.3. qPCR to quantify FGSC in grain 133
4.3.5.3.1. DNA extraction 133
4.3.5.3.2. qPCR 133
4.3.6. Mycotoxins associated with FGSC in grain 133
4.3.7. Statistical analysis 134

4.4. RESULTS 134
4.4.1. Soil properties 134
4.4.1.1. Nutrient levels and pH 134
4.4.1.2. Microbial activity 135
4.4.2. Root rot assessment 135
4.4.2.1. Root visual ratings and root mass 135
4.4.2.2. Evaluation of ergosterol content to determine fungal biomass in roots 136
4.4.2.3. qPCR to quantify FGSC in roots 136
4.4.3. Mycotoxin accumulation in roots associated with FGSC 136

4.4.4. Grain mold assessment 137
4.4.4.1. Grain visual rating and grain yield 137
4.4.4.2. Evaluation of ergosterol content to determine fungal biomass in grain 137
4.4.4.3. qPCR to quantify FGSC in grain 138
4.4.5. Mycotoxin accumulation in grain associated with FGSC 138
4.4.6. Relationships between variables 139

4.5. DISCUSSION 140

4.6. CONCLUSION 144

CHAPTER 5 167

SORGHUM FUNGICIDE SPRAY REGIME EFFECTS ON SORGHUM GRAIN MOLD AND ROOT ROTS 167

5.1. Abstract 167

5.2. INTRODUCTION 168

5.3. MATERIALS AND METHODS 169
5.3.1. Field trials 169
5.3.2. Evaluation of ergosterol content 170
5.3.3. qPCR to quantify Fusarium graminearum species complex 171
5.3.3.1. DNA extraction 171
5.3.3.2. qPCR 172
5.3.4. Mycotoxin quantification 172
5.3.5. Data analysis 173

5.4. RESULTS 173
5.4.1. Field trials 173
5.4.1.1. Root visual ratings 173
5.4.1.2. Grain visual ratings 174
5.4.1.3. Root mass per plant 175
5.4.1.4. Effective root mass 175
5.4.1.5. Grain yield 176
5.4.1.6. Weather variables 176
5.4.2. Evaluation of ergosterol content to determine fungal biomass in grain and roots 176
5.4.2.1. Ergosterol levels in roots 176
5.4.2.2. Ergosterol levels in grain 177
5.4.3. qPCR to quantify Fusarium graminearum species complex in sorghum grain 177
5.4.4. Mycotoxin accumulation in grain associated with F. graminearum 178

5.5. DISCUSSION 179

5.6. CONCLUSION 183

5.7. REFERENCES 183

SUMMARY 213

DECLARATION

I hereby declare that this thesis submitted by me for the degree of *Philosophiae Doctor* in Plant Pathology at the University of the Free State is entirely my own work and has not previously been submitted by me at other higher education institutions. I furthermore cede all copyright of this thesis to the University of the Free State.

30 January 2019

DANELLE VAN ROOYEN **DATE**

ACKNOWLEDGEMENTS

I would like to thank the following persons, who contributed greatly to the success of this project:

My promoter, Prof. N.W. McLaren, thank you Prof for your valuable insights and for being the McLaren in Team McLaren! It was such a privilege to work with you! Thank you for being such a good sport and an encourager through this journey!

Thank you to my co-promoter, Prof. G. J. Marais for support and enthusiasm, to Prof. B. Visser, Dr. L. Mohase in the Department of Plant Sciences and Dr. G. Kemp from the Microbiology, Biochemical and Food-Biotechnology Department for technical assistance. A big thank you to L. A. Coetzee-Rothmann for support and assistance throughout this project and M. Bester for encouragement along the way.

Thank you to the National Research Foundation for financial assistance and The Sorghum Trust and the N.E. Borlaug Institute for funding this project.

Thank you to Dr. A. Schoeman, Dr. M. Craven, Dr. B. Janse van Rensburg, Ms. G. Khali, Mrs. V. Knight-Coetzee and Mr. J. Bow for technical assistance and thank you to the Agricultural Research Council – Grain Crops Institute in Potchefstroom and Cedara, Texas AgriLife Research and Pannar Seed in Greytown for providing research facilities.

To all my friends, for love, support and motivation, for being there when I needed you most - how blessed I am to have you!

To my husband, for not complaining when he could have! And my brother, mother and father – I love you!

And finally, special thanks to my Heavenly Father, who still never failed to answer a prayer when I was at my weakest.

DEDICATION

I dedicate this thesis to all the strong, courageous women out there, in particularly my grandmother, J. M. W. Van Rooyen (1922 - 2017) who taught me to appreciate the finer things in life and whose influence contributed towards making me the woman I am today.

PREFACE

This thesis consists of five chapters, including a literature review. The main focus of this study was to evaluate plant and grain characteristics of sorghum lines that contribute to grain mold resistance. Cultural control measures, obtained through rotation systems and fungicide spray regimes, were also examined.

The first chapter is a literature review of grain mold of sorghum, starting with a general introduction on sorghum, including production, history, environmental requirements and genes that influence specific traits. This is followed by a review of causal organisms and related mycotoxins, as well as management of grain mold using abiotic and biotic factors and specific reference to plant and grain characteristics that influence the severity thereof.

In Chapter 2 the relationship between grain and plant characteristics in 22 sorghum lines and genotype response to grain mold was determined. Sorghum lines were planted at Cedara and Alma in eight environments over a 5 year period. Visual grain ratings and ergosterol analyses were used to quantify resistance of lines to grain mold pathogens and colonisation by fungi, respectively. Colonisation of grain by members of the *F. graminearum* species complex was quantified using qPCR and mycotoxin concentrations using LC-MS-MS. AMMI analyses were used to examine the response of genotype to the respective evaluation criteria and stability over environments.

In Chapter 3 resistance traits from the 22 sorghum lines used in Chapter 2 were examined, with specific attention to phenolic compounds and antifungal proteins. Florets collected during anthesis and threshed grain were used to determine total phenolic compounds. Subsequently phenolic compounds were separated on a HPLC at five different wavelengths. Total protein concentration, followed by glucanase and chitinase activity were also assessed.

In Chapter 4 the effects of legume rotation systems on soil properties and the health of sorghum plants, with respect to root rot and grain mold over a two year period were assessed. Rotation systems included fallow, monoculture sorghum and legumes comprising of soybean, drybean

and cowpea. Soil nutrient status, pH and soil biodiversity were determined, followed by examination of roots and grain using visual ratings, root and grain mass, ergosterol analyses using HPLC, qPCR to quantify *F. graminearum* species complex pathogens and mycotoxin concentrations using LC-MS-MS. Regression analysis was used to determine relationships between disease parameters and nutrient levels.

In Chapter 5 the efficacy of two fungicides used prophylactically to control leaf blight of sorghum, in the control of grain mold and reduced mycotoxin accumulation, was determined. Four cultivars were planted at three localities over a two year period. Roots and grain were examined to establish general plant health. This included disease assessment through visual ratings, root mass, yield and ergosterol analyses. Furthermore, the grain was assessed to quantify *F. graminearum* species complex pathogens and subsequent mycotoxins.

CHAPTER 1

A REVIEW OF GRAIN MOLD OF SORGHUM

1.1. INTRODUCTION

One of the many difficulties facing mankind in an ever changing environment, is the growing demand for a sustainable food source. Bearing in mind the current population growth rate, the amount of food being produced will have to increase with 60% by 2050 if we are to have a sufficient food supply (Alexandratos and Bruinsma, 2012). This is particularly important in semi-arid-tropics such as the African savanna, Sahel and parts of Australia, Asia and the Americas, where billions of people inhabit approximately 20 million square kilometers (De Wet, 1986). Some of the most important cereals such as rice, wheat and maize, are not adapted to survive in these harsh, unpredictable environments where inadequate rainfall and nutrient poor soils are the norm. In these areas the principal cereals that are best adapted to these conditions include pearl millet, foxtail millet and sorghum (De Wet, 1986).

Sorghum (*Sorghum bicolor* (L.) Moench) production in 2015 worldwide was 60.27 million tons (Anonymous, 2016a). South Africa contributed a mere 88 500 tons from a production area of 48 500 hectares, mostly covering areas of the Free State, Mpumalanga and Limpopo Provinces (Anonymous, 2016b). The many uses of sorghum differ throughout the world and in Asia and Africa, sorghum is mainly grown as grain for human consumption as either pure flour for bread and porridge or fermented for alcohol production. The dried leaves and stalks are used for animal feed, fuel production or other domestic uses (Tarr, 1962).

Sorghum belonging to the grass family Gramineae, is a resilient plant capable of surviving and thriving in warmer areas normally associated with variable climatic and environmental conditions (Du Plessis, 2008). As a result it is considered one of the first grasses to be cultivated in the Mediterranean region by the earliest civilizations. On the banks of the River Tigris, situated in the ancient capital of Assyria near Nineveh, a carving representing a field of sorghum was found in the palace of King Sennacherib dating back to around 704 – 681 B.C. (Tarr, 1962). Linnaeus first described sorghum in 1753 under the name of *Holcus,* but the genus was separated later by Moench into *Sorghum* and *Holcus* (Clayton, 1961, cited by Dahlberg, 2000).

The survival of sorghum in hot areas is mainly due to the ability of the plant to remain dormant without killing the developing flowers and to resume growth when conditions become favourable. High resistance towards desiccation is acquired through the waxy layer covering the impervious epidermis, low transpiration ratio by in-rolled leaves and an extensive root system (Tarr, 1962).

Environmental requirements for optimal sorghum growth include (Kimber, 2000; Du Plessis, 2008):

- ❖ Day length: This is the most important environmental factor influencing maturity in sorghum (Rooney, 2000). Many traditional, photo-period sensitive varieties require at least a 12 hour day length to initiate floral development. If longer photoperiods continue, vegetative growth is continued.
- ❖ Rainfall: Sorghum is versatile and can grow in both rainy weather and semi-arid regions, where rainfall of 400 mm - 800 mm will suffice. It usually flowers after rain events and in tropical areas completes its life cycle during the dry season.
- ❖ Altitude: Sorghum can grow up to 3000 m above sea level.
- ❖ Temperature: Frost and a temperature at freezing point kill sorghum plants. Sorghum seeds germinate between 10°C and 35°C. The optimum temperature for growth and development of sorghum ranges between 27°C and 30°C. Very high temperatures decrease yield by delaying flower initiation.
- ❖ Soil type: Sorghum can tolerate different soil types and is able to grow in both clay and sandy soils, although performance is poorer in sandy soils. A 10 to 30% clay content is optimal. Sorghum is more tolerant of alkaline salts than many other crops and a pH varying from 5 to 8.5 is sufficient for cultivation.

Numerous genes have been reported to determine specific traits in sorghum since the first report of genetic inheritance. These traits include the following (Doggett, 1970; Rooney, 2000):

- ❖ Maturity: Genes influence maturity of sorghum and this is mostly done by regulating the number of leaves and hence number of nodes produced before flowering. Four loci have been identified with the primary locus Ma_1 influencing photoperiod response.

- Plant height: Plant height is determined by four major genes Dw_1, Dw_2, Dw_3, Dw_4 that affect internode length and tall is partially dominant to short. Recessive *dw* genes have also been linked to yield losses. Several other independent genes also contribute to plant height by controlling node number, panicle- and peduncle length.
- Sterility: Multiple genes determine sterility by either ensuring the absence or termination of pollen production. Female sterility (Fs_1, Fs_2) is caused by the development of a simple stigma, style and ovary. Male sterility ($ms_1 – ms_3$) is caused by the development of anthers without functional pollen. A dominant restoration gene Rf_1 can restore pollen fertility. Lines with cytoplasmic sterility conditioned through msc_1 and msc_2 loci (referred to as A-lines) are used in commercial hybrid seed production as female lines and maintained by isogenic B-lines. Restorer lines (R-lines) are used as male counterpart (Ali *et al.,* 2014).
- Seedling phenotype: Genes responsible for seedling phenotypes restrict the production of chlorophyll resulting in albino (*wp*, $w_1 – w_{31}$), yellowish ($ys_1 – ys_{10}$), or light green (*pgp*, $pg_1 – pg_8$) seedlings with a low survival rate or postponed maturity.
- Leaves: The colour, presence of hairs, shape, midribs and angles are all determined by approximately 45 gene loci. Dark green leaves are dominant to lighter shades of green.
- Plant Colour: Tan (*pq* or *pQ*), red purple (*PQ*) and black purple (*Pq*) pigmentation of the stems are primarily the result of two genes *P* and *Q* and their effects are mostly visible when injury occurs. At the *Q* locus, the presence of a third recessive allele q^r causes typical red plants (Pq^r).
- Panicle: The shape of the panicle is affected by various genes. In short, a loose panicle (Pa_1) is dominant to a compact panicle.
- Glume: Glume colour is influenced by plant colour and the plant pigmentation genes *PQ* indirectly affect glume colour. Purple and red plants usually tend to have darker coloured glumes.
- Sorghum grain: The colour of the grain is usually affected by genes that determine the colour and thickness of the pericarp, absence or presence of a testa, and the structure and colour of the endosperm. Pericarp colour is determined by a combination of *R* and *Y* genes. *R_yy or rryy* results in a white or colourless, *rrY_* in lemon yellow or *R_Y_* a red pericarp colour (Waniska and Rooney, 2000). When only the pericarp is coloured, the grain is generally yellow or red. If both the pericarp and testa are coloured, the grain has a more brownish colour (Du Plessis, 2008). Pericarp thickness differs in mature caryposis as a result of the *Z* gene and this is related to testa colour. A thick

pericarp has homozygous recessive *zz* (resulting in a thick mesocarp) with starch granules giving it a chalky appearance. This conceals the colours of the testa and endosperm. A pigmented testa resulting from both dominant B_1_ and B_2_ genes, contains tannins, especially proanthocyanidins (Waniska and Rooney, 2000).

- Phenolic compounds: Phenolic compounds affect the colour of the plants and grain, and their concentrations are influenced by the absence or presence of a pigmented testa and the thickness of the pericarp. Therefore the genes responsible for these traits, also control the expression of phenolic compounds (Dykes and Rooney, 2006).

Environmental effects can determine the expression of these genes referred to above. Temperature, for example, can influence maturity by altering the behaviour of the recessive ma_4 locus, where flowering is initiated 20 days later than usual, which is similar to the behaviour of the dominant *Ma4* genotype. On the other hand, while natural growing conditions affect plant height, the general response of height genes remain constant over different environments. Plant height is affected by other genetic factors which also influence other phenotypic characters, including leaf number and size, peduncle length and panicle size (Rooney, 2000). The rate of leaf production in different varieties is almost similar under the same environmental conditions (Doggett, 1970).

Sorghum as we know it today, differs only slightly from those first cultivated and its earlier wild ancestors on a genetic level. It is clear that for sorghum to have survived through the ages, it had to have some form of avoidance, resistance or tolerance towards plant pathogens and in particular grain mold pathogens (Frederiksen, 2000). This literature study will examine grain mold of sorghum, including causal organisms and mycotoxins associated with these pathogens. Biotic and abiotic factors influencing the severity of the disease are also discussed to provide a better understanding of the management of grain mold, especially the role of plant and grain characteristics that enhance resistance.

1.2. SORGHUM GRAIN MORPHOLOGY

Sorghum grain consists primarily of three components (Waniska and Rooney, 2000):

- Pericarp (Outer layer)
- Endosperm (Storage tissue)
- Germ (Embryo)

The pericarp can be divided into three parts: epicarp, mesocarp and endocarp (Rooney and Murty, 1982). The outer epicarp consists of two to three layers with long, rectangular cells containing wax or sometimes pigments. A thick mesocarp usually contains small starch granules and gives the grain a chalky appearance. A pearly grain has a thin mesocarp without starch granules. The inner endocarp contains cross and tube cells which contribute to the movement of moisture (Rooney and Murty, 1982).

The testa is situated just beneath the pericarp, but is not present in all grains. The testa contains a bad tasting tannin-like substance which deters the eating thereof. In the absence of a testa, bird damage is considerably higher (Du Plessis, 2008).

Just beneath the testa is the endosperm. The endosperm ensures that seedlings have sufficient nutrients during germination until they are able to absorb their own nutrients (Du Plessis, 2008). The endosperm consists of an aleurone layer, peripheral endosperm, corneous endosperm and floury endosperm. The block-like aleurone layer has spherical bodies containing protein, minerals, vitamins, enzymes, phytin and high levels of oil. The peripheral endosperm consists of small square cells with starch granules fixed in a protein rich matrix of prolamins (alcohol soluble) and glutelins (alkali soluble) (Rooney and Murty, 1982).

The fraction of the corneous endosperm to the floury endosperm influences kernel texture. Kernels vary from being corneous to floury and this usually depends on genotype and environment. The kernel texture is important for sorghum quality when considering milling for food production (Rooney and Murty, 1982).

1.3. SORGHUM GRAIN MOLD ETIOLOGY

Pathogens can cause great financial losses in crops worldwide and grain mold is rated as one of the most economically important diseases on sorghum, influencing production, marketing and utilization (Das *et al.,* 2012). “Grain mold” refers to the deteriorating condition of sorghum grain as a result of fungal spoilage and is a major concern when wet weather conditions continue from flowering to grain maturity (Bandyopadhyay *et al.,* 2000).

Grain mold has been considered as either, resulting from infection and colonisation of grains by saprophytic or parasitic fungi during anthesis and harvesting stages, or as a result of infection and colonisation of florets by a few fungi before grain maturity. Colonisation of grain after maturity tends to be superficial and is considered to be general grain deterioration, usually restricted to the outer pericarp that only influences appearance and thus, affects financial gain (Bandyopadhyay *et al.,* 2000).

1.3.1. Grain mold causal organisms

Numerous fungal species have been associated with deterioration of sorghum grain. These include *Fusarium* spp., *Bipolaris* spp. (*B. australiensis*), *Alternaria* spp. (*A. alternata*), *Colletotrichum* spp. (*C. graminicola*), *Phoma* spp. (*P. sorghina*), *Curvularia* spp. (*C. lunata* and *C. clavata*), *Cladosporium* spp., *Exserohilum* spp. (*E. turcicum*), *Aspergillus* spp., *Penicillium* spp. and *Drechslera sorghicola* (Thakur *et al*., 2006; Tarekegn *et al.,* 2006) with *Fusarium thapsinum* and *Curvularia lunata* being the most prevalent (Frederiksen, 2000). Other *Fusarium* spp. associated with grain mold include *F. verticillioides*, *F. pallidoroseum, F. nygamai* and *F. pseudonygamai* (Thakur *et al*., 2006), as well as *F. graminearum sensu lato*, *F. andiyazi, F. proliferatum, F. equiseti, F. sacchari, F. thapsinum* (Sharma *et al.,* 2010; Bodoci *et al.,* 2013). The frequency of these pathogens depends on environmental conditions, therefore varying across and within localities and growing seasons (Thakur *et al*., 2006). Some saprophytes can also colonize the mature grain and consume it under warm, humid conditions (Frederiksen, 2000).

Germination, an indication of seed viability and quality, can be severely affected by grain mold pathogens (Das *et al*., 2012). Deteriorated seed has reduced germination (Rana *et al.,* 1977). Seed with a higher germination rate is considered more resistant to colonisation by grain pathogens than seed with a lower germination rate and this has been correlated with higher seed density (Rao *et al.,* 2013).

Fusarium spp. were discovered more than 200 years ago by Link (Glenn, 2007) and have been shown to be the most damaging and economically important fungi in the sorghum grain mold complex (Das *et al*., 2012). *Fusarium* spp. are such a diverse group of pathogens with molecular, morphological and metabolic differences that in-depth knowledge is needed to ensure healthy, quality and quantity grain through appropriate control measures (Glenn, 2007).

Fusarium spp. have been closely associated with sorghum, commencing from seedlings throughharvesting and storage of the grain (Das *et al*., 2012). *Gibberella zeae* (*F. graminearum sensu lato*) can infect sorghum seedlings at an early stage and progressively continue to infect other tissues as an endophyte. This early colonisation gives *Gibberella zeae* an edge over other saprophytic *Fusarium* spp. invaders (Quazi *et al.,* 2010). In a study conducted by Bodoci *et al.* (2013), *F. subglutinans, F. solani, F. verticillioides* and *F. graminearum sensu stricto* were tested for pathogenicity and applied as a spray inoculum to sorghum and broomcorn during flowering in a field trial. *F. graminearum sensu stricto* had the highest pathogenicity on sorghum grain, followed by *F. solani, F. verticillioides* and lastly *F. subglutinans.*

Gibberella zeae/*F. graminearum sensu lato* was redefined by O'Donnell *et al.* (2000; 2004) based on molecular phylogenetic analyses of 11 nuclear genes. Nine distinct species were initially identified. The first lineage was *F. austroamericanum* followed by *F. meridionale* (lineage 2), *F. boothii* (lineage 3), *F. mesoamericanum* (lineage 4), *F. acaciae-mearnsii* (lineage 5), *F. asiaticum* (lineage 6), *F. graminearum sensu stricto* (lineage 7), *F. cortaderiae* (lineage 8) and *F. brasilicum* (lineage 9) (Glenn, 2007). Today 16 species are known (Van der Lee *et al.,* 2015) including *F. gerlachii*, *F. vorosii, F. aethiopicum, F. ussurianum*, *F. nepalense* and *F. louisianense* (O'Donnell *et al.,* 2008; Yli-Mattila *et al.,* 2009; Sarver *et al.,* 2011). Such diversity among *Fusarium* spp. will result in new species belonging to the *Fusarium graminearum* species complex, being continually identified (Glenn, 2007). *F. meridionale, F. acaciae-mearnsii* and *F. cortaderiae* have been associated with sorghum grain in South Africa (Mavhunga, 2013).

1.3.2. Infection pathway and symptoms associated with grain mold

Infection can occur as either initial infection on the apical floral tissues or infection of the developing grain. The former is revealed by early signs of discolouring of the lemma, glumes, palea and lodicules, which are usually cultivar dependent (Bandyopadhyay *et al.,* 2000). The symptoms can vary depending on the grain development stage, the pathogen and the severity of the infection.

After entering apical floral tissues, mycelial growth of the grain mold pathogen continues towards the base of the flower or into the surrounding spaces (Bandyopadhyay *et al.,* 2000)

and, depending on severity, to the anthers and filaments. Mycelial growth can be observed within five days of infection, with most of the growth focused around lodicules, which seem to be a vital energy source. Subsequently the infection spreads towards the aleurone layer of the endosperm and finally the pericarp (Das *et al.,* 2012). Under favourable environmental conditions, fungal growth pushes through the pericarp exposing the grain to a fungal mass that can cover the grain entirely (Das *et al.,* 2012). Early infection during anthesis is mainly responsible for low grain quality, including grain filling, endosperm texture and kernel production (Little and Magill, 2003) and results in blasted florets, poor seed set and tiny shrunken grains (Thakur *et al*., 2006).

Infection of the developing grain starts at the base close to the pedicel and superficial growth of the fungus can be seen at the hilar end of the grain extending along the pericarp surface. The spread of fungal growth to grain surfaces is subsequently dependent on the characteristics of the glumes. Large papery glumes that cover grain prevent pathogen entry (Williams and Rao, 1980) and when combined with higher PR-10 protein content, pathogen growth is restricted in resistant plants (Katile *et al.,* 2010). Where grain is not covered by glumes, infection is dependent on favourable weather conditions (Bandyopadhyay *et al.,* 2000). The fungal mycelium penetrates the pericarp and continues through the cross and tube cells of the endocarp to colonize the grain tissue within five to ten days (Thakur *et al*., 2006).

Colonisation of mature grain usually occurs due to a vast range of field fungi that infect exposed, non-living tissues (Das *et al.,* 2012) and infection may be restricted to that specific point of entrance (Navi *et al*., 2005). Infection of mature grain is mostly caused by weather-dependent saprophytes (Govardhan and Das, 2015). A reduction in kernel size is inevitable (Bandyopadhyay *et al.,* 2000). Highly infected grain may disintegrate (Thakur *et al*., 2006).

Grain with a high level of infection can be completely covered with mould, while grain partially infected can appear normal with few discolourations on the surface. Discolourations are more visible on white grain than on brown or red coloured grains (Thakur *et al*., 2006). Slightly infected grain can appear symptomless (Thakur *et al*., 2006). Infected grain can also appear symptomless when environmental conditions change suddenly after flowering, permitting the fungal pathogen to reside within the grain without causing visible symptoms on the pericarp (Navi *et al*., 2005).

The fungi associated with grain mold give grain a specific colour and texture. *C. lunata* can be seen as shiny, fluffy, velvety black growth on the grain surface (Thakur *et al*., 2006). Castor and Frederiksen (1977, cited by Das *et al.,* 2012) reported that infection by *C. lunata* affects translocation of carbohydrates to immature grains, giving rise to small, lightweight seeds. *Fusarium* spp. produce a pinkish-white and fluffy mycelium. Pin-like small, round black pycnidia embedded in the grain with a thick dirty black crust, can be observed with infection by *P. sorghina* (Thakur *et al*., 2006). *A. alternata* gives the grain a dull appearance with a grey-black mycelium. *B. australiensis* produces a black mycelium and *C. graminicola* produces acervuli that are black and studded with clusters of setae, creating concentric rings (Thakur *et al*., 2006).

1.4. MYCOTOXIN PRODUCTION

Severely infected sorghum grain is not suitable for human and animal consumption (Navi *et al*., 2005). Grain mold fungi can produce secondary metabolites that are dangerous to animal and human health when ingested. These secondary metabolites, also referred to as "mycotoxins", a term which literally means "poison from a fungus" (Reddy *et al.,* 2010b) are associated with growth defects, cellular toxicity and cancer in humans and animals (Glenn, 2007).

Many of these secondary metabolites are also produced to assist fungi in surviving in a hostile environment and to improve pathogen fitness (Fox and Howlett, 2008). Secondary metabolites produced by the soil fungus *Aspergillus nidulans* deter feeding by the fungivorous springtail *Folsomia candida* (Rohlfs *et al.,* 2007). Zearalenone, produced by *F. graminearum sensu lato* and *F. culmorum*, can also boosts sporulation of the fungus by inciting perithecia development (Wolf and Mirocha, 1977). Zearalenone and its derivative, α-zearalenone, can inhibit the growth of other filamentous fungi, including *Sordaria fimicola, Epicoccum purpurascens, Cladosporium herbarum* and *Alternaria alternata*, thereby reducing competition for a specific host so as to benefit its producer (Utermark and Karlovsky, 2007). Fungi fortunate enough to counteract the production of zearalenone, such as *Gliocladium roseum* that produces lactonase, are found to co-inhibit the host with the zearalenone producer, as observed with *G. roseum* and *F. culmorum* in wheat (Utermark and Karlovsky, 2007). Other mycotoxins are specifically produced to assist with disease development by interfering with cellular processes in hosts (Brosch *et al.,* 1995).

Over 400 mycotoxins have been recorded (Reddy *et al.,* 2010b) since their discovery in the 1960's (Summerell and Leslie, 2011). The mycotoxins with the greatest economic impact and effect on human and animal well-being, are those produced by *Fusarium* spp., *Penicillium* spp. and *Aspergillus* spp. (Reddy *et al.,* 2010b). Each of these fungal species has a specific climatic requirement necessary for the production of mycotoxins. Aflatoxin is ideally produced in hot and dry environments, in contrast with fumonisins that are produced in hot, wet environments. The production of zearalenone and trichothecenes occur mostly in cold, damp environments (Council for Agricultural Science and Technology, 2003; Reddy *et al.,* 2010b), except nivalenol, which is produced in hot and dry conditions (Yazar and Omurtag, 2008).

Mycotoxins produced by *Fusarium* spp. include fumonisins, moniliformin, zearalenone, fusaric acid, fusarins, fusaproliferin, gibberellic acids, beauvericin and the trichothecenes, specifically diacetoxyscirpenol, deoxynivalenol, nivalenol, T-2 toxin and HT-2 toxin (Schothorst and Van Egmond, 2004; Thakur *et al*., 2006; Glenn, 2007) and these are mostly associated with mouldy grain and diseased plants (Kumar *et al.,* 2008). The presence of mycotoxins are therefore, a global agricultural concern that limits grain quality and production (Reddy *et al*., 2010a). The most important mycotoxins produced by *Fusarium* spp. and associated with health issues are fumonisins, trichothecenes, zearalenone and moniliformin (D'Mello and Macdonald, 1997) and are therefore discussed below.

1.4.1. Fumonisins

The chemical structure of fumonisins was first described by Bezuidenhout *et al.* (1988) and consists of long-chain amino polyalcohols (Desjardins and Proctor, 2007). Fifteen fumonisin-related compounds have been identified with fumonisin B being the most abundant group (Kumar *et al.,* 2008). Fumonisin-producing fungi include *Aspergillus niger* and a few *Fusarium* spp. (Yazar and Omurtag, 2008) such as *F. verticillioides* (previously *F. moniliforme*), *F. proliferatum* (Rheeder *et al.,* 2002), *F. nygamai* (Glenn, 2007) and *F. anthophilum* (Yazar and Omurtag, 2008). Temperature has no effect on the levels of fumonisin B_1 present when cultures of *F. verticillioides* are boiled for 30 minutes and dried for 24 hours at 60°C indicating stability of fumonisin B1 to heat variations (Alberts *et al.,* 1990).

Fumonisin B_1 intake by humans and animals has been associated with initial generation of cancer tumors and progression thereof in much the same way as any carcinogen. It has also been associated with esophageal cancer in humans (Rheeder *et al.,* 1992; Bandyopadhyay *et al.,* 2000) and birth defects (Desjardins and Proctor, 2007). In a study conducted over six seasons from 1976 to 1989, higher oesophageal cancer rates in humans were correlated with higher fumonisin B_1 and B_2 levels produced by *F. verticillioides* in maize in the Eastern Cape Province of South Africa (Rheeder *et al.,* 1992). Esophageal lesions combined with wart-like growths in the lower part of the esophagus were observed in five week old piglets exposed to 100 mg/kg fumonisin B_1 for ten days and 190 mg/kg thereafter for 83 days (Casteel *et al.,* 1993). In depth studies indicated fumonisin B_1 affecting normal appearance of the nucleus, nucleolus and mitochondria in cells, as well as damaging the membranes, thus leading to oesophageal cancer (Myburg *et al.,* 2009).

After four weeks some of the piglets treated with fumonisin B_1 vomited blood or excreted blood in their feces and the carcinogenic effects observed were also associated with liver tumor formation which affected liver function (Casteel *et al.,* 1993). After rat hepatocytes (cell tissue of the liver) were exposed to fumonisin B_1, cell changes were observed that ultimately lead to cell death. This was explained by regenerative nodule formation, distorted liver, extensive fibrosis (Gelderblom *et al.,* 1991) and changes to cellular membrane phospholipids (Gelderblom *et al.,* 1996). Lesions in lungs and kidneys were also observed (Gelderblom *et al.,* 1991), as well as altered gene expression (Bhandari and Sharma, 2002).

Fumonisins also cause leukoencephalomalacia, a brain lesion fatal to horses after exposure to contaminated grain (Kellerman *et al.,* 1990). Forty-one farms containing 506 different breeds of horses of varying ages with a 17% fatality rate, were surveyed for toxicity. Feed samples were found to be contaminated with *F. moniliforme* (*F. verticillioides*) and further tests on ducklings were conducted to indicate toxicity (Wilson *et al.,* 1990). Twenty-five to a hundred percent fatality rate was recorded when one day old pekin ducklings were fed on contaminated maize kernels mixed with commercial chicken feed (1:1) and this was related to fumonisin levels (Leslie *et al.,* 1996).

The health effects observed are explained by fumonisins bearing a resemblance to sphinganine responsible for sphingolipid metabolism and are therefore able to disrupt and alter the so-called cell lipid membranes, sphingolipids (Yazar and Omurtag, 2008). These allow electrical signals

to be conducted through the central nervous system (Soriano *et al.,* 2005). Currently the acceptable fumonisin B_1 and B_2 level established by the World Health Organization in maize, is determined at 1000 µg/kg (Reddy *et al.,* 2010b) and that of The Food and Drug Administration (2011) between 2000 µg/kg to 4000 µg/kg depending on the testing produce. In South Africa, legislation since 2016 established acceptable levels for fumonisin B_1 and B_2 in maize to be between 2000 µg/kg and 4000 µg/kg, depending on the usage (National Department of Health, 2016).

1.4.2. Trichothecenes

The trichothecenes consist of a large group of tricyclic sesquiterpenes produced by a number of fungal groups including *Fusarium* spp. (Desjardins and Proctor, 2007). These can be divided into four groups based on the absence or presence of characteristic functional groups (Yazar and Omurtag, 2008). Type A and B are considered more significant. Type A includes T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolaniol, while type B includes deoxynivalenol and its 3-acetyl and 15-acetyl derivatives, nivalenol, 4-acetyl nivalenol and fusarenon-X (Placinta *et al.,* 1999; Van der Lee *et al.,* 2015). Deoxynivalenol, diacetoxyscirpenol, nivalenol, HT-2 toxin and T-2 toxin are significant on cereals, but the occurrence of the latter is supposedly less frequent (Schothorst and Van Egmond, 2004; Kumar *et al.,* 2008). In a study conducted by the European Commission to establish trichothecene levels in cereal grain from twelve European Union countries between 2002 and 2003 (Schothorst and Van Egmond, 2004), deoxynivalenol was most abundant, occuring in 57% of samples, nivalenol in 16%, T-2 toxin in 20% and HT-2 toxin in 14% of samples. Maize was the most susceptible cereal to deoxynivalenol contamination compared to wheat barley, oats and rye.

Diversity within fungal species plays a role in the type of trichothecene that is produced. Type A for instance, is mainly produced by *F. sporotrichioides* and *F. poae*, whereas Type B is produced by *F. culmorum* and *F. graminearum sensu lato* (Placinta *et al.,* 1999). Deoxynivalenol (also referred to as vomitoxin) is associated with *F. graminearum sensu stricto* and *F. culmorum* causing *Fusarium* head blight in wheat (Creppy, 2002). Nivalenol is produced by *F. acacia-mearnsii*, *F. cortaderiae* and *F. meridionale* on sorghum (Mavhunga, 2013). The same *Fusarium* spp. can also produce different trichothecenes and occasionally even in combination with other mycotoxins (Perkowski *et al.,* 1997) e.g. deoxynivalenol and

zearalenone that are both produced by *F. graminearum* and *F. culmorum*, can be found co-existing in grain (Reddy *et al*., 2010b).

T-2 toxin ingestion caused weight loss, beak lesions and poor feathering in poultry and produced permanent digestive problems and extensive tissue and organ damage in livestock (Bandyopadhyay *et al.,* 2000). The ingestion of deoxynivalenol in food or feed produced nausea, vomiting and diarrhea in humans and farm animals, with weight loss and food refusal recorded in the latter (Rotter *et al.,* 1996). Nivalenol affects immune responses in mice (Choi *et al.,* 2000). Diacetoxyscirpenol can influence the human haematopoietic system resulting in nausea, vomiting, confusion, hypotension, diarrhea, chills and fever (Lautraite *et al.,* 1997). In plants, germination was inhibited after barley embryos were exposed to diacetoxyscirpenol, resulting in restricted coleoptile and root development (Schapira *et al.,* 1989).

It is believed that the effects of these mycotoxins are the result of obstructions in protein synthesis by preventing peptidyltransferase and by the binding of deoxynivalenol to ribosomes (Glenn, 2007), thus interfering with elongation termination (Rotter *et al.,* 1996) in DNA and RNA synthesis (Yazar and Omurtag, 2008). The same mode of action is associated with nivalenol and T-2 toxin, with the latter affecting other cellular processes (Yazar and Omurtag, 2008). Furthermore, nivalenol also inhibits interleukin-4 production that regulates antibody synthesis by the immune system (Choi *et al.,* 2000). Diacetoxyscirpenol inhibits protein synthesis at ribosomal level (D'Mello *et al.,* 1999).

Trichothecene-producing pathogens contain a transformed ribosomal protein L3 that safeguards them from the effects of the produced trichothecenes (Rocha *et al.,* 2005). This could possibly explain how some of the mycotoxin producers can co-exist in the same host. Trichothecenes are stable and not effected by heating (Yazar and Omurtag, 2008).

The advised maximum level for deoxynivalenol in human products by The Food and Drug Administration (2011) is 1000 µg/kg, the European Union 1250 µg/kg (Bhat *et al.,* 2010) and in South Africa between 1000 µg/kg and 2000 µg/kg depending on the produce (National Department of Health, 2016). Total dietary intake for nivalenol is 0.70 µg/kg bodyweight and for both T-2 toxin and HT-2 toxin 0.06 µg/kg bodyweight (Schothorst and Van Egmond, 2004).

1.4.3. Zearalenone

Zearalenone is a non-steroidal estrogenic mycotoxin (Desjardins and Proctor, 2007) produced by a number of *Fusarium* spp. although limited to trichothecene producers (Glenn, 2007), including *F. graminearum sensu lato*, *F. culmorum, F. equiseti* and *F. cerealis* (*F. crookwellense*) (Kumar *et al.,* 2008). Zearalenone is relatively stable at high temperatures (Yazar and Omurtag, 2008), although variations in pH levels can metabolize zearalenone into its metabolite counterparts i.e. α-zearalenone or β-zearalenone (Zinedine *et al.,* 2007).

The main concern associated with zearalenone in grain is its effect on reproductive systems in animals and humans (Reddy *et al.,* 2010b). Breeding difficulties in farm animals as a result of mycotoxicoses have been noted. Swine, for instance, develop a reproductive disorder termed vulvovaginitis, resulting in swelling and reddening of the vulva (Prodanov *et al.,* 2009). Piglets exposed to zearalenone developed vulvoganitis and neonatal diarrhoea which leads to small, weak piglets. Fatalities indicated pneumonia, pleuropneumonia and bleeding of the mucosal surface layering the digestive track. Older pigs prepared for market developed bronchopneumonia, vulvovaginitis, rectal prolapses and bloody diarrhoea. Sows with suckling piglets developed endometritis, agalactia and nourishment refusal (Prodanov *et al.,* 2009). Loss of weight was not solely related to feed refusal, but also impaired immune responses and damage to lymphoid organs, (Hueza *et al.,* 2014). In humans, premature puberty in girls and cervical cancer can occur (Reddy *et al.,* 2010b).

The way in which zearalenone or its metabolites affect reproductive systems is through interactions with estrogen receptors (Zinedine *et al.,* 2007). The Joint Committee FAO/WHO on Food Additives established a maximum tolerable intake for ingestion of zearalenone at 0.50 μg/kg bodyweight (Zinedine *et al.,* 2007).

1.4.4. Moniliformin

Moniliformin is widely dispersed over a range of crops, but in relatively small amounts (Von Bargen, 2012). It is produced by *Fusarium subglutinans* (Marasas *et al.,* 1986), *F. acuminatum sensu lato, F. concolor* (Rabie *et al.,* 1982), *F. anthophilum, F. proliferatum* (Marasas *et al.,* 1986), *F. oxysporum,* (Abbas *et al.*, 1990) and *F. fujikuroi sensu lato* (Kubena *et al.,* 1997), *F. temperatum* (Janse van Rensburg, 2018). Moniliformin was first discovered by Cole *et al.*

(1973) and subsequently characterized (Von Bargen, 2012). Since then, toxicity has been established in rats (Abbas *et al.,* 1990), ducklings (Marasas *et al.,* 1991; Leslie *et al.,* 1996) and broiler chickens (Harvey *et al.,* 1997).

Rats fed with contaminated rice and a complete rat diet (1:1 or 1:9) were exposed to different levels of moniliformin. Rats exposed to levels higher than 1000 µg/g, died within 16 hours of ingestion. Post-mortem observations indicated hemorrhage of the small intestines (Abbas *et al.,* 1990). Deaths of ducklings fed on contaminated maize kernels mixed with commercial chicken feed (1:1) were attributed to production of moniliformin, fumonisin and other unidentified compounds detected, although one specific toxic sample tested contained only moniliformin (Leslie *et al.,* 1996). Five out of six *Fusarium* spp. tested positive for duckling toxicity and from these, 96% of strains produced significant moniliformin levels associated with duckling fatalities (Marasas *et al.,* 1991). Contaminated feed of 100 mg/kg resulted in body weight loss of 25% and heart weight gain with cardiac damage and a mortality rate of 17% in broiler chickens (Harvey *et al.,* 1997). It is important to note that above-mentioned experiments were conducted with higher mycotoxin dosages than levels that occur naturally (Harvey *et al.,* 1997) and therefore no direct associations have been made with disease epidemics among animals (Glenn, 2007). Moniliformin can suppress immune responses in humans, thereby possibly predisposing humans to more infections (Ficheux *et al.,* 2013). Currently no daily limit has been established.

1.5. ECONOMIC IMPORTANCE

Pest and pathogen outbreaks can cause annual crop losses of 10 to 40%, depending on topographical environment (Repellin *et al.,* 2001). Sorghum crop losses due to grain mold can be as high as 30 to 100% depending on environmental conditions, sorghum genotype and time of flowering (Singh and Bandyopadhyay, 2000, cited by Thakur *et al,* 2006). In 1992, losses were estimated at approximately US$ 130 million by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Das and Patil, 2013).

Financial loss caused by grain mold is difficult to determine because loss would have to be determined from production, marketing and usage of the grain and seed (Thakur *et al*., 2006).

1.6. FACTORS INFLUENCING SORGHUM GRAIN MOLD AND THEIR ROLE IN DISEASE MANAGEMENT

Epidemiology of sorghum grain mold is highly complex due to the numerous fungal pathogens responsible for disease (Bandyopadhyay *et al.,* 2000) and the extensive diversity between and within species (Summerell and Leslie, 2011). The interactions among these pathogenic fungi in a natural habitat also involve either inhibition, antagonism or co-habitation strategies. These usually coincide with the production of secondary metabolites (Müller *et al.,* 2012) and are dependent on specific environmental parameters which may deter or encourage their production or influence the pathogen itself (Thakur *et al*., 2006). Therefore, no single control method has been found to be effective against the vast diversity of fungal pathogens causing grain mold of sorghum (Thakur *et al*., 2006).

1.6.1. Weather

Weather conditions play a significant role in grain mold development. In countries where sorghum originated, i.e. Egypt, Sudan and Ethiopia, tall landraces are grown without the threat of developing grain mold, even with rainfall. These plants have evolved through natural selection and human intervention to develop photosensitivity and therefore only flower during the dry season, which coincides with shorter autumn days. In the process, these varieties escape grain mold development (Frederiksen, 2000) and the pathogen is most likely restricted to the hilar end of the grain (Govardhan and Das, 2015) where it survives until conditions change. Moisture combined with warm temperatures during flowering and grain development will greatly increase the risk of grain mold severity (Thakur *et al*., 2006).

Grain mold severity was higher under wetter weather conditions compared to the same period of the previous drier year in a study conducted by Prom *et al*. (2003). Higher moisture and increasing minimum temperatures following flowering, promote grain mold development (Tarekegn *et al.,* 2006). A maximum relative humidity lower than 90% resulted in disease severity remaining fairly low, compared to RH greater than 95% where a noticeable increase in the level of grain mold on sorghum genotypes was observed (Bandyopadhyay *et al.,* 2000). Coetzee (2015) reported that high maximum relative humidity during flowering stages, 82 to 95 days after sowing, resulted in higher *F. graminearum* species complex colonisation and mycotoxin production. Grain moisture content is also a factor and fungi usually invade kernels

with a 20% to 22% moisture content combined with a relative humidity of more than 90% (Castor and Frederiksen, 1982).

Not all grain mold fungi respond in the same way to wetness. *C. lunata* requires at least 48 to 72 hours of wetness for 100% infection rate, compared to *Fusarium* spp., *P. sorghina* and *B. australiensis* that need approximately 40 hours. *P. sorghina* needs only 16 hours of wetness to initiate high levels of infection (Navi *et al*., 2005). *P. sorghina* is also able to infect kernels at hard dough growth stage or maturity without moisture treatment in a dew chamber (Bandyopadhyay *et al.,* 2000), indicating that reduced wetness duration, or none as in this case, was needed during later grain development stages compared to early development stages (Navi *et al*., 2005). The duration of wetness required at specific growth stages differs for each pathogen, indicating that each pathogen has a specific window of opportunity to initiate infection (Thakur *et al*., 2006).

Optimum temperatures can also vary with different pathogens in the same host. *Fusarium culmorum* produced more head blight symptoms on wheat at 20°C compared to *F. graminearum* with 95% humidity. At 16°C the reverse was observed, indicating species temperature preferences (Brennan *et al.,* 2005). *In vitro* growth rates for *F. poae, F. culmorum* and *F. graminearum* increased between 10°C to 25°C and decreased between 25°C to 30°C (Brennan *et al.,* 2003). Optimum growth temperature of *F. poae* and *F. graminearum* were determined at 25°C and that of *F. culmorum* ranging from 20 to 25°C (Brennan *et al.,* 2003), hereby indicating that pathogenic aggressiveness could be affected by specific temperature or that the plant is more susceptible to infection by that particular pathogen at that specific temperature (Brennan *et al.,* 2005).

In maize ear rot, *F. verticillioides* requires a wider range of temperatures for germination and growth than *F. graminearum sensu stricto* (Czembor *et al.,* 2015). The optimum temperatures for *F. graminearum* growth ranged from 26°C to 28°C and that of *F. verticillioides* from 28°C to 30°C (Reid *et al.,* 1999). *F. verticillioides* was also able to grow twice as fast at 24°C compared with *F. graminearum*, indicating its ability to thrive at sub-optimal temperatures. In general *F. verticillioides* was a faster growing pathogen than *F. graminearum* indicating its potential to out-compete the latter (Reid *et al.,* 1999).

1.6.2. Chemical environment

1.6.2.1. Pesticides

Adult head bugs and their nymphs feed on developing grains and provide an entry point for grain mold fungi. Leakage of plant sap from punctures provides a nutrient source for fungal establishment (Bandyopadhyay *et al.,* 2000). This can result in even the most resistant sorghum cultivars becoming susceptible. Thus, even the smallest injury can provoke a severe grain mold outbreak. Efficient pest control is therefore important to reduce the risk of grain mold (Bandyopadhyay *et al.,* 2000). Insecticide application decreases thrips and corn earworm activity, resulting in a lower risk of developing *Fusarium* ear rot of maize (Parsons, 2008).

1.6.2.2. Fungicides

Grain mold protection can be provided to some extent by chemical applications, but this is generally, not practical nor economical (Bandyopadhyay *et al.,* 2000). Until recently fungicides have only been used to reduce grain weathering. Studies on the application of fungicides to sorghum florets at 80% flowering were conducted by Govardhan and Das (2015). Propiconazole was found to be more effective against seed-borne pathogens compared to mancozeb and carbendazim, with a reduction of up to 65% in *Fusarium* spp., 89% in *Curvularia* spp. and 67% total infection (including *Bipolaris* spp. and *Alternaria* spp.), thereby reducing grain mold severity on mature kernels. Audilakshmi *et al.* (2007) reported that propiconazole sprayed three times from flowering and at subsequent 10 day intervals, reduced sorghum grain mold by almost half. Propiconazole is a systemic fungicide capable of interfering with ergosterol biosynthesis of a broad range of fungi (Audilakshmi *et al.,* 2007).

In vitro tests with carbendazim (Pearl®), hexaconazole (Cotaf®) and micronised sulphur (Thiovit®) fungicides completely inhibited the growth of *F. graminearum sensu stricto* and *F. poae,* the causal pathogens of *Fusarium* head blight of wheat. In a glasshouse trial, head blight was reduced to different extents, with micronised sulphur being the most effective fungicide (Musyimi *et al.,* 2012). The efficacy of fungicides in reducing head blight of wheat is dependent on abiotic factors, including temperature and water availability and the interactions among these factors with the fungicide used and dosages applied. At 25°C, as water availability declined, efficacy of fungicides increased with more prevalent growth inhibition of *F.*

graminearum. At 15°C the growth reducing ability of the fungicides was less noticeable (Ramirez *et al.,* 2004).

Tebuconazole (Folicur 430SC) applied in half and full dosages at flower initiation and throughout flowering, reduced the number of wheat ears that were predominately infected with *F. graminearum sensu lato*, as well as the number of spikelets infected per ear (Cromey *et al.,* 2001). Tebuconazole reduced the incidence of ear rot by up to 50% and azoxystrobin and carbendazim by up to 33%. It was proposed that spraying at mid-flowering is more effective than spraying fully developed ears to control *Fusarium* spp. Grain weight per 1000 kernels was also increased by all three fungicides used (Cromey *et al.,* 2001).

The overall efficacy of fungicides for grain mold reduction is still unclear. A study conducted by Ramirez *et al.* (2004) indicated a decrease in mycelial growth with fungicides, i.e. tebuconazole (Folicur®), propioconazole (Tilt®), epoxyconazole (Opus®), prochloraz (Sportak®), azoxystrobin (Amistar®) when applied to *F. graminearum sensu stricto in vitro.* However, an increase in deoxynivalenol levels was observed. This was explained as a last attempt by *F. graminearum* to defend itself by producing secondary metabolites in a deadly environment.

The widespread use of fungicides can result in resistance build-up in pathogens. In Brazil, the development of less-sensitive *F. graminearum* species complex isolates was attributed to either the inherent ability of the isolates to withstand chemical treatment or the continuous usage of metconazole for multiple wheat diseases that resulted in a selection pressure and subsequent fungicide resistance in the pathogen population (Spolti *et al.,* 2012.). Rotating fungicides from different FRAC groups is therefore important.

The above studies and observations indicate that control of grain mold pathogens through fungicide application needs to consider: fungicides used (ensuring rotation to reduce resistance build-up), application timing (flowering), repeated applications required and weather interactions (temperature and relative humidity). Overall the efficacy and economic expediency of chemical application to control grain mold have yet to be determined (Thakur *et al*., 2006).

1.6.3. Biotic environment

1.6.3.1. Biological control of sorghum grain mold

Increasing costs of fungicide development and growing environmental concerns have stimulated research on alternate control methods for grain mold, including biological control. Biological control can be defined as application of antagonistic microorganisms displaying suppressiveness towards pathogens, in order to improve plant health (Handelsman and Stabb, 1996). Micro-organisms such as bacteria from *Bacillus* spp. and *Pseudomonas* spp. and fungi from *Trichoderma* spp., *Gliocladium* spp. and *F. oxysporum* have been reported to be antagonistic to a range of pathogens (Handelsman and Stabb, 1996; Olivain *et al.,* 2006). Biological control has received considerable attention due to its low environmental impact, sustainability and compatibility with other control methods, especially in the case of *Fusarium* head blight of wheat (Musyimi *et al.* 2012). Until recently the main focus of biological control in wheat head blight was to reduce overall disease severity. Currently the focus has moved to include the ability of the biological control agent to reduce the production of pathogen mycotoxins (Matarese *et al.,* 2012; Nguyen *et al.,* 2017).

Trichoderma spp., *Epicoccum* spp. and *Alternaria* spp. were found to be antagonistic to *F. graminearum sensu stricto* and *F. poae*, causal agents of *Fusarium* head blight, but to varying extents. Radial growths were reduced *in vitro* by up to 53.4% and 64.9% respectively, but when tested in the glasshouse, only *F. graminearum* was significantly inhibited by all antagonists with *Trichoderma* spp. being the prevalent inhibitor (Musyimi *et al.,* 2012). Other biological agents tested for efficacy against *F. graminearum* in a field environment included *Bacillus subtilis* and *Brevibacillus* sp. (Palazzini *et al.,* 2016). These bacteria not only reduced disease severity by up to 62 to 76% and 42 to 58% respectively, but also reduced deoxynivalenol to undetectably low levels, even when applied in small dosages. The mode of action was presumably the production of antibiotics or lipopeptides or even induced resistance in the host plants (Palazzini *et al.,* 2016).

Trichoderma spp. able to flourish in the presence of deoxynivalenol, were selected for testing against *F. graminearum* and *F. culmorum*, causal agents of *Fusarium* head blight. *Trichoderma gamsii* and *T. velutinum* reduced the growth of both pathogens by overgrowing the *Fusarium* colony *in vitro* and by coiling around the hyphae. Deoxynivalenol levels

produced by *F. graminearum* were also reduced by between 60 to 92% (Matarese *et al.,* 2012). *T. viride*, *T. harzianum* and *Pseudomonas fluorescens* have also shown antagonistic activity against sorghum grain mold pathogens. There were also beneficial effects on the germination rate of infected seeds and increased seedling vigour (Thakur *et al*., 2006).

Biopesticides, under the trade name Aflasafe, have been developed for use on groundnut and maize in African countries, including Nigeria, Senegal and Kenya. Aflasafe products contain vegetative compatible native strains of *Aspergillus flavus* able to reduce aflatoxin contamination by up to 75% in crops. Bird mortality from feeding was also reduced by 70% indicating the beneficial non-target effect of Aflasafe on the environment. Further research is underway to determine the exact mechanisms involved and to exploit these to their full capacity in order to ensure registration of the commercial product in more countries (Bandyopadhyay *et al.*, 2016).

1.6.4. Cultural practices

1.6.4.1. Avoidance

Photosensitive cultivars only initiate flowering and grain filling during drier periods and by doing so, escape grain mold which requires wetter conditions during flowering stages (Bandyopadhyay *et al.,* 2000). Avoidance is one of the most important traditional control methods (Thakur *et al*., 2006) and is of great importance in seed production which is usually carried out under irrigation in regions with dry seasons. Avoidance is obtained through either delaying sowing dates or growing cultivars that mature only after the rainy season.

Delaying sowing may not be practical as irrigation systems are needed and this is not always available in poorer countries (Bandyopadhyay *et al.,* 2000). Also, adjusting sowing dates is unrealistic in some regions due to the limited length of growing seasons. Other problems can intensify grain mold development (Thakur *et al*., 2006), such as severe attacks by head bugs and midge (Bandyopadhyay *et al.,* 2000) which create wounds that are potential infection sites for grain mold pathogens including *F. verticillioides* (Parsons, 2008). Applying insecticides during delayed planting seasons could lower severity of *Fusarium* ear rot of maize even further (Parsons, 2008).

1.6.4.2. Rotation/tillage

The uninterrupted cultivation of a specific crop year after year will lead to the establishment of plant pathogen populations specific to that crop. To avoid the accumulation of pathogens, crop rotation is applied where unrelated plant species are alternated in the cultivated field (Janvier *et al*., 2007).

Crop residues left from previous wheat, maize and soybean rotation crops, were the most abundant in no-tillage (65%), compared to chisel plowing (31%) and moldboard plowing (9%). Wheat and maize residues on the soil surface were similar (41/42%), compared to soybeans (23%). *F. graminearum sensu lato* was isolated from these maize and wheat residues (Dill-Macky and Jones, 2000) which served as potential sources of inoculum (Matarese *et al.,* 2012). No-tillage practices resulted in higher *Fusarium* head blight severity in wheat due to crop and weed residues that harboured the pathogen.

A three year rotation system with soybean and maize was sufficient to reduce deoxynivalenol levels in wheat, in particular when the previous rotation crop was soybean (Schaafsma *et al.,* 2001. Deoxynivalenol levels were 25% lower in soybean/wheat rotation compared to wheat/wheat and 50% lower than maize/wheat (Dill-Macky and Jones, 2000).

F. graminearum sensu stricto can be hosted on weed species surrounding the field. In areas where weather conditions favour disease outbreaks, weeds can serve as the initial source of inoculum (Mourelos *et al.,* 2014). Rotating unrelated species to reduce initial inoculum, as well as tillage practices to remove stubble and weeds are important factors that could reduce survival of the pathogen.

Sorghum needs sufficient nitrogen because of its high yield potential (Holford, 1989) and can easily drain nitrogen reserves in the soil (Doughton and Mackenzie, 1984). Nitrogen deficiency affects soil fertility and consequently plant vigour (Arnon, 1964; Bado *et al.,* 2013). Fertilizers may not be sufficient to sustain the required nitrogen levels, while the form of fertilizers applied, i.e. ammonium nitrate, could create physiological stress resulting in more severe wheat head blight (Edwards, 2004). Methods are therefore, needed to increase and maintain healthy soil (Arnon, 1964). Planting legumes in rotation with sorghum increases soil fertility and adds 30 to 50% more moisture to the soil than in the crop proceeding sorghum (Arnon, 1964). This

effect can be attributed to the release of nitrogen by the legumes (Bagayoko *et al.,* 2000) leading to improved growth and higher yields, hereby potentially counteracting effects of diseased tissues.

1.6.4.3. Harvesting

At physiological maturity sorghum grain develops the so-called black layer at the hilar end of the sorghum kernel. The black layer detaches the kernel from the sorghum plant and transportation of photosynthates from the plant ceases immediately. This initiates the drying down phase as the kernel loses moisture (Bandyopadhyay *et al.,* 2000). Harvesting at this time with artificial drying will prevent superficial saprophytic fungal growth and reduce grain damage (Thakur *et al*., 2006). Delaying harvesting to maturity increases the chances of grain mold and combined with wet environmental conditions, grain mold development is unavoidable (Bandyopadhyay *et al.,* 2000).

1.6.4.4. Dehulling (Pearling)

Pearling of deteriorated grain is done to improve the appearance and increase the market price using a pearling (dehulling) device where the discoloured pericarp is removed by grinding stones. Up to 16% increase in market price can be obtained with only about 5% loss in grain weight (Audilakshmi *et al.,* 2007). Seeds are usually pre-conditioned with moisture and subsequently sun dried to reduce the breakage of grain during the polishing process (Audilakshmi *et al.,* 2007). Red sorghum cultivars resistant to grain mold fungi can also be used as a food source after pearling since the dehulled sorghum can be milled into white flour (Thakur *et al*., 2006). In the preparation of culinary products, pearling is a requirement (Audilakshmi *et al.,* 2007).

Infection by members of the *F. graminearum* species complex occurs deep within the endosperm of the sorghum kernel (Coetzee, 2015). Dehulling for three to four minutes was required to remove 30% grain mass to reach the infected area. Thus, this is not an economical method of removing all the infected tissue. On the other hand, pearling can reduce zearalenone and deoxynivalenol levels by removing the outer layer of the kernel, where these mycotoxins were located. Nivalenol is concentrated more deeply in the endosperm and dehulling of 22% was required to reduce levels in commercial sorghum grain (Coetzee, 2015).

1.6.4.5. Post-harvest storage

In order to prevent post-harvest grain mold development, sorghum grain needs less than 10 to 12% moisture content. Farmers in South Africa tend to leave grain for longer in the field to dry before harvest, but this can be a problem when grain is exposed to rain, especially where wetness persists (Bandyopadhyay *et al.,* 2000). Artificial drying methods can prevent post-harvest grain mold development by enhancing grain quality and subsequently increase the value (Thakur *et al*., 2006) while anti-heating chemicals, e.g. acetic acid, sprayed onto the wet panicles after harvesting can improve the quality of the produce. A 4% acetic acid treatment reduced the growth of mold pathogens and saprophytes, although seed vigour and germination rates were also reduced. Anti-heating chemicals are recommended only for improving grain quality rather than application in seed multiplication trials (Audilakshmi *et al.,* 2007).

Storage of grain at low temperatures and low humidity will help to maintain the quality of grain. Post-harvest mould is usually the result of *Aspergillus* spp. and *Penicillium* spp. and these mold fungi can also produce harmful mycotoxins (Castor and Frederiksen, 1982) i.e. aflatoxin, ochratoxin and patulin (Reddy *et al.,* 2010b). Post-harvest fungi are able to increase the temperature of grain. *A. flavus* can increase the temperature by up to 55°C which decreases grain quality. Other chemical processes may further raise the temperature which can ultimately lead to ignition of the grain. Mouldiness and caking are the last phases of spoilage, making the grain unusable as food source (Castor and Frederiksen, 1982).

1.7. GRAIN MOLD RESISTANCE IN SORGHUM

The economic constraints of chemical control and the limited efficacy (Prom *et al*., 2003), as well as the limited proven success of biological control in on-farm systems (Thakur *et al*., 2006), makes resistance the most effective method to control grain mold (Prom *et al*., 2003). Grain mold resistance is considered a multigenic trait due to the complexity of the disease in relation to variable causal pathogens and their association with specific growing conditions (Little and Magill, 2003) that favour a particular pathogen to cause infection (Thakur *et al*., 2006). Breeding for these specific traits is therefore not easy, as each trait could be inherited differently in diverse environments. Therefore, for accuracy, evaluation and selection of

resistance in lines must be conducted over a range of diverse environments to ensure reliable results (Rodriguez-Herrera *et al.,* 2000).

Sorghum plant and grain characteristics, either physical or chemical, have been associated with host response to grain mold pathogens, but no one trait has been distinctly linked to resistance. Sufficient levels of resistance can only be obtained through combinations of more than one trait (Rooney, 2000), but progress has been limited by a lack of understanding of the genetics of specific traits and the interactions of these traits to promote grain mold resistance in sorghum. Another constraint limiting the understanding of these traits is the vast range of plant characteristics available that are associated with resistance, e.g. flower and panicle structure, grain hardness and phenolic compounds (Thakur *et al*., 2006). In general, sorghum with an open panicle, large tight glumes, thin pericarp caryopsis, corneous endosperm and condensed tannins, is usually considered more resistant to grain mold pathogens (Waniska *et al*., 1989, cited by Waniska and Rooney, 2000).

1.7.1. Physical properties

1.7.1.1. Panicle compactness

The canopy of a crop influences the microclimate and duration of wetness through features such as height, density and panicle structures (Navi *et al*., 2005). In areas with higher rainfall or humidity, an open panicle ensures more rapid drying of the sorghum head which minimizes the risk of fungal infection of grain and subsequent grain weathering due to grain mold pathogens (Ayana and Bekele, 1998). Grain mold resistant sorghum lines are also primarily tall, thus preventing high humidity levels in the canopy that predispose the plants to infection (Audilakshmi *et al.,* 1999). A compact panicle may also harbour insects such as head bugs and aphids. Aphids exude honeydew that is a sticky fluid that drips from severely infested plants, aiding in the establishment of grain mold pathogens that enter the plants through wounds (Doggett, 1970).

1.7.1.2. Grain hardness

Grain hardness influences the susceptibility of a cultivar to both grain mold and head bug infestation, especially during maturity. By including this trait in a specific cultivar and

ensuring its sustainability throughout the entire season, could ensure improved resistance towards grain mold and head bugs in wetter seasons (Bandyopadhyay *et al.,* 2000). Grain with a corneous, hard endosperm tends to be more resistant and restricts the growth of mould pathogens on both the grain surface and internal tissue (Katile *et al.,* 2010). This was confirmed by Coetzee (2015) where harder sorghum grain had a lower biomass of *F. graminearum* species complex and lower levels of mycotoxins. Endosperm of hard grain contains more proteins (Reddy *et al.,* 2000), presumably chitinases (Little and Magill, 2003) that are directly antagonistic to the fungal pathogen (Chandrashekar and Satyanarayana, 2006).

Usually grain hardness and glume colour are the only resistance factors available in white sorghum (Thakur *et al*., 2006). However, even though hard grains, combined with a corneous endosperm, can stop infection within, it cannot prevent the subsequent infection of the pericarp by *Curvularia* spp., *Alternaria* spp. or *Cladosporium* spp. (Bandyopadhyay *et al.,* 2000) that can still lead to the appearance of the grain being infected. Grain with a very high degree of hardness is not desirable as a food source, and a balance is needed between hardness and digestibility to ensure good quality grain (Thakur *et al*., 2006). This balance will be dependent upon the specific end-use of the sorghum grain.

1.7.1.3. Glumes

White grain sorghum lacks phenolic substances and is thus, generally more susceptible to grain mold than coloured grains with antagonistic phenolic substances. Fungi usually colonise the part of the kernel not covered by glumes (Castor and Frederiksen, 1982) and therefore, large papery glumes that can cover the entire kernel are associated with resistance, particularly in the low phenolic content grains (Williams and Rao, 1980).

Coloured glumes and moderately hard white grains have proven to be more resistant to grain mold. This observed resistance was associated with glume phenol and glume flavan-4-ol content linked to colour. Glume antifungal proteins are also associated with resistance. Resistant sorghum cultivars have higher levels of PR-10 proteins (Katile *et al.,* 2010) and chitinases can accumulate rapidly after infection to restrict grain mold pathogen growth (Little and Magill, 2003).

1.7.1.4. Waxy layer

A waxy layer on the grain surface of sorghum is associated with resistance towards *F. verticillioides* (Rodriguez-Herrera *et al.,* 2000). Sampietro *et al.* (2009) found that removal of the waxy layer on the surface of maize kernels by emerging them in chloroform, predisposes them to infection by *F. verticillioides* and fumonisin accumulation, and therefore suggested that the waxy layer restricts infection.

1.7.1.5. Dormancy

Pre-harvest sprouting of the grain as a result of the germination of the embryo during wet environmental conditions, can lead to the splitting of the pericarp over the hilar end. In turn, the endosperm is digested by α-amylase that predisposes the grain to colonization by mould fungi, particularly *Curvularia* spp. and *Fusarium* spp. This may occur before the hard dough stage is initiated. Pre-harvest sprouting can be overcome by seed dormancy, which restricts germination by more than 30 days after grain maturity is reached (Bandyopadhyay *et al.,* 2000).

1.7.2. Chemical properties

1.7.2.1. Phenolic compounds

More than 8000 phenolic compounds are known to be produced throughout the plant kingdom where they can be found in either free form or bound to carbohydrates in the vacuole. All sorghum plants contain phenolic compounds, but to different extents throughout the plant. The amount present is dependent on the specific genotype and prevailing environmental conditions in which the plant finds itself (Dicko *et al*., 2006). Sorghum grain contains phenolic compounds that affect several factors including appearance, colour and nutritional value of the grain (Hahn *et al.,* 1984). These phenolic compounds also inhibit growth of fungal pathogens and are thus, associated with resistance to pre- and post-maturity grain mold (Hahn *et al*., 1984).

Plants with purple/red plant colour have higher levels of total phenolic compounds compared to plants with a tan colour (Dykes *et al*., 2005). Phenolic compounds can be found in the pericarp, inner and outer layers of the testa and cell walls of the aleurone layer (Hahn *et al*.,

1984; McDonough *et al*., 1986). Only tightly bound phenolic compounds are seated in the endosperm (Hahn *et al*., 1984). Phenolic compounds can be divided into three groups, i.e. phenolic acids, flavonoids and condensed tannins (Dykes and Rooney, 2006). Phenolic acids are found in all sorghums, with just a few containing flavonoids and only brown, bird-resistant varieties containing condensed tannin (Hahn *et al*., 1984).

Flavonoids are situated in the outer layers of the grain and differences in the colour and thickness of the pericarp as well as the presence or absence of a testa, influence the concentration and composition of the different flavonoids (Dykes *et al.,* 2005; Taleon *et al*., 2012). Grain with a thick pericarp and pigmented testa has higher flavonoid levels compared to grain with a thin pericarp without a testa (Dykes *et al*., 2005).

The most well-known flavonoid, 3-deoxyanthocyanin, is abundant in black pericarp sorghum. Red pericarp sorghum contains a class of flavonoids i.e. flavan-4-ol compounds (luteoforol and apiforol) produced from flavones and are the highest in purple/red plants (Dykes *et al*., 2005; Dykes and Rooney, 2006). White pericarp sorghums have no fungal growth restricting phenolic compounds (Thakur *et al*., 2006), therefore they are more susceptible to grain mold infections (Ambekar *et al.,* 2011) unless other defence characters such as antifungal proteins are associated with them.

Non-damaged maize kernels and kernels wounded by piercing of the pericarp with a gauge needle, were dipped in an inoculum suspension of *F. verticillioides* and prepared for fumonisin determination. Wounded kernels showed an increase in fumonisin levels, compared to the undamaged kernels, suggesting that resistance factors exist in the pericarp layer that block the initial fungal penetration (Sampietro *et al.*, 2009). These resistance factors were associated with phenolic compounds in the pericarp. Sekhon *et al.* (2006) indicated that 3-deoxyanthocyanidins accumulated in the pericarp of a resistant maize line after infection by *F. verticillioides.*

Condensed tannins are only found in sorghum with a pigmented testa (Dykes *et al*., 2005) and are shown to inhibit fungal growth either by inhibiting the production of extracellular enzymes (Scalbert, 1991) or depriving the pathogen of much needed iron (Mila *et al.,* 1996).

Sorghum seedlings inoculated with *Fusarium thapsinum* and *F. proliferatum* had significantly increased concentrations of total phenolics, particularly luteolinidin and apigeninidin. That increase wasespecially apparent in the scutellum of these seedlings which appeared to be the pathogen's point of entry as determined by microscopic examination. Higher levels of phenolics were produced in response to infection with *F. thapsinum* compared to *F. proliferatum*, probably as a result of more severe infection from a more aggressive pathogen, (Huang and Backhouse, 2004). Luteolinidin and 5-methoxyluteolinidin were produced only in mesocotyl tissues of resistant sorghum cultivars. Apigeninidin and the caffeic acid ester arabinosyl 5-O-apigeninidin levels were higher in resistant sorghum cultivars during the first 72 hours after infection, compared to susceptible cultivars. After this time, levels in susceptible cultivars rapidly increased and exceeded those in resistant cultivars to counteract colonisation by the pathogen (Lo *et al.,* 1999). Resistance is therefore linked to timing, where resistant cultivars produce phenolic compounds upon pathogen accumulation on plant surface while in susceptible cultivars this only occurs after pathogen establishment in the first 24 hours (Lo *et al.,* 1999).

1.7.2.2. Antifungal proteins

Proteins located in the endosperm of sorghum have been associated with inhibition towards fungal growth (Waniska and Rooney, 2000). These plant defense proteins include glucanase, sormatin, chitinase and ribosome-inactivating proteins and play an important part in primary infection (Das *et al.,* 2012).

Studies conducted by Seetharaman *et al.* (1996) revealed a significant increase in sormatin levels of resistant sorghum cultivars, from caryopsis formation until maturation, during which a higher risk of grain mold is expected. Synthesis of antifungal proteins was induced in resistant sorghum cultivars after pathogenic attack (Bueso *et al.,* 2000). Levels of sormatin and chitinase were also higher in resistant sorghum lines during all stages of grain development indicating their role in suppressing grain mold infection (Rodriguez-Herrera *et al.,* 2006). After infection with *Fusarium thapsinum* and *F. proliferatum*, sorghum seedlings displayed higher β-1,3-glucanase activity (Huang and Backhouse, 2004). It is suggested that β-1,3-glucanase has a protective function (Rodriguez-Herrera *et al.,* 2006) by weakening fungal cell walls and ultimately leading to cell death (Prom and Egilla, 2011). This activity appears even more

important after grain maturity (Rodriguez-Herrera *et al.,* 2006). PR-10 proteins were also higher in the glumes of resistant cultivars (Katile *et al.,* 2010).

White grain sorghum displaying grain mold resistance had higher levels of chitinase and ribosome-inactivating proteins. This suggests the existence of other defense mehcanisms like antifungal proteins in sorghum plants whose grain lacks both a pigmented testa and coloured pericarp (Little and Magill 2003; Rodriguez-Herrera *et al.,* 1999). Levels of ribosome-inactivating proteins increase as the sorghum plant matures (Rodriguez-Herrera *et al.,* 2006), while chitinase accumulation is initiated rapidly after initial infection (Little and Magill 2003). Chitinase is not only found during grain development or maturation, but may also occur in floral tissues (Little and Magill 2003). Chitinases possess the ability to degrade fungal cell walls *in vitro* (Little, 2000).

1.8. CONCLUSION

Grain mold of sorghum is a serious problem, especially when considering health related issues associated with mycotoxin production and economic losses as a result of reduced grain quality and yield through grain deterioration and poor appearance. Therefore grain mold control measures are needed, whether technically, by implementing certain farming practices, or genetically by influencing the expression of genes linked to certain plant and grain characteristics associated with disease resistance.

Research is needed to identify the most important and desirable plant and grain characteristics conferring resistance to grain mold and how to incorporate those genetic components into sorghums in future breeding programmes. The bitterness of high tannin sorghum grain lowers bird damage, but that bitterness and an associated reduction in nutritional value (Kobue-Lekalake, *et al.,* 2007) negates its use in food source sorghums. Thus, knowledge about specific traits along with assessment of their individual and additive value can assist in selecting those most worth exploiting. The future goal should be development of area-adapted grain mold resistant sorghums that consistently produce high quality grain even under disease-conducive environments.

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CHAPTER 2

PLANT AND GRAIN CHARACTERISTICS THAT INFLUENCE GRAIN MOLD SEVERITY IN SORGHUM

2.1. Abstract

Sorghum is one of the most important crops produced in areas not suitable for other cereals. Sorghum grain mold is considered the most important disease threatening yield and quality of the grain. Pathogens associated with grain mold include *Fusarium* spp. that produce mycotoxins that could affect the health of humans and animals. Breeding for resistance is considered the most cost-effective control method. Field trials were conducted at Cedara and Alma to evaluate grain mold resistance in twenty-two sorghum lines representing the range of plant and grain characteristics observed in commercially planted cultivars. Visual grain mold ratings at maturity on a 0 - 5 scale ranged from 1.43 in RTX 436 to 3.33 in Hegari with higher ratings generally associated with purple plant colour. Ergosterol levels, representing total fungal biomass in the grain, ranged from 318.08 µg/g in SC 630-11Eii to 1178.06 µg/g in TX 2911. *F. graminearum* species complex (FGSC) DNA levels, quantified using qPCR ranged from 18.46 µg/g in RTam 428 to 203.89 µg/g in RTX 2917. Deoxynivalenol (DON) levels ranged from 1.82 µg/kg in RTam 428 to 174.06 µg/kg in BTX 635. Nivalenol (NIV) levels ranged from 18.24 µg/kg in RTam 428 to 243.04 µg/kg in BTX 635. Zearalenone (ZEA) levels ranged from 4.14 µg/kg in SCAY 16 to 341.49 µg/kg in SC 748-5. No specific host trait, linked to grain colour or texture, was consistently associated with resistance to colonisation or mycotoxin production and resistance appeared to be associated with complex interactions. Cedara, the wetter location, had higher visual ratings, FGSC DNA and mycotoxin levels, compared to the dryer Alma location, which had higher ergosterol levels. AMMI analysis of visual ratings indicated genotypic stability through changing environments with genotype being the main driving variable. Ergosterol, as rating criterion indicated significant genotype adaptations to the different environments, with environment being the main driving variable. Genotypes differed in tolerance to colonisation by FGSC pathogens and associated mycotoxin production with variable rates of resistance breakdown in relation to the mycotoxigenic potential of the respective environments, as indicated using regression analysis. Results emphasize the need for a multi-variable approach to the evaluation of sorghum genotype responses to grain mold pathogens.

Keywords: colonisation, *Fusarium graminearum* species complex, genotypic stability, mycotoxins, resistance characters, sorghum

2.2. INTRODUCTION

The most important crops for food and feed utilization worldwide include sorghum, barley, maize, rice, sugarcane and wheat (Jwa *et al.,* 2006). Sorghum (*Sorghum bicolor* (L.) Moench) is the most important of these in the arid and semi-arid regions of the world (Rao *et al.,* 2012). This is due to the ability of the crop to thrive in areas not suitable for most other cereal crops (De Wet, 1986). This attribute could be important in view of a growing population and the need for increased productivity, which is currently more dire than ever throughout history (Rao *et al.,* 2012).

Sorghum grain mold is considered the most important of the sorghum diseases (Rao *et al.,* 2012) and can lead to large yield and quality crop losses whenever rainy conditions persist from flowering through maturity (Bandyopadhyay *et al.,* 2000). Furthermore, its negative effect can have post-harvest repercussions on marketing and use of grain. Mouldy grain in India can result in a 40% decrease in market price (Das and Patil, 2013).

Pathogens associated with sorghum grain mold include *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Curvularia* spp., *Drechslera* spp., *Fusarium* spp., *Olpitrichum* spp. and *Phoma* spp. (Das *et al.,* 2012) and many members of these genera can produce mycotoxins that are dangerous to humans and animals when infected grains are ingested (Rao *et al*., 2012). The pathogens can survive on stubble from the previous growing season and when favourable conditions prevail, conidia are wind dispersed onto emerging anthers (Reddy *et al.,* 2010) or onto the floret itself from where they enter the apical floral tissues (Bandyopadhyay *et al.,* 2000).

Fusarium spp. closely associated with sorghum grain mold include members of the *F. graminearum* species complex (FGSC)*, F. andiyazi, F. nygamai, F. proliferatum, F. thapsinum*, *F. verticillioides*, *F. equiseti* and *F. sacchari* (Sharma *et al.,* 2010; Das *et al.,* 2012; Bodoci *et al.,* 2013). Members of the FGSC appear to be host specific with *F. graminearum sensu stricto* occurring predominately on wheat, *F. boothii* on maize and *F. meridionale, F. acaciae-mearnsii* and *F. cortaderiae* on sorghum (Boutigny *et al.,* 2011b; Mavhunga, 2013). The trichothecene mycotoxins, in particularly deoxynivalenol (DON), also referred to as vomitoxin and nivalenol (NIV), are produced by several *Fusarium* spp.. DON and its derivative, 15-ADON, are produced by *F. graminearum sensu stricto* and *F. boothii.* Another

FGSC member, *F. brasilicum* from wheat, is associated with 3-ADON chemotype (Boutigny *et al.,* 2011b). DON and these derivatives are associated with vomiting, diarrhea, immune suppression, weight loss, dizziness and headaches (Bandyopadhyay *et al.,* 2000; Reddy *et al.,* 2010). NIV is produed by *F. acacia-mearnsii*, *F. cortaderiae* and *F. meridionale* on sorghum (Mavhunga, 2013) and results in slower food intake or complete food refusal (D'Mello *et al.,* 1999). Zearalenone (ZEA) is also mainly produced by *F. graminearum sensu stricto* and *F. culmorum* and is associated with estrogenic complications, reproduction difficulties in animals and cervical cancer (Bandyopadhyay *et al.,* 2000; Reddy *et al.,* 2010).

Breeding for resistance is considered the most cost-effective control method against sorghum grain mold (Rao *et al.,* 2012). Exploitation of traits such as panicle compactness, plant height (Navi *et al*., 2005), grain hardness (Bandyopadhyay *et al.,* 2000), glume coverage (Williams and Rao, 1980), waxy layer on the grain surface (Rodriguez-Herrera *et al.,* 2000), dormancy (Bandyopadhyay *et al.,* 2000), phenolic compounds related to plant colour (Dykes *et al*., 2005; Chandrashekar and Satyanarayana, 2006) and antifungal proteins (Das *et al.,* 2012) are factors shown to influence resistance of sorghum to grain mold pathogens.

The aim of this study was to determine the relationship between grain and plant characteristics in sorghum lines and the response to grain mold pathogens under field conditions. The stability of the observed disease responses under a range of environmental conditions was also assessed. Similarly, mycotoxin production was compared to identify potential role of host x pathogen interactions in mycotoxin levels in the grain. The mycotoxins DON, NIV and ZEA are the most regularly reported toxic metabolites (Müller *et al.,* 2012) and were therefore examined in this study. Janse van Rensburg (2012) found very low levels of fumonisin and aflatoxin in sorghum grain from a range of localities in South Africa and these were thus, not included in the current study.

2.3. MATERIALS AND METHODS

2.3.1. Field trials

Twenty-two sorghum lines (Table 2.1) received from Texas AgriLife (Lubbock) were evaluated for grain mold resistance. Lines differed in plant and grain characteristics and evaluation was aimed at determining traits that contribute to resistance or susceptibility to grain

mold pathogens. Lines were planted at Cedara (29.5477°S, 30.2667°E), KwaZulu-Natal Province, during the following growing seasons: 2009/2010 (mid-November), 2010/2011 (early and late November) and 2011/2012 (early and late November). Lines were also planted at Alma (24.4841°S, 28.0750°E), Limpopo Province, during the following growing seasons: 2012/2013 (early and late November) and 2013/2014 (early November). These evaluation plots provided a range of environments and thus, differing disease potentials.

The lines were planted in 10 m, single-row plots with a 0.75 m inter-row and 0.1 m intra-row spacing. Trials were replicated three times in a randomized block experimental design. Prior to planting during each season, fields were fertilized with 600 kg 2:3:2 (N:P:K). Dual Gold® (metolachlor, 915 g/l) was applied directly after planting at a rate of 500 ml/ha for pre-emergence grass control, followed by Basagran® (Bendioxide - thiadiazine 480 g/l) post-emergence as required at 1.5 l/ha for broadleaf weeds. Decis® (deltamethrin, 25 g/l) was applied at 250 ml/ha for pest control, including stalk borer and aphids. Additional weeding by hand was done as needed. Trials were maintained until harvesting which was done post-maturity at approximately 12.5% grain moisture content.

2.3.2. Grain mold assessment

2.3.2.1. Grain visual ratings

Visual rating based on percent visible kernel surface deterioration on harvested grain was conducted on a 1 to 5 scale according to Audilakshmi *et al.* (2007) where 1 = 0% visible deterioration; 2 = 10%, 3 = 11–25%, 4 = 26–50% and 5 = ≥50%.

2.3.2.2. Evaluation of ergosterol content to determine total fungal biomass in sorghum grain

Fungal biomass in harvested grain was determined from ergosterol content using the modified method of Jambunathan *et al.* (1991). Sorghum grain was ground into a fine meal using a coffee grinder. Extraction commenced by placing 5 g grain meal into 25 ml HPLC-grade methanol (Merck) and mixing at 1470 rpm for 30 minutes using a Heidolph Multi Reax Shaker (Labotec). The suspension was allowed to settle and 12.5 ml of the clear supernatant was added to 1.5 g potassium hydroxide (KOH) in a screw cap test tube. This was vortexed on a Multi

Reax Shaker to dissolve the KOH. N-hexane (5 ml) was then added and the mixture was incubated for 30 min in a 75°C water bath and allowed to cool to room temperature. Distilled water (2.5 ml) was added and the suspension was again thoroughly vortexed and centrifuged at 3000 rcf (BHG Optima) for 5 min. The upper hexane layer was transferred to a new test tube. N-hexane (5 ml) was added to the residual aliquot which was again vortexed and centrifuged. The upper hexane layer was removed and added to the earlier aliquot. This n-hexane step was repeated. The collective hexane extracts were evaporated until dry in a 75°C water bath and resuspended in 2.5 ml methanol. Extracts were filtered through 0.45 µl Pall Acrodisc syringe filter and placed in glass vials for analysis using a Perkin Elmer high performance liquid chromatograph (HPLC) with a SIL-20A auto sampler. The extracts were loaded onto a reverse phase column (Phenomenex, C18 125 A 10 µm particle size, 150 mm x 4.6 mm) at 50°C. The mobile phase consisted of methanol:water (96:4) at a flow rate of 1.2 ml/min. Standard ergosterol (Sigma) was used to develop a standard curve in the range of 7500 to 29 µg/g. Ergosterol was determined from the peak area at 282 nm with a retention time of approximately 7 min.

2.3.2.3. qPCR to quantify FGSC in sorghum grain

Equal grain volumes from every line replicate in the repective environments were pooled for DNA extraction to optimise resources.

2.3.2.3.1. DNA extraction

DNA extraction was done using a Wizard® Genomic DNA Purification Kit (Promega) according to manufacturer's instructions. *Fusarium graminearum* MRC 4712 and *F. pseudograminearum* MRC 4927 were obtained from the Plant Protection Research Institute of the Agricultural Research Council and the Promec Unit of the Medical Research Council and included as reference strains. Sorghum grain from lines harvested from the respective field trials were ground with a coffee grinder into a fine meal. Meal (40 mg) was placed in a 1.5 ml Eppendorf tube. Nuclei Lysis solution (600 µl) was added and vortex mixed (Vortex Genie 2, Scientific Industries) for a few seconds. The tubes were incubated for 15 minutes in a 65°C water bath. RNase solution (3 µl) was added and mixed by gently inverting tubes. This was followed by incubation for 15 minutes in a 37°C water bath, after which the tubes were left to cool to room temperature for 5 minutes. Protein precipitation solution (200 µl) was added and

vortexed for 20 seconds. The tubes were centrifuged for 3 minutes at 14 000 rcf on a Hermle centrifuge (Lasec) and the supernatant was removed and transferred to a clean tube containing 600 µl isopropanol at room temperature. The tubes were mixed by inverting them until thread-like strands of DNA were visible. The tubes were centrifuged for 1 minute at 14 000 rcf. The supernatant was decanted and 600 µl 70% (v/v) ethanol (Merck) at room temperature was added. Tubes were inverted gently to wash the DNA and centrifuged at 14 000 rcf for 1 minute. The ethanol was aspirated and the pellet was left to air-dry for approximately 30 minutes until all ethanol had evaporated. DNA Rehydration solution (100 µl) was added and the tube was left overnight at 4°C. The DNA concentrations of the samples were determined using a NanoDrop Spectrophotometer. Samples were diluted to 10 ng/µl and stored at -20°C until qPCR analysis was conducted.

2.3.2.3.2. qPCR

Quantitative polymerase chain reaction (qPCR) analysis was conducted according to Nicolaisen *et al.* (2009). Species specific primers used for FGSC detection were (5'-3'):

- FgramB379 fwd (nucleotide sequence: CCATTCCCTGGGCGCT)
- FgramB411 rev (nucleotide sequence: CCTATTGACAGGTGGTTAGTGACTGG) (Inqaba Biotechnical Industries).

qPCR was conducted in a 10 µl total volume reaction, consisting of 5 µl iTaq^{TM} Universal SYBR$^{®}$ Green (BIO-RAD), 1 µl Primer mix (0.5 µl FgramB379 and 0.5 µl FgramB411), 3.2 µl Nuclease-free water (Promega) and 0.8 µl template DNA. A no template control to detect contamination, FGSC positive control and matrix matched standards diluted 4, 16, 64, 256 times were included for the standard curve. Reactions were done in triplicate for accuracy. qPCR was conducted on a Bio-Rad C1000 thermal cycler with a CFX96 real-time attachment (Bio-Rad, Hercules, USA) containing a 96-well reaction plate with the following cycling conditions: 10 minutes at 95°C, 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds and 72°C for 15 seconds. A melt curve was included at 72°C to 95°C with 1°C increments. Efficiency was determined and ranged from 90 to 110%.

2.3.3. Mycotoxin accumulation in sorghum grain associated with FGSC

Mycotoxin extraction was conducted on each pooled sample using the modified method of Small *et al.* (2012). Sorghum grain from the respective field trials was ground into fine meal using a coffee grinder. Sorghum meal (5 g) was added to test tubes containing 20 ml 70% methanol (Merck). The test tubes were shaken in a Heidolph Multi Reax Shaker (Labotec) at 1470 rpm for 30 minutes. The tubes were centrifuged at 3000 rcf (BHG Optima) for 10 minutes, the solution was filtered through a 0.45 µl Pall Acrodisc syringe filter into 2 ml Eppendorf tubes and left overnight at 4°C. The following day the Eppendorf tubes were centrifuged (Hermle, Lasec) for 10 minutes at 14 000 rcf. The content of tubes prepared for NIV and ZEA were diluted ten fold, while DON was left undiluted (Coetzee, 2015). The samples were placed in 1.8 ml vials. Matrix matched standards with the range of 100 µg/kg to 12500 µg/kg were developed with mycotoxin standards obtained from Sigma-Aldrich and an identified "mycotoxin free" sorghum grain sample.

A 4000 QTRAP hybrid triple quadrupole ion trap mass spectrometer (AB Sciex), Shimadzu UFLC stack with LC-20AB binary pump and SIL-20A HT autosampler was used to analyze the mycotoxin samples in collaboration with Dr. G. Kemp, Biochemistry and Microbiology Department of the University of the Free State. The samples were separated on a C18 (150 x 2.1 mm x 5µ, Discovery C18, Supelco) column. The mobile phase consisted of 10 mM ammonium acetate (mobile phase A) and methanol/10 mM ammonium acetate (mobile phase B) with a flow rate of 300 µl/min using a stepwise gradient between the different mobile phases, starting with 2% mobile phase B increasing rapidly to 75%, holding it for 4 minutes and returning to 2% for a total runtime of 9 minutes. A negative electrospray mode with a 4500 V ion spray voltage, 500°C heater temperature, 60 psi nebuliser gas, 60 psi heater gas and 25 psi curtain gas was used to ionize eluting analytes. The injection volume for each sample was 20 µl. The instrument used a Multiple Reaction Monitoring workflow to analyse the sample in which the instrument is used in triple quadrupole mode. Fragment masses were produced by ionized analytes eluting off the column and a set of masses created a transition. The instrument jumped between different transitions in a Multiple Reaction Monitoring transition during a cycle. For each transition detected, the ion intensity value was plotted. Data outputs were processed using Analyst 1.5 (AB Sciex) software.

2.3.4. Data analysis

Data were analysed using NCSS (Hintze, 2001) by means of Analysis of Variance (ANOVA). Means separation was done using Fisher's Protected LSD (P<0.05). AMMI analyses were conducted on grain visual ratings and ergosterol levels using GenStat (18th Edition) to indicate stability of genotype responses over environments. Unreplicated ANOVA was conducted on FGSC and mycotoxin concentrations using Excel 2013. Regression analysis to determine the FGSC and mycotoxin responses of lines to changing environmental conditions, was conducted according to McLaren (1992) and Flett and McLaren (1994) using the model $y=ax^b$ where y=observed value in a specific line, x=FGSC mycotoxin potential and a and b are regression parameters.

2.4. RESULTS

2.4.1. Grain mold assessment

2.4.1.1. Grain visual ratings

All sorghum lines were susceptible to grain mold in all environments in which field trials were conducted. Mean visual grain ratings across all environments ranged from 1.43 in RTX 436 to 3.33 in Hegari (Table 2.2). Based on means separation using Fisher's Protected LSD, lines RTX 436, SCAY 14, SCAY 21, SCAY 13, BTX 635 and SC 630-11Eii fell into a distinctly less susceptible group across all environments. Hegari, RTAM 2566, Dobbs, RTX 430, TX 2911 and RTAM 428 fell into a distinctly more susceptible group. Noteworthy, is that most of the latter group displayed a purple plant colour compared to tan plants in the former group. No specific grain characteristics could be associated with the response of the grain to grain mold based on visual grain ratings.

Although visual ratings differed significantly in the environments used, all environments were conducive to grain mold development. Higher visual ratings were observed at Cedara compared with Alma. The genotype x environment interaction was significant (P<0.05), indicating differential responses of genotypes to grain mold pathogens at the different environments. Additive main effects and multiplicative interaction (AMMI) analysis indicated significant interaction principle component axis effects, i.e. IPCA1 (interaction principal

component analysis) and IPCA2, however the respective IPCA values <1 suggest that the scale of the interactions is relatively small and that genotype responses over the environments tested remained relatively stable. Sum of squares indicated that genotypes had the biggest influence on visual rating (39.67%), followed by the genotype x environment interaction (26.33%) and environment (8.25%). This was also indicated in the AMMI biplots where all but two genotypes were stable in most environments (Figure 2.1, Figure 2.2). IPCA1 and IPCA2 contributed 47.60% and 18.90% to sum of squares of genotype x environment interaction, indicating that complex interactions are involved in visual grain mold development. IPCA1 and IPCA2 were plotted against one another to further indicate genotype stability (Figure 2.2). Stability remained intact within changing environments with IPCA scores >-1 or <1.

2.4.1.2. Evaluation of ergosterol content to determine fungal biomass in sorghum grain

Ergosterol was recorded in grains from all sorghum lines and mean ergosterol levels ranged from 318.08 µg/g in SC 630-11Eii to 1178.06 µg/g in TX 2911 (Table 2.3). Means separation using Fisher's Protected LSD indicated that SC 630-11Eii, Hegari and BTX378 were a distinctly more resistant group compared with Tx2911 and RTX 436 at the opposite end of the spectrum. This ranking was in contrast to visual ratings and no relationship was observed between visual rating and ergosterol levels ($R^2=0.32^{n/s}$). In contrast to visual ratings, purple plants displayed lower mean ergosterol levels (493.03 µg/g) compared to tan plants (666.08 µg/g), supporting the notion that visual rating does not provide a true representation of grain colonization by pathogens. Ergosterol levels at the different environments ranged from a mean of 107.11 µg/g at Alma 12/13 (2) to 1606.41 µg/g at Alma 13/14 with no significant differences between the former, Cedara 11/12 (1) and Cedara 11/12 (2). Also, in contrast with visual rating, Alma had the highest ergosterol levels (728.21 µg/g) compared to Cedara (499.27 µg/g). A significant genotype x environment interaction ($P<0.05$) was recorded indicating response differences in lines in the different environments. Based on the sum of squares of ANOVA, the observed ergosterol levels were mostly influenced by environments (35.75%), followed by the genotype x environment interaction (27.60%) and genotype (10.73%).

AMMI analysis indicated that genotype responses were unstable across environments with IPCA1 scores -1< or >1. AMMI biplots also indicated that the genotypes were adapted to different environments (Figure 2.3, Figure 2.4). Seven genotypes were unstable and adapted to higher grain mold potential environments with increased colonisation (Quadrant II and III).

Fourteen genotypes were unstable and adapted to lower grain mold potential environments with decreased colonisation (Quadrant I and IV). Only RTX 436 was stable in high potential environments. IPCA1 and IPCA2 contributed 48.08% and 28.34% to sum of squares of genotype x environment respectively. IPCA1 and IPCA2 were plotted to further examine genotype stability. This indicated that lines reacted significantly to the second principle component.

2.4.1.3. qPCR to quantify FGSC in sorghum grain lines

All the sorghum lines tested were susceptible to colonisation by the FGSC. Analysis of Variance for unreplicated data indicated significant differences in DNA levels that ranged from 18.46 µg/g in RTam 428 to 203.89 µg/g in RTX 2917 (Table 2.4). FGSC DNA concentrations were generally low in most lines, with higher levels of colonisation in RTX 2917, BTX 635, TX2911, SC109-14E, RTX 436 and RTAM 2566. Mean FGSC DNA levels differed significantly across environments and ranged from 5.22 µg/g at Alma 13/14 to 302.09 µg/g at Cedara 09/10 (Table 2.4). No specific plant or grain colour and texture or combination of these traits were associated with levels of grain colonisation by FGSC fungi. Based on the sum of squares of the ANOVA, the environment accounted for 45.18% of variation in FGSC DNA levels compared to genotypes with only 11.96%.

Since replications were pooled in the analysis of FGSC DNA levels, the stability of line responses to changing environments and grain colonisation by FGSC (as indicated by FGSC DNA level) were determined using regression analysis with mean FGSC DNA levels over all lines associated with an environment as an independent variable (termed FGSC DNA potential) and observed responses within lines at those environments as dependent variable (McLaren, 1992; Flett and McLaren, 1994). A linear relationship between disease potential and observed line colonisation by the FGSC potential is indicated with $b=1$. Where $b<1$, susceptibility is indicated despite low FGSC DNA potential, while $b>1$ indicates resistance despite a higher FGSC potential (Table 2.4). Four lines had a linear, twelve lines a susceptible and six lines a resistant response to FGSC DNA potential. The range of responses is illustrated in Figure 2.5. The FGSC DNA potential required to induce colonisation of 25 µg/g of FGSC DNA (as arbitrary value) was calculated by substitution, with the rate of colonisation an indicator of resistance breakdown calculated using $dY/dX = AbX^{(b-1)}$. The onset potential for colonisation at 25 µg/g varied between 4.61 for BTX 635 and 97.83 for SCAY 13. Rate of resistance

breakdown varied from 0.13 µg/g per potential unit in RTam 428 to 4.29 µg/g per potential unit in BTX 635

2.4.2. Mycotoxin accumulation in sorghum grain lines associated with FGSC

Significant differences in the mycotoxin levels within grain of sorghum lines were recorded, although levels were generally low. Mean DON (Table 2.5) and NIV levels (Table 2.6) over all environments ranged from 1.82 µg/kg and 18.24 µg/kg in RTam 428 respectively, to 174.06 µg/kg and 243.04 µg/kg in BTX 635. Variation within DON levels was high due to the low and zero levels recorded in some of the lines. A limited number of differences in the levels of the mycotoxin were recorded between line means. BTX 635 was particularly susceptible to DON accumulation compared to the remaining lines. A trend was recorded with NIV levels in which all lines but two i.e. BTX 635 and RTX 436, did not differ significantly from one another, the latter two lines forming a distinct susceptible group. ZEA levels (Table 2.7) ranged from 4.14 µg/kg in SCAY 16 to 341.49 µg/kg in SC 748-5. With the exception of SC 748-5 and Hegari which formed a distinct susceptible group, remaining lines did not differ significantly in ZEA levels.

No significant differences were observed between the DON and ZEA levels produced in the Alma environments, although levels were significantly different between Alma and Cedara environments. Trials conducted at Cedara had the highest DON, NIV and ZEA levels (69.52, 95.16, 108.02 µg/kg respectively) compared to Alma (5.72, 8.54, 5.56 µg/kg respectively), which coincides with recorded DNA levels (Table 2.4). NIV levels at Cedara 09/10 were significantly different from all the other environments. Alma trial 12/13 (1) was the only season in which DON was not detected in 15 of the lines, zero detected NIV in all but one (SC 103-12E) and zero detected ZEA in any of the lines. No specific plant or grain characteristics could be associated with the levels of mycotoxin recorded. Based on the sum of squares of ANOVA, the environment contributed the most to the observed DON and NIV levels (25.67% and 30.65% respectively) compared to the genotypes (12.00% and 11.84%), whereas with ZEA the lines contributed most with 17.17% and the environment 15.29%.

Stability of mycotoxin response in lines to changing environment was determined using regression analysis as applied above, using the relationship between the respective mycotoxin potential associated with a specific environment and observed mycotoxin level in lines. Onset

mycotoxin level (arbitrarily taken at 100 µg/kg) and rate of mycotoxin accumulation indicated potential resistance to mycotoxin accumulation. Significant regression relationships were recorded in thirteen lines for DON. Six lines yielded a b<1 indicating a more rapid response of lines to mycotoxin potential, while seven lines yielded b>1 indicating slower response of lines to changing mycotoxin potential. The onset potential at 100 µg/kg varied between 93.20 for BTX 3197 and 2192.14 for TX 2911. Mycotoxin accumulation rate varied from 0.02 per potential unit for TX 2911 to 3.99 per potential unit for SCAY 14.

Significant relationships between observed NIV accumulation in lines and mycotoxin potential were recorded in 20 lines. One line (BTX 378) with b=1 indicated a linear relationship between observed and potential, thirteen lines with b<1 indicated a more rapid response in mycotoxin accumulation with changing mycotoxin potential and six lines with b>1 indicated a slower response to mycotoxin potential. The onset potential at 100 µg/kg varied between 12.59 for RTX 2917 and 1029.80 for SCAY 16. Mycotoxin accumulation rate varied from 0.04 per potential unit for SCAY 16 to 11.94 per potential unit for RTX 2917.

Nineteen significant relationships were recorded for ZEA, with one line yielding a b=1 (RTam 428), fourteen lines b<1 and four lines with b>1. The onset potential at 100 µg/kg varied between 13.95 for Hegari and 152311.98 for SC719-11E. Mycotoxin accumulation rate varied from 0.00 per potential unit for three lines (BTX 635, RTX 2917 and SC 719-11E) to 10.37 per potential unit for SC 748-5.

2.5. DISCUSSION

Visual rating of pericarp discolourations can be considered when rapid screening of multiple samples need to be conducted (Bandyopadhyay and Mughogho, 1988). However, these are not always effective in portraying the entire level of grain colonisation. Visual ratings provide an indication of surface infection, visible to the naked eye and therefore, not sufficient for determining internal colonisation of the grain. In some cases, infection that occurs during favourable conditions between flower initiation and soft dough stages, followed by subsequent unfavourable conditions until maturity, may not show any visible symptoms as these fungi remain hidden within the grain (Navi *et al.,* 2005). It can therefore be assumed that some colonisation by fungi may not produce visible symptoms and this could account for the absence

of a relationship between visual ratings and ergosterol levels detected (the latter indicating total fungal biomass).

Sorghum genotypes, particularly those with a purple plant colour, contain pigments in the pericarp associated with "purple wound response" which are produced in a protective state when *Fusarium* spp. invade developing grains (Funnell, 2006). This could explain the higher apparent symptom development observed in purple plants (Hegari, RTam 2566, Dobbs and RTX 430) compared to tan plants when visual ratings are applied. Van Rooyen (2012) questions whether observed discolourations on colonised sorghum root tissues are an indication of a resistance/defense response to colonisation by pathogens or a sign of tissue degradation. Purple/red coloured plants have higher levels of total phenolic compounds compared to tan coloured plants (Dykes *et al*., 2005) and many of these phenolic compounds have been associated with resistance to pre- and post-maturity grain mold (Hahn *et al*., 1984). This further confounds a question as to the value of visual ratings.

Three Cedara environments with highest environmental means, indicated eleven genotypes with lower than the population mean visual grain mold ratings, indicating the presence of, at least superficial resistance in these high risk areas. AMMI analysis also indicated stability of the genotype responses across varying environments, hereby supporting the notion that genotype contributed more to visual grain mold development than the environment. No specific grain characters were associated with resistance. Seed colour of lines with low grain ratings varied from white, yellow to red, while in high grain rating lines seed colour varied from white, red to brown, combined with either a chalky or pearly texture. This suggest that more complex interactions are involved in resistance rather than simple relationships with the grain characteristics considered in the current study. Grain mold resistance has been observed in darker pericarp colours and darker glumes, which are correlated with enhanced tannin and phenolic compound levels (Doherty *et al.,* 1987), while resistance in white grain has been attributed to the presence of proteins (Rodriguez-Herrera *et al.,* 1999). Harder grain also contains proteins (Reddy *et al.,* 2000) that are antagonistic to fungi (Chandrashekar and Satyanarayana, 2006) and include glucanase, sormatin, chitinase and ribosome-inactivating proteins (Das *et al.,* 2012).

Ergosterol as a measure of fungal biomass was used as an effective indication of colonisation by fungi and thus resistance to fungal colonisation. Jambunathan *et al.* (1991) suggested that

sorghum cultivars with ergosterol levels lower than 30 µg/g could be considered resistant to grain mold pathogens. In our study this would only amount to 5.11% of all the lines x environment combinations tested. In this study, the environment had the biggest influence on the observed ergosterol levels, with higher levels observed in drier Alma region than the wetter Cedara region. Prom *et al.* (2003) reported that only cultivars treated with *Fusarium thapsinum* in drier environments displayed high grain mold severities and germination difficulties due to an absence of competitive activities from other grain colonisers more adapted to wetter conditions. This was in contrast with higher visual grain ratings from the wetter Cedara environment, which coincide with reports by Bandyopadhyay and Mughogho (1988). Prom *et al.* (2003) also observed higher superficial grain mold severity when sorghum was exposed to prolonged wetness periods, which led to the notion that wetter conditions favoured superficial colonisation. It is clearly evident that the epidemiologies of grain colonisers differ and a more detailed component analysis of grain mold epidemiology is required.

In contrast to visual ratings, AMMI analysis using ergosterol as evaluation criterion indicated that the response of lines to grain mold pathogens was unstable and adapted to different environments. Rodriguez-Herrera *et al.,* (2000) indicated that environments favour a specific resistance mechanism which results in differential response of the genotypes to grain pathogens across environments. Only RTX 436 yielded a stable response, although this stability was associated with susceptibility to grain mold pathogens in high risk areas.

Fifteen lines had mean lower ergosterol levels than the environmental means indicating that although environmental stability was a factor, resistance selection may be possible. Jambunathan *et al.* (1991) could only relate lower ergosterol levels to flavan-4-ols contents in coloured grains, and not in white grains. Other resistance traits were suggested, such as antifungal proteins (Rodriguez-Herrera *et al.,* 1999). Funnell (2006), observed that white pericarp kernels on tan plants were more susceptible to *Fusarium* spp. infection than red pericarp kernels on purple plants. This was consistent with line SC 630-11Eii which is a red grain and purple plant colour that displayed the lowest ergosterol levels. However, the highest ergosterol levels were also from a red grain line (TX 2911), which indicates that in the current study no single grain characteristic could be associated with reduced colonisation by grain mold pathogens. This is in agreement with Rao *et al.* (2012) where no particular pericarp colour was linked to lower levels of ergosterol. This strengthens the notion of complex interactions involved in grain mold resistance in contrast to visual grain ratings in the current study. Purple

plants displayed lower ergosterol levels compared to tan plants raising the question as to the potential role of translocates (Dodd, 1980) from plant tissues to grains in the suppression of internal grain mold pathogens.

In contrast with ergosterol levels, FGSC levels were higher in the wetter Cedara than Alma environments. Prom *et al.* (2003) and Navi *et al.* (2005) reported that infection levels fluctuated during prolonged wet weather conditions with different grain mold fungi, hereby indicating environmental preferences of pathogens. It was suggested that fungi took advantage of different windows of opportunity for maximum infection occurrence (Prom *et al.,* 2003; Navi *et al.,* 2005), which could explain the differences in ergosterol levels (total fungal biomass) vs. specific FGSC DNA levels. RTam 2566 had zero FGSC DNA in the first Alma trial (2012/13), although a high ergosterol level of 1019.70 µg/g was measured. This could indicate colonisation by other fungi before FGSC had an opportunity to do so in the drier environment. In inoculated plots, Reid *et al.* (1999) compared DNA levels of *F. graminearum sensu lato* and *F. verticillioides* with ergosterol levels and found that fungal biomass was not always correlated with the anticipated pathogen and that the faster growing *F. verticillioides,* could out-compete *F. graminearum sensu lato* in drier climates.

No plant or grain characteristics were associated with levels of colonisation by FGSC, which was mostly attributed to environmental influence, hereby emphasizing the need for quantification of G x E interactions in selecting genotype adaptation to pathogen-favourable conditions (Sabaghnia *et al.,* 2008). The "stability" of a genotype can be determined by obtaining a comparative constant yield or disease resistance under variable environmental conditions without being dependent on specific environmental requirements (Sabaghnia *et al.,* 2008). Emphasis on these interactions has contributed to selection of ergot escape resistance in sorghum (McLaren, 1992) and *Stenocarpella maydis* ear rot in maize (Flett and McLaren, 1994). This approach could be considered when dealing with sorghum grain mold.

Many mycotoxins are produced by fungal pathogens to disable plant defense mechanisms for disease establishment (Brosch *et al.,* 1995). Chemotypes produced within the FGSC include NIV and DON and derivatives of these i.e. 4-acetylnivalenol, 3-acetyldeoxy-nivalenol and 15-acetyldeoxynivalenol (Wang *et al.*, 2011). The two DON chemotypes, 15-acetyldeoxynivalenol and 3-acetyldeoxy-nivalenol, are found within *F. graminearum sensu stricto* (Boutigny *et al.,* 2011b) pathogens causing *Fusarium* head blight of wheat (Tralamazza

et al., 2016) and barley (Astolfi *et al.,* 2011; Boutigny *et al.,* 2011a), with the 15-acetyldeoxynivalenol chemotype being the most abundant (Boutigny *et al.,* 2011b). *F. boothii*, causal pathogen of *Gibberella* ear rot on maize (Desjardins and Proctor, 2011) also belongs to the 15-acetyldeoxynivalenol chemotype (Boutigny *et al.,* 2011b). NIV is produced by *F. acacia-mearnsii*, *F. cortaderiae* and *F. meridionale* on sorghum (Mavhunga, 2013). Differences between lines in the accumulation of DON, NIV and ZEA were limited and no specific grain or plant characteristic could be associated with reduced mycotoxin levels in the grain. DON levels in grains other than sorghum are generally much higher compared to NIV and ZEA (Placinta *et al.,* 1999). DON and ZEA are both produced by *F. graminearum sensu stricto* and *F. culmorum*, and therefore they can be found coexisting in the same samples (Reddy *et al.,* 2010). In this study DON levels were lowest which coincides with Coetzee (2015) who also found lower levels of DON and higher levels of NIV in sorghum grain. These observations further support the view that the primary fungal pathogens within the FGSC in sorghum grain are the NIV producers *F. meridionale* and *F. acacia-mearnsii* (Mavhunga, 2013).

Although environmental conditions at Cedara promoted mycotoxin production, none of the DON levels detected were higher than the advised maximum level of 1000 µg/kg set by the Food and Drug Administration (2011) for human products or 1250 µg/kg set by the European Union (Bhat *et al.,* 2010). The differences in the allowed amounts indicate the variations between safety standards of different countries. Only 17.05% of all the lines recorded lower levels of NIV than the total dietary intake set at 0.70 µg/kg bodyweight by the Scientific Committee on Food of the European Commission (Schothorst and Van Egmond, 2004). A similar trend was recorded with ZEA where only 32.95% of lines had lower levels than the maximum tolerable intake of 0.50 µg/kg as set by The Joint Committee FAO/WHO on Food Additives (Zinedine *et al.,* 2007). These high mycotoxin levels indicate that although some level of resistance to grain mold exist, resistance to mycotoxin accumulation still needs to be addressed.

2.6. CONCLUSION

Although no particular plant or grain characteristic was associated with grain mold resistance, the environment in which the sorghum plant was cultivated and the differential G x E interactions contributed to the severity of infection recorded. The measurement of host response to grain mold pathogens was further confounded by the rating criterion applied as indicated by the absence of correlations between the various criteria used in the current study. Environmental conditions, such as prolonged wetness, favoured the growth of FGSC, while the drier Alma conditions favoured colonisation by other pathogens not quantified in the study, but indicated by ergosterol levels in the grain. It is therefore important to consider multiple factors when screening for resistance and to examine the stability of measured responses through testing in diverse environments. AMMI analysis and regression analysis can be applied to identify genotypes that are more stable to changing environments. Continuous updating of assessment protocols is necessary for choosing grain mold resistance characters worth persuing ie in future breeding programs.

2.7. REFERENCES

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Table 2.1 Twenty-two sorghum lines representing a range of plant and grain characteristics used for evaluating grain mold resistance and genotype x environment interaction.

Line No	Line	Plant	Pericarp	Mesocarp	Testa	Spreader	Plant colour	Seed colour
1	BTX 378	PPQQ	RRYYII	zz	b1b1B2B2	SS	purple	red, chalky
2	BTX 3197	PPQQ	RRyyii	zz	b1b1B2B2	SS	purple	white, chalky
3	RTX 430	PPQQ	Rryyll	ZZ	b1b1b2b2	SS	purple	white, pearly, yellow endosperm
4	Rtam 428	PPqq	rryyll	ZZ	b1b1B2B2	ss	red	white, pearly
5	RTX 436	ppQQ	Rryyii	ZZ	B1B1b2b2	--	tan	white
6	BTX 635	ppQQ	Rryyii	ZZ	--	--	tan	white pearly food grad sorghum
7	BTX ARG-1	ppQQ	Rryyii	ZZ	--	--	tan	waxy endosperm
8	RTX 2917							
9	SC 630-11EII	PPQQ	RRYYII	ZZ	b1b1B2B2	SS	purple	dark red, pearly
10	SC 630-11Eii	PPQQ	RRYYii	ZZ	b1b1B2B2	SS	purple	light red, pearly
11	SC 748-5	PPQQ	rrYYIl	ZZ	b1b1B2B2	ss	purple	lemon yellow, pearly
12	SC 109-14E	PPQQ	Rryy--	zz	B1B1B2B2	ss	purple	white pearly, purple testa
13	SC719-11E	PPQQ	RRYYii	zz	B1B1B2B2	ss	purple	red, chalky,
14	SC103-12E	PPQQ	RRYYII	zz	B1B1B2B2	SS	purple	dark brown-red, chalky
15	Dobbs	PPQQ	Rryyii	zz	B1B1B2B2	SS	purple	brown, chalky
16	Hegari	PPQQ	Rryyii	zz	B1B1B2B2	ss	purple	white, chalky
17	Rtam 2566	PPQQ	RRYYII	ZZ	B1B1B2B2	ss	purple	dark brown-red, pearly
18	TX 2911							
19	SCAY 13						tan	lemon-yellow
20	SCAY 16							
21	SCAY 21						tan	red
22	SCAY 14						tan	lemon-yellow

Table 2.2 Grain visual ratings and AMMI analysis for twenty-two sorghum lines representing a range of plant and grain characters evaluated at Cedara and Alma for grain mold resistance over eight environments.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Total	527	407.10	0.77		
Treatments	175	302.20	1.73	5.82	<1.00E-03
Genotypes	21	161.50	7.69	25.91	<1.00E-03
Environments	7	33.60	4.79	14.71	<1.00E-03
Block	16	5.20	0.33	1.10	0.36
Interactions	147	107.20	0.73	2.46	<1.00E-03
IPCA1	27	47.60	1.76	5.94	<1.00E-03
IPCA2	25	18.90	0.76	2.54	<1.00E-03
IPCA3	23	15.90	0.69	2.33	<1.00E-03
IPCA4	21	9.70	0.46	1.55	0.06
Residuals	51	15.10	0.30	1.00	0.48
Error	336	99.70	0.30		

Genotype	**Line**	**Cedara**					**Alma**			**Mean**	**IPCA scores - genotypes**			
		09/10	**10/11 (1)**	**10/11 (2)**	**11/12 (1)**	**11/12 (2)**	**12/13 (1)**	**12/13 (2)**	**13/14**		**1**	**2**	**3**	**4**
G1	BTX 378	2.95	2.27	2.61	2.27	2.23	2.45	2.28	2.88	**2.49**^{cd}	0.08	0.18	-0.15	-0.09
G2	BTX 3197	2.20	1.63	1.60	1.49	1.61	2.29	2.24	3.77	**2.10**^{ef}	-0.71	0.20	0.07	-0.13
G3	RTX 430	1.95	3.18	2.88	2.54	2.77	2.53	2.35	3.81	**2.75**^{bc}	-0.17	-0.56	0.31	0.41
G4	RTam 428	2.18	2.67	2.56	2.34	2.44	2.30	2.15	3.44	**2.51**^{cd}	-0.14	-0.28	0.11	0.30
G5	RTX 436	1.43	1.31	1.17	1.58	1.44	0.93	0.92	2.65	**1.43**^{j}	-0.27	-0.06	-0.19	0.63
G6	BTX 635	2.30	0.87	1.27	1.11	0.94	1.19	1.45	3.12	**1.53**^{hij}	-0.59	0.29	-0.53	-0.09
G7	BTX ARG-1	1.30	1.74	1.39	1.17	1.65	2.99	2.18	2.28	**1.84**^{fgh}	-0.17	0.26	0.97	-0.36
G8	RTX 2917	2.11	1.86	1.79	1.47	1.76	2.82	2.48	3.36	**2.21**^{de}	-0.50	0.23	0.41	-0.35
G9	SC 630-11EII	2.27	1.83	2.36	1.17	1.21	1.36	1.86	3.83	**1.99**^{efg}	-0.63	-0.38	-0.53	-0.30
G10	SC 630-11Eii	2.15	1.89	2.76	1.07	1.18	1.56	1.46	1.36	**1.68**^{ghij}	0.52	-0.24	-0.24	-0.65
G11	SC 748-5	2.04	3.19	3.51	2.14	2.37	2.16	1.96	2.37	**2.47**^{cd}	0.51	-0.72	0.09	-0.06
G12	SC 109-14E	2.46	2.30	2.13	2.54	2.56	2.75	2.19	2.60	**2.44**^{cd}	0.16	0.31	0.31	0.28
G13	SC 719-11E	2.76	2.24	2.45	2.22	2.23	2.56	2.28	2.80	**2.44**^{cd}	0.06	0.21	0.01	-0.08
G14	SC 103-12E	3.22	1.49	1.96	2.37	2.03	2.10	1.69	1.52	**2.05**^{ef}	0.53	0.80	-0.33	0.08
G15	Dobbs	3.29	2.98	3.46	2.95	2.84	2.65	2.30	2.14	**2.83**^{b}	0.75	0.04	-0.17	0.03
G16	Hegari	3.92	2.94	3.23	3.25	3.10	3.16	3.06	3.95	**3.33**^{a}	-0.05	0.31	-0.28	0.08
G17	RTam 2566	3.28	3.47	3.73	3.29	3.27	3.04	2.64	2.71	**3.18**^{a}	0.64	-0.11	0.02	0.16
G18	TX 2911	2.78	2.75	2.93	2.63	2.67	2.79	2.31	2.33	**2.65**^{bc}	0.48	0.09	0.15	0.03
G19	SCAY 13	1.39	1.38	1.31	1.19	1.24	1.26	1.31	3.10	**1.52**^{ij}	-0.53	-0.15	-0.05	0.23
G20	SCAY 16	1.73	1.79	1.82	1.57	1.67	1.90	1.57	2.23	**1.78**^{ghi}	0.05	0.01	0.18	0.05
G21	SCAY 21	1.50	1.72	2.20	0.83	1.01	1.24	1.30	2.19	**1.50**^{ij}	-0.01	-0.44	-0.14	-0.35
G22	SCAY 14	1.56	1.35	1.43	1.34	1.32	1.32	1.13	2.06	**1.44**^{j}	-0.02	0.04	-0.03	0.18
Mean		**2.31**^{a}	**2.31**^{a}	**2.13**^{ab}	**2.30**^{a}	**1.93**^{c}	**1.98**^{bc}	**2.15**^{ab}	**1.96**^{bc}	**LSD(P<0.05)**	**Genotypes =**		**0.31**	
IPCA scores – Environments											**Environments=**		**0.19**	
	1	0.21	0.38	0.68	0.42	0.34	0.01	-0.33	-1.71		**GxE interaction**		**0.87**	
	2	0.81	-0.78	-0.83	0.31	0.08	0.54	0.23	-0.35					
	3	-0.97	0.26	-0.42	-0.13	0.28	0.93	0.30	-0.26					
	4	-0.30	0.08	-0.49	0.82	0.59	-0.41	-0.48	0.20					

Table 2.3 Ergosterol concetrations recorded in sorghum grain and AMMI analysis for twenty-two sorghum lines representing a range of plant and grain characters evaluated at Cedara and Alma for grain mold resistance over eight environments.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Total	527	348015227.00	660370.00		
Treatments	175	257806414.00	1473180.00	6.56	<1.00E-03
Genotypes	21	37340548.00	1778121.00	7.91	<1.00E-03
Environments	7	124427084.00	17775298.00	19.32	<1.00E-03
Block	16	14723641.00	920228.00	4.10	<1.00E-03
Interactions	147	96038783.00	653325.00	2.91	<1.00E-03
IPCA1	27	46172680.00	1710099.00	7.61	<1.00E-03
IPCA2	25	27222113.00	1088885.00	4.85	<1.00E-03
IPCA3	23	14005067.00	608916.00	2.71	<1.00E-03
IPCA4	21	6536926.00	311282.00	1.39	0.12
Residuals	51	2101996.00	41216.00	0.18	1.00
Error	336	75485171.00	224658.00		

Genotype	**Line**	**Cedara**					**Alma**			**Mean**	**IPCA scores - genotypes**			
		09/10	**10/11 (1)**	**10/11 (2)**	**11/12 (1)**	**11/12 (2)**	**12/13 (1)**	**12/13 (2)**	**13/14**		**1**	**2**	**3**	**4**
G1	BTX 378	270.50	574.70	508.40	91.40	95.70	135.40	70.30	961.00	**338.43**^{lm}	6.17	2.17	6.07	7.21
G2	BTX 3197	195.80	369.90	404.70	194.20	208.30	190.80	183.60	1139.00	**360.79**^{k}	3.24	5.82	10.94	7.57
G3	RTX 430	651.30	1028.30	1016.80	144.40	303.20	414.70	76.60	3096.00	**841.41**^{c}	-21.28	-2.68	-3.20	1.02
G4	RTam 428	110.20	517.70	349.10	32.00	80.30	174.20	7.20	1530.00	**350.09**^{kl}	-3.06	5.28	4.96	3.48
G5	RTX 436	2128.20	589.70	3041.60	105.20	13.30	1254.70	155.30	2066.00	**1169.25**^{a}	-1.00	-42.64	17.90	-6.62
G6	BTX 635	523.30	775.60	775.80	146.40	180.60	27.80	98.50	1105.00	**454.13**^{i}	5.49	-2.09	2.86	11.95
G7	BTX ARG-1	574.30	1322.80	978.90	345.10	216.40	1527.00	406.50	1384.00	**844.38**^{c}	9.16	1.72	3.08	-19.81
G8	RTX 2917	1666.40	2626.70	2151.50	136.00	160.20	443.40	19.80	1429.00	**1079.13**^{b}	12.25	-25.33	-27.03	6.19
G9	SC 630-11EII	182.80	607.10	457.30	79.30	205.80	309.60	38.20	2600.00	**560.01**^{f}	-17.60	5.83	2.76	0.33
G10	SC 630-11 Eii	246.70	552.10	453.00	146.90	139.50	114.10	128.30	764.00	**318.08**^{n}	9.12	3.68	7.31	8.88
G11	SC 748-5	207.60	1050.40	417.00	96.70	122.70	258.30	57.40	1254.00	**433.01**^{j}	3.85	5.35	-3.27	2.76
G12	SC 109-14E	709.70	1601.90	1081.50	49.50	36.10	597.80	10.90	1342.00	**678.68**^{d}	6.92	-6.64	-10.72	-3.63
G13	SC 719-11E	322.80	965.70	547.00	91.50	184.00	65.30	25.60	1758.00	**494.99**^{g}	-4.14	2.85	-3.36	7.47
G14	SC 103-12E	16.70	1107.20	276.10	96.90	36.70	888.30	118.80	1213.00	**469.21**^{h}	6.03	9.74	-1.29	-13.15
G15	Dobbs	99.40	710.30	352.70	134.70	99.40	627.70	150.40	1189.00	**420.45**^{j}	4.54	7.59	5.73	-5.04
G16	Hegari	334.20	655.70	554.80	151.20	124.80	140.10	133.30	580.00	**334.26**^{m}	12.57	1.69	6.20	8.99
G17	RTam 2566	245.30	1879.30	505.20	160.00	103.30	1019.70	151.60	1272.00	**667.05**^{d}	9.44	7.53	-14.07	-14.94
G18	TX 2911	754.70	1139.10	1155.10	228.30	545.00	504.50	119.80	4978.00	**1178.06**^{a}	-47.53	-1.12	-7.15	-1.18
G19	SCAY 13	258.40	533.30	557.60	134.50	138.40	536.70	140.60	1575.00	**484.31**^{g}	-1.60	3.69	8.52	-1.95
G20	SCAY 16	127.40	824.40	354.70	88.60	54.50	411.50	85.20	861.00	**350.91**^{kl}	8.98	6.01	2.43	-0.58
G21	SCAY 21	152.40	1591.00	271.90	91.20	176.30	19.20	11.72	1394.00	**461.79**^{hi}	2.88	8.34	-15.83	6.54
G22	SCAY 14	318.40	650.90	648.20	157.20	164.80	703.50	166.70	1851.00	**582.59**^{e}	-4.43	3.21	7.18	-5.49
	Mean	**458.93**^{d}	**985.17**^{b}	**766.31**^{c}	**131.87**^{e}	**154.06**^{e}	**471.10**^{d}	**107.11**^{e}	**1606.41**^{a}	**LSD(P<0.05)**	**Genotypes =**		**14.90**	
IPCA scores – Environments														
	1	6.27	15.40	4.39	8.52	2.59	10.70	9.87	-57.76		**Environments=**		**69.32**	
	2	-25.81	5.66	-37.95	16.38	17.38	5.14	16.42	2.78		**GxE interaction**		**324.94**	
	3	2.55	-41.41	5.02	8.71	6.05	11.79	11.90	-4.61					
	4	8.32	-2.78	1.88	9.04	13.59	-32.98	5.79	-2.87					

Table 2.4 FGSC DNA levels recorded in twenty-two sorghum lines evaluated at Cedara and Alma for grain resistance and the relationship between FGSC potential and observed FGSC colonisation over eight environments.

Source of Variation	d.f.	s.s.	m.s.	F	P-value	F crit
Genotypes	21	497405.57	23685.98	1.95	0.01	1.63
Environments	7	1878693.45	268384.78	22.13	3.05E-20	2.07
Error	147	1782548.48	12126.18			
Total	175	4158647.49				

Genotype	**Line**	**Cedara**					**Alma**			**Mean**	A-parm	B-parm	R^2	Onset Potential (25 µg/g)	Rate at 25pg	Area under disease potential curve
		09/10	**10/11 (1)**	**10/11 (2)**	**11/12 (1)**	**11/12 (2)**	**12/13 (1)**	**12/13 (2)**	**13/14**							
G1	BTX 378	97.00	102.50	103.80	18.90	13.20	5.80	2.70	10.20	**44.26**^{cd}	4.85	0.56	0.85	19.08	0.73	22226.13
G2	BTX 3197	82.00	76.10	98.70	12.70	9.90	1.80	1.20	0.70	**35.39**^{cd}	2.99	0.62	0.86	31.16	0.50	18762.71
G3	RTX 430	340.00	191.20	121.90	26.40	3.10	4.30	3.70	3.60	**86.78**^{bcd}	1.24	0.97	0.90	21.94	1.11	48816.42
G4	RTam 428	53.00	36.10	14.90	24.10	16.20	0.50	0.60	2.30	**18.46**^{d}	2.54	0.50	0.66	97.54	0.13	8722.52
G5	RTX 436	487.00	188.80	178.20	33.80	10.10	3.90	5.10	2.60	**113.69**^{abc}	0.48	1.21	0.95	26.82	1.12	63904.38
G6	BTX 635	574.20	290.20	672.00	23.20	7.90	13.10	10.80	15.60	**200.88**^{a}	7.48	0.79	0.88	4.61	4.29	114036.00
G7	BTX ARG-1	257.00	71.80	135.00	22.30	20.20	6.50	4.20	9.20	**65.78**^{cd}	0.38	1.14	0.98	39.65	0.72	35859.50
G8	RTX 2917	1216.00	184.10	178.20	9.50	24.80	6.60	5.50	6.40	**203.89**^{a}	5.38E-05	2.96	0.95	81.67	0.91	102817.20
G9	SC 630-11EII	451.30	30.90	111.90	8.70	3.20	6.40	5.70	5.20	**77.91**^{bcd}	2.83E-04	2.50	0.97	95.14	0.66	41942.76
G10	SC 630-11Eii	453.20	28.00	132.10	15.10	1.30	3.70	3.30	2.30	**79.88**^{bcd}	9.53E-04	2.30	0.98	85.39	0.67	44144.10
G11	SC 748-5	83.00	114.00	82.00	25.60	1.20	1.80	1.40	0.50	**38.69**^{cd}	4.62	0.55	0.73	22.19	0.61	19994.19
G12	SC 109-14E	487.30	160.00	334.10	33.40	1.20	13.80	13.60	11.90	**131.91**^{abc}	1.51	1.02	0.99	15.87	1.60	74573.14
G13	SC 719-11E	134.00	158.00	169.00	27.60	4.00	3.70	3.20	2.70	**62.78**^{cd}	6.30	0.58	0.80	10.62	1.37	33135.90
G14	SC 103-12E	133.00	131.60	30.00	34.10	5.30	10.50	10.00	9.30	**45.48**^{cd}	4.74	0.56	0.61	19.15	0.73	22514.76
G15	Dobbs	190.00	163.00	228.00	27.10	7.00	3.50	3.10	2.60	**78.04**^{bcd}	5.61	0.65	0.87	9.83	1.66	42399.86
G16	Hegari	137.00	103.60	162.10	36.10	8.10	11.80	11.50	10.40	**60.08**^{cd}	5.55	0.59	0.89	12.70	1.17	30555.52
G17	RTam 2566	374.00	158.00	235.90	22.90	8.20	0.00	8.10	0.90	**101.00**^{abcd}	1.47	0.97	0.99	18.48	1.31	57816.32
G18	TX 2911	530.00	121.90	691.90	12.20	84.70	3.70	4.80	3.20	**181.55**^{ab}	4.84	0.85	0.77	6.85	3.12	102799.80
G19	SCAY 13	258.00	17.90	82.00	31.90	14.80	7.50	7.40	5.60	**53.14**^{cd}	1.98E-03	2.06	0.94	97.83	0.53	26531.67
G20	SCAY 16	127.00	75.90	186.90	31.20	2.90	6.30	6.10	4.70	**55.13**^{cd}	3.90	0.65	0.79	17.14	0.95	29465.95
G21	SCAY 21	86.00	44.00	40.90	20.60	3.90	1.10	37.20	2.00	**29.46**^{cd}	2.37	0.61	0.77	48.32	0.31	14130.68
G22	SCAY 14	96.00	16.90	52.90	29.90	2.30	4.90	4.90	3.00	**26.35**^{cd}	0.21	1.07	0.89	89.04	0.30	13507.94
Mean		**302.09**^{a}	**112.02**^{c}	**183.75**^{b}	**23.97**^{d}	**11.52**^{d}	**5.51**^{d}	**7.00**^{d}	**5.22**^{d}							

LSD ($P<0.05$)	**Genotypes =**	**107.92**
	Environments=	**23.01**

Table 2.5 DON levels recorded in twenty-two sorghum lines evaluated for grain resistance at Cedara and Alma and the relationship between FGSC potential and mycotoxin accumulation over eight environments.

Source of Variation	d.f.	s.s.	m.s.	F	P-value	F crit
Genotypes	21	258749.80	12321.42	1.35	0.15	1.63
Environments	7	553417.70	79059.67	8.65	7.22E-09	2.07
Error	147	1343785.00	9141.39			
Total	175	2155952.00				

Genotype	Line	Cedara					Alma			Mean	A-parm	B-parm	R^2	Onset Potential (100 µg/kg)	Rate at 25pg	Ymax
		09/10	**10/11 (1)**	**10/11 (2)**	**11/12 (1)**	**11/12 (2)**	**12/13 (1)**	**12/13 (2)**	**13/14**							
1	BTX 378	0.00	160.00	38.70	8.70	0.00	5.80	0.00	0.00	**26.66^{bc}**	-	-	-	-	-	160.00
2	BTX 3197	115.00	58.90	97.50	6.50	0.00	0.00	5.20	1.20	**35.54^{bc}**	3.30E-01	1.26	0.91	93.20	1.35	115.00
3	RTX 430	61.40	48.30	1.30	0.00	0.00	0.00	2.80	0.00	**14.23^{c}**	-	-	-	-	-	61.40
4	RTam 428	0.00	0.70	0.80	8.30	4.70	0.00	0.00	0.00	**1.82^{c}**	-	-	-	-	-	8.30
5	RTX 436	306.00	48.60	0.00	0.00	4.80	0.00	0.00	22.00	**47.67^{bc}**	4.13E-05	2.56	0.98	314.48	0.81	306.00
6	BTX 635	689.00	313.50	344.00	0.00	0.00	9.70	33.50	2.80	**174.06^{a}**	-	-	-	-	-	689.00
7	BTX ARG-1	208.00	16.10	118.00	60.30	102.00	24.50	2.00	6.20	**67.13^{bc}**	4.40E+00	0.68	0.72	98.17	0.69	208.00
8	RTX 2917	152.00	0.00	96.50	52.50	4.00	0.00	3.30	0.00	**38.54^{bc}**	2.58E+00	0.57	0.70	592.42	0.10	152.00
9	SC 630-11EII	864.00	0.00	0.90	13.70	2.00	0.00	23.50	3.20	**113.43^{ab}**	1.0E-07	3.74	0.98	253.63	1.48	864.00
10	SC 630-11Eii	553.00	46.10	3.80	0.00	3.90	5.60	19.90	0.00	**79.03^{bc}**	4.61E-08	3.79	0.99	288.99	1.31	553.00
11	SC 748-5	5.50	2.00	48.50	0.00	2.00	5.10	39.70	0.00	**12.85^{c}**	-	-	-	-	-	48.50
12	SC 109-14E	443.00	2.00	170.00	14.30	0.00	0.00	0.00	7.70	**79.62^{bc}**	2.79E-05	2.68	0.99	278.93	0.96	443.00
13	SC 719-11E	8.40	256.00	44.60	83.80	0.00	0.00	0.00	0.00	**49.10^{bc}**	-	-	-	-	-	256.00
14	SC 103-12E	0.00	279.00	3.30	28.90	4.30	54.50	5.00	18.80	**49.22^{bc}**	-	-	-	-	-	279.00
15	Dobbs	38.60	48.10	80.00	49.10	2.50	0.00	0.00	20.60	**29.86^{bc}**	6.10E+00	0.43	0.70	688.41	0.06	80.00
16	Hegari	20.50	80.10	106.20	6.80	1.10	10.80	0.00	4.50	**28.75^{bc}**	2.06E-01	1.18	0.67	188.13	0.63	106.20
17	RTam 2566	147.00	92.60	87.10	7.90	33.50	0.00	0.00	2.70	**46.35^{bc}**	2.34E+00	0.69	0.94	224.48	0.31	147.00
18	TX 2911	67.70	51.90	49.30	24.50	0.00	0.00	0.00	4.10	**24.69^{bc}**	3.86E+00	0.42	0.68	2192.14	0.02	67.70
19	SCAY 13	71.80	81.80	67.40	19.20	0.00	0.00	4.50	9.90	**31.84^{bc}**	-	-	-	-	-	81.80
20	SCAY 16	48.50	67.40	110.00	34.00	6.50	0.00	2.10	10.20	**34.84^{bc}**	2.24E+00	0.73	0.88	188.82	0.38	110.00
21	SCAY 21	0.00	10.90	4.10	17.60	11.90	0.00	0.00	1.30	**5.72^{c}**	-	-	-	-	-	17.60
22	SCAY 14	72.50	0.00	3.30	15.90	0.00	0.00	4.80	0.00	**12.05^{c}**	4.37E-07	4.15	0.94	103.83	3.99	72.50
Mean		**176.00^{a}**	**75.64^{b}**	**67.06^{b}**	**20.56^{bc}**	**8.32^{c}**	**5.27^{c}**	**6.65^{c}**	**5.24^{c}**							

LSD ($P<0.05$)	**Genotypes =**	**93.70**
	Environments=	**56.50**

Table 2.6 NIV levels recorded in twenty-two sorghum lines evaluated for grain resistance at Cedara and Alma and the relationship between FGSC potential and mycotoxin accumulation over eight environments.

Source of Variation	d.f.	s.s.	m.s.	F	P-value	F crit
Genotypes	21	532334.50	25349.26	1.44	0.11	1.63
Environments	7	1377327.00	196761.00	11.19	2.60E-11	2.07
Error	147	2584720.00	17583.13			
Total	175	4494382.00				

Genotype	Line	Cedara					Alma			Mean	A-parm	B-parm	R^2	Onset Potential (100µg/kg)	Rate at 25pg	Ymax
		09/10	10/11 (1)	10/11 (2)	11/12 (1)	11/12 (2)	12/13 (1)	12/13 (2)	13/14							
1	BTX 378	124.90	81.00	104.80	19.70	10.40	0.00	10.00	6.30	**44.64[c]**	9.44E-01	1.02	0.92	98.43	1.03	124.90
2	BTX 3197	402.00	54.50	50.80	20.10	24.10	0.00	7.10	3.40	**70.25[bc]**	-	-	-	-	-	402.00
3	RTX 430	105.00	74.00	55.30	22.90	4.70	0.00	13.80	18.70	**36.80[c]**	3.65E+00	0.57	0.98	320.44	0.18	105.00
4	RTam 428	62.90	26.40	16.70	23.90	10.50	0.00	0.00	5.50	**18.24[c]**	2.87E-01	1.34	0.94	78.14	1.72	62.90
5	RTX 436	1380.00	49.30	0.00	0.00	1.80	0.00	11.20	9.00	**181.41[ab]**	1.77E-08	4.05	0.99	254.93	1.59	1380.00
6	BTX 635	870.00	419.00	530.00	66.40	24.10	0.00	27.70	7.10	**243.04[a]**	8.59E+00	0.68	0.87	36.73	1.85	870.00
7	BTX ARG-1	149.00	1.00	13.30	58.20	7.40	0.00	17.00	4.30	**31.28[c]**	1.33E-07	3.73	0.79	241.57	1.54	149.00
8	RTX 2917	963.00	54.90	52.90	14.60	18.00	0.00	16.90	6.00	**140.79[abc]**	2.22E+00	1.50	0.99	12.59	11.94	963.00
9	SC 630-11EII	604.00	42.80	30.10	82.10	20.90	0.00	26.40	10.80	**102.14[bc]**	3.22E-03	1.99	0.97	182.38	1.09	604.00
10	SC 630-11Eii	413.00	29.80	27.70	0.00	0.00	0.00	28.20	13.90	**64.08[bc]**	9.46E-04	2.12	0.98	232.54	0.91	413.00
11	SC 748-5	106.80	55.90	18.40	35.70	35.80	0.00	3.00	12.50	**33.51[c]**	-	-	-	-	-	106.80
12	SC 109-14E	114.00	57.00	98.90	55.20	20.30	0.00	8.20	4.20	**44.73[c]**	4.06E+00	0.54	0.88	370.07	0.15	114.00
13	SC 719-11E	116.80	80.20	90.40	58.10	25.70	0.00	26.20	0.00	**49.68[c]**	1.08E+01	0.43	0.85	169.61	0.26	116.80
14	SC 103-12E	141.00	80.30	49.70	90.10	59.20	27.60	21.40	4.60	**59.24[bc]**	1.22E+01	0.47	0.64	92.87	0.50	141.00
15	Dobbs	91.90	21.00	83.70	41.10	5.10	0.00	9.40	6.00	**32.28[c]**	3.46E+00	0.58	0.70	372.72	0.15	91.90
16	Hegari	91.90	21.90	66.50	27.60	7.50	0.00	11.50	7.20	**29.26[c]**	6.82E-01	0.94	0.76	201.60	0.47	91.90
17	RTam 2566	152.00	137.00	32.90	33.40	6.50	0.00	31.90	6.50	**50.03[c]**	7.81E+00	0.47	0.66	219.52	0.22	152.00
18	TX 2911	82.80	31.40	269.00	0.00	0.00	0.00	17.30	11.90	**51.55[bc]**	8.31E-11	0.41	0.97			269.00
19	SCAY 13	103.00	46.70	37.70	15.90	16.50	0.00	13.00	4.90	**29.71[c]**	2.69E+00	0.65	0.85	257.96	0.25	103.00
20	SCAY 16	40.90	31.20	46.40	35.50	0.00	0.00	21.00	7.20	**22.78[c]**	5.24E+00	0.43	0.80	1029.80	0.04	46.40
21	SCAY 21	154.20	133.00	74.20	48.20	18.20	0.00	18.70	9.50	**57.00[bc]**	2.52E+00	0.93	0.78	53.16	1.74	154.20
22	SCAY 14	39.90	25.30	32.70	14.20	3.80	0.00	21.80	15.10	**19.10[c]**	5.84E+00	0.42	0.66	922.94	0.05	39.90
Mean		**286.77[a]**	**67.89[b]**	**71.91[b]**	**34.68[b]**	**14.57[b]**	**1.25[b]**	**16.44[b]**	**7.94[b]**							
LSD (P<0.05)	**Genotypes =**			**129.99**												
	Environments=			**78.36**												

Table 2.7 ZEA levels recorded in twenty-two sorghum lines evaluated for grain resistance at Cedara and Alma and the relationship between FGSC potential and mycotoxin accumulation over eight environments.

Source of Variation	d.f.	s.s.	m.s.	F	P-value	F crit
Genotypes	21	1467548.00	69883.25	1.53	0.08	1.64
Environments	6	1306681.00	217780.20	4.76	2.12E-04	2.17
Error	126	5770741.00	45799.53			
Total	153	8544970.00				

Genotype	Line	Cedara					Alma			Mean	A-parm	B-parm	R²	Onset Potential (100 µg/kg)	Rate at 25pg	Ymax
		09/10	10/11 (1)	10/11 (2)	11/12 (1)	11/12 (2)	12/13 (1)	12/13 (2)	13/14							
1	BTX 378	124.10	301.00	158.00	0.00	0.40	0.00	0.70	0.60	**73.10**[bc]	4.95E-11	6.28	0.84	91.10	6.89	301.00
2	BTX 3197	486.30	559.00	390.00	0.00	0.30	0.00	2.40	76.70	**189.34**[abc]	7.05E+00	0.94	0.86	16.86	5.57	559.00
3	RTX 430	216.00	237.40	71.70	0.40	3.00	0.00	0.50	70.00	**74.88**[bc]	3.46E+00	0.73	0.79	101.01	0.72	237.40
4	RTam 428	16.20	18.90	9.60	1.80	1.50	0.00	0.40	4.50	**6.61**[c]	3.70E-01	0.98	0.64	308.35	0.32	18.90
5	RTX 436	132.50	124.00	0.00	0.00	1.40	0.00	1.80	15.20	**34.36**[bc]	6.00E-01	0.88	0.67	341.56	0.26	132.50
6	BTX 635	18.60	14.10	5.80	0.20	0.30	0.00	2.10	7.50	**6.08**[c]	1.02E+00	0.40	0.00	103016.67	0.00	18.60
7	BTX ARG-1	43.80	29.80	34.50	0.50	1.00	0.00	2.50	0.90	**14.13**[bc]	8.16E-01	0.74	0.88	693.81	0.11	43.80
8	RTX 2917	29.70	22.60	17.30	0.60	0.30	0.00	0.40	7.90	**9.85**[c]	1.74E+00	0.41	0.83	18642.81	0.00	29.70
9	SC 630-11EII	144.60	98.20	132.00	0.40	1.20	0.00	0.20	60.30	**54.61**[bc]	1.50E+01	0.39	0.71	127.37	0.31	144.60
10	SC 630-11Eii	69.00	39.20	46.60	0.00	0.60	0.00	0.90	1.30	**19.70**[bc]	3.87E+00	0.48	0.85	876.47	0.05	69.00
11	SC 748-5	2.60	2490.00	237.00	0.10	0.40	0.00	0.90	0.90	**341.49**[a]	1.71E-13	7.86	0.99	75.77	10.37	2490.00
12	SC 109-14E	184.30	122.00	130.00	0.30	0.60	0.00	1.80	4.30	**55.41**[bc]	1.79E+00	0.75	0.93	211.01	0.36	184.30
13	SC 719-11E	2.40	11.70	7.50	10.80	1.60	0.00	0.70	0.00	**4.34**[c]	1.27E+00	0.37	0.44	152311.98	0.00	11.70
14	SC 103-12E	0.30	8.10	0.60	25.10	0.30	0.00	0.50	33.60	**8.56**[c]	-	-	-	-	-	33.60
15	Dobbs	187.30	286.20	720.00	61.30	0.30	0.00	1.10	39.90	**162.01**[abc]	2.16E-08	4.46	0.91	147.56	3.02	720.00
16	Hegari	322.40	791.00	410.00	245.00	1.00	0.00	0.30	1.20	**221.36**[ab]	1.65E+01	0.68	0.61	13.95	4.90	791.00
17	RTam 2566	466.00	146.00	210.00	7.40	0.30	0.00	0.30	1.10	**103.89**[bc]	6.15E-02	1.51	0.99	135.59	1.11	466.00
18	TX 2911	8.80	28.50	0.90	0.00	0.00	0.00	0.40	0.40	**4.88**[c]	-	-	-	-	-	28.50
19	SCAY 13	326.50	215.30	204.00	41.50	0.70	0.00	0.40	2.10	**98.81**[bc]	1.10E+01	0.62	0.71	35.80	1.73	326.50
20	SCAY 16	8.70	10.10	0.30	0.20	0.00	0.00	0.90	12.90	**4.14**[c]	-	-	-	-	-	12.90
21	SCAY 21	91.60	116.30	76.90	16.40	1.20	0.00	0.90	0.80	**38.01**[bc]	2.48E+00	0.84	0.62	81.62	1.03	116.30
22	SCAY 14	13.20	2.00	12.00	12.30	0.80	0.00	1.20	3.20	**5.59**[c]	9.32E-01	0.61	0.78	2159.20	0.03	13.20
Mean		**131.59**[ab]	**257.79**[a]	**130.67**[b]	**19.29**[bc]	**0.78**[c]	**0.00**[c]	**0.97**[c]	**15.70**[bc]							

LSD (P<0.05) **Genotypes =** **209.73**

Environments= **126.47**

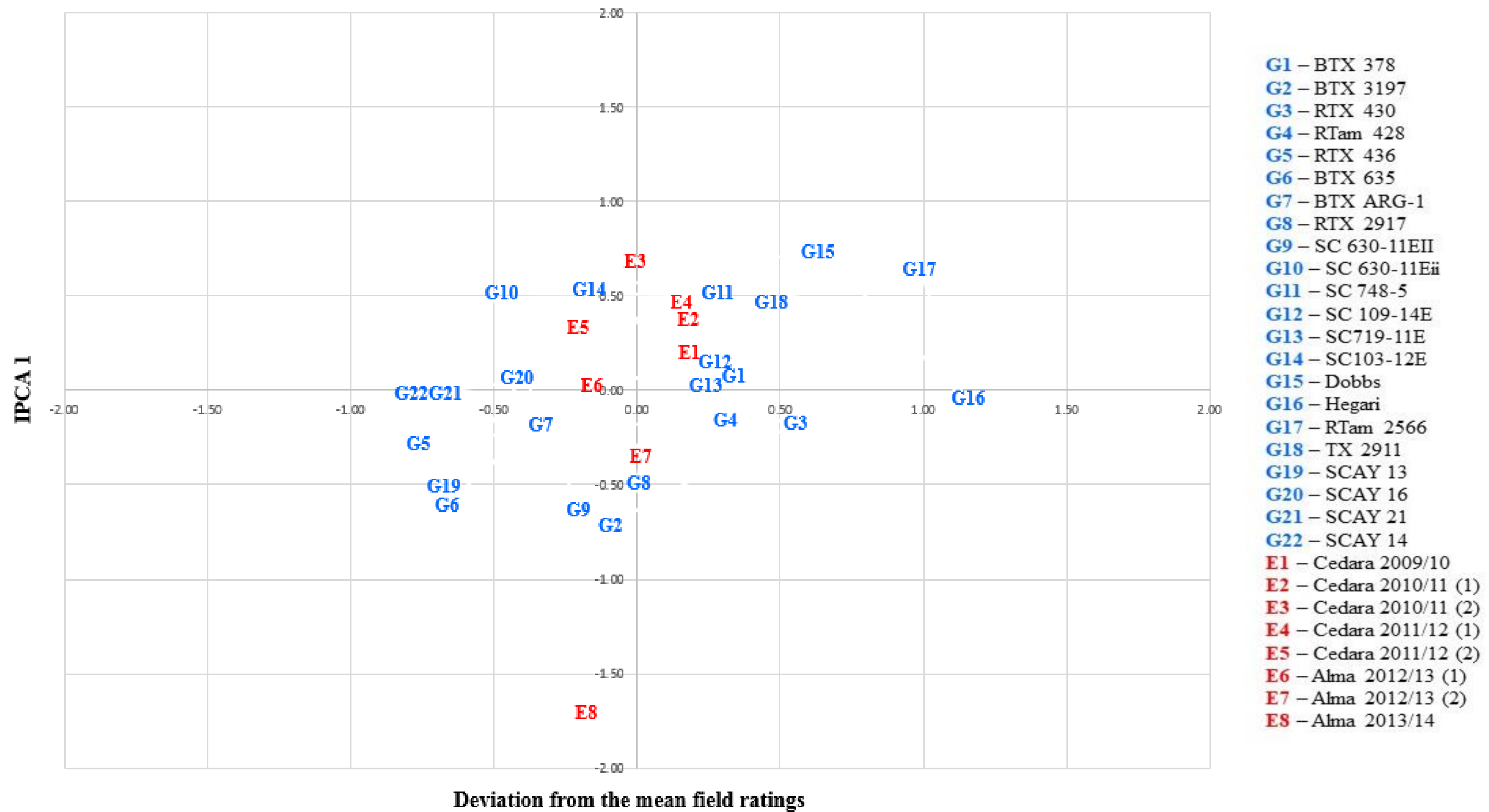

Figure 2.1 AMMI biplot of mean field visual ratings for sorghum grain from twenty two sorghum lines evaluated at Cedara and Alma for grain mold resistance over eight environments.

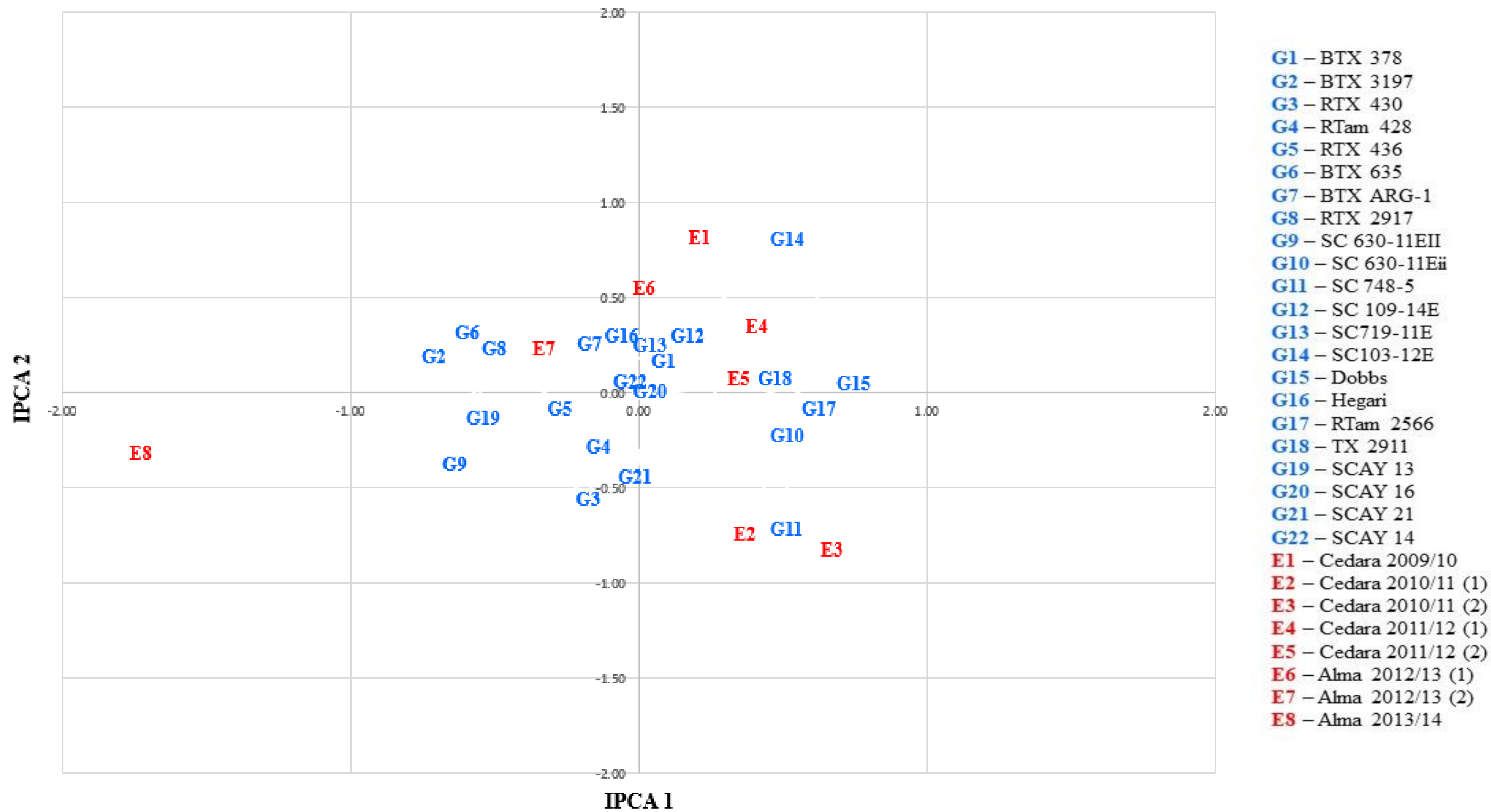

Figure 2.2 AMMI biplot to indicate the interaction between principle components of field visual ratings for sorghum grain from twenty two sorghum lines evaluated at Cedara and Alma for grain mold resistance over eight environments

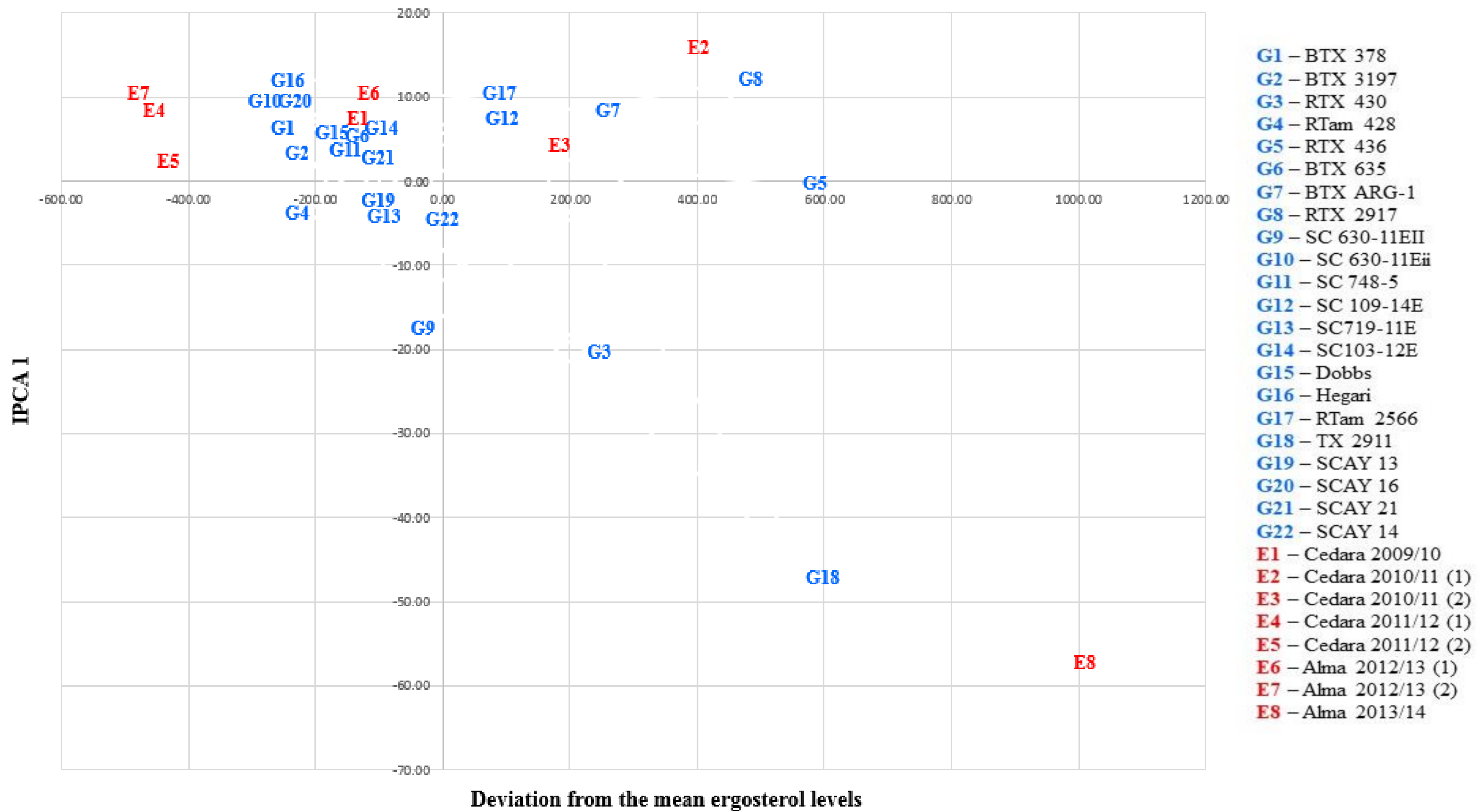

Figure 2.3 AMMI biplot of mean ergosterol levels for sorghum grain from twenty two sorghum lines evaluated at Cedara and Alma for grain mold resistance over eight environments.

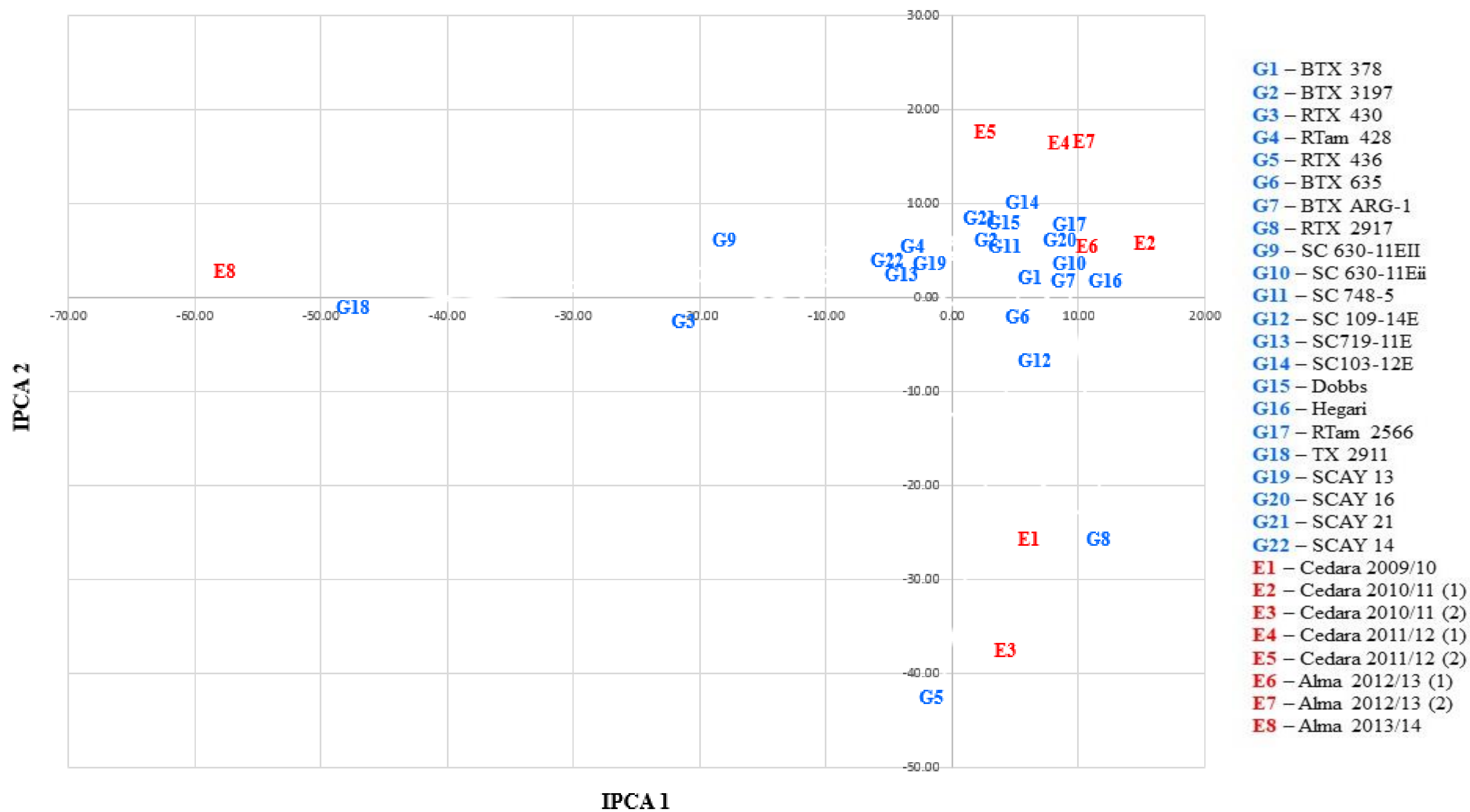

Figure 2.4 AMMI biplot to indicate the interaction between principle components of ergosterol levels for sorghum grain from twenty two sorghum lines evaluated at Cedara and Alma for grain mold resistance over eight environments.

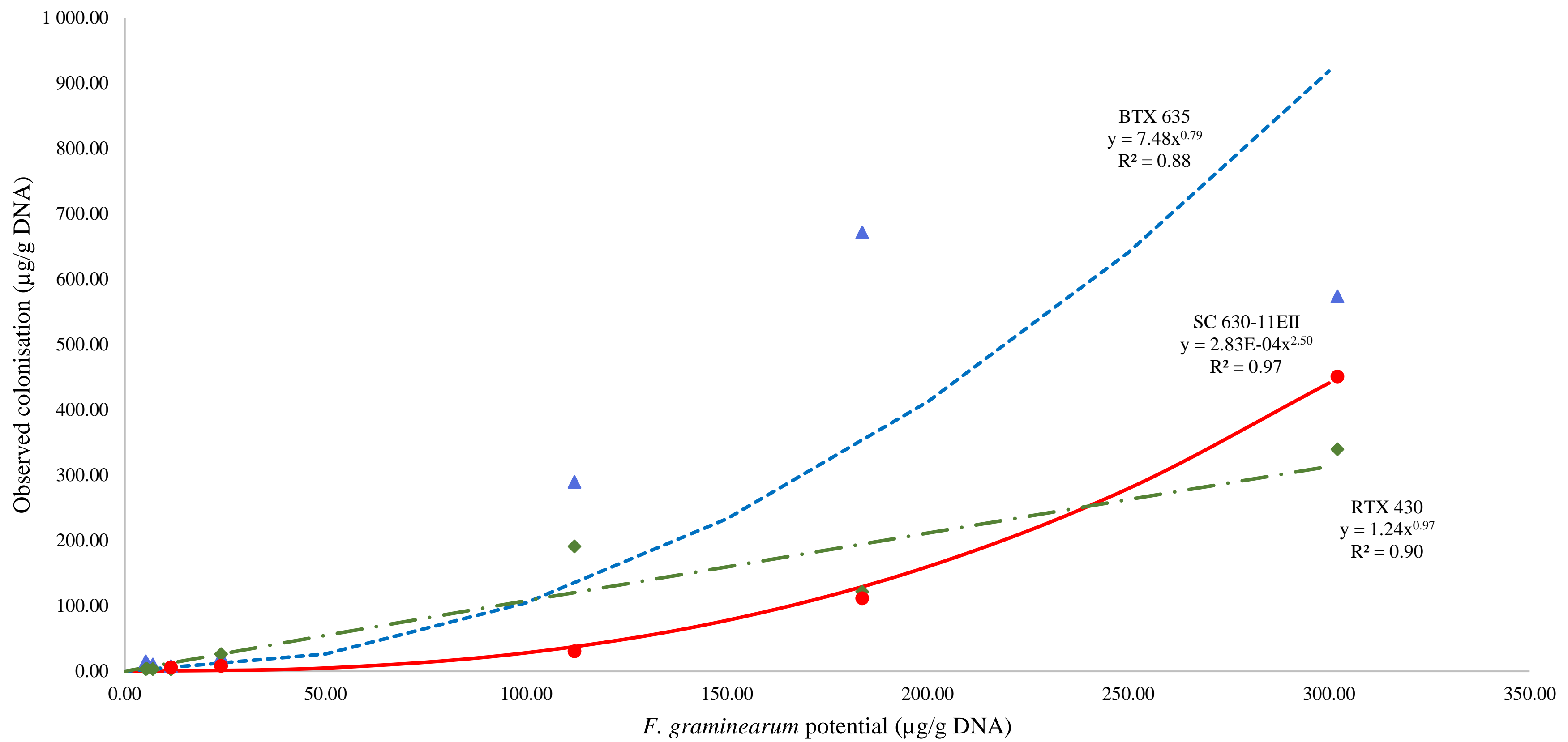

Figure 2.5 Relationships between FGSC potential and observed colonisation of sorghum grain in twenty two sorghum lines evaluated at Cedara and Alma for grain resistance over eight environments.

CHAPTER 3

SORGHUM GRAIN METABOLITES IN DEFENSE AGAINST SORGHUM GRAIN MOLD

3.1. Abstract

Grain mold, caused by a complex of fungal pathogens, is a disease of sorghum with serious repercussions on the quality and quantity of grain produced. Breeding for resistance is one of the most economical and effective control methods and includes the exploitation of host physical and chemical traits. Grain of 22 sorghum lines representing a range of plant and grain characteristics were harvested from field trials conducted at Cedara and Alma from 2009/2010 to 2013/2014. Grains were evaluated for chemical properties that may influence grain mold resistance. Total phenolic content of florets during anthesis ranged from 50.18 µg/g in BTX 3197 to 203.89 µg/g in BTX 378 and that of the grain from 37.64 µg/g in BTX 3197 to 450.04 µg/g in TX 2911. Lowest levels of total phenolic compounds were associated with white pericarp sorghum and the highest with red pericarp sorghum. AMMI analysis indicated that total phenolic content of eleven lines remained stable across environments while the content of remaining lines reacted to environmental changes. HPLC analysis of a range of phenolic compounds produced inconsistent results with the exception of gallic acid which also displayed the only significant relationship with disease parameters, this indicating a possible role in grain mold resistance. Total protein levels ranged from 0.70 mgprot/ml in BTX 635 to 0.98 mgprot/ml in TX 2911 and these were more abundant in coloured pericarp grain with a tan plant colour. Chitinase levels ranged from 0.73 mgchitin/mgprot/hour in SC 748-5 to 2.97 mgchitin/mgprot/hour in SC 109-14E and glucanase levels from 4.16 mggluc/mgprot/hour in TX 2911 to 5.89 mggluc/mgprot/hour in SC 719-11E. Variation in the recorded enzyme levels were not associated with specific plant or grain characteristics. The resistance mechanism appears far more complex and most certainly involves other processes and metabolites that need further exploration.

Keywords: chitinase, gallic acid, glucanase, grain mold, phenolic compounds, proteins, sorghum

3.2. INTRODUCTION

Grain mold of sorghum affects the quality, quantity (Rao *et al.,* 2012) and viability of the grain (Ambekar *et al.,* 2011) and can cause substantial financial losses (Thakur *et al.,* 2006). Breeding for disease resistance to improve cultivar yield has received considerable attention (Rao *et al.,* 2012). Although the process itself is not easy due to the weather-dependent nature of the disease (Seetharaman *et al.,* 1996), and the diversity of causal organisms (Little and Magill, 2003), many successful efforts have led to the identification of resistant genotypes for inclusion in breeding programs (Ambekar *et al.,* 2011).

Resistance in genotypes to grain molds can be attributed to physical (Navi *et al*., 2005) or chemical traits (Chandrashekar and Satyanarayana, 2006). Physical traits include grain hardness (Bandyopadhyay *et al.,* 2000), waxy layer on the grain surface (Rodriguez-Herrera *et al.,* 2000), grain coverage by elongated glumes (Williams and Rao, 1980) and dormancy (Bandyopadhyay *et al.,* 2000). Other physical disease avoidance factors include plant height and panicle compactness that reduce humidity levels in the canopy (Ayana and Bekele, 1998; Audilakshmi *et al.,* 1999).

Chemical traits include secondary metabolites (Bennett and Wallsgrove, 1994) such as phenolic compounds associated with the pericarp and plant colour (Dykes *et al*., 2005) and the synthesis of antifungal proteins (Das *et al.,* 2012). In sorghum three classes of phenolic compounds are found and include phenolic acids, flavonoids and condensed tannins (Dykes and Rooney, 2007). Phenolic acids can be divided into hydroxybenzoic acids and hydroxycinnamic acids while flavonoids include flavonols, flavones, flavanones, flavanols and anthocyanins (Dykes and Rooney, 2007).

Phenolic compounds and antifungal proteins may be natural constituents of the plant, while others are produced in response to pathogen attack (Rodriguez-Herrera *et al.,* 2006). The antifungal efficacy of the compound is related to the tempo and duration of production subsequent to infection and not the concentration present beforehand (Bennett and Wallsgrove, 1994). Resistance may therefore be associated with the production of phenolic compounds upon pathogen infection, which limit fungal growth and development (Lo *et al.,* 1999). These phenolic compounds are usually associated with darker pericarp colours (Menkir *et al.,* 1996). Flavan-4-ol phenolics (Jambunathan *et al.*, 1991), gallic acid, protocatechuic acid, *p-*

hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid and cinnamic acid have been associated with grain mold resistance in sorghum (Hahn *et al.,* 1983). Sorghum seedlings challenged by *Fusarium thapsinum* and *F. proliferatum* produced phenolic compounds, specifically anthocyanins, apigeninidin, luteolinidin near infection sites (Huang and Backhouse, 2004).

Proteins located in the endosperm of sorghum grain have shown antifungal activity (Waniska and Rooney, 2000) and include peroxidase, chitinase, β-1,3-glucanase (Huang and Backhouse, 2004) sormatin and ribosomal-inactivating proteins (Rodriguez-Herrera *et al.,* 2006). Higher levels of β-1,3-glucanases and chitinases accumulate in resistant sorghum floral tissues after infection with *F. thapsinum* and *Curvularia lunata* (Little and Magill, 2003). β-1,3-glucanases weaken fungal cell walls resulting in cell death (Prom and Egilla, 2011). Chitinase has the ability to degrade fungal cell walls of various species (Little, 2000).

The aim of this study was to determine chemical resistance traits, with specific attention to phenolics and antifungal proteins that may contribute to grain mold resistance in sorghum lines representing a range of plant and grain characteristics.

3.3. MATERIALS AND METHODS

3.3.1. Total phenolic content of sorghum florets at anthesis and grain at harvest

Twenty-two sorghum lines representing a range of plant and grain characteristics (Table 3.1) were evaluated for total phenolic compounds over a range of planting dates at Cedara (29.5477^{o}S, 30.2667^{o}E), KwaZulu-Natal Province and Alma (24.4841^{o}S, 28.0750^{o}E), Limpopo Province over an range of seasons and planting dates (Table 3.3). Samples were collected from experimental plots that consisted of 10 m single-row plots with a 0.75 m inter-row and 0.1 m intra-row spacing. Trials were replicated three times in a randomized block experimental design. Prior to planting, fields were fertilized with 600 kg 2:3:2 (N P K (28%)). Dual Gold® (metolachlor, 915 g/l) was applied directly after planting at a rate of 500 ml/ha for pre-emergence grass control, followed by Basagran® (Bendioxide - thiadiazine 480 g/l) post-emergence as required, at a rate of 1.5 l/ha for broadleaf weeds. Decis® (deltamethrin, 25 g/l) was applied at a rate of 250 ml/ha for pest control, including stalk borer and aphids. Additional weeding by hand was done as needed.

Florets from 5 plants were collected at anthesis during 2011/2012 (early November planting) at Cedara, KwaZulu-Natal Province and during 2014/2015 (early November planting) at Alma, Limpopo Province. Grains from 5 plants were harvested at approximately 12.5% moisture content during 2009/2010 (mid-November planting), 2010/2011 (early [1] and late [2] November plantings) and 2011/2012 (early [1] and late [2] November plantings) at Cedara and during 2012/2013 (early [1] and late [2] November plantings), 2013/2014 (early November planting) at Alma.

Florets from the respective trial replicates were ground into a fine powder in liquid nitrogen using a mortar and pestle. Grain from the respective trial replicates were ground into a fine powder using a coffee grinder as described previously. The respective ground samples (0.125 g) were vortex mixed for two hours in 6.25 ml of a solution comprised of 75% aqueous acetone in 50 ml Falcon test tubes using a Heidolph Multi Reax Shaker (Labotec) (Khadambi, 2007). The samples were centrifuged at 3 000 rcf (BHG Optima) for approximately six minutes. The supernatant (1 ml) was transferred to a volumetric flask containing 70 ml de-ionized water and 5 ml Folin-Ciocalteu phenol reagent (Merck). A 20% sodium carbonate solution (15 ml) was added within 1 to 8 min and the flask was filled with de-ionized water to the 100 ml mark (Waterman and Mole, 1994). After being left at room temperature for two hours, a 2 ml aliquot was assayed for total phenolic content at 760 nm using a T60 UV VIS spectrophotometer (PG Instruments).

3.3.2. HPLC analysis of phenolic compounds

Extraction of phenolic compounds from sorghum grain was conducted using a modified method of Vidovic *et al.* (2015). Sorghum grain obtained from experimental plots at Cedara and Alma was ground into a fine powder with a coffee grinder. Sorghum grain meal (0.5 g) was suspended in 2 ml methanol (Merck) containing 0.1% HCL in 50 ml Falcon test tubes and kept overnight in a refrigerator at 4°C. The samples were shaken for 30 minutes on a Heidolph Multi Reax Shaker (Labotec) and centrifuged (BHG Optima) for 6 minutes at 3 000 g. The supernatant (600 µl) was transferred into a new Eppendorff tube containing 400 µl double distilled water and 600 µl chloroform. The tubes were shaken on a Heidolph Multi Reax Shaker in the dark for 45 minutes and centrifuged for five minutes on a Hemle Centrifuge (Lasec) at 14 000 rcf. The supernatant was hydrolyzed in 600 µl 2 mol dm^{-3} HCL in a 85°C water bath

for 40 minutes. The fluid was aliquoted into five HPLC vials with cone inserts for analyses at five different wavelengths, i.e. 280 nm, 320 nm, 340 nm, 360 nm and 520 nm. The vials were kept frozen at -80°C until analyses on a Perkin Elmer HPLC.

Gallic acid standards were prepared and ranged from 29-7500 µg/g and phenolic acids were expressed as gallic acid equivalents.

The extracts were loaded onto a column (C18, 5 µm particle size, 250 x 4.6 mm) at 25°C. Mobile phase A consisted of acetonitrile and mobile phase B acetic acid/acetonitrile/phosphoric acid/distilled water (10: 5: 0.1: 84.9) at a flow rate of 1.0 ml/min with injection volume of 10 µl for the samples and 50 µl for the gallic acid standards (Vidovic *et al.,* 2015). The procedure was as follows: 0-5 min, 100% mobile phase B (isocratic step); 5-25 min, 100–80% mobile phase B (linear gradient); 25-35 min, 80-60% mobile phase B (linear gradient); 35-40 min, 60-100% mobile phase B (linear gradient).

3.3.3. Protein extraction

Protein extraction and analyses were conducted in collaboration with Dr. L. Mohase, Department of Plant Sciences, University of the Free State. Grain from Cedara and Alma was ground into a fine powder using a coffee grinder. The respective meals (0.3 g) were homogenised with sand purified by HCL acid (Merck) in 3 ml of 100 mM potassium phosphate buffer with a pH of 7.0. This buffer was specifically chosen to protect the proteins during extraction and to remove excess phenolics. The buffer also contained 2mM ethylenediamine tetra-acetic-acid, 1% polyvinylpyrrolidone, and 1 mM phenylmethylsulfonyl fluoride. The samples were centrifuged at 12 000 g for 10 minutes at 4°C. The supernatants were stored at -20°C until further use as extracts for total protein-, glucanase- and chitinase analysis.

3.3.4. Total protein analysis in sorghum grain

The dye-binding assay technique of Bradford (1976) was used to determine total protein concentrations. In each well of the microplate, the mixture consisted of 10 µl enzyme extract, 40 µl BioRad protein reagent and 150 µl distilled water. In the blank samples, the enzyme extract was replaced with 10 µl distilled water and in the standard, 10 µl γ-globulin. Each

sample was repeated in triplicate and assayed at a wavelength of 595 nm in a Biorad microplate reader (Zenyth 3100, Anthos).

3.3.5. *Chitinase activity in sorghum grain*

Chitinase activity was determined using a modified method of Wirth & Wolf (1990) as described by Steyn (2015). This method relies on perceptibility of carboxymethyl-chitin-remazol brilliant violet 5R (CM-chitin-RBV; Loewe Biochemica GmbH) with hydrochloric acid in a sodium acetate buffer.

Enzyme extract (10 µl) obtained during protein extraction, 0.1 M sodium acetate buffer with a pH of 5.2 (600 µl) and 200 µl CM-chitin-RBV (2 mg/ml) were added to an Eppendorf tube. This mixture was incubated at 37°C in a water bath for 30 minutes. The reaction was stopped with the addition of 200 µl 2 N Hydrochloric acid. The reaction mixture was left on ice for 10 minutes and centrifuged at 14 000 rcf (Hemle, Lasec) for 5 minutes. Dilutions of 1, 2 and 4 folds were prepared to optimize detection and absorbance was measured at 550 nm using a T60 UV VIS spectrophotometer (PG Instruments). Chitinase was expressed as mg^{-1} protein $hour^{-1}$.

3.3.6. *Glucanase activity in sorghum grain*

β-1,3-glucanase activity was assayed using a modified technique of Fink *et al*. (1988). Aliquots containing enzyme extract (10 µl) obtained during protein extraction, 250 µl Laminarin (2 mg/ml) and 240 µl 50mM sodium acetate buffer (pH 4.5) were incubated for 10 minutes at 37°C in a glass test tube. Somogyi's reagent (500 µl) was added and test tubes were placed in a boiling water bath for 10 minutes. The test tubes were cooled in a cool water bath and 500 µl Nelson's reagent was added. The absorbance was measured at 540 nm using a T60 UV VIS spectrophotometer (PG Instruments) and the concentration of glucose produced was expressed as mg glucose mg^{-1} protein $hour^{-1}$.

3.3.7. *Data analysis*

Data were analysed by means of Analysis of Variance (ANOVA) using NCSS (Hintze, 2001) and means separation was done using Fisher's Protected LSD ($P<0.05$). AMMI analyses were performed on total phenolic content using GenStat (18th Edition) to indicate phenolic content

stability over environments. Regression analysis was conducted according to McLaren (1992) and Flett and McLaren (1994) to indicate relationships between disease parameters recorded in Chapter 2 and resistance metabolites from the current chapter.

3.4. RESULTS

3.4.1. Total phenolic content of sorghum florets during anthesis and grain at harvest

Analysis of variance (ANOVA) indicated that total phenolic content in the sorghum florets differed significantly at both localities and across lines. Mean total phenolic content in florets ranged from 50.18 µg/g in BTX 3197 to 203.89 µg/g in BTX 378 (Table 3.2). Total phenolics in eight lines did not differ significantly from BTX 3197 while another eight lines were comparable with the high levels in BTX 378. No significant line x locality interaction was recorded. Florets from red pericarp grain (BTX 378, SCAY 21 and SC 719-11 E) had a higher phenolic content, compared to florets from white pericarp grain (BTX 3197, SC 109-14E and RTX 430). No specific colour with respect to plant characteristics, was evident in either high or low phenolic floral contents. No significant relationship was recorded between total phenolic content of the florets and disease parameters, i.e. grain visual ratings, ergosterol, *Fusarium graminearum* species complex (FGSC), deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) levels presented in Chapter 2 (Table 3.14).

ANOVA indicated that total phenolic content produced in grain from sorghum lines differed significantly (Table 3.3). The mean total phenolic content ranged from 37.64 µg/g in BTX 3197 to 450.04 µg/g in TX 2911, with the latter containing significant higher total phenolic content compared to all other lines. High phenolic levels were recorded in SC 719-11E and SC 103-12E, and these were also significantly different from other lower phenolic concentration lines. As recorded in florets, red pericarp grain (SC 719-11E and SC 103-12E) also had higher phenolic contents than white pericarp grain (BTX 3197, RTam 428 and BTX 635). No specific relationship with plant colour was evident in grain containing either high or low phenolic concentration. AMMI analysis indicated that the total phenolic compound responses of eleven lines across eight environments from which grain was collected were unstable with IPCA's -1< or >1 (Table 3.3). However genotype had the biggest influence on variation within total phenolic content at 60.31%, followed by the genotype x environment interaction at 15.04% and lastly environment at 2.06% based on ANOVA sum of squares.

AMMI biplot (Figure 3.1) indicated that five lines were adapted to lower potential environments for decreased phenolic compound production (Quadrant I and IV) and six lines were adapted to higher potential environments for increased phenolic compound production (Quadrant II and III). Sum of squares indicated that IPCA 1 contributed 48.24% to genotype x environment interaction, indicating complex interactions were involved in the production of phenolic compounds. The only significant relationship ($P<0.05$) with disease parameters from Chapter 2 (Table 3.11 – Table 3.19) was recorded between total phenolic content and DON levels ($R^2 = 0.45$*) (Figure 3.2) in grain from Cedara 2011/2012 (1) (Table 3.14).

3.4.2. HPLC analysis of phenolic compounds

Initial analyses were conducted on grain from Cedara 11/12 (Table 3.4) and Alma 12/13 (Table 3.5). Specific phenolic compound levels were inconsistent across lines with the exception of gallic acid. Gallic acid was therefore, further analysed in all the remaining seasons. Specific flavonoid and phenolic acids detected are indicated in Table 3.6 with their respective concentrations in Table 3.14 and Table 3.16 for Cedara 11/12 and Alma 12/13 respectively. All the lines, except Dobbs from Cedara 11/12, produced flavonoid and phenolic acid classes of phenolic compounds (Table 3.6). Phenolic acids from the hydroxy-benzoic acid group, were detected in all the lines from both Cedara 11/12 and Alma 12/13. This group included gallic acid detected at 280 nm. Gallic acid content ranged from 1357.81 µg/g in RTX 436 to 2956.51 µg/g in SC 103-12E (Table 3.7). No significant differences in gallic acid levels were recorded between the lines. However, a tendency for lower levels of gallic acid (1357.81 µg/g and 1769.57 µg/g) was associated with white pericarp grain in combination with a tan plant colour (RTX 436 and BTX 635 respectively). Significant differences in gallic acid content were associated with environments, with the mean lowest gallic acid content in Alma 13/14 (1053.05 µg/g) and the highest in Cedara 09/10 (3156.05 µg/g). No significant relationships ($P<0.05$) between gallic acid content and disease parameters from Chapter 2 in grain from respective localities were recorded (Table 3.11 – Table 3.18). However, positive albeit poor relationships were recorded (Figure 3.3) with the means over all localities (Table 3.19). Higher gallic acid content was associated with lower grain fungal colonisation ($R^2 = 0.30$*), lower FGSC colonisation ($R^2 = 0.25$*) and lower nivalenol production ($R^2 = 0.40$*).

3.4.3. Total protein analysis in sorghum grain

Significant differences in total protein levels were recorded in the lines and ranged from 0.70 mgprot/ml in BTX 635 to 0.98 mgprot/ml in TX 2911 (Table 3.8). No significant differences were recorded within the low protein containing lines BTX 635, BTX 3197, Hegari, BTX 378, RTX 436 and RTam 428 or within the high protein containing lines SC 748-5, SCAY 13, RTX 2917, SCAY 14, SCAY 21 and TX 2911 and these two groups differed significantly from one another. A significant relationship ($P<0.05$) between mean total protein levels and general fungal colonisation determined by ergosterol levels in lines (Table 3.19) was recorded, although this relationship was weak ($R^2 = 0.20$*) (Figure 3.3). Higher total protein levels were associated with higher ergosterol levels. A coloured pericarp in combination with a tan plant colour (SCAY 13, SCAY 14 and SCAY 21 respectively) was associated with higher protein levels.

3.4.4. Chitinase activity in sorghum grain

No significant differences in chitinase content of grain from sorghum lines was recorded (Table 3.9), although the mean chitin levels recorded in lines from the wetter Cedara (1.66 mgchitin/mgprot/hour) were almost double those recorded in lines from the drier Alma (0.77 mgchitin/mgprot/hour). No plant or grain characteristics were associated with the chitin levels recorded. No significant relationship was recorded between disease parameters from Chapter 2 and chitinase levels at each locality (Table 3.11 – Table 3.18) or the line means (Table 3.19) recorded.

3.4.5. Glucanase activity in sorghum grain

Mean glucanase levels in grain from sorghum lines were significantly different and ranged from 4.16 mggluc/mgprot/hour in TX 2911 to 5.89 mggluc/mgprot/hour in SC 719-11E (Table 3.10). No significant differences were recorded within the low glucanase containing lines TX 2911, BTX 378, BTX 635 and Dobbs or the high glucanase containing lines RTX 436, SCAY 13, SCAY 16 and SC 719-11E, and these two groups differed significantly from one another. As recorded for chitinase levels, no relationship between plant or grain characteristics, nor significant relationships between disease parameters from Chapter 2 and glucanase levels were recorded (Table 3.11 – Table 3.19).

3.5. DISCUSSION

Grain mold resistance is considered a multigenic trait, complicated by the diversity of causal pathogens (Little and Magill, 2003) and their specific environmental requirements to cause infection (Thakur *et al*., 2006). Phenolic compounds associated with plant and grain characteristics were responsible for some observed variation in grain mold evaluation parameters in the current study with the highest levels in red pericarp grain compared to lower levels in white pericarp grain in both florets during anthesis and in the post-harvest grain. Phenolic compounds produced during both these stages can inhibit the growth of fungal pathogens and include three major classes, i.e. tannins, flavonoids and phenolic acids (Hahn *et al*., 1984). Higher levels of phenolics were found during anthesis compared with post-harvest grain in the current study, which coincides with Doherty *et al.* (1987), where higher tannin and phenolic compound levels were recorded during sorghum development compared with mature grain. Similarly, Maurya *et al.* (2007) recorded increases in phenolic acids in leaves and stems in early post-infection periods with *Sclerotium rolfsii* subsequently declining in concentration. During anthesis sorghum florets are more susceptible to grain mold fungi (Thakur *et al*., 2006) resulting in a more severe grain mold outbreak (Little and Magill, 2003), hereby indicating the greater importance of phenolic compounds during these growth stages if fungal infection is to be suppressed. The positive correlation between total phenolic content and deoxynivalenol levels of grain from Cedara 11/12(1) could indicate a rapid response of the plants to counteract fungal attack (Lo *et al.,* 1999).

Lines SC 719-11E and SCAY 21, both red pericarp grain, contained very high levels of total phenolic content in florets and grain, as well as various flavonoids and phenolic acids detected. These lines also displayed resistance towards colonisation, specifically FGSC fungi and mycotoxin production (measured in Chapter 2). Literature has attributed resistance found in red pericarp grain to the presence of flavan-4-ol compounds (Dykes *et al*., 2005) and the role of these compounds in lowering grain mold colonisation has been reported (Jambunathan *et al.,* 1991). Plant colour is also affected by specific phenolic compounds. Purple sorghum plants contain high levels of 3-deoxyanthocyanin (Dykes *et al*., 2009) belonging to the anthocyanidin group of flavonoids (Dykes and Rooney, 2007). The purple plant SC 719-11E contained all six of the anthocyanidins tested in both environments, compared to the tan plant SCAY 21 that only contained two in Cedara and four in Alma. Tan plants however, have more

flavone groups of flavonoids (Dykes *et al*., 2009) which was confirmed in SCAY 21 containing all three types of flavones detected in Alma, compared to SC 719-11E with none detected in either environment. The differences in flavonoid groups produced in both these resistant lines were consistent with a report by Hahn *et al.,* (1983) who also indicated differences in phenolic content in resistant genotypes by HPLC analyses and suggested different resistant mechanisms for sorghum varieties.

Although red pericarp grain are considered to be more resistant, the red pericarp line TX 2911 (Awika and Rooney, 2004) was susceptible to colonisation by multiple fungi, specifically FGSC fungi, irrespective of the high levels of phenolic compounds recorded. Prom *et al.* (2003) reported that resistance and susceptibility of sorghum lines to pathogens varied for different fungal species. Another concern is the potential of fungi to overcome defense mechanisms. Specialist pathogens have evolved to overcome defense mechanisms of their hosts (Bennett and Wallsgrove, 1994) by producing mycotoxins (Brosch *et al.,* 1995) and secreting enzymes, such as in the case with *F. verticillioides* and *C. lunata* that can degrade grain endosperm tissues and obstruct translocation of carbohydrates (Castor and Frederiksen, 1980).

Alternatively, line BTX 635, a tan plant with a white pericarp also displayed fairly low resistance as indicated by high FGSC fungal colonisation and high mycotoxin levels. This line's susceptibility was attributed to low levels of total phenolic compounds, gallic acid and proteins. White pericarp grain contained no antifungal phenolic compounds (Thakur *et al*., 2006) and are therefore more prone to *Fusarium* spp. infections, and even more so when white pericarp grain are combined with a tan plant colour (Funnell, 2006). Contradictory to this, was line RTAM 428, a white pericarp grain with a red plant colour that proved resistant to fungal colonisation, including FGSC fungi and mycotoxin production. This line has low total phenolic compounds, with the exception of high gallic acid levels. Other phenolic compounds analysed in this study varied in their presence and levels and could not be directly linked to grain mold resistance in this line. Hahn *et al*. (1983) could also not correlate specific phenolic acids with resistance in sorghum, thereby conveying the notion of complex interactions involved in resistance.

Negative relationships between gallic acid and fungal colonisation, by particularly FGSC pathogens and nivalenol production were indicated. Gallic acid contains antimicrobial

properties (Chanwitheesuk *et al.,* 2007) and can disrupt ergosterol biosynthesis in fungal membranes by decreasing enzyme activity (Li *et al.,* 2017). A phenolic extract mostly consisting of gallic acid, reduced the growth of FGSC fungi, deoxynivalenol and nivalenol production and enzyme activity *in vitro* on culture media (Pagnussatt *et al.,* 2014). In a similar study, gallic acid reduced *F. oxysporum* growth *in vitro* and mycotoxin production, but stimulated pathogenic enzyme activity, i.e. pectinase, proteinase and cellulase (Wu *et al.,* 2009) indicating a narrow inhibition range of pathogen related activities (Pagnussatt *et al.,* 2014). The enhanced enzyme activity could be produced in a defensive response.

Line RTam 428, apart from having high gallic acid concentrations in grain, also had relatively high chitinase levels, which indicated the likely contribution of antifungal proteins to resistance in white pericarp grain (Rodriguez-Herrera *et al.,* 1999). This is in agreement with Little and Magill (2003) who found higher levels of chitinase and ribosome-inactivating proteins in resistant white pericarp grain sorghum. SCAY 14 and SCAY 21, both tan plants with a coloured pericarp (yellow and red respectively), yielded high levels of total proteins, with lower visual ratings, lower FGSC fungal colonisation and lower mycotoxin production. A positive significant relationship was recorded between total proteins and ergosterol levels recorded in Chapter 2, probably indicating their production in response to fungal colonisation. Little and Magill (2003) recorded rapid chitinase accumulation after pathogen infection, indicating the influence of pathogen pressure (Rodriguez-Herrera *et al.,* 2006). Glucanase levels could not be connected to resistance in this study. This was probably due to sorghum genotype resulting in the expression of β-1,3-glucanases throughout grain development (Little and Magill, 2003) or that β-1,3-glucanases work in collaboration with other antifungal proteins, i.e. chitinases, to develop resistance. Protein extracts containing a combination of β-1,3-glucanase and chitinase were capable of inhibiting various fungal pathogens, including *Fusarium solani* f.sp. *phaseoli*, *F. solani* f.sp. *pisi in vitro.* When tested individually, only chitinase inhibited fungal growth, but to a lesser extent than in combination with β-1,3-glucanase (Mauch *et al.,* 1988).

Genotype and pathogen pressure are not the only contributing factors that influence the levels of phenolic compounds or proteins produced. The environment also plays a role in the expression of these metabolites (Dicko *et al*., 2006). Total phenolic content was higher in grain from the wetter Cedara environment, while higher total protein content with higher glucanases was found in grain from the drier Alma environment. In the separation of specific phenolic compounds, more phenolic compounds from both groups of flavonoids and phenolic acids,

were detected in grain from Alma and it seemed as if particular environmental conditions favoured the variations recorded (Rodriguez-Herrera *et al.,* 2000). AMMI analysis revealed stability of half the lines in their responses to produce phenolic compounds, compared to the other lines where the environment influenced the production thereof. In a low humidity and low temperature environment, higher levels of hydroxycinnamic acid groups of phenolic acids were detected in currant berries by HPLC analysis (Yang *et al.,* 2013).

3.6. CONCLUSION

The role of plant and grain characteristics in grain mold disease resistance could only be linked to total phenolic content, where higher levels were generally found in red pericarp grain compared to lower levels in white pericarp grain from flowering to post-harvest. When the phenolic compounds were analyzed separately, only gallic acid could be linked to disease resistance, as indicated in the negative relationships with grain colonisation by especially nivalenol-producing FGSC pathogens. Higher total protein levels were associated with higher general fungal colonisation and were presumably produced in response to pathogen attack. The highest and lowest producing chitinase and glucanase lines included resistant and susceptible genotypes and were irrespective of pericarp or plant colour. Environmental factors contributed to the levels of metabolites produced, with some of the lines adapted to specific environments. The complexity of these processes intertwined with one another to acquire resistance, supported the notion that we only understand a fraction of the mechanisms. It is likely that other metabolites not explored in this study, contributed to the observed resistance, but with such an abundance of metabolites found in nature, a more in-depth study to fully understand the processes involved, will not be an easy task.

3.7. REFERENCES

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Table 3.1 Twenty-two sorghum lines representing a range of plant and grain characteristics used in the evaluation of resistance mechanisms.

Line No	Line	Plant	Pericarp	Mesocarp	Testa	Spreader	Plant colour	Seed colour
1	BTX 378	PPQQ	RRYYII	zz	b1b1B2B2	SS	purple	red, chalky
2	BTX 3197	PPQQ	RRyyii	zz	b1b1B2B2	SS	purple	white, chalky
3	RTX 430	PPQQ	Rryyll	ZZ	b1b1b2b2	SS	purple	white, pearly, yellow endosperm
4	Rtam 428	PPqq	rryyll	ZZ	b1b1B2B2	ss	red	white, pearly
5	RTX 436	ppQQ	Rryyii	ZZ	B1B1b2b2	--	tan	white
6	BTX 635	ppQQ	Rryyii	ZZ	--	--	tan	white pearly food grad sorghum
7	BTX ARG-1	ppQQ	Rryyii	ZZ	--	--	tan	waxy endosperm
8	RTX 2917							
9	SC 630-11EII	PPQQ	RRYYII	ZZ	b1b1B2B2	SS	purple	dark red, pearly
10	SC 630-11Eii	PPQQ	RRYYii	ZZ	b1b1B2B2	SS	purple	light red, pearly
11	SC 748-5	PPQQ	rrYYIl	ZZ	b1b1B2B2	ss	purple	lemon yellow, pearly
12	SC 109-14E	PPQQ	Rryy--	zz	B1B1B2B2	ss	purple	white pearly, purple testa
13	SC719-11E	PPQQ	RRYYii	zz	B1B1B2B2	ss	purple	red, chalky,
14	SC103-12E	PPQQ	RRYYII	zz	B1B1B2B2	SS	purple	dark brown-red, chalky
15	Dobbs	PPQQ	Rryyii	zz	B1B1B2B2	SS	purple	brown, chalky
16	Hegari	PPQQ	Rryyii	zz	B1B1B2B2	ss	purple	white, chalky
17	Rtam 2566	PPQQ	RRYYII	ZZ	B1B1B2B2	ss	purple	dark brown-red, pearly
18	TX 2911							
19	SCAY 13						tan	lemon-yellow
20	SCAY 16							
21	SCAY 21						tan	Red
22	SCAY 14						tan	lemon-yellow

Table 3.2 Total phenolic content (µg/g) of florets during anthesis at Cedara and Alma in twenty-two sorghum lines representing a range of plant and grain characters.

Genotype	Line	Cedara 11/12 (1)	Alma 14/15	Line mean
G1	BTX 378	322.59	85.18	**203.89**^{a}
G2	BTX 3197	63.70	36.66	**50.18**^{g}
G3	RTX 430	82.58	-	**82.58**^{efg}
G4	RTam 428	134.07	37.62	**85.84**^{defg}
G5	RTX 436	154.07	44.84	**99.46**^{cdefg}
G6	BTX 635	125.93	52.06	**88.99**^{defg}
G7	BTX ARG-1	84.44	76.30	**80.37**^{efg}
G8	RTX 2917	197.41	70.35	**133.88**^{bcde}
G9	SC 630-11EII	167.58	52.96	**110.27**^{bcdefg}
G10	SC 630-11Eii	212.59	78.52	**145.55**^{abcde}
G11	SC 748-5	184.82	101.11	**142.97**^{abcde}
G12	SC 109-14E	93.34	25.93	**59.63**^{fg}
G13	SC 719-11E	228.52	113.33	**170.93**^{ab}
G14	SC 103-12E	208.52	101.85	**155.18**^{abc}
G15	Dobbs	79.80	46.51	**63.15**^{fg}
G16	Hegari	222.96	47.62	**135.29**^{bcde}
G17	RTam 2566	213.70	77.06	**145.38**^{abcde}
G18	TX 2911	225.56	114.83	**170.20**^{ab}
G19	SCAY 13	175.36	73.65	**124.51**^{bcdef}
G20	SCAY 16	240.74	58.15	**149.44**^{abcd}
G21	SCAY 21	263.33	80.74	**172.04**^{ab}
G22	SCAY 14	153.70	93.73	**123.71**^{bcdef}
Locality mean		**174.33**^{a}	**69.95**^{b}	

Table 3.3 AMMI analysis of total phenolic content (µg/g) in twenty-two sorghum grain lines representing a range of plant and grain characters planted at Cedara and Alma.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Total	527.00	5359693.00	10170.00		
Treatments	175.00	4148632.00	23706.00	5.51	<0.001
Genotypes	21.00	3232314.00	153920.00	35.74	<0.001
Environments	7.00	110267.00	15752.00	2.53	0.02
Block	14.00	87115.00	6222.00	1.44	0.13
Interactions	144.00	806051.00	5598.00	1.30	0.03
IPCA 1	27.00	388841.00	14402.00	3.34	<0.001
IPCA 2	25.00	220297.00	8812.00	2.05	0.00
IPCA 3	23.00	91178.00	3964.00	0.92	0.57
IPCA 4	21.00	56724.00	2701.00	0.63	0.90
Residuals	48.00	49010.00	1021.00	0.24	1.00
Error	261.00	1123946.00	4306.00		

Genotype	**Line**	**Cedara**					**Alma**			**Line mean**	**IPCA scores - genotypes**			
		09/10	**10/11 (1)**	**10/11 (2)**	**11/12 (1)**	**11/12 (2)**	**12/13 (1)**	**12/13 (2)**	**13/14**		**1**	**2**	**3**	**4**
G1	BTX 378	123.53	72.41	101.01	90.74	93.53	83.34	90.74	100.00	**94.41** ^{def}	1.34	1.19	-0.17	0.38
G2	BTX 3197	47.68	32.22	44.76	32.96	32.41	25.19	27.78	58.15	**37.64** ^{i}	0.66	2.89	-0.17	0.07
G3	RTX 430	50.75	40.74	61.60	41.11	53.53	51.11	66.30	62.59	**53.47** ^{ghi}	-0.11	1.46	0.44	-0.67
G4	RTam 428	57.41	45.18	53.70	36.67	46.86	46.67	50.37	61.11	**49.75** ^{hi}	0.35	2.22	-0.31	0.53
G5	RTX 436	96.57	66.57	153.34	45.19	29.90	70.00	88.53	51.85	**75.24** ^{efgh}	-1.03	0.26	2.53	3.14
G6	BTX 635	51.01	41.11	61.60	41.85	50.19	46.67	58.52	49.26	**50.03** ^{hi}	0.08	1.20	0.50	-0.27
G7	BTX ARG-1	50.75	56.67	47.04	37.41	40.75	41.85	37.78	86.30	**49.82** ^{hi}	0.37	4.10	-0.33	0.15
G8	RTX 2917	158.53	125.87	131.42	89.63	275.46	73.34	94.81	77.04	**128.26** ^{d}	3.44	-1.84	-10.43	-2.04
G9	SC 630-11EII	67.97	77.78	91.11	69.63	74.35	76.30	94.44	69.26	**77.60** ^{efgh}	-0.68	1.34	0.74	1.19
G10	SC 630-11Eii	78.53	88.15	95.56	67.03	56.30	81.11	112.96	107.04	**85.83** ^{efgh}	-1.48	2.58	1.34	0.64
G11	SC 748-5	82.41	97.41	86.67	62.59	94.64	89.63	107.78	90.37	**88.94** ^{efg}	-0.96	1.92	-1.46	0.89
G12	SC 109-14E	56.30	79.26	111.48	55.56	47.42	40.74	51.11	76.31	**64.77** ^{fghi}	0.74	2.36	1.47	-0.08
G13	SC 719-11E	342.97	260.00	439.50	386.67	318.53	322.96	339.63	238.52	**331.10** ^{b}	2.86	-5.84	6.33	-0.40
G14	SC 103-12E	383.53	174.07	415.61	310.00	371.86	275.93	282.22	296.67	**313.74** ^{b}	6.34	-5.02	1.79	-8.19
G15	Dobbs	384.08	155.93	269.26	223.70	229.64	181.48	129.26	107.78	**210.14** ^{c}	9.82	-5.64	-0.41	5.47
G16	Hegari	56.30	66.30	393.66	41.11	47.42	43.70	113.33	100.74	**107.82** ^{de}	-2.23	2.09	1.07	-1.33
G17	RTam 2566	90.75	98.52	138.09	85.92	106.86	85.56	172.22	70.37	**106.04** ^{de}	-2.58	-1.69	-0.07	0.34
G18	TX 2911	129.64	152.59	182.12	123.70	2324.52	130.00	455.56	102.22	**450.04** ^{a}	-12.57	-8.42	-1.60	-0.34
G19	SCAY 13	122.41	79.63	77.04	92.96	105.19	82.22	112.96	46.30	**89.84** ^{efg}	0.40	-1.19	-1.99	3.15
G20	SCAY 16	89.08	101.48	107.40	88.89	86.86	80.37	94.44	197.78	**105.79** ^{de}	-0.39	6.45	0.49	-3.13
G21	SCAY 21	66.31	80.74	101.48	74.07	74.64	54.07	200.00	81.11	**91.55** ^{def}	-5.01	-1.30	0.61	-0.42
G22	SCAY 14	98.53	77.04	100.87	91.11	95.20	87.78	92.59	78.89	**90.25** ^{efg}	0.65	0.89	-0.39	0.94
Locality mean		**122.05** ^{cd}	**94.08** ^{e}	**148.38** ^{b}	**99.48** ^{de}	**211.64** ^{a}	**94.09** ^{e}	**130.61** ^{bc}	**100.44** ^{de}	**LSD(P<0.05)**		**Genotypes =**	**37.49**	
IPCA scores – Environments														
	1	8.91	-3.38	4.05	3.16	3.30	0.51	-15.16	-1.40			**Environments=**	**22.61**	
	2	-4.13	6.16	-4.66	-0.70	-3.49	2.41	-6.92	11.31			**GxE interaction**	**106.03**	
	3	-1.18	-3.67	6.34	3.99	-9.75	2.57	0.51	1.19					
	4	4.29	6.21	-4.00	1.27	-4.88	2.82	-0.13	-5.57					

Table 3.4 Phenolic compounds in grain from twenty-two sorghum lines planted at Cedara during 11/12 (1) and detected at five wavelengths. Data are expressed as gallic acid equivalent (µg/g).

Phenolic compounds	Wave Length (nm)	Ret Time (min)	Lines										
			BTX 378	BTX 3197	RTX 430	RTam 428	RTX 436	BTX 635	BTX ARG-1	RTX 2917	SC 630-11EII	SC 630-11Eii	SC 748-5
gallic acid	280	3.39	1526.94	1226.38	2764.47	4349.08	2101.93	1160.77	1235.58	4031.77	2957.37	3010.24	2945.27
protocatechuic acid	280	5.17			0.07	0.18							
(+)-catechin	280	5.71			0.11	0.28	0.22			0.12			
p-hydroxybenzoic acid	280	8.70			0.18		0.07		4.59	8.80			
(−)-epicatechin	280	9.43			0.09	0.18	0.10						
syringic acid	280	12.33			0.06	0.09	0.12						
naringenin-7-O-rutinoside	280	23.06			0.07		0.61			56.75			
benzoic acid	280	24.61					0.27						
daidzein	280	29.57		0.05			0.28	1.81		153.07			
naringenin	280	34.31							2.06	22.74			
genistein	280	34.54											
chlorogenic acid	320	7.16									0.08	0.07	0.04
caffeic acid	320	10.39											
p-coumaric acid	320	16.34		0.06									0.07
ferulic acid	320	18.96											
sinapic acid	320	19.66					0.85						
m-coumaric acid	320	20.82									0.06		0.16
caffeic methyl ester	320	25.58					0.22						0.05
resveratrol	320	26.72	0.06									0.08	7.26
p-coumaric methyl ester	320	32.55	0.10				1.02		2.57		0.06		
ferulic methyl ester	320	33.16											
sinapic methyl ester	320	33.31	0.07										
m-coumaric methyl ester	320	33.67					66.27		0.56				
apigenin	320	35.25									0.06		
apigenin-7-O-glucoside	340	24.36			0.06		0.07		32.58				
luteolin	340	32.01			0.14				0.13				
ellagic acid	360	17.45											
quercetin-3-O-rutinoside	360	19.43											
quercetin-3-O-glucoside	360	20.01					0.06						
myricetin	360	24.85							0.05				
quercetin	360	31.36	0.52		0.12		0.06			0.03			
kaempferol	360	35.88											
cyanidin-3-O-glucoside	520	8.48				0.13					0.09		0.08
cyanidin-3-O-rutinoside	520	9.98			0.53					0.04	0.13	0.10	0.19
pelargonidin-3-O-glucoside	520	11.69			0.15		0.07				0.12	0.05	0.23
delphinidin	520	12.71			0.16	0.09	0.25				0.16	0.06	
cyanidin	520	16.73				2.74				0.23	0.20	0.05	0.23
pelargonidin	520	20.87			0.10	0.06				0.04	0.13		0.59
Line mean			**305.54**	**408.83**	**197.59**	**483.73**	**127.79**	**581.29**	**159.77**	**427.36**	**268.95**	**430.09**	**268.56**

Table 3.4 Continued.

Phenolic compounds	Wave Length (nm)	Ret Time (min)	Lines											Phenolic compound mean
			SC 109-14E	SC 719-11E	SC 103-12E	Dobbs	Hegari	R-Tam 2566	TX 2911	SCAY 13	SCAY 16	SCAY 21	SCAY 14	
gallic acid	280	3.39	2434.48	2850.89	4662.66	4825.59	2688.11	2256.57	2700.37	2404.44	3676.13	3277.62	3727.25	**2855.18**
protocatechuic acid	280	5.17												**0.13**
(+)-catechin	280	5.71												**0.18**
p-hydroxybenzoic acid	280	8.70												**3.41**
(−)-epicatechin	280	9.43												**0.12**
syringic acid	280	12.33												**0.09**
naringenin-7-O-rutinoside	280	23.06						0.14						**14.39**
benzoic acid	280	24.61												**0.27**
daidzein	280	29.57						0.08		6.01			87.31	**35.52**
naringenin	280	34.31												**12.40**
genistein	280	34.54												**12.40**
chlorogenic acid	320	7.16				0.14								**0.08**
caffeic acid	320	10.39												
p-coumaric acid	320	16.34												**0.06**
ferulic acid	320	18.96	0.07					0.13						**0.10**
sinapic acid	320	19.66	0.09											**0.47**
m-coumaric acid	320	20.82	0.05	0.06	0.06									**0.08**
caffeic methyl ester	320	25.58	0.10											**0.12**
resveratrol	320	26.72												**2.47**
p-coumaric methyl ester	320	32.55												**0.94**
ferulic methyl ester	320	33.16												
sinapic methyl ester	320	33.31												**0.07**
m-coumaric methyl ester	320	33.67			0.09									**22.31**
apigenin	320	35.25												**0.06**
apigenin-7-O-glucoside	340	24.36						0.15						**8.21**
luteolin	340	32.01						0.11		0.12				**0.13**
ellagic acid	360	17.45										0.06		**0.06**
quercetin-3-O-rutinoside	360	19.43						0.05						**0.05**
quercetin-3-O-glucoside	360	20.01	0.05					0.14	0.08					**0.08**
myricetin	360	24.85						0.05			0.08			**0.06**
quercetin	360	31.36	0.08		0.12			0.09					0.05	**0.13**
kaempferol	360	35.88						0.07						**0.07**
cyanidin-3-O-glucoside	520	8.48	0.14	0.26				0.05	0.17			0.05		**0.12**
cyanidin-3-O-rutinoside	520	9.98	0.49	0.08	0.10				0.19	0.09	0.05			**0.18**
pelargonidin-3-O-glucoside	520	11.69	0.43	0.11				0.07	0.17		0.27	0.47	0.43	**0.21**
delphinidin	520	12.71	0.18	0.19				0.23	0.32		0.22			**0.19**
cyanidin	520	16.73	0.27	0.19	0.19				0.13		0.11		0.06	**0.40**
pelargonidin	520	20.87	0.05	0.25	0.07		0.10		0.19					**0.16**
Line mean			**187.42**	**356.50**	**666.18**	**2412.87**	**1344.11**	**161.28**	**337.70**	**602.67**	**612.81**	**819.55**	**763.02**	**84.53**

Table 3.5 Phenolic compounds of grain from twenty-two sorghum lines planted at Alma during 12/13 (1) and detected at five wavelengths. Data are expressed as gallic acid equivalent (µg/g).

Phenolic compounds	**Wave Length (nm)**	**Ret Time (min)**	**Lines**										
			BTX 378	**BTX 3197**	**RTX 430**	**RTam 428**	**RTX 436**	**BTX 635**	**BTX ARG-1**	**RTX 2917**	**SC 630-11EII**	**SC 630-11Eii**	**SC 748-5**
gallic acid	280	3.39	3931.80	3141.53	2627.40	2966.83	2797.33	765.08	2410.95	2987.10	2474.55	2584.04	2960.00
protocatechuic acid	280	5.17					0.11				0.14		0.08
(+)-catechin	280	5.71					0.07	0.05	2.06		0.15		0.32
p-hydroxybenzoic acid	280	8.70	0.14				0.11		12.58	0.14	0.04	0.42	0.24
(−)-epicatechin	280	9.43	0.09				0.07			0.25	0.15		0.15
syringic acid	280	12.33	0.07									0.10	0.13
naringenin-7-O-rutinoside	280	23.06	0.15		0.07						0.07	0.08	0.07
benzoic acid	280	24.61	0.17		0.03		0.07		49.84		0.26	0.13	0.69
daidzein	280	29.57				0.11					0.13	0.56	0.21
naringenin	280	34.31	0.06		0.11		0.17		0.14			0.14	0.08
genistein	280	34.54	0.15									0.08	
chlorogenic acid	320	7.16	0.20						0.24	0.27	0.06		
caffeic acid	320	10.39	0.10	0.11			0.20			0.12			
p-coumaric acid	320	16.34	0.23	0.08		0.06	0.07	0.12	0.09	0.10	0.07		
ferulic acid	320	18.96			0.06					0.16			
sinapic acid	320	19.66											
m-coumaric acid	320	20.82				0.21	0.05	0.15	0.16	0.15		0.16	
caffeic methyl ester	320	25.58							0.69		0.14	0.11	0.09
resveratrol	320	26.72	0.07			0.11	0.07			0.06			
p-coumaric methyl ester	320	32.55			0.12				1.87	0.16	0.12		0.07
ferulic methyl ester	320	33.16								0.18			
sinapic methyl ester	320	33.31	0.09				0.13				0.11		
m-coumaric methyl ester	320	33.67	0.10		0.10		0.27						
apigenin	320	35.25	0.07		0.24			0.20		0.13			
apigenin-7-O-glucoside	340	24.36	0.08	0.09	0.22	0.09		0.49	0.11	0.10	30.47	0.12	43.13
luteolin	340	32.01		0.07	0.27	0.11	0.07	0.23		0.12	0.48	0.10	
ellagic acid	360	17.45	0.09	0.06	0.06	0.12	0.06	0.10	0.05	0.10	0.11	0.11	0.24
quercetin-3-O-rutinoside	360	19.43		0.16		0.14	0.09	0.11			0.04	0.11	0.22
quercetin-3-O-glucoside	360	20.01		0.04		0.35		0.07			0.12	0.07	0.18
myricetin	360	24.85		0.05		0.06		0.16	0.08	0.07	0.04	0.09	0.31
quercetin	360	31.36	0.06	0.28		0.09	0.08	0.06		0.09	0.08	0.15	0.07
kaempferol	360	35.88	0.08	0.04				0.09			0.16	0.06	
cyanidin-3-O-glucoside	520	8.48	0.08	0.26							0.20		0.05
cyanidin-3-O-rutinoside	520	9.98	0.35	0.13	0.11	0.08	0.07		0.05	0.37	0.17	0.11	0.03
pelargonidin-3-O-glucoside	520	11.69		0.11						0.23	0.05	0.10	0.21
delphinidin	520	12.71	0.05	0.11	0.12			0.05			0.24		
cyanidin	520	16.73		0.10	0.05	0.18	0.24	0.19			0.42	0.41	0.21
pelargonidin	520	20.87	0.06					0.16			0.25	0.15	0.09
Line mean			**171.06**	**196.45**	**187.78**	**212.04**	**147.33**	**48.00**	**177.07**	**157.36**	**92.92**	**117.61**	**130.73**

Table 3.5 Continued.

Phenolic compounds	Wave Length (nm)	Ret Time (min)	Lines											Phenolic compound mean
			SC 109-14E	SC 719-11E	SC 103-12E	Dobbs	Hega-ri	R-Tam 2566	TX 2911	SCAY 13	SCAY 16	SCAY 21	SCAY 14	
gallic acid	280	3.39	4047.56	4248.45	3793.34	3913.01	2648.38	2955.53	2629.71	1755.46	4416.01	2804.26	2609.24	**2975.80**
protocatechuic acid	280	5.17			0.14									**0.12**
(+)-catechin	280	5.71	0.09					0.13	0.14			0.10	0.16	**0.33**
p-hydroxybenzoic acid	280	8.70	0.22	0.13								0.08		**1.41**
(−)-epicatechin	280	9.43	0.05	0.16	0.19			0.12	0.13					**0.14**
syringic acid	280	12.33	0.25	0.10					0.10					**0.13**
naringenin-7-O-rutinoside	280	23.06	0.12		0.09			0.09	0.26			0.10		**0.11**
benzoic acid	280	24.61	0.17	0.10	0.09				0.11					**4.70**
daidzein	280	29.57		0.08	2.71		0.05		0.07		0.10	0.07	1.34	**0.50**
naringenin	280	34.31						0.22				0.13		**0.13**
genistein	280	34.54												**0.11**
chlorogenic acid	320	7.16	0.06		0.16		0.09			0.10	0.12	0.07	0.14	**0.14**
caffeic acid	320	10.39			0.08	0.09	0.03	0.13		0.27	0.09		0.08	**0.12**
p-coumaric acid	320	16.34					0.05	0.10		0.50	0.23	0.12	11.11	**0.92**
ferulic acid	320	18.96	0.13							0.17	0.09	0.10	88.09	**12.69**
sinapic acid	320	19.66	0.04					0.10	0.05	0.26	0.09		0.11	**0.11**
m-coumaric acid	320	20.82			0.14		0.11			0.09		0.10	5.57	**0.62**
caffeic methyl ester	320	25.58			0.09	0.20	0.05		0.08	0.22	0.46		375.33	**34.32**
resveratrol	320	26.72	0.09		0.08	0.07			0.12	0.23	0.08	0.13	6.32	**0.62**
p-coumaric methyl ester	320	32.55	0.14		0.06					0.31	0.12	0.10	0.65	**0.34**
ferulic methyl ester	320	33.16										0.25		**0.22**
sinapic methyl ester	320	33.31								0.08	0.07	0.14	0.18	**0.12**
m-coumaric methyl ester	320	33.67								0.37	0.07		15.64	**2.76**
apigenin	320	35.25			0.08		0.06			0.54	0.13	0.07	0.07	**0.16**
apigenin-7-O-glucoside	340	24.36	0.16		0.43	0.07	0.08	0.11		0.12	0.28	0.46	0.06	**4.04**
luteolin	340	32.01	0.09			0.09	0.08	0.13		0.13	0.14	0.11		**0.15**
ellagic acid	360	17.45	0.11	0.17		0.22	0.16		0.15		0.13			**0.12**
quercetin-3-O-rutinoside	360	19.43	0.13	0.07		0.37	0.08	0.04			0.04	0.22		**0.13**
quercetin-3-O-glucoside	360	20.01	0.16	0.04	0.09	0.08	0.05	0.17	0.09	0.08	0.11	0.04		**0.11**
myricetin	360	24.85	0.25	0.20	0.13	0.14	0.19		0.04		0.04		0.06	**0.12**
quercetin	360	31.36	0.04	0.12	0.04	0.03	0.12		0.17		0.07	0.03	0.10	**0.09**
kaempferol	360	35.88	0.05	0.04	0.07	0.27	0.05	0.07	0.09		0.03	0.12	0.06	**0.08**
cyanidin-3-O-glucoside	520	8.48	0.11	0.04	0.46		0.06	0.07	0.15					**0.15**
cyanidin-3-O-rutinoside	520	9.98	0.10	0.14	0.27	0.13		0.07				0.05		**0.14**
pelargonidin-3-O-glucoside	520	11.69		0.09					0.12		0.08	0.10	0.08	**0.12**
delphinidin	520	12.71		0.10	0.05		0.17		0.05			0.09		**0.10**
cyanidin	520	16.73	0.30	0.13								0.27	0.05	**0.21**
pelargonidin	520	20.87		0.07	0.45		0.04	0.07						**0.15**
Line mean			**176.11**	**236.12**	**172.69**	**301.14**	**139.47**	**184.82**	**146.20**	**109.93**	**200.85**	**112.29**	**148.31**	**162.10**

Table 3.6 Summary table indicating the detected phenolic compounds divided into their respective groups in grain from 22 sorghum lines at Cedara 11/12 and Alma 12/13.

Genotype	**Line**	**Cedara 11/12**								**Alma 12/13**								**Line total**
		Flavonoids						**Phenolic acids**		**Flavonoids**						**Phenolic acids**		
		Flavan ol	Iso-flavo-noids	Flava-nones	Fla-vones	Fla-vonols	Antho-cyani-dins	Hydroxy-benzoic acid	Hydroxy-cinnamic acid	Flavan-ol	Iso-flavo-noids	Flava-nones	Fla-vones	Fla-vonols	Antho-cyani-dins	Hydroxy-benzoic acid	Hydroxy-cinnamic acid	
G1	BTX 378					1		1	2	1	1	2	3	2	4	5	5	**27**
G2	BTX 3197		1					1	1				2	5	5	2	2	**19**
G3	RTX 430	2		1	2	1	4	4				2	3	3		3	3	**28**
G4	RTam 428	2					4	3			1		2	4	2	2	2	**22**
G5	RTX 436	2	1	1	1	2	2	4	4	2		1	1	2	2	5	5	**35**
G6	BTX 635		1					1		1			3	5	3	2	2	**18**
G7	BTX ARG-1			1	2	1		2	2	1		1	1	1	1	4	6	**23**
G8	RTX 2917	1	1	2		1	3	2		1			3	2	2	3	8	**29**
G9	SC 630-11EII				1		6	1	3	2	1	1	2	5	6	5	6	**39**
G10	SC 630-11Eii						4	1	1		2	2	2	5	4	5	2	**28**
G11	SC 748-5						5	1	4	2	1	2	1	4	5	6	2	**33**
G12	SC 109-14E					2	6	1	4	2		1	2	5	3	5	4	**35**
G13	SC 719-11E						6	1	1	1	1			5	6	5		**26**
G14	SC 103-12E					1	3	1	2	1	1	1	2	4	4	3	5	**28**
G15	Dobbs							1	1				2	5	1	2	2	**14**
G16	Hegari						1	1			1		3	5	3	2	5	**21**
G17	RTam 2566		1	1	2	5	3	1	1	2		2	2	3	3	1	3	**30**
G18	TX 2911					1	6	1		2	1	1		4	3	4	2	**25**
G19	SCAY 13		1		1		1	1					3	1		1	10	**19**
G20	SCAY 16					1	4	1			1		3	5	1	2	9	**27**
G21	SCAY 21						2	2		1	1	2	3	4	4	2	7	**28**
G22	SCAY 14		1			1	2	1		1	1		2	3	2	1	10	**25**
Total		**7**	**7**	**6**	**9**	**17**	**62**	**33**	**26**	**20**	**13**	**18**	**45**	**82**	**64**	**69**	**100**	

Table 3.7 Gallic acid content (µg/g) in grain from twenty-two sorghum lines representing a range of plant and grain characters planted at Cedara and Alma and detected at 280 nm.

Genotype	Line	Cedara			Alma		Line mean
		09/10	10/11 (1)	11/12 (1)	12/13 (1)	13/14	
G1	BTX 378	2991.36	3269.52	1526.94	3931.80	510.41	**2446.01**
G2	BTX 3197	3065.73	3285.84	1226.38	3141.53	1167.67	**2377.43**
G3	RTX 430	3395.21	958.63	2764.47	2627.40	1004.41	**2150.02**
G4	RTam 428	3363.74	1714.16	4349.08	2966.83	960.12	**2670.79**
G5	RTX 436	114.06	750.59	2101.93	2797.33	1025.15	**1357.81**
G6	BTX 635	3402.56	2611.08	1160.77	765.08	908.34	**1769.57**
G7	BTX ARG-1	4230.75	2258.76	1235.58	2410.95	956.88	**2218.59**
G8	RTX 2917	1906.60	938.61	4031.77	2987.10	977.09	**2168.23**
G9	SC 630-11EII	3689.75	1314.19	2957.37	2474.55	1051.77	**2297.53**
G10	SC 630-11Eii	3063.70	1216.90	3010.24	2584.04	1576.93	**2290.36**
G11	SC 748-5	3604.74	553.85	2945.27	2960.00	1677.42	**2348.26**
G12	SC 109-14E	4084.39	1686.12	2434.48	4047.56	1498.40	**2750.19**
G13	SC 719-11E	1613.25	1360.75	2850.89	4248.45	1103.65	**2235.40**
G14	SC 103-12E	3311.76	1985.30	4662.66	3793.34	1029.47	**2956.51**
G15	Dobbs	1927.98	1302.81	4825.59	3913.01	1080.66	**2610.01**
G16	Hegari	3020.39	981.34	2688.11	2648.38	1168.47	**2101.34**
G17	RTam 2566	3226.67	905.58	2256.57	2955.53	1054.94	**2079.86**
G18	TX 2911	2592.97	1190.52	2700.37	2629.71	774.38	**1977.59**
G19	SCAY 13	4193.04	790.78	2404.44	1755.46	608.40	**1950.43**
G20	SCAY 16	3864.13	361.27	3676.13	4416.01	1112.02	**2685.91**
G21	SCAY 21	4559.81	691.14	3277.62	2804.26	977.49	**2462.06**
G22	SCAY 14	4210.60	797.04	3727.25	2609.24	943.05	**2457.44**
Locality mean		**3156.05**[a]	**1405.67**[b]	**2855.18**[a]	**2975.80**[a]	**1053.05**[b]	

Table 3.8 Total protein content (mgprot/ml) in grain from twenty-two sorghum lines representing a range of plant and grain characters planted at Cedara and Alma.

Genotype	**Line**	**Cedara**			**Alma**		**Line mean**
		09/10	**10/11 (1)**	**11/12 (1)**	**12/13 (1)**	**13/14**	
G1	BTX 378	0.65	0.85	0.63	0.85	0.82	**0.76^{fghi}**
G2	BTX 3197	0.51	0.79	0.66	0.89	0.73	**0.72^{hi}**
G3	RTX 430	0.66	0.94	0.87	0.96	0.84	**0.86^{bcdef}**
G4	RTam 428	0.47	0.85	0.82	0.94	0.84	**0.78^{efghi}**
G5	RTX 436	0.67	0.92	0.68	0.91	0.69	**0.77^{fghi}**
G6	BTX 635	0.50	0.84	0.74	0.74	0.70	**0.70^{i}**
G7	BTX ARG-1	0.75	0.83	0.94	0.91	0.78	**0.84^{bcdef}**
G8	RTX 2917	0.85	0.97	0.82	1.01	0.89	**0.91^{abcd}**
G9	SC 630-11EII	0.67	0.96	0.73	1.00	0.87	**0.85^{bcdef}**
G10	SC 630-11Eii	0.68	0.83	0.87	0.99	0.75	**0.83^{cdefg}**
G11	SC 748-5	0.67	0.97	0.86	1.02	0.89	**0.88^{abcde}**
G12	SC 109-14E	0.64	0.89	0.79	1.01	0.81	**0.83^{cdefg}**
G13	SC 719-11E	0.77	0.86	0.88	0.96	0.65	**0.82^{cdefg}**
G14	SC 103-12E	0.78	0.67	0.93	1.02	0.87	**0.85^{bcdef}**
G15	Dobbs	0.66	0.77	0.87	0.90	0.92	**0.82^{cdefg}**
G16	Hegari	0.59	0.54	0.83	0.89	0.78	**0.73^{ghi}**
G17	RTam 2566	0.63	0.92	0.67	0.97	0.87	**0.81^{defgh}**
G18	TX 2911	0.90	1.02	0.98	1.11	0.89	**0.98^{a}**
G19	SCAY 13	0.72	0.96	1.03	1.00	0.76	**0.89^{abcd}**
G20	SCAY 16	0.60	0.90	0.94	0.93	0.79	**0.83^{cdef}**
G21	SCAY 21	0.78	1.00	0.97	1.15	0.80	**0.94^{ab}**
G22	SCAY 14	0.73	0.99	0.96	1.01	0.91	**0.92^{abc}**
Locality mean		**0.68^{d}**	**0.88^{b}**	**0.84^{bc}**	**0.96^{a}**	**0.81^{c}**	

Table 3.9 Chitinase content (mgchitin/mgprot/hour) in grain from twenty-two sorghum lines representing a range of plant and grain characters planted at Cedara and Alma.

Genotype	**Line**	**Cedara**			**Alma**		**Line mean**
		09/10	**10/11 (1)**	**11/12 (1)**	**12/13 (1)**	**13/14**	
G1	BTX 378	2.31	1.88	0.29	0.47	0.69	**1.13**
G2	BTX 3197	1.97	1.48	3.13	0.29	0.18	**1.41**
G3	RTX 430	2.47	1.89	0.17	1.05	0.53	**1.22**
G4	RTam 428	2.47	1.59	1.87	0.54	0.56	**1.41**
G5	RTX 436	1.98	1.66	0.23	0.45	1.82	**1.23**
G6	BTX 635	3.01	2.31	0.22	0.36	0.19	**1.22**
G7	BTX ARG-1	1.86	2.34	0.08	1.52	0.29	**1.22**
G8	RTX 2917	1.40	1.71	4.62	0.41	3.07	**2.24**
G9	SC 630-11EII	2.60	1.02	0.63	0.18	1.02	**1.09**
G10	SC 630-11Eii	3.16	0.49	0.45	0.06	4.04	**1.64**
G11	SC 748-5	2.33	0.09	0.74	0.06	0.42	**0.73**
G12	SC 109-14E	3.39	8.51	0.56	2.38	0.01	**2.97**
G13	SC 719-11E	1.11	1.55	0.73	2.10	0.27	**1.15**
G14	SC 103-12E	1.56	1.99	0.77	0.26	0.30	**0.98**
G15	Dobbs	2.15	1.47	1.14	0.12	0.14	**1.00**
G16	Hegari	1.97	2.14	2.17	0.51	0.11	**1.38**
G17	RTam 2566	2.52	1.44	0.11	0.70	0.68	**1.09**
G18	TX 2911	2.15	0.05	0.28	2.16	0.02	**0.93**
G19	SCAY 13	1.70	1.68	0.72	0.10	1.16	**1.07**
G20	SCAY 16	2.84	1.72	0.63	0.39	0.30	**1.18**
G21	SCAY 21	2.03	1.08	0.84	1.46	0.15	**1.11**
G22	SCAY 14	1.31	1.58	1.35	2.13	0.23	**1.32**
Locality mean		**2.20**[a]	**1.80**[a]	**0.99**[b]	**0.80**[b]	**0.74**[b]	

Table 3.10 Glucanase content (mggluc/mgprot/hour) in grain from twenty-two sorghum lines representing a range of plant and grain characters planted at Cedara and Alma.

Genotype	**Line**	**Cedara**			**Alma**		**Line mean**
		09/10	**10/11 (1)**	**11/12 (1)**	**12/13 (1)**	**13/14**	
G1	BTX 378	2.98	5.03	4.47	4.46	4.20	**4.23^{ef}**
G2	BTX 3197	3.99	4.60	5.22	5.38	5.52	**4.94^{abcdef}**
G3	RTX 430	4.01	5.51	4.05	5.46	4.15	**4.64^{cdef}**
G4	RTam 428	3.77	5.52	4.04	5.84	5.22	**4.88^{bcdef}**
G5	RTX 436	5.08	5.84	4.93	6.05	5.32	**5.44^{abc}**
G6	BTX 635	3.05	4.72	3.98	5.36	4.41	**4.30^{ef}**
G7	BTX ARG-1	3.89	5.07	3.75	5.08	4.91	**4.54^{cdef}**
G8	RTX 2917	4.31	5.73	6.16	5.98	4.54	**5.34^{abc}**
G9	SC 630-11EII	4.09	4.99	5.23	5.93	4.53	**4.95^{abcdef}**
G10	SC 630-11Eii	4.04	5.83	5.14	6.09	5.36	**5.29^{abcd}**
G11	SC 748-5	4.59	5.14	5.29	5.75	3.90	**4.94^{abcdef}**
G12	SC 109-14E	4.04	5.79	4.35	6.03	5.20	**5.08^{abcdef}**
G13	SC 719-11E	5.43	6.14	5.88	6.68	5.34	**5.89^{a}**
G14	SC 103-12E	3.85	5.32	4.75	6.05	4.84	**4.96^{abcdef}**
G15	Dobbs	3.55	3.25	4.99	5.35	4.70	**4.37^{def}**
G16	Hegari	2.84	5.08	4.90	5.62	5.04	**4.69^{bcdef}**
G17	RTam 2566	3.96	5.65	5.23	5.79	5.07	**5.14^{abcde}**
G18	TX 2911	4.97	4.70	5.49	0.00	5.65	**4.16^{f}**
G19	SCAY 13	4.40	5.94	5.75	6.08	5.45	**5.53^{ab}**
G20	SCAY 16	4.67	6.00	5.82	6.88	4.78	**5.63^{ab}**
G21	SCAY 21	4.67	3.99	5.58	6.15	5.24	**5.13^{abcdef}**
G22	SCAY 14	3.58	5.41	5.66	5.57	4.72	**4.99^{abcdef}**
Locality mean		**4.08^{c}**	**5.24^{ab}**	**5.03^{b}**	**5.53^{a}**	**4.91^{b}**	

Table 3.11 Disease parameters and resistance metabolites from 22 sorghum lines representing a range of plant and grain characteristics planted at Cedara 09/10.

				Cedara 09/10										
Genotype	**Line**	**Plant colour**	**Grain colour**	**Mean visual rating**	**Mean ergos-terol** (µg/g)	**Mean FGSC** (µg/g)	**Mean DON** (µg/kg)	**Mean NIV** (µg/kg)	**Mean ZEA** (µg/kg)	**Mean total pheno-lics** (µg/g)	Mean total protein mgprot/ ml	Mean gallic acid (µg/g)	**Mean chiti-nase** (ch/prot/ hr)	**Mean gluca-nase** (gl/prot/ hr)
G1	BTX 378	Purple	Red	2.95	270.50	97.00	0.00	124.90	124.10	123.53	0.65	2991.36	2.31	2.98
G2	BTX 3197	Purple	White	2.20	195.80	82.00	115.00	402.00	486.30	47.68	0.51	3065.73	1.97	3.99
G3	RTX 430	Purple	White	1.95	651.30	340.00	61.40	105.00	216.00	50.75	0.66	3395.21	2.47	4.01
G4	RTam 428	Red	White	2.18	110.20	53.00	0.00	62.90	16.20	57.41	0.47	3363.74	2.47	3.77
G5	RTX 436	Tan	White	1.43	2128.2	487.00	306.00	1380.0	132.50	96.57	0.67	114.06	1.98	5.08
G6	BTX 635	Tan	White	2.30	523.30	574.20	689.00	870.00	18.60	51.01	0.50	3402.56	3.01	3.05
G7	BTX ARG-1	Tan		1.30	574.30	257.00	208.00	149.00	43.80	50.75	0.75	4230.75	1.86	3.89
G8	RTX 2917			2.11	1666.4	1216.0	152.00	963.00	29.70	158.53	0.85	1906.60	1.40	4.31
G9	SC 630-11EII	Purple	Dark red	2.27	182.80	451.30	864.00	604.00	144.60	67.97	0.67	3689.75	2.60	4.09
G10	SC 630-11Eii	Purple	Light red	2.15	246.70	453.20	553.00	413.00	69.00	78.53	0.68	3063.70	3.16	4.04
G11	SC 748-5	Purple	Lemon yellow	2.04	207.60	83.00	5.50	106.80	2.60	82.41	0.67	3604.74	2.33	4.59
G12	SC 109-14E	Purple	White	2.46	709.70	487.30	443.00	114.00	184.30	56.30	0.64	4084.39	3.39	4.04
G13	SC 719-11E	Purple	Red	2.76	322.80	134.00	8.40	116.80	2.40	342.97	0.77	1613.25	1.11	5.43
G14	SC 103-12E	Purple	Dark brown-red	3.22	16.70	133.00	0.00	141.00	0.30	383.53	0.78	3311.76	1.56	3.85
G15	Dobbs	Purple	Brown	3.29	99.40	190.00	38.60	91.90	187.30	384.08	0.66	1927.98	2.15	3.55
G16	Hegari	Purple	White	3.92	334.20	137.00	20.50	91.90	322.40	56.30	0.59	3020.39	1.97	2.84
G17	RTam 2566	Purple	Dark brown-red	3.28	245.30	374.00	147.00	152.00	466.00	90.75	0.63	3226.67	2.52	3.96
G18	TX 2911			2.78	754.70	530.00	67.70	82.80	8.80	129.64	0.90	2592.97	2.15	4.97
G19	SCAY 13	Tan	Lemon yellow	1.39	258.40	258.00	71.80	103.00	326.50	122.41	0.72	4193.04	1.70	4.40
G20	SCAY 16			1.73	127.40	127.00	48.50	40.90	8.70	89.08	0.60	3864.13	2.84	4.67
G21	SCAY 21	Tan	Red	1.50	152.40	86.00	0.00	154.20	91.60	66.31	0.78	4559.81	2.03	4.67
G22	SCAY 14	Tan	Lemon yellow	1.56	318.40	96.00	72.50	39.90	13.20	98.53	0.73	4210.60	1.31	3.58
Mean				**2.31**	**458.96**	**302.09**	**176.00**	**286.77**	**131.59**	**122.05**	**0.68**	**3156.05**	**2.20**	**4.08**

Table 3.12 Disease parameters and resistance metabolites from 22 sorghum lines representing a range of plant and grain characteristics planted at Cedara 10/11 (1).

				Cedara 10/11 (1)										
Genotype	**Line**	**Plant colour**	**Grain colour**	**Mean visual rating**	**Mean ergos-terol** (µg/g)	**Mean FGSC** (µg/g)	**Mean DON** (µg/kg)	**Mean NIV** (µg/kg)	**Mean ZEA** (µg/kg)	**Mean total pheno-lics** (µg/g)	**Mean total protein** mgprot/ ml	**Mean gallic acid** (µg/g)	**Mean chiti-nase** (ch/prot/ hr)	**Mean gluca-nase** (gl/prot/ hr)
G1	BTX 378	Purple	Red	2.27	574.70	102.50	160.00	81.00	301.00	72.41	0.85	3269.52	1.88	5.03
G2	BTX 3197	Purple	White	1.63	369.90	76.10	58.90	54.50	559.00	32.22	0.79	3285.84	1.48	4.60
G3	RTX 430	Purple	White	3.18	1028.3	191.20	48.30	74.00	237.40	40.74	0.94	958.63	1.89	5.51
G4	RTam 428	Red	White	2.67	517.70	36.10	0.70	26.40	18.90	45.18	0.85	1714.16	1.59	5.52
G5	RTX 436	Tan	White	1.31	589.70	188.80	48.60	49.30	124.00	66.57	0.92	750.59	1.66	5.84
G6	BTX 635	Tan	White	0.87	775.60	290.20	313.50	419.00	14.10	41.11	0.84	2611.08	2.31	4.72
G7	BTX ARG-1	Tan		1.74	1322.8	71.80	16.10	1.00	29.80	56.67	0.83	2258.76	2.34	5.07
G8	RTX 2917			1.86	2626.7	184.10	0.00	54.90	22.60	125.87	0.97	938.61	1.71	5.73
G9	SC 630-11EII	Purple	Dark red	1.83	607.10	30.90	0.00	42.80	98.20	77.78	0.96	1314.19	1.02	4.99
G10	SC 630-11Eii	Purple	Light red	1.89	552.10	28.00	46.10	29.80	39.20	88.15	0.83	1216.90	0.49	5.83
G11	SC 748-5	Purple	Lemon yellow	3.19	1050.40	114.00	2.00	55.90	2490.00	97.41	0.97	553.85	0.09	5.14
G12	SC 109-14E	Purple	White	2.30	1601.9	160.00	2.00	57.00	122.00	79.26	0.89	1686.12	8.51	5.79
G13	SC 719-11E	Purple	Red	2.24	965.70	158.00	256.00	80.20	11.70	260.00	0.86	1360.75	1.55	6.14
G14	SC 103-12E	Purple	Dark brown-red	1.49	1107.20	131.60	279.00	80.30	8.10	174.07	0.67	1985.30	1.99	5.32
G15	Dobbs	Purple	Brown	2.98	710.30	163.00	48.10	21.00	286.20	155.93	0.77	1302.81	1.47	3.25
G16	Hegari	Purple	White	2.94	655.70	103.60	80.10	21.90	791.00	66.30	0.54	981.34	2.14	5.08
G17	RTam 2566	Purple	Dark brown-red	3.47	1879.30	158.00	92.60	137.00	146.00	98.52	0.92	905.58	1.44	5.65
G18	TX 2911			2.75	1139.1	121.90	51.90	31.40	28.50	152.59	1.02	1190.52	0.05	4.70
G19	SCAY 13	Tan	Lemon yellow	1.38	533.30	17.90	81.80	46.70	215.30	79.63	0.96	790.78	1.68	5.94
G20	SCAY 16			1.79	824.40	75.90	67.40	31.20	10.10	101.48	0.90	361.27	1.72	6.00
G21	SCAY 21	Tan	Red	1.72	1591.0	44.00	10.90	133.00	116.30	80.74	1.00	691.14	1.08	3.99
G22	SCAY 14	Tan	Lemon yellow	1.35	650.90	16.90	0.00	25.30	2.00	77.04	0.99	797.04	1.58	5.41
Mean				**2.31**	**985.17**	**112.02**	**75.64**	**67.89**	257.79	**94.08**	**0.88**	**1405.67**	**1.80**	**5.24**

Table 3.13 Disease parameters and total phenolic content from 22 sorghum lines representing a range of plant and grain characteristics planted at Cedara 10/11 (2)

				Cedara 10/11 (2)						
Genotype	**Line**	**Plant colour**	**Grain colour**	**Mean visual rating**	**Mean ergos-terol** (µg/g)	**Mean FGSC** (µg/g)	**Mean DON** (µg/kg)	**Mean NIV** (µg/kg)	**Mean ZEA** (µg/kg)	**Mean total pheno-lics** (µg/g)
G1	BTX 378	Purple	Red	2.61	508.40	103.80	38.70	104.80	158.00	101.01
G2	BTX 3197	Purple	White	1.60	404.70	98.70	97.50	50.80	390.00	44.76
G3	RTX 430	Purple	White	2.88	1016.80	121.90	1.30	55.30	71.70	61.60
G4	RTam 428	Red	White	2.56	349.10	14.90	0.80	16.70	9.60	53.70
G5	RTX 436	Tan	White	1.17	3041.60	178.20	0.00	0.00	0.00	153.34
G6	BTX 635	Tan	White	1.27	775.80	672.00	344.00	530.00	5.80	61.60
G7	BTX ARG-1	Tan		1.39	978.90	135.00	118.00	13.30	34.50	47.04
G8	RTX 2917			1.79	2151.50	178.20	96.50	52.90	17.30	131.42
G9	SC 630-11EII	Purple	Dark red	2.36	457.30	111.90	0.90	30.10	132.00	91.11
G10	SC 630-11Eii	Purple	Light red	2.76	453.00	132.10	3.80	27.70	46.60	95.56
G11	SC 748-5	Purple	Lemon yellow	3.51	417.00	82.00	48.50	18.40	237.00	86.67
G12	SC 109-14E	Purple	White	2.13	1081.50	334.10	170.00	98.90	130.00	111.48
G13	SC 719-11E	Purple	Red	2.45	547.00	169.00	44.60	90.40	7.50	439.50
G14	SC 103-12E	Purple	Dark brown-red	1.96	276.10	30.00	3.30	49.70	0.60	415.61
G15	Dobbs	Purple	Brown	3.46	352.70	228.00	80.00	83.70	720.00	269.26
G16	Hegari	Purple	White	3.23	554.80	162.10	106.20	66.50	410.00	393.66
G17	RTam 2566	Purple	Dark brown-red	3.73	505.20	235.90	87.10	32.90	210.00	138.09
G18	TX 2911			2.93	1155.10	691.90	49.30	269.00	0.90	182.12
G19	SCAY 13	Tan	Lemon yellow	1.31	557.60	82.00	67.40	37.70	204.00	77.04
G20	SCAY 16			1.82	354.70	186.90	110.00	46.40	0.30	107.40
G21	SCAY 21	Tan	Red	2.20	271.90	40.90	4.10	74.20	76.90	101.48
G22	SCAY 14	Tan	Lemon yellow	1.43	648.20	52.90	3.30	32.70	12.00	100.87
Mean				**2.13**	**766.31**	**183.75**	**67.06**	**71.91**	**130.67**	**148.38**

Table 3.14 Disease parameters and resistance metabolites from 22 sorghum lines representing a range of plant and grain characteristics planted at Cedara 11/12 (1).

				Cedara 11/12 (1)															
Genotype	**Line**	**Plant colour**	**Grain colour**	**Mean visual rating**	**Mean ergos-terol** (µg/g)	**Mean FGSC** (µg/g)	**Mean DON** (µg/kg)	**Mean NIV** (µg/kg)	**Mean ZEA** (µg/kg)	**Mean total pheno-lics (grain)** (µg/g)	**Mean total protein** mgprot /ml	**Mean gallic acid** (µg/g)	**Mean chiti-nase** (ch/pr ot/hr)	**Mean gluca-nase** (gl/prot /hr)	**No flavo-noids**	**Total [flavo-noids]**	**No pheno-lic acids**	**Total [pheno-lic acids]**	**Mean total pheno-lics (florets)**
G1	BTX 378	purple	red	2.27	91.40	18.90	8.70	19.70	0.00	90.74	0.63	1526.94	0.29	4.47	1	0.52	3	1527.17	322.59
G2	BTX 3197	purple	white	1.49	194.20	12.70	6.50	20.10	0.00	32.96	0.66	1226.38	3.13	5.22	1	0.05	2	1226.44	63.70
G3	RTX 430	purple	white	2.54	144.40	26.40	0.00	22.90	0.40	41.11	0.87	2764.47	0.17	4.05	10	1.53	4	2764.78	82.58
G4	RTam 428	red	white	2.34	32.00	24.10	8.30	23.90	1.80	36.67	0.82	4349.08	1.87	4.04	6	3.48	3	4349.35	134.07
G5	RTX 436	tan	white	1.58	105.20	33.80	0.00	0.00	0.00	45.19	0.68	2101.93	0.23	4.93	9	1.72	8	2170.75	154.07
G6	BTX 635	tan	white	1.11	146.40	23.20	0.00	66.40	0.20	41.85	0.74	1160.77	0.22	3.98	1	1.81	1	1160.77	125.93
G7	BTX ARG-1	tan		1.17	345.10	22.30	60.30	58.20	0.50	37.41	0.94	1235.58	0.08	3.75	4	34.82	4	1243.30	84.44
G8	RTX 2917			1.47	136.00	9.50	52.50	14.60	0.60	89.63	0.82	4031.77	4.62	6.16	8	233.02	2	4040.57	197.41
G9	SC 630-11EII	purple	dark red	1.17	79.30	8.70	13.70	82.10	0.40	69.63	0.73	2957.37	0.63	5.23	7	0.89	4	2957.57	167.58
G10	SC 630-11Eii	purple	light red	1.07	146.90	15.10	0.00	0.00	0.00	67.03	0.87	3010.24	0.45	5.14	4	0.26	2	3010.39	212.59
G11	SC 748-5	purple	lemon yellow	2.14	96.70	25.60	0.00	35.70	0.10	62.59	0.86	2945.27	0.74	5.29	5	1.32	5	2952.85	184.82
G12	SC 109-14E	purple	white	2.54	49.50	33.40	14.30	55.20	0.30	55.56	0.79	2434.48	0.56	4.35	8	1.69	5	2434.79	93.34
G13	SC 719-11E	purple	red	2.22	91.50	27.60	83.80	58.10	10.80	386.67	0.88	2850.89	0.73	5.88	6	1.08	2	2850.95	228.52
G14	SC 103-12E	purple	dark brown-red	2.37	96.90	34.10	28.90	90.10	25.10	310.00	0.93	4662.66	0.77	4.75	4	0.48	3	4662.81	208.52
G15	Dobbs	purple	brown	2.95	134.70	27.10	49.10	41.10	61.30	223.70	0.87	4825.59	1.14	4.99	0	0.00	2	4825.73	79.80
G16	Hegari	purple	white	3.25	151.20	36.10	6.80	27.60	245.00	41.11	0.83	2688.11	2.17	4.90	1	0.10	1	2688.11	222.96
G17	RTam 2566	purple	dark brown-red	3.29	160.00	22.90	7.90	33.40	7.40	85.92	0.67	2256.57	0.11	5.23	12	1.23	2	2256.70	213.70
G18	TX 2911			2.63	228.30	12.20	24.50	0.00	0.00	123.70	0.98	2700.37	0.28	5.49	7	1.25	1	2700.37	225.56
G19	SCAY 13	tan	lemon yellow	1.19	134.50	31.90	19.20	15.90	41.50	92.96	1.03	2404.44	0.72	5.75	3	6.22	1	2404.44	175.36
G20	SCAY 16			1.57	88.60	31.20	34.00	35.50	0.20	88.89	0.94	3676.13	0.63	5.82	5	0.73	1	3676.13	240.74
G21	SCAY 21	tan	red	0.83	91.20	20.60	17.60	48.20	16.40	74.07	0.97	3277.62	0.84	5.58	2	0.52	2	3277.68	263.33
G22	SCAY 14	tan	lemon yellow	1.34	157.20	29.90	15.90	14.20	12.30	91.11	0.96	3727.25	1.35	5.66	4	87.85	1	3727.25	153.70
Mean				**2.30**	**131.87**	**23.97**	**20.56**	**34.68**	**19.29**	**99.48**	**0.84**	**2855.18**	**0.99**	**5.03**	**4.91**	**17.30**	**2.68**	**2859.50**	**174.33**

Table 3.15 Disease parameters and total phenolic content from 22 sorghum lines representing a range of plant and grain characteristics planted at Cedara 11/12 (2)

				Cedara 11/12 (2)						
Genotype	**Line**	**Plant colour**	**Grain colour**	**Mean visual rating**	**Mean ergos-terol** (µg/g)	**Mean FGSC** (µg/g)	**Mean DON** (µg/kg)	**Mean NIV** (µg/kg)	**Mean ZEA** (µg/kg)	**Mean total pheno-lics** (µg/g)
G1	BTX 378	Purple	Red	2.23	95.70	13.20	0.00	10.40	0.40	93.53
G2	BTX 3197	Purple	White	1.61	208.30	9.90	0.00	24.10	0.30	32.41
G3	RTX 430	Purple	White	2.77	303.20	3.10	0.00	4.70	3.00	53.53
G4	RTam 428	Red	White	2.44	80.30	16.20	4.70	10.50	1.50	46.86
G5	RTX 436	Tan	White	1.44	13.30	10.10	4.80	1.80	1.40	29.90
G6	BTX 635	Tan	White	0.94	180.60	7.90	0.00	24.10	0.30	50.19
G7	BTX ARG-1	Tan		1.65	216.40	20.20	102.00	7.40	1.00	40.75
G8	RTX 2917			1.76	160.20	24.80	4.00	18.00	0.30	275.46
G9	SC 630-11EII	Purple	Dark red	1.21	205.80	3.20	2.00	20.90	1.20	74.35
G10	SC 630-11Eii	Purple	Light red	1.18	139.50	1.30	3.90	0.00	0.60	56.30
G11	SC 748-5	Purple	Lemon yellow	2.37	122.70	1.20	2.00	35.80	0.40	94.64
G12	SC 109-14E	Purple	White	2.56	36.10	1.20	0.00	20.30	0.60	47.42
G13	SC 719-11E	Purple	Red	2.23	184.00	4.00	0.00	25.70	1.60	318.53
G14	SC 103-12E	Purple	Dark brown-red	2.03	36.70	5.30	4.30	59.20	0.30	371.86
G15	Dobbs	Purple	Brown	2.84	99.40	7.00	2.50	5.10	0.30	229.64
G16	Hegari	Purple	White	3.10	124.80	8.10	1.10	7.50	1.00	47.42
G17	RTam 2566	Purple	Dark brown-red	3.27	103.30	8.20	33.50	6.50	0.30	106.86
G18	TX 2911			2.67	545.00	84.70	0.00	0.00	0.00	2324.52
G19	SCAY 13	Tan	Lemon yellow	1.24	138.40	14.80	0.00	16.50	0.70	105.19
G20	SCAY 16			1.67	54.50	2.90	6.50	0.00	0.00	86.86
G21	SCAY 21	Tan	Red	1.01	176.30	3.90	11.90	18.20	1.20	74.64
G22	SCAY 14	Tan	Lemon yellow	1.32	164.80	2.30	0.00	3.80	0.80	95.20
Mean				**1.93**	**154.06**	**11.52**	**8.32**	**14.57**	**0.78**	**211.64**

Table 3.16 Disease parameters and resistance metabolites from 22 sorghum lines representing a range of plant and grain characteristics planted at Alma 12/13 (1).

				Alma 12/13 (1)														
Genotype	**Line**	**Plant colour**	**Grain colour**	**Mean visual rating**	**Mean ergos-terol** (µg/g)	**Mean FGSC** (µg/g)	**Mean DON** (µg/kg)	**Mean NIV** (µg/kg)	**Mean ZEA** (µg/kg)	**Mean total pheno-lics** (µg/g)	**Mean total protein** mgprot /ml	**Mean gallic acid** (µg/g)	**Mean chiti-nase** (ch/prot/hr)	**Mean gluca-nase** (gl/prot /hr)	**No flavo-noids**	**Total [flavo-noids]**	**No pheno-lic acids**	**Total [pheno-lic acids]**
G1	BTX 378	Purple	Red	2.45	135.40	5.80	5.80	0.00	0.00	83.34	0.85	3931.80	0.47	4.46	13	1.28	10	3933.06
G2	BTX 3197	Purple	White	2.29	190.80	1.80	0.00	0.00	0.00	25.19	0.89	3141.53	0.29	5.38	12	1.44	4	3141.78
G3	RTX 430	Purple	White	2.53	414.70	4.30	0.00	0.00	0.00	51.11	0.96	2627.40	1.05	5.46	8	1.19	6	2627.77
G4	RTam 428	Red	White	2.30	174.20	0.50	0.00	0.00	0.00	46.67	0.94	2966.83	0.54	5.84	9	1.21	4	2967.33
G5	RTX 436	Tan	White	0.93	1254.7	3.90	0.00	0.00	0.00	70.00	0.91	2797.33	0.45	6.05	8	0.86	10	2798.47
G6	BTX 635	Tan	White	1.19	27.80	13.10	9.70	0.00	0.00	46.67	0.74	765.08	0.36	5.36	12	1.86	4	765.45
G7	BTX ARG-1	Tan		2.99	1527.0	6.50	24.50	0.00	0.00	41.85	0.91	2410.95	1.52	5.08	5	2.44	10	2476.47
G8	RTX 2917			2.82	443.40	6.60	0.00	0.00	0.00	73.34	1.01	2987.10	0.41	5.98	8	1.36	11	2988.54
G9	SC 630-11EII	Purple	Dark red	1.36	309.60	6.40	0.00	0.00	0.00	76.30	1.00	2474.55	0.18	5.93	17	33.22	11	2475.60
G10	SC 630-11Eii	Purple	Light red	1.56	114.10	3.70	5.60	0.00	0.00	81.11	0.99	2584.04	0.06	6.09	15	2.33	7	2585.07
G11	SC 748-5	Purple	Lemon yellow	2.16	258.30	1.80	5.10	0.00	0.00	89.63	1.02	2960.00	0.06	5.75	15	45.33	8	2961.54
G12	SC 109-14E	Purple	White	2.75	597.80	13.80	0.00	0.00	0.00	40.74	1.01	4047.56	2.38	6.03	13	1.65	9	4048.77
G13	SC 719-11E	Purple	Red	2.56	65.30	3.70	0.00	0.00	0.00	322.96	0.96	4248.45	2.10	6.68	13	1.28	5	4248.95
G14	SC 103-12E	Purple	Dark brown-red	2.10	888.30	10.50	54.50	27.60	0.00	275.93	1.02	3793.34	0.26	6.05	13	5.06	8	3794.18
G15	Dobbs	Purple	Brown	2.65	627.70	3.50	0.00	0.00	0.00	181.48	0.90	3913.01	0.12	5.35	8	1.18	4	3913.59
G16	Hegari	Purple	White	3.16	140.10	11.80	10.80	0.00	0.00	43.70	0.89	2648.38	0.51	5.62	12	1.03	7	2648.87
G17	RTam 2566	Purple	Dark brown-red	3.04	1019.70	0.00	0.00	0.00	0.00	85.56	0.97	2955.53	0.70	5.79	12	1.29	4	2955.86
G18	TX 2911			2.79	504.50	3.70	0.00	0.00	0.00	130.00	1.11	2629.71	2.16	0.00	11	1.31	6	2630.32
G19	SCAY 13	Tan	Lemon yellow	1.26	536.70	7.50	0.00	0.00	0.00	82.22	1.00	1755.46	0.10	6.08	4	0.87	11	1758.06
G20	SCAY 16			1.90	411.50	6.30	0.00	0.00	0.00	80.37	0.93	4416.01	0.39	6.88	10	1.02	11	4417.56
G21	SCAY 21	Tan	Red	1.24	19.20	1.10	0.00	0.00	0.00	54.07	1.15	2804.26	1.46	6.15	15	1.96	9	2805.35
G22	SCAY 14	Tan	Lemon yellow	1.32	703.50	4.90	0.00	0.00	0.00	87.78	1.01	2609.24	2.13	5.57	9	1.98	11	3112.46
Mean				**1.98**	**471.10**	**5.51**	**5.27**	**1.25**	**0.00**	**94.09**	**0.96**	**2975.80**	**0.80**	**5.53**	**11**	**5.05**	**7.73**	**3002.50**

Table 3.17 Disease parameters and total phenolic content from 22 sorghum lines representing a range of plant and grain characteristics planted at Alma 12/13 (2)

Genotype	Line	Plant colour	Grain colour	Alma 12/13 (2) Mean visual rating	Mean ergos-terol (µg/g)	Mean FGSC (µg/g)	Mean DON (µg/kg)	Mean NIV (µgk/g)	Mean ZEA (µg/kg)	Mean total pheno-lics (µg/g)
G1	BTX 378	Purple	Red	2.28	70.30	2.70	0.00	10.00	0.70	90.74
G2	BTX 3197	Purple	White	2.24	183.60	1.20	5.20	7.10	2.40	27.78
G3	RTX 430	Purple	White	2.35	76.60	3.70	2.80	13.80	0.50	66.30
G4	RTam 428	Red	White	2.15	7.20	0.60	0.00	0.00	0.40	50.37
G5	RTX 436	Tan	White	0.92	155.30	5.10	0.00	11.20	1.80	88.53
G6	BTX 635	Tan	White	1.45	98.50	10.80	33.50	27.70	2.10	58.52
G7	BTX ARG-1	Tan		2.18	406.50	4.20	2.00	17.00	2.50	37.78
G8	RTX 2917			2.48	19.80	5.50	3.30	16.90	0.40	94.81
G9	SC 630-11EII	Purple	Dark red	1.86	38.20	5.70	23.50	26.40	0.20	94.44
G10	SC 630-11Eii	Purple	Light red	1.46	128.30	3.30	19.90	28.20	0.90	112.96
G11	SC 748-5	Purple	Lemon yellow	1.96	57.40	1.40	39.70	3.00	0.90	107.78
G12	SC 109-14E	Purple	White	2.19	10.90	13.60	0.00	8.20	1.80	51.11
G13	SC 719-11E	Purple	Red	2.28	25.60	3.20	0.00	26.20	0.70	339.63
G14	SC 103-12E	Purple	Dark brown-red	1.69	118.80	10.00	5.00	21.40	0.50	282.22
G15	Dobbs	Purple	Brown	2.30	150.40	3.10	0.00	9.40	1.10	129.26
G16	Hegari	Purple	White	3.06	133.30	11.50	0.00	11.50	0.30	113.33
G17	RTam 2566	Purple	Dark brown-red	2.64	151.60	8.10	0.00	31.90	0.30	172.22
G18	TX 2911			2.31	119.80	4.80	0.00	17.30	0.40	455.56
G19	SCAY 13	Tan	Lemon yellow	1.31	140.60	7.40	4.50	13.00	0.40	112.96
G20	SCAY 16			1.57	85.20	6.10	2.10	21.00	0.90	94.44
G21	SCAY 21	Tan	Red	1.30	11.72	37.20	0.00	18.70	0.90	200.00
G22	SCAY 14	Tan	Lemon yellow	1.13	166.70	4.90	4.80	21.80	1.20	92.59
Mean				**2.15**	**107.11**	**7.00**	**6.65**	**16.44**	**0.97**	**130.61**

Table 3.18 Disease parameters and resistance metabolites from 22 sorghum lines representing a range of plant and grain characteristics planted at Alma 13/14

				Alma 13/14										
Genotype	**Line**	**Plant colour**	**Grain colour**	**Mean visual rating**	**Mean ergos-terol** (µg/g)	**Mean FGSC** (µg/g)	**Mean DON** (µg/kg)	**Mean NIV** (µg/kg)	**Mean ZEA** (µg/kg)	**Mean total pheno-lics** (µg/g)	**Mean total protein** mgprot/ml	**Mean gallic acid** (µg/g)	**Mean chiti-nase** (ch/prot/hr)	**Mean gluca-nase** (gl/prot/hr)
G1	BTX 378	Purple	Red	2.88	961.00	10.20	0.00	6.30	0.60	100.00	0.82	510.41	0.69	4.20
G2	BTX 3197	Purple	White	3.77	1139.00	0.70	1.20	3.40	76.70	58.15	0.73	1167.67	0.18	5.52
G3	RTX 430	Purple	White	3.81	3096.00	3.60	0.00	18.70	70.00	62.59	0.84	1004.41	0.53	4.15
G4	RTam 428	Red	White	3.44	1530.00	2.30	0.00	5.50	4.50	61.11	0.84	960.12	0.56	5.22
G5	RTX 436	Tan	White	2.65	2066.00	2.60	22.00	9.00	15.20	51.85	0.69	1025.15	1.82	5.32
G6	BTX 635	Tan	White	3.12	1105.00	15.60	2.80	7.10	7.50	49.26	0.70	908.34	0.19	4.41
G7	BTX ARG-1	Tan		2.28	1384.00	9.20	6.20	4.30	0.90	86.30	0.78	956.88	0.29	4.91
G8	RTX 2917			3.36	1429.00	6.40	0.00	6.00	7.90	77.04	0.89	977.09	3.07	4.54
G9	SC 630-11EII	Purple	Dark red	3.83	2600.00	5.20	3.20	10.80	60.30	69.26	0.87	1051.77	1.02	4.53
G10	SC 630-11Eii	Purple	Light red	1.36	764.00	2.30	0.00	13.90	1.30	107.04	0.75	1576.93	4.04	5.36
G11	SC 748-5	Purple	Lemon yellow	2.37	1254.00	0.50	0.00	12.50	0.90	90.37	0.89	1677.42	0.42	3.90
G12	SC 109-14E	Purple	White	2.60	1342.00	11.90	7.70	4.20	4.30	76.31	0.81	1498.40	0.01	5.20
G13	SC 719-11E	Purple	Red	2.80	1758.00	2.70	0.00	0.00	0.00	238.52	0.65	1103.65	0.27	5.34
G14	SC 103-12E	Purple	Dark brown-red	1.52	1213.00	9.30	18.80	4.60	33.60	296.67	0.87	1029.47	0.30	4.84
G15	Dobbs	Purple	Brown	2.14	1189.00	2.60	20.60	6.00	39.90	107.78	0.92	1080.66	0.14	4.70
G16	Hegari	Purple	White	3.95	580.00	10.40	4.50	7.20	1.20	100.74	0.78	1168.47	0.11	5.04
G17	RTam 2566	Purple	Dark brown-red	2.71	1272.00	0.90	2.70	6.50	1.10	70.37	0.87	1054.94	0.68	5.07
G18	TX 2911			2.33	4978.00	3.20	4.10	11.90	0.40	102.22	0.89	774.38	0.02	5.65
G19	SCAY 13	Tan	Lemon yellow	3.10	1575.00	5.60	9.90	4.90	2.10	46.30	0.76	608.40	1.16	5.45
G20	SCAY 16			2.23	861.00	4.70	10.20	7.20	12.90	197.78	0.79	1112.02	0.30	4.78
G21	SCAY 21	Tan	Red	2.19	1394.00	2.00	1.30	9.50	0.80	81.11	0.80	977.49	0.15	5.24
G22	SCAY 14	Tan	Lemon yellow	2.06	1851.00	3.00	0.00	15.10	3.20	78.89	0.91	943.05	0.23	4.72
Mean				**1.96**	**1606.41**	**5.22**	**5.24**	**7.94**	**15.70**	**100.44**	**0.81**	**1053.05**	**0.74**	**4.91**

Table 3.19 Mean disease parameters and mean resistance metabolites from twenty-two sorghum lines representing a range of plant and grain characters.

Genotype	Line	Total Phenolics (µg/g)	Gallic acid (µg/g)	Total proteins (mgprot/ ml)	Chitinase (mgchitin/ mgprot/ hour)	Glucanase (mggluc/ mgprot/ hour)	Line mean	Visual rating	Ergosterol levels (µg/g)	DNA levels (µg/g)	DON Levels (µg/kg)	NIV levels (µg/kg)	ZEA levels (µg/kg)	Line mean
G1	BTX 378	94.41	2446.01	0.76	1.13	4.23	**509.31**	2.49	338.43	44.26	26.66	44.64	73.1	**88.26**
G2	BTX 3197	37.64	2377.43	0.72	1.41	4.94	**484.43**	2.1	360.79	35.39	35.54	70.25	189.34	**115.57**
G3	RTX 430	53.47	2150.02	0.86	1.22	4.64	**442.04**	2.75	841.41	86.78	14.23	36.8	74.88	**176.14**
G4	RTam 428	49.75	2670.79	0.78	1.41	4.88	**545.52**	2.51	350.09	18.46	1.82	18.24	6.61	**66.29**
G5	RTX 436	75.24	1357.81	0.77	1.23	5.44	**288.10**	1.43	1169.25	113.69	47.67	181.41	34.36	**257.97**
G6	BTX 635	50.03	1769.57	0.70	1.22	4.30	**365.16**	1.53	454.13	200.88	174.06	243.04	6.08	**179.95**
G7	BTX ARG-1	49.82	2218.59	0.84	1.22	4.54	**455.00**	1.84	844.38	65.78	67.13	31.28	14.13	**170.76**
G8	RTX 2917	128.26	2168.23	0.91	2.24	5.34	**461.00**	2.21	1079.13	203.89	38.54	140.79	9.85	**245.74**
G9	SC 630-11EII	77.60	2297.53	0.85	1.09	4.95	**476.40**	1.99	560.01	77.91	113.43	102.14	54.61	**151.68**
G10	SC 630-11Eii	85.83	2290.36	0.83	1.64	5.29	**476.79**	1.68	318.08	79.88	79.03	64.08	19.7	**93.74**
G11	SC 748-5	88.94	2348.26	0.88	0.73	4.94	**488.75**	2.47	433.01	38.69	12.85	33.51	341.49	**143.67**
G12	SC 109-14E	64.77	2750.19	0.83	2.97	5.08	**564.77**	2.44	678.68	131.91	79.62	44.73	55.41	**165.47**
G13	SC 719-11E	331.10	2235.40	0.82	1.15	5.89	**514.87**	2.44	494.99	62.78	49.1	49.68	4.34	**110.56**
G14	SC 103-12E	313.74	2956.51	0.85	0.98	4.96	**655.41**	2.05	469.21	45.48	49.22	59.24	8.56	**105.63**
G15	Dobbs	210.14	2610.01	0.82	1.00	4.37	**565.27**	2.83	420.45	78.04	29.86	32.28	162.01	**120.91**
G16	Hegari	107.82	2101.34	0.73	1.38	4.69	**443.19**	3.33	334.26	60.08	28.75	29.26	221.36	**112.84**
G17	RTam 2566	106.04	2079.86	0.81	1.09	5.14	**438.59**	3.18	667.05	101	46.35	50.03	103.89	**161.92**
G18	TX 2911	450.04	1977.59	0.98	0.93	4.16	**486.74**	2.65	1178.06	181.55	24.69	51.55	4.88	**240.56**
G19	SCAY 13	89.84	1950.43	0.89	1.07	5.53	**409.55**	1.52	484.31	53.14	31.84	29.71	98.81	**116.56**
G20	SCAY 16	105.79	2685.91	0.83	1.18	5.63	**559.87**	1.78	350.91	55.13	34.84	22.78	4.14	**78.26**
G21	SCAY 21	91.55	2462.06	0.94	1.11	5.13	**512.16**	1.50	461.79	29.46	5.72	57.00	38.01	**98.91**
G22	SCAY 14	90.25	2457.44	0.92	1.32	4.99	**510.98**	1.44	582.59	26.35	12.05	19.1	5.59	**107.85**
Mean		**125.09**	**2289.15**	**0.83**	**1.31**	**4.96**	**484.27**	**2.19**	**585.05**	**81.39**	**45.59**	**64.16**	**69.60**	**141.33**

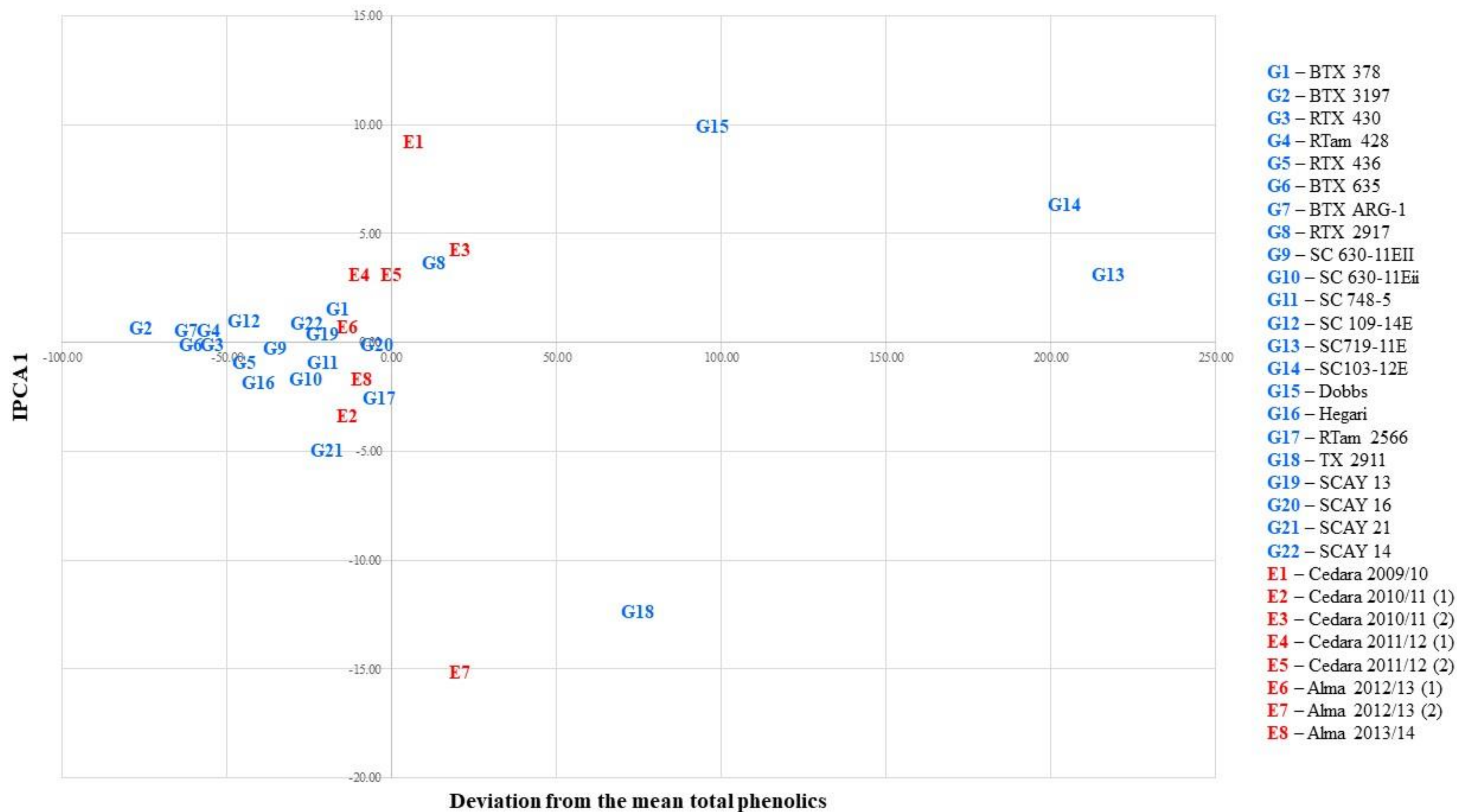

Figure 3.1 AMMI biplot of mean total phenolics in twenty two sorghum lines planted in Cedara and Alma.

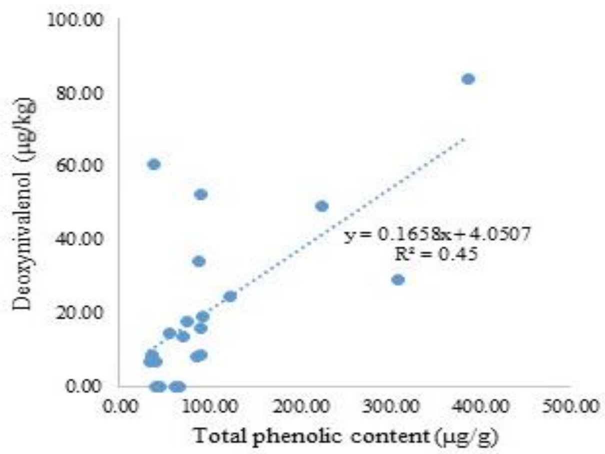

Figure 3.2 Relationship between total phenolic content and deoxynivalenol levels in grain from the first planting in Cedara during 2011/2012.

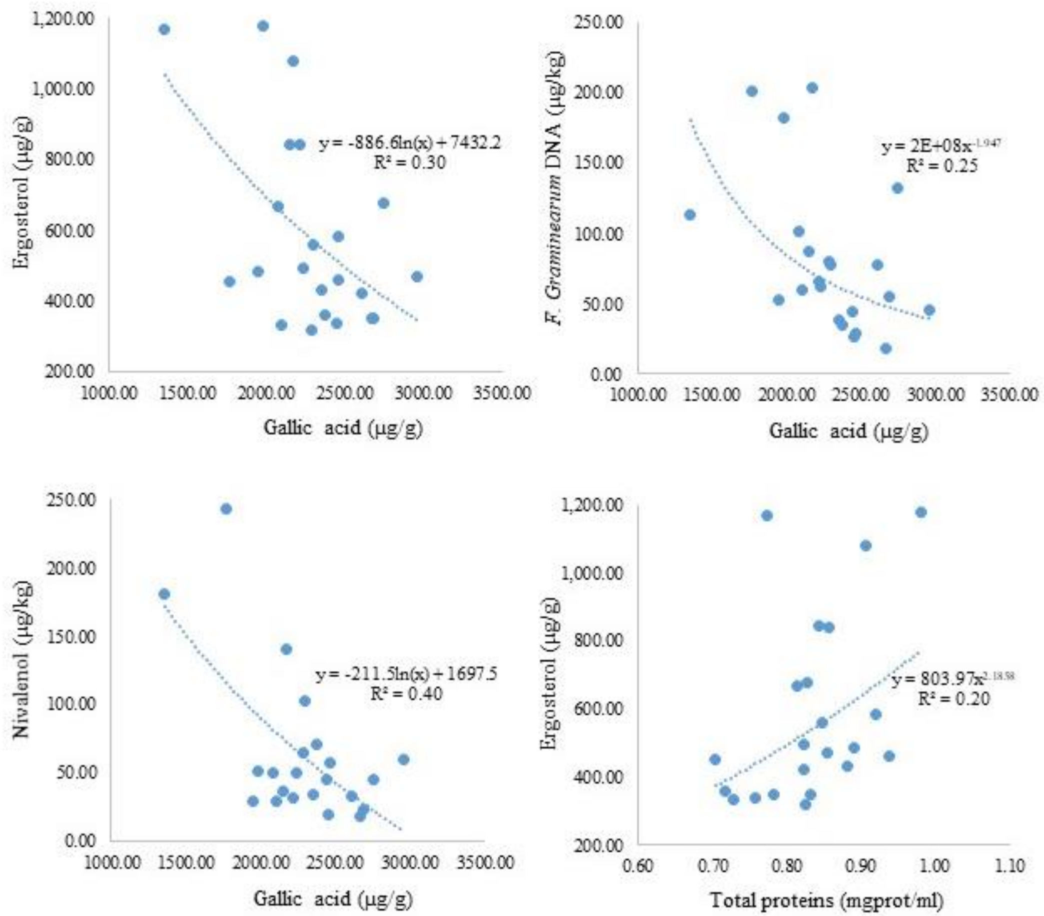

Figure 3.3 Relationships between mean disease parameters and mean levels of resistance metabolites from 22 sorghum lines to indicate their role in resistance.

CHAPTER 4

THE EFFECT OF ROTATION SYSTEMS ON GRAIN MOLD AND ROOT ROT OF SORGHUM

4.1. Abstract

Grain mold affects yield and quality of sorghum and the fungi responsible can produce mycotoxins that affect both human and animal health when ingested. Many grain mold pathogens also cause root rot of sorghum and the translocation of mycotoxins from roots to grain may occur. Rotation systems with legumes (soybean, drybean and cowpeas) were introduced into a field trail at Alma over a three year period to evaluate the effect on sorghum grain mold and root rot severity in a white-tan and high-tannin-brown cultivar, i.e. PAN 8706W and NS 5511 respectively. Monoculture and fallow systems were also included for comparison. Seasonal changes in soil nutrient status and biodiversity using fluorescein diacetate analyses were measured. Visual ratings of root rot and grain mold were assessed at soft dough and maturity growth stages respectively. Root mass and yield were recorded at the respective growth stages. Effective root mass was calculated as an integration of root rot rating and root mass. Ergosterol as an indicator of total fungal biomass was recorded in roots and grains using HPLC analysis while quantification of *Fusarium graminearum* species complex (FGSC) was conducted using real-time PCR. The mycotoxins, deoxynivalenol, nivalenol and zearalenone were also quantified using tandem mass spectrometry. In the season following the initial rotation cropping system, the fallow plot resulted in the lowest nutrient levels, apart from iron. In the second measured season, nutrients were the lowest in plots where sorghum followed sorghum, no matter the preceding system, with the highest calcium, magnesium and potassium detected in double drybean systems. Nitrogen fixation by legumes could not be detected in either seasons. Increased root mass and yield of sorghum was recorded with legume systems in both seasons with restricted plant growth in monoculture and fallow systems. The lowest masses were recorded in double fallow and monoculture sorghum for roots and yield respectively. Treatments differed in their effect on soil biodiversity, root and grain colonisation by FGSC, total fungal biomass and mycotoxin production. Cultivars also differed in their response to rotation systems. NS 5511 was more resistant to grain mold and more susceptible to root rot, compared with PAN 8706W, although the latter had almost double the levels of deoxynivalenol in the roots in the second season. Throughout the seasons, beneficial effects of exposure to legume treatments on sorghum growth and resistance to root rot were observed although response to grain pathogens was variable.

Keywords: legumes, nutrient, root rot, rotation systems, sorghum grain mold

4.2. INTRODUCTION

Due to increasing population growth and a greater need for food security, ways of improving grain quality and quantity in crops, are being explored. The limited area of productive farmland available (Marschner *et al.,* 2004) and other yield-limiting factors, including environmental conditions and diseases, are a growing concern. Root rot and grain mold are diseases of sorghum that affect yield and grain quality in South Africa (Schoeman and Greyling-Joubert, 2017). Root rot can lead to yield losses of up to 25% (Tarr, 1962) and grain mold 30% to 100% (Singh and Bandyopadhyay, 2000). Both these diseases are caused by a complex of pathogens with the major causal organisms of root rot being *Fusarium* spp., *Colletotrichum graminicola*, *Macrophomina phaseolina, Periconia circinata* and *Pythium* spp. (Mughogho, 1984) and those of grain mold, *Fusarium* spp., *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., *Curvularia* spp., *Drechslera* spp., *Olpitrichum* spp. and *Phoma* spp. (Das *et al.,* 2012). Many of these fungi can produce heat-stable mycotoxins that affect the health of humans and animals when consumed (Zinedine and Mañes, 2009) and the translocation of mycotoxins from roots to grain may occur.

Disease management strategies have been confounded by the diversity of fungal pathogens responsible for these diseases (Thakur *et al*., 2006). Breeding for resistance and cultural control strategies that reduce inoculum in the field, remain some of the best management strategies for both root rot and grain mold pathogens (Dill-Macky and Jones, 2000; Prom *et al*., 2003; Blandino *et al.,* 2012).

The continuous use of monocropping in farming systems has led to a build-up of pathogens, as well as a loss of organic matter that has resulted in the collapse of soil structure, higher compaction, surface runoff and water erosion of, in particular, sandy soils (Dominy *et al.,* 2002). This can be alleviated by the introduction of rotation systems (Dominy *et al.,* 2002; Janvier *et al*., 2007), although the efficacy in the prevention of plant diseases, depends on the type of crops planted, as well as the amount of subsequent crop residue (Dill-Macky and Jones, 2000). Rotating unrelated plant species can reduce diseases such as *Sclerotinia* stem rot of soybean caused by *S. sclerotiorum* where a reduction in disease severity was observed in a three year soybean-maize rotation system (Rousseau *et al.,* 2007).

The high cost of commercial fertilizers versus the required nitrogen levels for rapid plant growth in five-leaf sorghum plants, led to the exploration of legume rotation systems for their added nitrogen fixation benefits and soil quality improvement (Whitney, 1998; Rochester *et al.,* 2001). In areas with predominantly sandy mineral-soils, legume-cereal rotations can also increase yield (Marschner *et al.,* 2004). This was attributed to:

- higher nitrogen released into the soil from decaying legume roots (Bagayoko *et al.,* 2000b);
- higher nitrogen and phosphorous levels in the soil which improve sorghum growth resulting in extra shoot and root biomass being produced, stimulating rapid colonisation by arbuscular mycorrhizae, which in turn assist with nutrient absorption (Bagayoko *et al.,* 2000b; Alvey *et al.,* 2001);
- increased pH levels which affect phosphorous availability by influencing solubility and equilibrium concentrations (Alvey *et al.,* 2001);
- reduced invasion by plant-parasitic nematodes associated with rotating host plants with non-host plants (Bagayoko *et al.,* 2000b) or the release of root exudates that deter nematode populations (Alvey *et al.,* 2001);
- a reduction in host-specific plant pathogens when cereals are substituted with unrelated legumes that result in healthier plants (Janvier *et al*., 2007).
- Less *Striga hermonthica* parasitic weeds by activating the suicidal germination of the seeds (Teka, 2014; Franke *et al*., 2018).

The aim of this study was to examine legume-sorghum rotation systems over a three year period and their effect on soil properties, sorghum grain molds and root rots in a Limpopo farming system from 2012/2013 to 2014/2015.

4.3. MATERIALS AND METHODS

4.3.1. Field trials

Cowpeas (*Vigna unguiculata* (L.) Walp), drybeans (*Phaseolus vulgaris* (L.)) and soybeans (*Glycine max* (L.) Merrill) were planted in rotation systems with monoculture sorghum (*Sorghum bicolor* (L.) Moench) and a fallow plot (Table 4.1) in a split plot design over a three year period to evaluate the effects of crop sequence on sorghum root rot and grain mold severity as well as yield quantity and quality. Systems were designed to provide permutations of single

rotation crop followed by single and double sorghum crops and double rotation crop followed by a single sorghum crop. During the first growing season, 180 m (length) x 25 m (width) plots were planted with the respective legumes and sorghum, while another plot remained fallow. Inter-row spacings were 0.75 m with population equivalents of 360 000 plants per hectare for legumes and 100 000 plants per hectare for sorghum. In the subsequent growing season, the initial plots were divided into 6 subplots of which three of these were split and planted to a high-tannin sorghum cultivar, NS 5511, or white sorghum cultivar, PAN 8706W. The remainder of plots were then planted with the respective legume rotation treatments or sorghum in the case of monoculture sorghum. In the third season all subplots were planted to NS 5511 and PAN 8706W. Subsequent to planting plots were treated with Frontier Optima (dimethenamid 75 g/l) for pre-emergence grass-weed control at a rate of 0.9 l/ha followed by Basagran® (Bendioxide - thiadiazine 480 g/l) post-emergence at a rate of 1.5 l/ha for broadleaf weeds. Stalk borer and aphid control was applied as required using Decis® (Deltamethrin 100 g/l) at 0.25 l/ha. Trials were maintained according to the best practices for the Limpopo region. At flowering 50 sorghum heads per plot were covered with fine mesh bags to limit bird damage.

4.3.2. Soil properties

4.3.2.1. Nutrient levels and pH

Soil samples from a depth of 15 cm were collected from each plot at the commencement of each growing season to measure soil uniformity, initially across the entire trial area and subsequently within plots. Soil nutrient analyses (Table 4.2 and Table 4.3) were conducted by the department of Soil Sciences at the University of the Free State.

4.3.2.2. Microbial activity

Soil samples for the assessment of microbial activity were collected prior to the commencement of the 2013/14 ie. the first season of sorghum subsequent to initial treatments. Samples at pre-plant and from sorghum root rhizospheres at early flowering were collected during the 2014/15 season. Total microbial activity using fluorescein diacetate (FDA) (Schnürer and Rosswall, 1982) was determined by Mr. H. Chung at the Department of Plant Sciences at the University of the Free State.

4.3.3. Root rot assessments

4.3.3.1. Root visual rating and root mass

Five plants per subplot were randomly selected at soft dough growth stage, dug up, severed at the crown and roots were rinsed in running tap water to remove soil. These roots were used in all the subsequent assessments. Root rot severity based on tissue discolouration was visually estimated on a percentage scale. Recovered root mass was recorded and effective root mass was calculated according to McLaren (1999) where E is effective root mass: E= [{(100-root rot severity)/100}*root mass].

4.3.3.2. Ergosterol content as a measure of fungal biomass in roots

A modified method of Jambunathan *et al.* (1991) was used. Sorghum roots were macerated into a powder using liquid nitrogen. Powdered root tissue (5 g) was added to 25 ml of methanol (Merck) and mixed with a Heidolph Multi Reax Shaker (Labotec) for 30 minutes. After allowing the solution to settle, 12.5 ml of the upper liquid layers were added to 1.5 g of potassium hydroxide (KOH) in a screw cap test tube and mixed using the Multi Reax Shaker to dissolve the KOH. N-hexane (5 ml) was added, the mixture was incubated for 30 min in a 75°C water bath and allowed to cool to ambient temperature. Distilled water (2.5 ml) was added, mixed thoroughly with the Heidolph Multi Reax Shaker and subsequently centrifuged at 3000 rcf (BHG Optima) for 1 min. The upper hexane layer was transferred to a new test tube. N-hexane (5 ml) was added to the residual aliquot, mixed and centrifuged. The upper hexane layer was removed once again and added to the earlier aliquot. This was repeated. The pooled hexane extracts were completely evaporated in a 75°C water bath. The residue was resuspended in 2.5 ml methanol and filtered through a 0.45 µl Pall Acrodisc syringe filter into a glass vial for analysis on a Perkin Elmer high performance liquid chromatograph (HPLC) with a SIL-20A auto sampler. The extracts were loaded onto a reverse phase column (Phenomenex, C18 125 A 10 µm particle size, 150 x 4.6 mm) at 50°C. The isocratic mobile phase was comprised of methanol:water (96:4) with a flow rate of 1.2 ml/min. Standard ergosterol (Sigma) was used to calibrate the equipment over a range of 7500.00 – 29.30 µg/g. Ergosterol concentration was recorded at 282 nm with a retention time of approximately 7 min.

4.3.3.3. *qPCR to quantify FGSC in roots*

4.3.3.3.1. *DNA extraction*

DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega) following manufacturer's instructions. Powdered roots (40 mg) were placed in a 1.5 ml Eppendorf tube. Nuclei Lysis solution (600 µl) was added and vortexed (Vortex Genie 2, Scientific Industries) for one to three seconds. The tubes were incubated for 15 minutes in a water bath set to 65°C. RNase solution (3 µl) was added. The tubes were lightly mixed by inverting them. A 37°C water bath was used to incubate the tubes for 15 minutes. The tubes were left to cool for 5 minutes to reach ambient temperature. Protein precipitation solution (200 µl) was added and the tubes were vortexed for 20 seconds. The supernatant was removed after centrifugation at 14 000 rcf for 3 minutes on a Hermle centrifuge (Lasec) and transferred to a clean tube with 600 µl isopropanol at ambient temperature. The tubes were mixed by inversion until thread-like DNA strands formed. The tubes were centrifuged for 1 minute at 14 000 rcf and the supernatant was decanted. Ethanol (70% v/v; 600 µl) at ambient temperature was added and the tubes were carefully inverted to wash the DNA. The tubes were centrifuged at 14 000 rcf for 1 minute, after which the ethanol was carefully removed. The pellet was left to air-dry for approximately 30 minutes. DNA Rehydration solution (100 µl) was added to dissolve the pellet and this was left overnight at 4°C. DNA concentrations were determined using a NanoDrop Spectrophotometer and diluted to 10 ng/µl DNA. The tubes were stored at -20°C until qPCR was performed.

4.3.3.3.2. *qPCR*

Quantitative polymerase chain reaction (qPCR) analysis was conducted according to Nicolaisen *et al.,* (2009). Species specific primers used for FGSC detection were (5'-3'):

- FgramB379 fwd (CCATTCCCTGGGCGCT)
- FgramB411 rev (CCTATTGACAGGTGGTTAGTGACTGG)
  (Inqaba Biotechnical Industries, Pretoria, RSA)

qPCR reactions consisted of 5 µl iTaq™ Universal SYBR® Green (BIO-RAD), 1 µl Primer mix (0.5 µl FgramB379 and 0.5 µl FgramB411), 3.2 µl Nuclease-free water (Promega) and 0.8 µl template DNA. A positive *F. graminearum* control, no template control and matrix matched

standards were included. These were diluted 4, 16, 64 and 256 fold. All reactions were done in triplicate. The cycling regime on a Bio-Rad C1000 thermal cycler with a CFX96 real-time attachment (Bio-Rad, Hercules, USA) containing a 96-well reaction plate was as follows: 10 minutes at 95°C, 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds and 72°C for 15 seconds. A melt curve was included at 72°C to 95°C with a 1°C increment at every step. Efficiency was determined using standard curves and ranged from 90 to 110%.

4.3.4. Mycotoxins associated with FGSC in roots

A modified method of Small *et al.* (2012) was used to extract mycotoxins from sorghum roots. Sorghum roots were ground into a fine powder using liquid nitrogen. Powdered root tissues (5 g) were added to 20 ml of 70% methanol (Merck) in test tubes and shaken on a Heidolph Multi Reax Shaker (Labotec) at 1470 rpm for 30 minutes. After centrifuging for 10 minutes, the supernatant was filtered through a 0.45 µl syringe filter and placed into 2 ml Eppendorf tubes, which were left overnight at 4°C. Deoxynivalenol (DON) samples were placed in 1.8 ml HPLC vials, whereas nivalenol (NIV) and zearalenone (ZEA) samples were diluted tenfold before placing them in the vials (Coetzee, 2015). Matrixed matched standards, over a range of 100 µg/kg to 12500 µg/kg, were prepared from standards attained from Sigma-Aldrich (JHB, RSA) and an established "mycotoxin free" sorghum grain sample.

A 4000 QTRAP hybrid triple quadrupole ion trap mass spectrometer (AB Sciex), Shimadzu UFLC stack with LC-20AB binary pump and SIL-20A HT autosampler was used to analyze the mycotoxin samples in collaboration with Dr. G. Kemp at the Biochemistry and Microbiology Department of the University of the Free State. The samples were separated on a C18 (150x2.1mmx5µ, Discovery C18, Supelco) column. The mobile phase consisted of 10mM ammonium acetate (mobile phase A) and methanol/10mM ammonium acetate (mobile phase B) with a flow rate of 300 µL/min using a stepwise gradient between the different mobile phases, starting with 2% mobile phase B increasing rapidly to 75%, maintaining it for 4 minutes and returning to 2% for a total runtime of 9 minutes. A negative electrospray mode with a 4500 V ion spray voltage, 500°C heater temperature, 60 psi nebuliser gas, 60 psi heater gas and 25 psi curtain gas was used to ionize eluting analytes. The injection volume for each sample was 20 µl. The instrument used a Multiple Reaction Monitoring workflow to analyse the sample in which the instrument is used in triple quadrupole mode. Fragment masses were produced by ionized analytes eluting off the column and a set of masses created a transition.

The instrument jumped between different transitions in a Multiple Reaction Monitoring transition during a cycle. For each transition detected, the ion intensity value was plotted. Data outputs were processed using Analyst 1.5 (AB Sciex) software (Coetzee, 2015).

4.3.5. Grain mold assessments

4.3.5.1. Grain visual rating and grain yield

Grain was harvested from bird-bag covered heads at maturity (12.5% moisture content), weighed and visually assessed for grain mold severity. Grain visual ratings were done on a 1 to 5 scale according to Audilakshmi *et al*. (2007) where 1 = 0% visible deterioration; 2 = 10%, 3 = 11–25%, 4 = 26–50% and 5 = ≥50%.

4.3.5.2. Evaluation of ergosterol content to determine fungal biomass in grain

Harvested grains were milled in a coffee grinder to a fine powder. Ergosterol analyses were conducted on 5 g samples as described above (Jambunathan *et al.,* 1991).

4.3.5.3. qPCR to quantify FGSC in grain

4.3.5.3.1. DNA extraction

DNA extraction was performed as described above using 40 mg fine sorghum meal prepared by using a coffee grinder.

4.3.5.3.2. qPCR

Quantitative polymerase chain reaction (qPCR) analysis was conducted as described above (Nicolaisen *et al.,* 2009).

4.3.6. Mycotoxins associated with FGSC in grain

Mycotoxin extractions were performed as described above on a 5 g ground grain sample (Small *et al.,* 2012).

4.3.7. Statistical analysis

Analysis of variance (ANOVA) was performed on all root and grain mold parameters to determine differences between treatment effects using NCSS (Hintze, 2001). Mean separation was calculated using Fishers Protected LSD ($P<0.05$). Relationships between disease and quality variables were quantified using Pearsons correlation analysis and linear and non-linear regression analysis using NCSS (Hintze, 2001).

4.4. RESULTS

4.4.1. Soil properties

4.4.1.1. Nutrient levels and pH

During the first season, subsequent to initial rotation systems (commencement of season two), levels of nitrogen (N), calcium (Ca), copper (Cu) and zinc (Zn) levels did not differ significantly ($P<0.05$) across the rotation systems. Carbon (C), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), manganese (Mn) and phosphorous (P) levels were significantly affected by rotation systems (Table 4.2). A general tendency for higher levels of these elements in legume systems compared to, in particularly fallow systems, was evident, with the exception of Fe. Na was also higher in monoculture systems than in legume systems. pH of soybean, drybean and monoculture systems were not significantly different ($P<0.05$) from one another, whereas drybean and monoculture were not significantly different from cowpea (Table 4.2).

In the subsequent season (commencement of season three), only N levels were not significantly different ($P<0.05$) in the rotation systems (Table 4.3). Legume rotation systems, in particular the drybean/drybean/sorghum system, resulted in higher Ca, Mg and K levels compared to the remaining legume systems with similar levels to one another. C and Na levels were significantly higher in monoculture and fallow systems respectively compared to legume rotations. P levels in legume rotation systems were variable. No significant differences were recorded between P levels of fallow, monoculture and drybean/sorghum/sorghum rotation systems, although the latter was similar to levels in cowpea/sorghum/sorghum rotation systems. No significant differences were recorded between levels in

soybean/soybean/sorghum, drybean/drybean/sorghum, cowpea/cowpea/sorghum and soybean/sorghum/sorghum rotations. Significantly higher (P<0.05) pH levels were recorded in soybean/sorghum/sorghum, compared to monoculture with the lowest pH levels.

4.4.1.2. Microbial activity

Significant differences (P<0.05) in rhizosphere biodiversity, as indicated by FDA levels, were recorded between legume rotation systems in the first season subsequent initial rotation systems (commencement of season two) and fallow systems. No significant differences were recorded between legume rotation systems (Table 4.2). The rhizosphere microbial activity levels in the legumes were, however, similar to those in monoculture rotations systems.

In the subsequent season (commencement of season three), significantly higher pre-season FDA levels were recorded in monoculture systems, with lower levels detected in legume rotation systems (Table 4.3) by in particularly cowpea systems. FDA levels determined later at the milk stage during grain set were not significantly different (P<0.05). When comparing the two FDA levels from each rotation system, only cowpea/cowpea/sorghum rotation systems indicated a significant increase in soil microbial activity from pre-planting to milk stage as indicated by a positive change proportion (P<0.05). The other rotation systems resulted in a decrease in microbial activity as indicated with a negative change proportion between the two FDA levels.

4.4.2. Root rot assessment

4.4.2.1. Root visual ratings and root mass

Root visual ratings in the first season subsequent the initiation of rotation systems (commencement of season two) were not significantly different, irrespective of fallow, monoculture or the legume rotation systems applied (Table 4.4). Root mass and effective root mass on the other hand, were significantly increased as a result of legume rotation systems (P<0.05), by in particularly drybean and cowpea systems, with the lowest recorded in monoculture and fallow systems. Mean root visual ratings ranged from 24.00% in PAN 8706W to 42.33% in NS 5511 indicating significant cultivar responses to root pathogens.

In the following season (commencement of season three), similar responses were recorded (Table 4.5). Root visual ratings were not significantly different (P<0.05) between any of the monoculture, fallow or legume systems applied. Root mass significantly increased with legume rotation systems, in particularly with the cowpea rotation system, indicating the stimulation of root growth by legume rotations. Cultivar responses were also similar to the previous season with root visual ratings that ranged from 26.77% in PAN 8706W to 41.60% in NS 5511. No significantly different (P<0.05) effective root mass was recorded between rotation systems.

4.4.2.2. Evaluation of ergosterol content to determine fungal biomass in roots

No significantly different (P<0.05) ergosterol levels were recorded between monoculture, fallow or legume rotation systems applied in the season subsequent to the initiation of rotation systems (commencement of season two) (Table 4.4) or the subsequent season (commencement of season three) (Table 4.5). Also no significantly different cultivar responses were recorded.

4.4.2.3. qPCR to quantify FGSC in roots

In the first season subsequent to the initiation of rotation systems (commencement of season two), significantly higher (P<0.05) FGSC levels were recorded in soybean rotation systems (Table 4.4), compared to the other legume, fallow and monoculture systems that had similar FGSC levels to one another. No significantly different cultivar responses were recorded.

In the subsequent season (commencement of season three), no significantly different FGSC levels (P<0.05) were recorded in the roots, although a tendency for lower levels in the legume rotation systems was observed (Table 4.5). Significantly different cultivar responses were also recorded and ranged from 0.01 µg/g in PAN 8706W to 0.37 µg/g in NS 5511.

4.4.3. Mycotoxin accumulation in roots associated with FGSC

In the first season subsequent to initial rotation systems (commencement of season two), legumes did not significantly affect (P<0.05) mycotoxin levels in the roots (Table 4.4). Significantly different cultivar responses were only observed with DON levels in the roots and ranged from 557.00 µg/kg in NS 5511 to 1054.73 µg/kg in PAN 8706W.

In the following season (commencement of season three), significantly increased DON levels (P<0.05) were recorded in cowpea rotation systems (Table 4.5). No significantly different DON levels were detected from the remaining monoculture, fallow or legumes systems. Significantly higher NIV levels (P<0.05) were recorded in fallow systems with lower levels in soybean rotation systems (Table 4.5). No significant differences (P<0.05) were recorded in ZEA levels (Table 4.5). Cultivar responses were not significantly different to any of the mycotoxins produced, although a tendency for higher mycotoxin production in PAN8706W was observed.

4.4.4. Grain mold assessment

4.4.4.1. Grain visual rating and grain yield

Analysis of variance indicated significant differences (P<0.05) in grain visual ratings from rotation systems in the first season subsequent to initial rotation systems (commencement of season two) with the highest grain visual rating from the monoculture system and the lowest from the fallow system (Table 4.6). A significant cultivar x rotation interaction was recorded indicating cultivar responses to rotation systems. Grain mass per 50 plants was significantly higher (P<0.05) in legume rotation systems, compared to the lowest grain mass from monoculture systems (Table 4.6).

In the 14/15 season (commencement of season three), significantly higher (P<0.05) grain visual ratings and grain masses were recorded in legume rotation systems, with the highest in soybean/sorghum/sorghum and the lowest in monoculture sorghum. A significant cultivar x rotation system interaction was recorded in grain mass per 50 plants indicating response of the cultivar to the rotation system applied.

4.4.4.2. Evaluation of ergosterol content to determine fungal biomass in grain

Ergosterol levels in the grain during the first season subsequent to the initiation of rotation systems (commencement of season two) differed significantly (P<0.05). Similarly low ergosterol levels (Table 4.6) were recorded in soybean, drybean and monoculture systems, with

higher ergosterol levels in grain from cowpea and fallow rotation systems. Significantly lower ergosterol levels were recorded in grain of cultivar NS 5511 compared with PAN 8706W.

No significant differences in ergosterol levels were recorded in the subsequent 14/15 season (commencement of season three) (Table 4.7). Also no significant differences in cultivar responses were recorded.

4.4.4.3. qPCR to quantify FGSC in grain

No significant differences were recorded in FGSC levels in grain from rotation systems in the first season (commencement of season two) subsequent to the initiation of rotation systems (Table 4.6). Only significant cultivar ($P<0.05$) responses that ranged from 5.00 µg/g in NS 5511 to 9.07 µg/g in PAN 8706W were recorded.

In the subsequent 14/15 season (commencement of season three), significantly different FGSC levels were recorded in rotation systems ($P<0.05$) with the highest recorded in cowpea systems and the lowest in soybean systems. FGSC levels in monoculture and fallow systems were similar to one another (Table 4.7). Significantly different ($P<0.05$) cultivar responses were also recorded. NS 5511 was more resistant to colonisation than PAN 8706W.

4.4.5. Mycotoxin accumulation in grain associated with FGSC

DON levels were not significantly different ($P<0.05$) in the first season (commencement of season two) subsequent to initial rotation systems (Table 4.6). Grain from drybean rotation systems contained significantly higher NIV levels, compared to the other rotation systems, with similar NIV levels to one another. A significant ($P<0.05$) cultivar x legume rotation was recorded with ZEA levels (Table 4.6) with the lowest ZEA levels of 0.89 µg/kg recorded in cultivar NS 5511 from soybean rotations and the highest levels of 72.69 µg/kg in PAN 8706W from drybean rotation systems. Significantly different ($P<0.05$) cultivar responses were recorded with higher NIV levels in PAN 8706W than in NS 5511.

In the subsequent 14/15 season (commencement of season three) (Table 4.7), DON levels differed significantly ($P<0.05$) with high levels recorded in legume systems, by in particularly soybean and drybean systems. Low levels of DON were found in grain from fallow systems.

No significant differences ($P<0.05$) were recorded in NIV levels in rotation systems. ZEA levels in fallow systems were significantly higher than the levels produced in all the legume rotation and monoculture systems. No significantly different cultivar responses were recorded in any of the rotations to any mycotoxins produced.

4.4.6. Relationships between variables

Significant relationships were recorded between mean disease parameters and nutrients, pH and FDA levels. During 13/14 (Figure 4.1) higher Na was associated with higher grain mold visual rating ($R^2 = 0.97$*) and lower NIV production in the roots ($R^2 = 0.89$*). Higher Fe was associated with reduced grain mold symptoms as indicated by visual rating ($R^2 = 0.70$*). Increased Cu levels suppressed fungal colonisation of the grain as indicated by lower ergosterol levels ($R^2 = 0.75$*). Higher Mg, P and FDA was associated with lower root rot visual rating ($R^2 = 0.71$*, $R^2 = 0.92$* and $R^2 = 0.94$* respectively). Higher P was also associated with increased fungal biodiversity in the rhizosphere determined through FDA levels ($R^2 = 0.77$*). Higher C and pH were associated with increased root colonisation ($R^2 = 0.79$* and $R^2 = 0.80$* respectively) by fungi. Higher N ($R^2 = 0.90$*) increased root colonisation by FGSC fungi (as indicated by root DNA), whereas higher Zn decreased FGSC root colonisation ($R^2 = 0.85$*).

In 14/15 (Figure 4.2) higher Mg levels were associated with increased grain mass ($R^2 = 0.50$*), root mass ($R^2 = 0.55$*) and root colonisation by fungi as determined through ergosterol analyses ($R^2 = 0.50$*). Higher Mg was also associated with decreased ZEA in grain ($R^2 = 0.49$*), FGSC colonisation of the roots (recorded as root DNA levels) ($R^2 = 0.58$*) and root NIV production ($R^2 = 0.61$*). Higher pH levels were associated with increased DON levels in grain ($R^2 = 0.41$*) and decreased NIV in roots ($R^2 = 0.69$*). Increased K levels were associated with increased root mass ($R^2 = 0.45$*), root colonisation by fungi as indicated with ergosterol ($R^2 = 0.52$*) and decreased grain ZEA levels ($R^2 = 0.48$*), root colonisation by FGSC ($R^2 = 0.60$*) and root NIV levels ($R^2 = 0.50$*). Higher Na was associated with higher grain ZEA ($R^2 = 0.75$*), FGSC root colonisation ($R^2 = 0.74$*) and lower root mass ($R^2 = 0.61$*) and root colonisation by fungi ($R^2 = 0.56$*). Higher biodiversity pre-planting was associated with lower root mass ($R^2 = 0.43$*), root colonisation by fungi ($R^2 = 0.44$*) and root DON ($R^2 = 0.53$*). Higher biodiversity during grain filling ($R^2 = 0.41$*) was associated with higher NIV in roots and lower P levels in the rhizosphere ($R^2 = 0.44$*).

4.5. DISCUSSION

The benefits of legume rotation systems are indicated in the current study. Increased availability of nutrients, growth promotion and disease suppression were evident in the various legume rotation systems.

Nitrogen, together with P, Ca, Mg, K and Si, form part of osmotic, structural, conformational and compositional processes of plants. Micronutrients, i.e. B, Cu, Fe, Mn and Zn act as catalysts, inhibitors or co-factors (Jordan *et al.,* 1984). These macro- and micronutrients affect disease development by interfering with plant physiology or influencing the causal pathogens directly (Dordas, 2008). Rhizosphere soil from the legume systems in the current study had increased levels of C, Mg, K, Mn and P, while monocropping and fallow rotation systems had increased levels of Na and Fe respectively, with levels of C and Na increasing towards the subsequent season. Double drybean rotation systems resulted in increased Ca, Mg and K.

Microbial activity was initially higher in rhizosphere soil from legume rotation systems. Only cowpea/cowpea/sorghum showed an increase in microbial activity from pre-planting to milk stage. The higher biodiversity was associated with lower root colonisation and DON production in roots. Marschner *et al.* (2004) indicated an increase in soil microbial organisms in groundnut rotation systems as a result of decomposing groundnut tissues in the soil. Strains of *Bacillus cereus* and *B. subtilis* from the rhizosphere can suppress sorghum root rot causal pathogens, *Fusarium oxysporum* and *Pythium ultimum*, through the production of antibiotics (Idris *et al.,* 2007; Idris *et al.,* 2008). Weinhold and Bowman (1968, cited by Cook *et al.,* 1978) showed that soybean residues were able to support the production of antibiotics by *B. subtilis.*

Higher pH levels were recorded in legume rotation systems, in particularly soybean rotation systems, followed by drybean systems. Marschner *et al.* (2004) found higher pH in soil from sorghum-groundnut rotation plots than from monoculture sorghum plots in Burkina Faso and Togo. Cowpea rhizoplane soil had a higher pH than soil from the surrounding field indicating the crops' ability to influence soil pH (Bagayoko *et al.,* 2000a). An increase in soil pH can influence plant growth by contributing to nutrient absorption and elimination of toxicities that limit the need for lime applications (Fageria and Zimmermann, 1998). Maize, wheat and common bean have increased uptake of N, P and K as soil pH rises (Fageria and Zimmermann, 1998). An improved uptake of Ca and Mg were observed at a higher pH in pearl millet planted

in rotation with cowpea. Mesocotyl and root rot development were reduced with a higher pH acquired through various fertilizer applications in KwaZulu Natal, leading to the assumption that sorghum plants are more predisposed to root rot at a lower pH (McLaren, 2004). Pathogens, such as *Fusarium* head blight pathogens, flourish under an acidic pH between 4.5 and 6.0 *in vitro*, with the exception of *F. graminearum* that can tolerate a wider range of pH fluctuations (Panwar *et al.,* 2016).

Throughout both seasons, the mass of sorghum roots and yield subsequent to legume rotation systems, was increased relative to monoculture sorghum or sorghum subsequent to fallow plots. Increased shoot and root biomass has been attributed to the release of N by legumes that enhances plant growth (Dill-Macky and Jones, 2000), although results from this study could not confirm this. Colder temperatures prior to pre-season sampling may have hindered the decomposition of surface stubble and N release may have only occurred subsequently to planting (Horst and Härdter, 1994). Higher nitrogen release through degradation of legume tissues (Venkatesh *et al.,* 2017) in the soil from legume rotation systems provides germinating sorghum with more favourable growth conditions compared to monoculture crops (Bagayoko *et al.,* 2000b). Lehmann *et al.* (1999) observed an increase in grain yield and general biomass with increased nitrogen uptake by sorghum. Marschner *et al.* (2004) reported that sorghum grown in pots containing soil from groundnut rotations, grew significantly faster than sorghum in monoculture soil. Increased root biomass and higher N content in sorghum shoots was also observed. Other benefits of improved N levels and legume rotation systems include enhanced water holding capacity of fields, thereby increasing water usage by 21% which results in yield increases (Zaongo *et al.,* 1997).

Root growth promotion was particularly evident in sorghum subsequent to drybean and cowpea rotation systems and persisted to subsequent seasons, with the best growth resulting from cowpea rotation systems. Soybean and drybean rotation systems increased yield the most. This is consistent with literature. Soybean rotations with wheat resulted in higher yields than corn-wheat or monoculture-wheat (Dill-Macky and Jones, 2000). Similar yield increases were recorded with soybean-maize rotation systems, although this rotation system was also linked to lower root rot incidence (Channon and Farina, 1991). Groundnut-sorghum rotations in Burkina Faso resulted in increased yield of up to 50% in the third year (Bagayoko *et al.,* 2000b) indicating the important role that legumes may play in ensuring yield stability.

Root rot and root colonisation by general root rot fungi seemed to be unaffected by legumes with similar ratings on roots from monoculture, fallow or legume rotation systems. However, relationships between nutrients and disease parameters revealed that increased availability of Mg and P in the rhizosphere from legume rotation systems resulted in lower effective root rot severity. P reduces the effect of root rot (Dordas, 2008) by promoting root growth (Alvey *et al.,* 2001) and hereby allowing the plant to escape the effect of root diseases (Huber and Graham, 1999). Magnesium found in the middle lamella of cell walls strengthens pectic substances, making tissues more tolerant to tissue-degrading pectolytic enzymes secreted by soft rot pathogens (Huber and Jones, 2013). Increased C, K and Mg either increased susceptibility of roots to colonisation by general fungi or stimulated pathogen activity in the rhizosphere. High levels of Na promoted FGSC root colonisation, while higher Mg, Zn and K deterred the growth of FGSC. Na affects plant growth (Subbarao *et al.,* 2003) and has been connected with host-pathogen relationships (Dordas, 2008), in particular due to stress under saline conditions associated with lowered water potential and therefore increased osmotic stress (Maathuis, 2014). This in turn increases susceptibility to diseases, such as common root rot of spring wheat and barley (Duczek, 1992). Zn is involved in protein and starch synthesis (Dordas, 2008) and can prevent the leakage of sugars from root plant cells that can stimulate pathogen activity (Valentine and Kleinert, 2006). Zn also preserves cellular function (Cakmak, 2000) and cellular membrane integrity (Sadeghzadeh, 2013) by detoxifying superoxide radicals that reduce oxidative toxicity (Cakmak, 2000). A Zn deficiency can increase root vulnerability to *Fusarium graminearu*m root diseases (Sadeghzadeh, 2013). K can lead to the development of thicker cell walls in the epidermis (Dordas, 2008) that decreases susceptibility to pathogens (Huber *et al.,* 2012). The different responses to nutrients can be related to the various pathogens responsible for root rot (Gupta *et al.,* 2017).

Initially sorghum root colonisation by FGSC was high in soybean rotation systems, but as the seasons progressed, low FGSC levels were detected in all systems, with an ultimate tendency for lower FGSC levels in legume rotation systems. This suggested a potential shift in fungal populations as a result of continuous rotation of crops (Janvier *et al*., 2007) that can lead to partial or total control of root rot fungi (Curl, 1963). Teich and Hamilton (1985) found that when maize was followed by winter wheat in Canada, increased head blight infection occurred, compared to when the preceding crop was either soybeans, barley or mixed small grains. This was attributed to the burial of stubble during plowing that prevented the dispersal of inoculum and exposing the buried spores to microbial degradation. FGSC can survive on stubble and

crop residues saprophytically and therefore survival of FGSC should be considered when introducing rotation systems as a control measure. DON levels were high compared to NIV and ZEA levels detected, with the highest in sorghum roots from cowpea systems, probably the result of a fungal survival strategy (Fox and Howlett, 2008) in a threatening environment (Ramirez *et al.,* 2004) or the translocation of DON from the grain. Low DON and NIV levels were detected in drybean and soybean systems. DON has been shown to translocate from infected stem tissues to other plant parts such as wheat heads (Covarelli *et al.,* 2012).

Currently there is a dearth of literature on the relationship between plant nutrition and grain mold and subsequent mycotoxin development. Some of the existing literature focus more on the effect of fertiliser with specific emphasis on N. In this study suppression of NIV in roots was recorded with soybean rotation systems and was associated with an increase in Mg and K levels. An increase in ZEA production in grain was recorded with fallow and drybean rotation systems and associated with an increase in Na levels. Additionally higher Fe was associated with lower grain mold and higher Cu with lower grain colonisation. Fe is a constituent of peroxidase (Dordas, 2008). Peroxidase and ferric iron accumulation at cell wall appositions in the plant following pathogen attack, can guide the production of H_2O_2 needed for an oxidative burst (Torres *et al.,* 2006; Greenshields *et al.,* 2007), a process where reactive oxygen species are produced (Huang, 2001) at infection sites (Torres *et al.,* 2006). These reactive oxygen species can damage pathogens resulting in deformed appressorial germtubes (Liu *et al.,* 2007). Cu plays a role in cell wall lignification (Valentine and Kleinert, 2006) hereby intensifying cell wall strength to prevent fungal penetration (Huang, 2001). Further research is required to clarify and confirm the relationships.

Another contributing factor to take into account is the cultivars planted. PAN 8706W was more resistant to root rot and specifically FGSC colonisation than NS 5511, whereas with the grain, NS 5511 was more resistant to FGSC colonisation than PAN 8706W. PAN 8706W contains high total phenolic content in the roots that are associated with root rot resistance (Van Rooyen, 2012). Coetzee (2015) indicated NS 5511 to be the most resistant cultivar to grain mold and grain mold colonisers.

4.6. CONCLUSION

The high cost of farming practices has steered agriculture into the direction of exploring other possibilities for reducing fungal diseases. Adequate plant nutrition promotes resistance by not only affecting the appearance and composition of the host, but by also depriving pathogens of much needed nutrients. Fertilizers can be costly too and, with the growing concern of the negative effects of field-applied chemicals on the environment, other more natural soil fertility methods are being explored. Crop rotations with legumes remains a favourite due to the benefits of prolonged exposure. The choice of legume remains challenging, as no single legume was connected to enhanced resistance in all disease parameters in the current study and effectiveness was dependent on specific disease parameter in question. Exposure to legume rotations generally increased nutrient availability, plant growth and yield and lowered pathogen colonisation and susceptibility, although the processes involved were more complicated and most likely involved the environment and choice of cultivar.

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Table 4.1 Rotation systems planted from 12/13 to 14/15 at Alma for the evaluation of their effects on sorghum grain mold and root rot severity.

2012/13//2013/14//2014/15	**2012/13//2013/14//2014/15**	**2012/13//2013/14//2014/15**	**2012/13//2013/14//2014/15**	**2012/13//2013/14//2014/15**
Fallow/Sorghum/Sorghum	Monoculture Sorghum	Cowpea/Sorghum/Sorghum	Soybean/Sorghum/Sorghum	Drybean/Sorghum/Sorghum
Fallow/Fallow/Sorghum	Monoculture Sorghum	Cowpea/Cowpea/Sorghum	Soybean/Soybean/Sorghum	Drybean/Drybean/Sorghum
Fallow/Sorghum/Sorghum	Monoculture Sorghum	Cowpea/Sorghum/Sorghum	Soybean/Sorghum/Sorghum	Drybean/Sorghum/Sorghum
Fallow/Fallow/Sorghum	Monoculture Sorghum	Cowpea/Cowpea/Sorghum	Soybean/Soybean/Sorghum	Drybean/Drybean/Sorghum
Fallow/Sorghum/Sorghum	Monoculture Sorghum	Cowpea/Sorghum/Sorghum	Soybean/Sorghum/Sorghum	Drybean/Sorghum/Sorghum
Fallow/Fallow/Sorghum	Monoculture Sorghum	Cowpea/Cowpea/Sorghum	Soybean/Soybean/Sorghum	Drybean/Drybean/Sorghum

Table 4.2 Soil status of legume, sorghum, monoculture and fallow rotation systems at the commencement of 13/14 season at Alma.

Rotation	N (mg/kg)	C (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	K (mg/kg)	Na (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	P (mg/kg)	pH (H_2O)	Pre-Season FDA (µg/ml)
Fallow	350.33	3096.67^{b}	479.33	61.33^{c}	57.33^{b}	18.00^{c}	14.14^{a}	0.43	1.82	4.74^{b}	12.51^{c}	6.61^{c}	3.26^{b}
Monoculture	330.67	3956.67^{a}	529.33	84.00^{ab}	79.33^{a}	25.33^{a}	9.96^{b}	0.49	1.89	6.27^{a}	13.49^{bc}	6.91^{ab}	3.48^{ab}
Drybean	312.00	4126.67^{a}	600.00	89.33^{ab}	82.00^{a}	20.00^{bc}	10.83^{b}	0.42	1.92	6.74^{a}	14.79^{ab}	6.96^{ab}	3.66^{a}
Cowpea	356.00	3610.00^{ab}	602.67	92.00^{a}	82.00^{a}	21.33^{b}	10.38^{b}	0.41	1.85	6.51^{a}	15.96^{a}	6.77^{bc}	3.80^{a}
Soybean	426.00	4336.67^{a}	502.00	76.67^{b}	64.00^{b}	20.67^{bc}	10.64^{b}	0.47	1.69	4.87^{b}	14.28^{abc}	7.07^{a}	3.82^{a}

Table 4.3 Soil nutrient status of legume, sorghum, monoculture and fallow rotation systems at the commencement of 14/15 season at Alma

Legume rotation	N (mg/kg)	C (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	K (mg/kg)	Na (mg/kg)	P (mg/kg)	pH (H_2O)	Pre-Season FDA (µg/ml)	Grain set FDA (µg/ml)	FDA change proportion
Fallow/Sorghum/Sorghum	420.43	3376.67^{abcd}	601.33^{b}	72.67^{d}	59.33^{e}	28.67^{a}	25.46^{ef}	6.63^{de}	3.86^{bc}	2.73	-0.29^{bc}
Fallow/Fallow/Sorghum	430.63	3386.67^{abcd}	595.33^{b}	75.33^{d}	69.33^{cde}	29.33^{a}	31.73^{bcde}	6.60^{de}	3.89^{bc}	2.48	-0.36^{c}
Monoculture Sorghum	451.53	3656.67^{ab}	468.67^{d}	75.33^{d}	72.67^{bcd}	22.67^{b}	26.37^{def}	6.58^{e}	4.25^{ab}	2.68	-0.37^{c}
Monoculture Sorghum	391.07	3786.67^{a}	522.67^{c}	78.67^{cd}	66.67^{de}	20.00^{bc}	28.61^{cdef}	6.66^{cd}	4.53^{a}	2.54	-0.43^{c}
Cowpea/Sorghum/Sorghum	416.20	3020.00^{cde}	598.67^{b}	87.33^{bc}	77.33^{bc}	17.33^{c}	21.00^{f}	6.71^{c}	2.92^{de}	2.80	-0.04^{ab}
Cowpea/Cowpea/Sorghum	402.67	2916.67^{de}	598.67^{b}	87.33^{bc}	78.67^{bc}	20.67^{bc}	35.69^{abc}	6.63^{cde}	2.53^{e}	2.83	0.12^{a}
Soybean/Sorghum/Sorghum	449.57	3176.67^{bcde}	614.67^{b}	86.67^{bc}	77.33^{bc}	19.33^{bc}	33.53^{abcd}	7.05^{a}	3.45^{cd}	2.59	-0.24^{bc}
Soybean/Soybean/Sorghum	377.43	2796.67^{e}	622.67^{b}	94.67^{ab}	80.67^{b}	18.00^{c}	40.09^{a}	6.82^{b}	3.17^{d}	2.19	-0.31^{c}
Drybean/Sorghum/Sorghum	428.80	3120.00^{cde}	642.00^{b}	90.00^{b}	82.00^{b}	21.33^{bc}	27.35^{def}	6.66^{cd}	3.91^{bc}	2.54	-0.35^{c}
Drybean/Drybean/Sorghum	503.13	3526.67^{abc}	718.00^{a}	102.00^{a}	93.33^{a}	23.33^{b}	37.17^{ab}	6.82^{b}	3.87^{bc}	2.38	-0.37^{c}

Table 4.4 The effect of legume rotation systems on root rot disease parameters of two sorghum cultivars in 13/14 at Alma.

Rotation	Root visual rating (%)		Rotation Mean	Root mass/5 plants (g)		Rotation Mean	Effective Root mass (g)		Rotation Mean	Root ergosterol (µg/g)		Rotation Mean
	NS 5511	**PAN 8706W**		**NS 5511**	**PAN 8706W**		**NS 5511**	**PAN 8706W**		**NS 5511**	**PAN 8706W**	
Fallow	51.67	28.33	**40.00**	142.83	145.87	**144.35**^{bc}	70.96	105.02	**87.99**^{bc}	235.63	299.67	**267.65**
Monoculture	38.33	31.67	**35.00**	95.63	112.00	**103.82**^{c}	61.16	79.76	**70.46**^{c}	597.67	520.33	**559.00**
Drybean	43.33	18.33	**30.83**	231.97	198.57	**215.27**^{a}	132.36	163.61	**147.99**^{a}	1374.00	239.33	**806.67**
Cowpea	41.67	16.67	**29.17**	313.17	182.93	**248.05**^{a}	176.40	149.23	**162.81**^{a}	67.00	411.67	**239.33**
Soybean	36.67	25.00	**30.83**	183.33	180.17	**181.75**^{ab}	118.35	137.47	**127.91**^{ab}	836.00	718.93	**777.47**
Cultivar Mean	**42.33**^{a}	**24.00**^{b}		**193.39**	**163.91**		**111.85**	**127.02**		**622.06**	**437.99**	

Table 4.4 Continued

Rotation	*F. graminearum* DNA (µg/g)		Rotation Mean	DON in roots (µg/kg)		Rotation Mean	NIV in roots (µg/kg)		Rotation Mean	ZEA in roots (µg/kg)		Rotation Mean
	NS 5511	**PAN 8706W**		**NS 5511**	**PAN 8706W**		**NS 5511**	**PAN 8706W**		**NS 5511**	**PAN 8706W**	
Fallow	0.09	0.40	**0.24**^{b}	0.00	1253.33	**626.67**	20.43	10.85	**15.64**	0.34	0.36	**0.35**
Monoculture	0.01	0.07	**0.04**^{b}	701.00	934.64	**817.82**	8.50	714	**7.82**	0.25	0.35	**0.30**
Drybean	0.11	0.07	**0.09**^{b}	811.33	1110.67	**961.00**	17.44	11.12	**14.28**	0.32	0.33	**0.32**
Cowpea	0.15	0.13	**0.14**^{b}	730.00	889.67	**809.83**	11.85	9.35	**10.60**	0.42	0.47	**0.45**
Soybean	0.82	0.80	**0.81**^{a}	542.67	1085.33	**814.00**	9.25	19.28	**14.27**	0.34	0.31	**0.32**
Cultivar Mean	**0.24**	**0.29**		**557.00**^{b}	**1054.73**^{a}		**13.50**	**11.55**		**0.33**	**0.36**	

Table 4.5 The effect of legume rotation systems on root rot disease parameters of two sorghum cultivars in 14/15 at Alma.

Rotation	Root visual rating (%)		Rotation Mean	Root mass/5 plants (g)		Rotation Mean	Effective Root mass (g)		Rotation Mean	Root ergosterol (µg/g)		Rotation Mean
	NS 5511	PAN 8706W		NS 5511	PAN 8706W		NS 5511	PAN 8706W		NS 5511	PAN 8706W	
Fallow/Sorghum/Sorghum	37.33	23.33	**30.33**	178.09	136.59	**157.34[cd]**	120.07	102.67	**111.37**	3.55	5.27	**4.41**
Fallow/Fallow/Sorghum	47.33	29.33	**38.33**	100.83	114.33	**107.58[d]**	53.14	80.88	**67.01**	5.21	7.22	**6.22**
Monoculture Sorghum	45.67	26.33	**36.00**	151.33	182.50	**166.92[cd]**	84.85	134.43	**109.64**	7.99	13.31	**10.65**
Monoculture Sorghum	42.67	24.33	**33.50**	217.50	154.83	**186.17[bcd]**	124.49	117.66	**121.08**	7.36	11.82	**9.59**
Cowpea/Sorghum/Sorghum	42.33	26.67	**34.50**	313.17	250.17	**281.67[a]**	179.03	184.77	**181.90**	26.18	23.68	**24.93**
Cowpea/Cowpea/Sorghum	46.67	27.67	**37.17**	329.83	231.00	**280.42[ab]**	180.32	169.93	**175.13**	27.36	19.01	**23.19**
Soybean/Sorghum/Sorghum	39.33	26.67	**33.00**	242.83	248.67	**245.75[abc]**	153.00	182.91	**167.95**	11.59	32.48	**22.03**
Soybean/Soybean/Sorghum	41.33	28.33	**34.83**	231.67	220.17	**225.92[abc]**	135.34	158.71	**147.03**	11.65	19.23	**15.44**
Drybean/Sorghum/Sorghum	39.33	27.67	**33.50**	257.67	229.67	**243.67[abc]**	164.71	172.56	**168.64**	21.00	22.14	**21.57**
Drybean/Drybean/Sorghum	34.00	27.33	**30.67**	254.17	247.33	**250.75[abc]**	170.48	180.24	**175.36**	20.07	15.24	**17.66**
Cultivar Mean	**41.60[a]**	**26.77[b]**		**227.71**	**201.53**		**136.54**	**148.48**		**14.20**	**16.94**	

Table 4.5 Continued

Rotation	*F. graminearum* DNA (µg/g)		Rotation Mean	DON in roots (µg/kg)		Rotation Mean	NIV in roots (µg/kg)		Rotation Mean	ZEA in roots (µg/kg)		Rotation Mean
	NS 5511	**PAN 8706W**		**NS 5511**	**PAN 8706W**		**NS 5511**	**PAN 8706W**		**NS 5511**	**PAN 8706W**	
Fallow/Sorghum/Sorghum	2.30	0.01	**1.16**	893.33	2409.18	**1651.26^{b}**	10.03	34.56	**22.29^{a}**	0.26	2.07	**1.17**
Fallow/Fallow/Sorghum	0.79	0.00	**0.39**	3280.00	1733.33	**2506.67^{b}**	30.20	19.23	**24.72^{a}**	0.74	0.35	**0.54**
Monoculture Sorghum	0.18	0.02	**0.10**	2606.67	2020.00	**2313.33^{b}**	14.13	22.27	**18.20^{abc}**	0.55	0.75	**0.65**
Monoculture Sorghum	0.27	0.00	**0.14**	2070.00	2300.00	**2185.00^{b}**	17.25	20.93	**19.09^{ab}**	0.70	0.61	**0.65**
Cowpea/Sorghum/Sorghum	0.02	0.00	**0.01**	4440.67	5403.33	**4922.00^{a}**	13.40	23.10	**18.25^{abc}**	1.80	3.47	**2.63**
Cowpea/Cowpea/Sorghum	0.02	0.01	**0.02**	5773.33	7060.00	**6416.67^{a}**	20.20	22.70	**21.45^{a}**	0.49	0.46	**0.48**
Soybean/Sorghum/Sorghum	0.00	0.02	**0.01**	2510.00	2006.67	**2258.33^{b}**	2.71	2.45	**2.58^{c}**	0.79	1.26	**1.02**
Soybean/Soybean/Sorghum	0.04	0.00	**0.02**	2576.67	2246.67	**2411.67^{b}**	2.80	1.99	**2.40^{c}**	0.65	26.74	**13.69**
Drybean/Sorghum/Sorghum	0.05	0.02	**0.04**	584.00	1646.67	**1115.33^{b}**	0.40	19.71	**10.05^{abc}**	0.81	0.43	**0.62**
Drybean/Drybean/Sorghum	0.02	0.05	**0.03**	2130.00	1646.67	**1888.33^{b}**	3.61	3.38	**3.50^{bc}**	0.54	0.33	**0.44**
Cultivar Mean	**0.37^{a}**	**0.01^{b}**		**2686.47**	**2847.25**		**11.47**	**17.03**		**0.73**	**3.65**	

Table 4.6 The effect of legume rotation systems on grain mold disease parameters of two sorghum cultivars in 13/14 at Alma.

Rotation	Grain visual rating		Rotation Mean	Grain mass/50 plants (g)		Rotation Mean	Grain ergosterol (µg/g)		Rotation Mean	*F. graminearum* DNA (µg/g)		Rotation Mean
	NS 5511	PAN 8706W		NS 5511	PAN 8706W		NS 5511	PAN 8706W		NS 5511	PAN 8706W	
Fallow	2.00	2.50	**2.25[c]**	2080.33	1998.67	**2039.50[b]**	164.67	511.00	**337.83[ab]**	2.52	5.78	**4.15**
Monoculture	3.67	3.33	**3.50[a]**	1825.33	1338.00	**1581.67[c]**	128.33	246.00	**187.17[bc]**	5.84	11.82	**8.83**
Drybean	2.17	3.00	**2.58[bc]**	3216.00	2920.00	**3068.00[a]**	174.00	346.00	**260.00[bc]**	8.99	10.21	**9.60**
Cowpea	2.50	3.33	**2.92[b]**	3032.33	2800.33	**2916.33[a]**	274.00	665.00	**469.50[a]**	4.30	6.56	**5.43**
Soybean	2.17	3.67	**2.92[b]**	3020.00	2976.33	**2998.17[a]**	187.67	155.67	**171.67[c]**	3.36	10.98	**7.17**
Cultivar Mean	**2.50[b]**	**3.17[a]**	**Interaction LSD (P<0.05) 42.56**	**2634.80**	**2406.67**		**185.73[b]**	**384.73[a]**		**5.00[b]**	**9.07[a]**	

Table 4.6 Continued

Rotation	DON in grain (µg/kg)		Rotation Mean	NIV in grain (µg/kg)		Rotation Mean	ZEA in grain (µg/kg)		Rotation Mean
	NS 5511	PAN 8706W		NS 5511	PAN 8706W		NS 5511	PAN 8706W	
Fallow	0.00	0.00	**0.00**	0.00	0.00	**0.00[b]**	3.34	11.26	**7.30**
Monoculture	0.00	0.00	**0.00**	15.75	14.09	**14.92[b]**	36.46	3.95	**20.20**
Drybean	0.00	8.05	**4.03**	12.05	73.93	**42.99[a]**	8.47	72.69	**40.58**
Cowpea	0.00	0.00	**0.00**	0.00	33.97	**16.98[ab]**	13.61	8.29	**10.95**
Soybean	0.00	0.00	**0.00**	0.00	11.58	**5.79[b]**	0.89	18.59	**9.74**
Cultivar Mean	**0.00**	**1.61**		**5.56[b]**	**26.71[a]**		**12.55**	**22.96**	**Interaction LSD (P<0.05) 42.56**

Table 4.7 The effect of legume rotation systems on grain mold disease parameters of two sorghum cultivars in 14/15 at Alma.

Rotation	Grain visual rating		Rotation Mean	Grain mass/50 plants (g)		Rotation Mean	Grain ergosterol (µg/g)		Rotation Mean	*F. graminearum* DNA (µg/g)		Rotation Mean
	NS 5511	PAN 8706W		NS 5511	PAN 8706W		NS 5511	PAN 8706W		NS 5511	PAN 8706W	
Fallow/Sorghum/Sorghum	2.67	1.68	**2.17^{d}**	2804.73	1649.41	**2227.07^{bc}**	19.49	15.21	**17.35**	1.33	7.61	**4.47^{bc}**
Fallow/Fallow/Sorghum	2.50	2.00	**2.25^{cd}**	1297.88	1138.73	**1218.30^{d}**	18.07	20.69	**19.38**	1.17	6.96	**4.07^{bc}**
Monoculture Sorghum	2.47	1.93	**2.20^{cd}**	2297.05	1784.35	**2040.70^{c}**	19.51	22.86	**21.18**	1.06	6.14	**3.60^{bc}**
Monoculture Sorghum	1.81	2.47	**2.14^{d}**	1164.95	1100.65	**1132.80^{d}**	6.50	30.75	**18.63**	0.85	8.43	**4.64^{bc}**
Cowpea/Sorghum/Sorghum	3.00	3.00	**3.00^{ab}**	2195.61	1841.34	**2018.48^{c}**	28.33	27.61	**27.97**	3.74	8.45	**6.09^{ab}**
Cowpea/Cowpea/Sorghum	2.67	3.17	**2.92^{ab}**	3242.79	2136.55	**2689.67^{ab}**	22.42	27.20	**24.81**	3.19	12.31	**7.75^{a}**
Soybean/Sorghum/Sorghum	3.17	3.17	**3.17^{a}**	4027.46	1995.22	**3011.34^{a}**	27.39	0.00	**13.70**	1.44	3.38	**2.41^{c}**
Soybean/Soybean/Sorghum	2.67	2.67	**2.67^{bc}**	3442.54	2063.20	**2752.87^{ab}**	25.06	3.61	**14.34**	1.22	5.55	**3.38^{bc}**
Drybean/Sorghum/Sorghum	2.83	3.43	**3.13^{ab}**	3569.99	1876.32	**2723.15^{ab}**	33.04	30.39	**31.71**	2.08	8.19	**5.14^{abc}**
Drybean/Drybean/Sorghum	2.50	2.83	**2.67^{bc}**	3642.91	2375.10	**3009.01^{a}**	19.01	14.06	**16.54**	2.57	8.96	**5.77^{ab}**
Cultivar Mean	**2.63**	**2.63**		**2768.59^{a}**	**1796.09^{b}**	**Interaction LSD ($P<0.05$) 833.73**	**21.88**	**19.24**		**1.86^{b}**	**7.60^{a}**	

Table 4.7 Continued

Rotation	DON in grain (µg/kg)		Rotation Mean	NIV in grain (µg/kg)		Rotation Mean	ZEA in grain (µg/kg)		Rotation Mean
	NS 5511	PAN 8706W		NS 5511	PAN 8706W		NS 5511	PAN 8706W	
Fallow/Sorghum/Sorghum	8.76	11.50	**10.13[cd]**	17.83	6.23	**12.03**	7.55	5.02	**6.28[a]**
Fallow/Fallow/Sorghum	11.67	39.80	**25.73[bc]**	24.00	13.65	**18.83**	7.67	5.44	**6.56[a]**
Monoculture Sorghum	3.11	4.81	**3.96[d]**	14.71	10.05	**12.38**	1.77	1.09	**1.43[b]**
Monoculture Sorghum	94.26	2.24	**48.25[a]**	0.00	4.69	**2.35**	0.14	2.54	**1.34[b]**
Cowpea/Sorghum/Sorghum	3.84	52.80	**28.32[abc]**	17.90	11.22	**14.56**	1.25	1.17	**1.21[b]**
Cowpea/Cowpea/Sorghum	4.00	42.40	**23.20[bcd]**	5.73	10.07	**7.90**	1.54	2.55	**2.04[b]**
Soybean/Sorghum/Sorghum	49.87	46.20	**48.03[a]**	9.15	15.66	**12.41**	0.96	0.60	**0.78[b]**
Soybean/Soybean/Sorghum	27.93	36.83	**32.38[ab]**	12.43	8.52	**10.48**	0.42	1.61	**1.02[b]**
Drybean/Sorghum/Sorghum	35.97	38.57	**37.27[ab]**	5.23	16.35	**10.79**	0.80	1.98	**1.39[b]**
Drybean/Drybean/Sorghum	42.43	32.70	**37.57[ab]**	8.73	16.09	**12.41**	0.92	0.99	**0.96[b]**
Cultivar Mean	**28.18**	**30.78**		**11.57**	**11.26**		**1.12**	**2.30**	

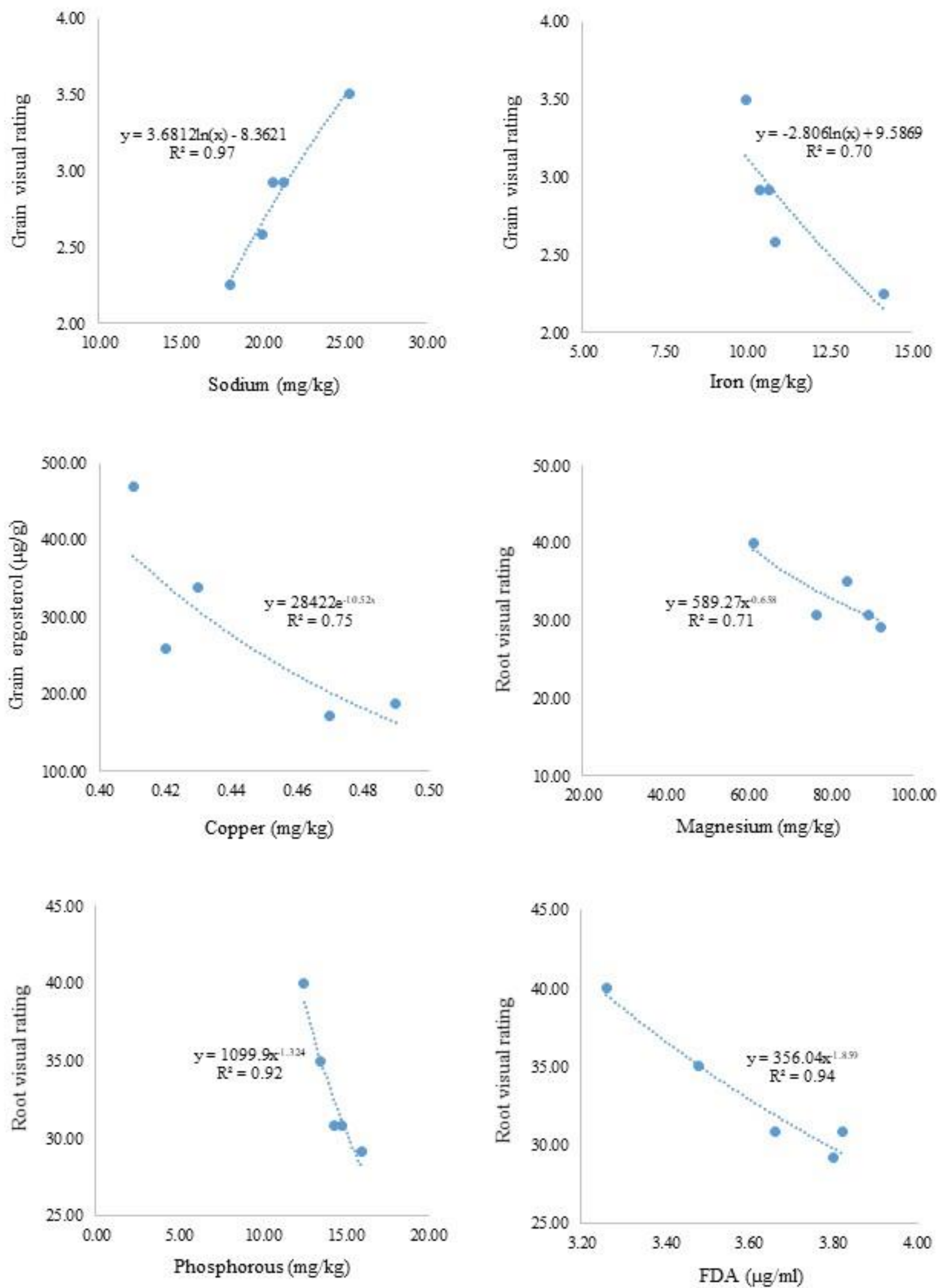

Figure 4.1 Relationships between disease parameters and soil characteristics of rotation systems in 13/14 at Alma.

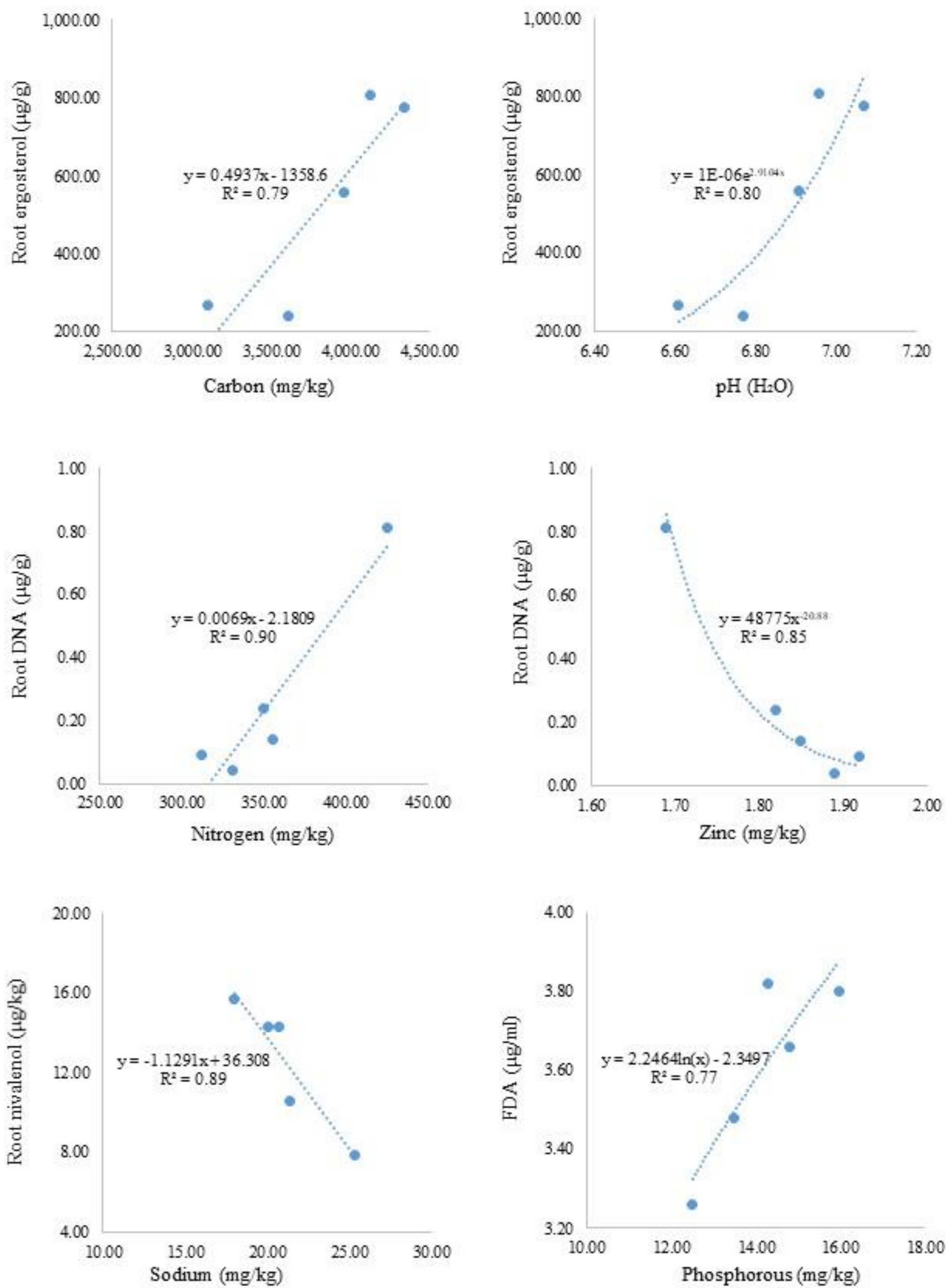

Figure 4.1 Continued

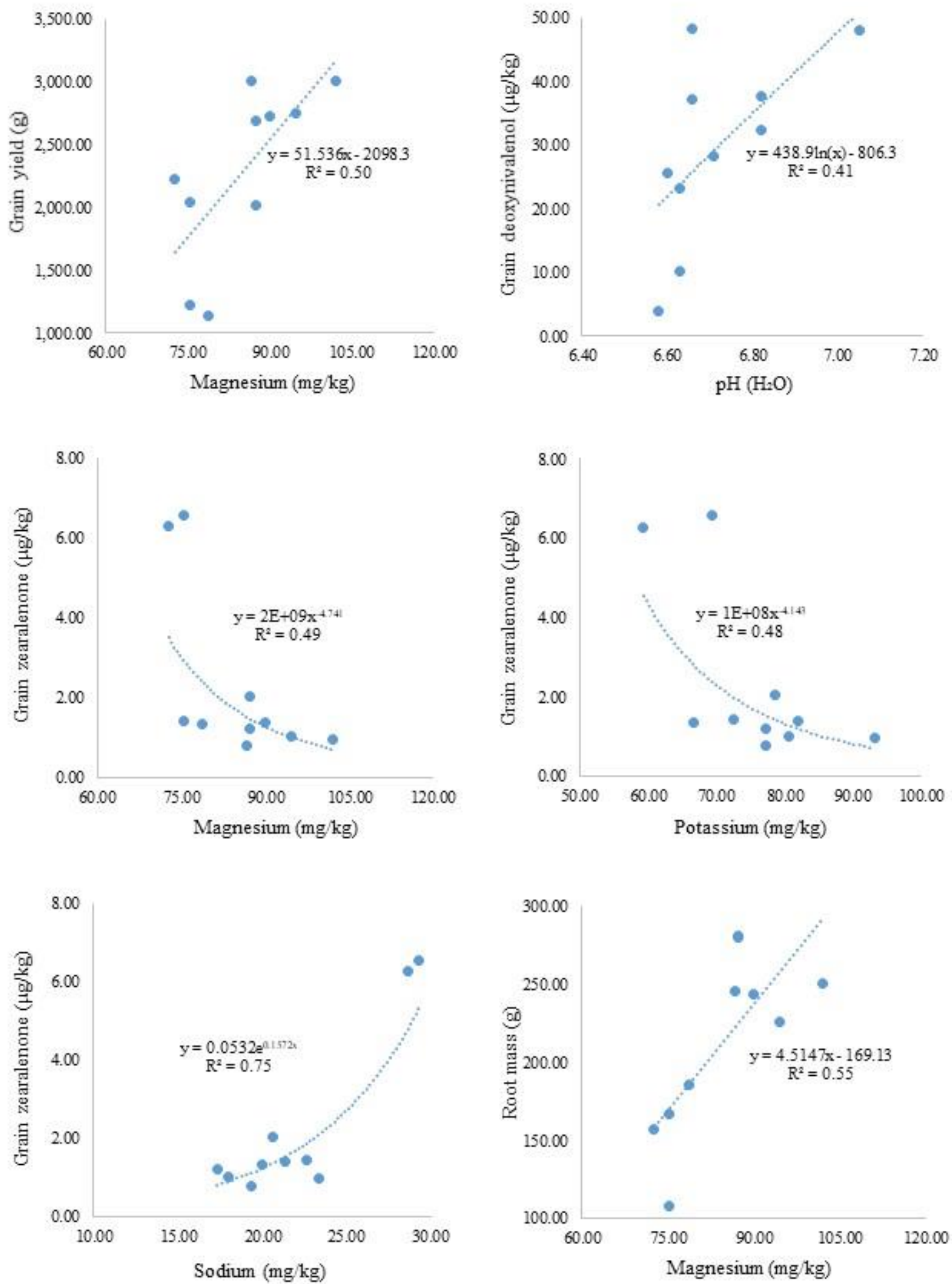

Figure 4.2 Relationships between disease parameters and soil characteristics of rotation systems in 14/15 at Alma.

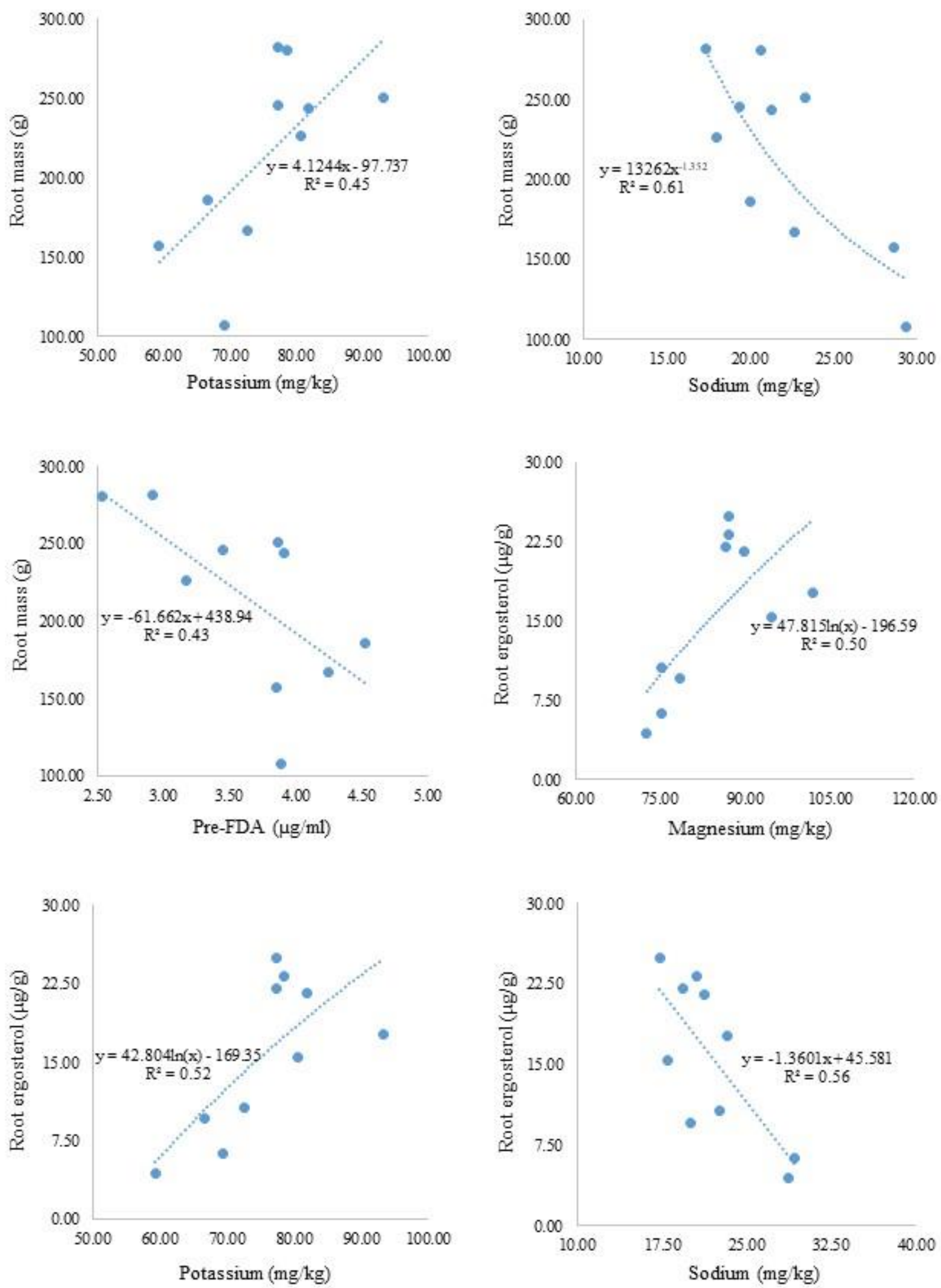

Figure 4.2 Continued

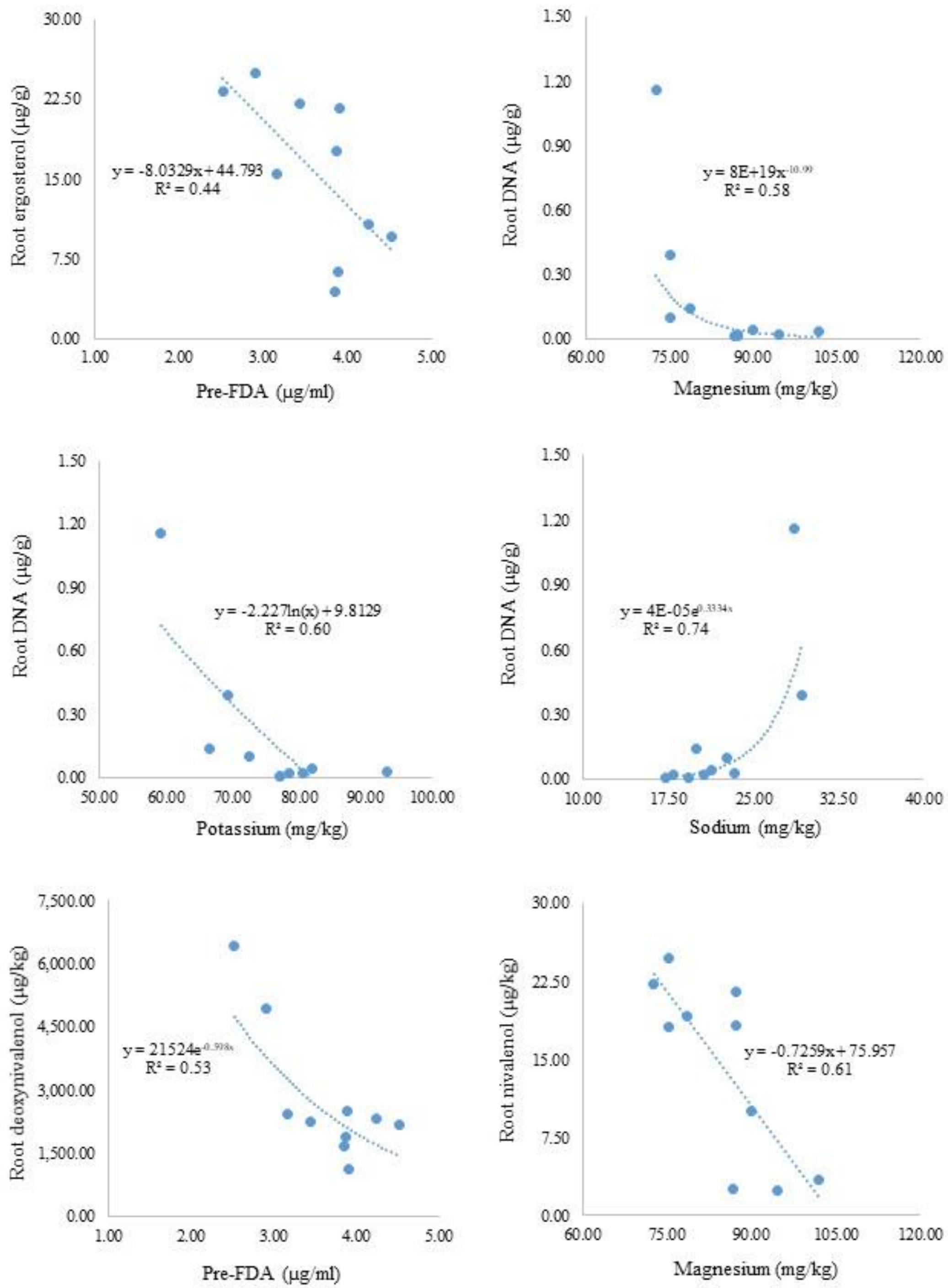

Figure 4.2 Continued

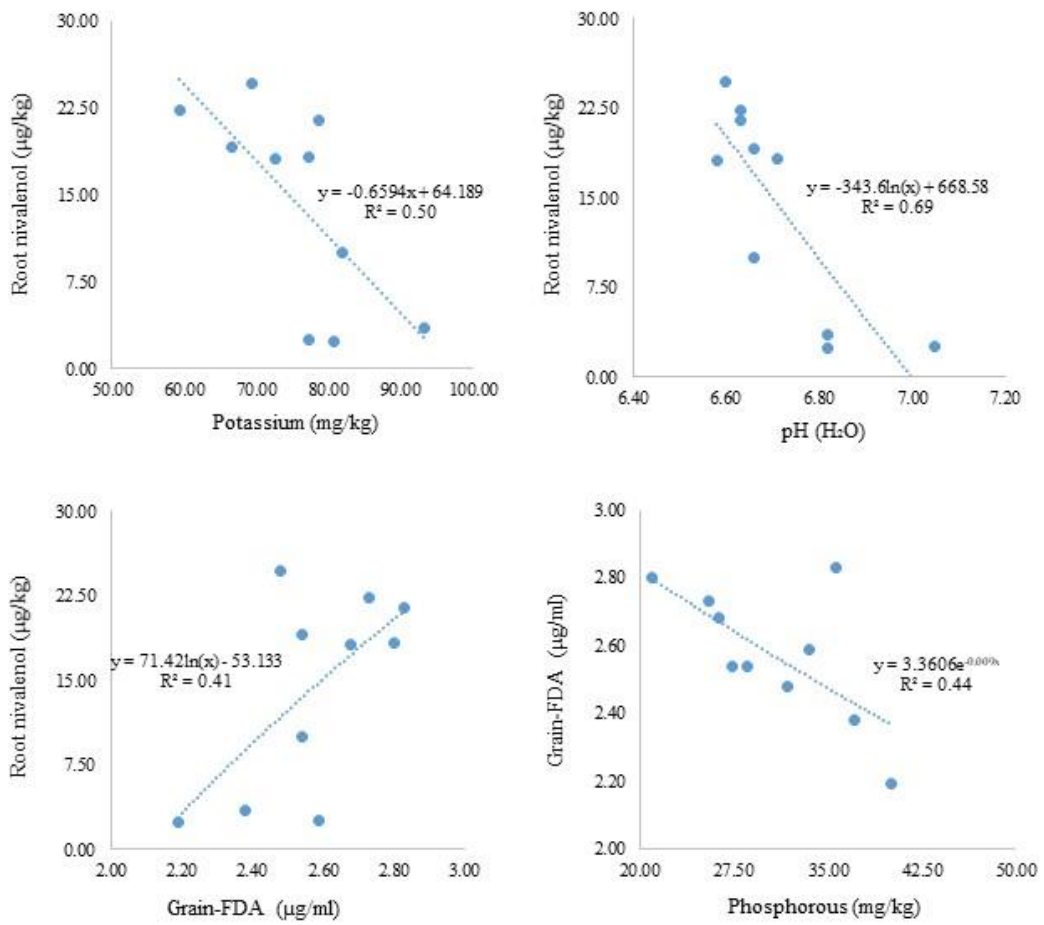

Figure 4.2 Continued

CHAPTER 5

SORGHUM FUNGICIDE SPRAY REGIME EFFECTS ON SORGHUM GRAIN MOLD AND ROOT ROTS

5.1. Abstract

Grain mold is an economically destructive disease that can influence both the yield and quality of sorghum grain. Grain colonizing *Fusarium* spp. produce mycotoxins that can cause serious illnesses in humans and animals. The potential control of grain molds by prophylactic spray regimes used to prevent photosynthetic leaf area loss due to sorghum leaf blight and to enhance the resistance of sorghum to other invasive pathogens was explored. The translocation of mycotoxins from roots to grain has been indicated in literature and root rots were therefore also included in the current study. Field trials were conducted over two years in Potchefstroom, Greytown and Standerton. Two fungicides, azoxystrobin/difenoconazole and epoxyconazole/pyraclostrobin were applied to four cultivars at 6, 8, 10, 6 + 8, 8 + 10 weeks after planting. Visual assessments of percentage root rot were made and root mass and grain yield were recorded, followed by grain mold assessment on harvested grain. Effective root mass was calculated as an integration of percentage of root rot and root mass. Ergosterol as an indicator of fungal biomass in grain and root tissues, was recorded and quantification of *Fusarium graminearum* species complex (FGSC) in grain was conducted using real-time PCR. The mycotoxins, deoxynivalenol, nivalenol and zearalenone in grain were analysed using HPLC-LC-MS-MS. The efficacy of leaf blight fungicides applied to control root rots and grain molds was variable. Both fungicides reduced visual root ratings compared to the control with epoxyconazole/pyraclostrobin being the most effective against accumulation of fungal biomass in roots. Only azoxystrobin/difenoconazole was more effective against grain mold compared to the control. Tissue specificity of fungicides was thus, apparent. Both fungicides stimulated root growth, although no effect on grain mass was recorded. Yield, FGSC colonisation and subsequent mycotoxin concentration were more influenced by localities, seasons and cultivars than by fungicide treatments.

Keywords: ergosterol, fungicides, *Fusarium graminearum* species complex, mycotoxins, sorghum grain mold

5.2. INTRODUCTION

Sorghum grain mold is one of the most important diseases of sorghum and affects both the yield and quality of the grain (Rao *et al.,* 2012). The complexity of this disease resides in the vast number of fungal species associated with grain mold (Bandyopadhyay *et al.,* 2000) and variation in their prevalence associated with locality and growing season (Thakur *et al.*, 2006). These fungi include *Fusarium* spp., *Alternaria* spp., *Aspergillus* spp*., Bipolaris* spp., *Curvularia* spp., *Colletotrichum* spp., *Cladosporium* spp., *Phoma* spp., *Exserohilum* spp., *Penicillium* spp., and *Drechslera* spp. (Tarekegn *et al.,* 2006; Thakur *et al*., 2006).

Many grain mold pathogens pathogens produce mycotoxins to assist in infection processes and overcoming host defenses (Bennett and Wallsgrove, 1994; Brosch *et al.,* 1995). These mycotoxins can be hazardous when ingested by humans and animals (Rao *et al*., 2012). The mycotoxin deoxynivalenol (DON) is produced by *F. graminearum* and *F. culmorum* and is associated with vomiting, diarrhea, immune suppression, weight loss, dizziness and headaches (Bandyopadhyay *et al.,* 2000; Creppy, 2002; Reddy *et al.,* 2010). Nivalenol (NIV) is associated with *F. acacia-mearnsii*, *F. cortaderiae* and *F. meridionale* on sorghum (Mavhunga, 2013) and results in slower food intake or inappetence (D'Mello *et al.,* 1999). Zearalenone (ZEA) is also produced by *F. graminearum sensu stricto* and *F. culmorum* and results in estrogenic complications, animal breeding difficulties and cervical cancer (Bandyopadhyay *et al.,* 2000; Reddy *et al.,* 2010).

Control of sorghum grain mold is difficult. The extensive diversity between and within fungal species complicates this (Summerell and Leslie, 2011). Breeding for resistance remains one of the most effective methods to control grain mold (Prom *et al*., 2003) but its effectiveness can be broken down by pathogen pressure (Bandyopadhyay *et al.,* 2000). Other methods have been explored and chemical control of grain mold of sorghum has been reported to be only somewhat effective (Bandyopadhyay *et al.,* 2000).

Several fungicides have been indicated for use against sorghum grain mold and *Fusarium* head blight of wheat, with different levels of efficacy. Propiconazole 25% EC, a systemic fungicide with a broad spectrum of activity, reduced grain mold of sorghum using a spray regime starting at 50% flowering, followed by two applications 10 days apart (Audilakshmi *et al.,* 2007). Tebuconazole and carbendazim reduced *Fusarium* head blight on wheat when applied at

flowering stage, compared to when the full ear was visible (Cromey *et al.,* 2001). Restricted growth of *Fusarium* spp. was associated with lowered levels of mycotoxins. In contrast, azoxystrobin was less effective, with little effect on fungal growth and mycotoxin accumulation (Cromey *et al.,* 2001).

Different modes of action exist within different groups of fungicides. Many work by interfering with ergosterol synthesis of fungal pathogens (Audilakshmi *et al.,* 2007) which leads to mycelial malformations, extensive branching or abnormal cell wall thickening (Ramirez *et al.,* 2004). Fungicides with this mode of action include propiconazole, tebuconazole and epoxiconazole fungicides from the triazole group (Ramirez *et al.,* 2004). Others, such as azoxystrobin belong to the strobilurin group of fungicides that act by blocking electron transport through the mitochondrial respiratory chain which effectively reduces aerobic energy available for fungal growth (Ramirez *et al.,* 2004).

In this study fungicide spray regimes used for prophylactic control of sorghum leaf blight were assessed for their effect on grain mold of sorghum and subsequent mycotoxin accumulation. Since *Fusarium* spp. are associated with sorghum root rots and a potential for mycotoxin translocation from roots to grain has been reported ((Sutton *et al.,* 1976; Mavhunga *et al.,* 2013), the effect of foliar fungicide regimes on root rots was also addressed.

5.3. MATERIALS AND METHODS

5.3.1. Field trials

Two fungicides, azoxystrobin/difenoconazole (Amistar®Top, Syngenta) and epoxyconazole/pyraclostrobin (Abacus®, BASF) were applied to four cultivars, PAN 8816, PAN 8906, PAN 8911 and NS 5511, at different time periods after planting (6, 8, 10, 6 + 8, 8 + 10 weeks). These fungicide treatments, as main plot effects, and cultivars as sub-plots, were planted at Greytown, Potchefstroom and Standerton in November 13/14 in a randomized split plot experimental design with single row plots. The trial was repeated in 14/15 and extended to include a fourth trial for root fungal biomass assessment at Potchefstroom, which was irrigated as needed to prevent drought stress. Due to not being available, the red PAN 8911 cultivar was replaced with another red cultivar, PAN 8625 during this season. The rows were 5 m (Potchefstroom and Standerton) and 4.5 m (Greytown) in length with 0.9 m inter-row

spaces. Trials were replicated three times. Prior to planting the fields were fertilized with 2:3:2 (N: P: K). Frontier Optima® (dimethenamid 75 g/l) was applied pre-emergence for weed control, followed by Basagran® (Bendioxide - thiadiazine 480 g/l) post-emergence. Karate® (ambda-cyhalothrin 5.5% 100 ml/ha) was applied for stalk borer control. Fungicides were applied at the different stages by means of a knapsack spray (Table 5.1).

At soft dough growth stage, roots of 5 randomly collected plants per row from Greytown and Potchefstroom, were removed and washed with tap water. Roots from Standerton trial were not available. Root rot severity based on visual tissue discolouration was estimated on a percentage scale. Recovered root mass was recorded and effective root mass was calculated according to McLaren (1999) where E is effective root mass: E= [{(100-root rot severity)/100}*root mass]. Grain was harvested at maturity from Greytown, Potchefstroom and Standerton and visually assessed. Grain visual ratings were done on a 1 to 5 scale according to Audilakshmi *et al.* (2007) where1 = 0% visible deterioration; 2 = 10%, 3 = 11–25%, 4 = 26–50% and 5 = ≥50%.

Automatic weather stations distributed around areas near the cultivated fields were used to obtain rainfall and minimum and maximum temperatures for the trial period.

5.3.2. Evaluation of ergosterol content

The modified method of Jambunathan *et al.* (1991) was used for ergosterol extraction as a measure of fungal biomass in roots and grain. Sorghum roots and grain from the three replicates were respectively pooled. Sorghum roots were ground with liquid nitrogen and sorghum grain was ground with a coffee grinder to produce a fine powder. Methanol (Merck) (25 ml) was added to 5 g of the respective powdered tissues and shaken with a Heidolph Multi Reax Shaker (Labotec) for 30 minutes. After allowing the suspension to settle, 12.5 ml of the upper fluid was added to 1.5 g of potassium hydroxide (KOH) in a clean test tube. The test tubes were shaken with the Multi Reax Shaker until the KOH had completely dissolved. Subsequently 5 ml N-hexane (Merck) was added, the mixture was incubated for 30 min in a 75°C water bath and permitted to cool to room temperature. Distilled water (2.5 ml) was added, mixed well with the Multi Reax Shaker, centrifuged (BHG Optima) at 3000 rcf for five minutes and the upper hexane layer was moved to a clean test tube. N-hexane (5 ml) was added to the remaining liquid, which was mixed and vortexed. After centrifugation, the upper hexane layer

was transferred once again and added to the previous aliquot. This step was repeated. The combined hexane extracts were evaporated until dry in a 75°C water bath and the residue was resuspended in 2.5 ml methanol. This was filtered through a 0.45 µl Pall Acrodisc syringe filter before being placed in glass vials to be analysed on a Perkin Elmer high performance liquid chromatograph (HPLC) with a SIL-20A auto sampler. The extracts were loaded onto a reverse phase column (C18 125 A 10 µm particle size, 150 x 4.6 mm) at 50°C. The mobile phase was methanol:water (96:4) at a flow rate of 1.2 ml/min. Standard ergosterol (Sigma) was used to develop a calibration curve. Ergosterol was recorded at 282 nm at a retention time of approximately 7 min.

5.3.3. qPCR to quantify Fusarium graminearum species complex

5.3.3.1. DNA extraction

DNA extraction was done on sorghum grain using a Wizard® Genomic DNA Purification Kit (Promega) according to manufacturer's instructions. Pooled sorghum grains from the three replicates, were ground into a fine powder with a coffee grinder. A 1.5 ml Eppendorf tube was filled with 40 mg fine powder, 600 µl Nuclei Lysis solution was added and vortex-mixed (Vortex Genie 2, Scientific Industries) for a few seconds. The Eppendorf tubes were incubated in a 65°C water bath for 15 minutes, after which 3 µl RNase solution was added. The tubes were mixed by slightly inverting the tubes and these were subsequently incubated in a 37°C water bath for 15 minutes. The tubes were left to cool for 5 minutes until room temperature had been reached. Protein precipitation solution (200 µl) was added to the tube and vortexed for 20 seconds. After centrifuging (Hermle, Lasec) the tubes for 3 minutes at 14 000 rcf, the supernatant was removed and transferred to a new tube with 600 µl isopropanol at room temperature. The tubes were mixed by inverting to form thread-like DNA strands. After centrifugation for 1 minute at 14 000 rcf, the supernatant was removed and 600 µl of 70% (v/v) ethanol (Merck) was added. The tubes were inverted gently and centrifuged at 14 000 rcf for 1 minute to wash the DNA. The ethanol was aspirated and the pellet was left to air-dry for approximately half an hour until all the ethanol evaporated. DNA Rehydration solution (100 µl) was added. The tubes were left at 4°C overnight. Concentrations of the DNA samples were determined using a NanoDrop Spectrophotometer. The DNA concentrations were diluted to 10 ng/µl and stored at -20°C until qPCR.

5.3.3.2. qPCR

Quantitative polymerase chain reaction (qPCR) analysis was conducted according to Nicolaisen *et al.* (2009). Species specific primers used for *F. graminearum* detection were (5'-3'):

- FgramB379 fwd with CCATTCCCTGGGCGCT nucleotide sequence.
- FgramB411 rev with CCTATTGACAGGTGGTTAGTGACTGG nucleotide sequence (Inqaba Biotechnical Industries).

qPCR reactions consisted of 5 µl iTaq™ Universal SYBR® Green (BIO-RAD), 1 µl Primer mix (0.5 µl FgramB379 and 0.5 µl FgramB411), 3.2 µl Nuclease-free water (Promega) and 0.8 µl template DNA. Matrix matched standards diluted 4, 16, 64 and 256 fold, a positive *F. graminearum* control and no-template control to detect contamination, were included. All reactions were done in triplicate on a Bio-Rad C1000 thermal cycler with a CFX96 real-time attachment (Bio-Rad, Hercules, USA) containing a 96-well reaction plate. The plates were incubated for 10 minutes at 95°C, followed by a further 40 cycles of 95°C for 15 seconds, 60°C for 15 seconds and 72°C for 15 seconds. A melt curve was included at 72°C to 95°C with an increase of 1°C at every stage. Efficiency was determined by using standard curves and ranged from 90 to 110%.

5.3.4. Mycotoxin quantification

Sorghum grain samples from each replicate were pooled for mycotoxin analyses. Mycotoxin extraction from grain was performed using a modified protocol of Small *et al.* (2012). Finely ground grain (5 g) was placed in a clean test tube and 20 ml 70% methanol (Merck) was added. The tubes were shaken on a Heidolph Multi Reax Shaker (Labotec) for 30 minutes at 1470 rpm.. The tubes were centrifuged for 10 minutes and the supernatant was filtered through a 0.45 µl syringe filter. The tubes were kept at 4°C overnight. Deoxynivalenol samples were placed in 1.8 ml vials, but nivalenol and zearalenone samples were diluted ten-fold before placing them into vials (Coetzee, 2015). Standards obtained from Sigma-Aldrich and a "mycotoxin free" sorghum grain sample were used for preparing matrix matched standards over a range of 100 µg/kg to 12500 µg/kg.

A 4000 QTRAP hybrid triple quadrupole ion trap mass spectrometer (AB Sciex), Shimadzu UFLC stack with LC-20AB binary pump and SIL-20A HT autosampler was used to analyze the mycotoxin samples in collaboration with Dr. G. Kemp at the Biochemistry and Microbiology Department of the University of the Free State. The samples were separated on a C18 (150x2.1mmx5µ, Discovery C18, Supelco) column. The mobile phase consisted of 10mM ammonium acetate (mobile phase A) and methanol/10mM ammonium acetate (mobile phase B) with a flow rate of 300 µL/min using a stepwise gradient between the different mobile phases, starting with 2% mobile phase B increasing rapidly to 75%, upholding it for 4 minutes and returning to 2% for a total runtime of 9 minutes. A negative electrospray mode with a 4500 V ion spray voltage, 500°C heater temperature, 60 psi nebuliser gas, 60 psi heater gas and 25 psi curtain gas was used to ionize eluting analytes. The injection volume for each sample was 20 µl. The instrument used a Multiple Reaction Monitoring workflow to analyse the sample in which the instrument is used in triple quadrupole mode. Fragment masses are produced by ionized analytes eluting off the column and a set of masses creates a transition. The instrument jumps between different transitions in a Multiple Reaction Monitoring transition during a cycle. For each transition detected, the ion intensity value is plotted. Data outputs were processed using Analyst 1.5 (AB Sciex) software.

5.3.5. Data analysis

All disease parameters were analysed using NCSS (Hintze, 2001) Analysis of Variance (ANOVA) and Fischer's Protected LSD ($P<0.05$) were used for means separation. Unreplicated ANOVA was conducted on ergosterol analysis data, FGSC and mycotoxin levels. Relationships between quality variables and root rot and grain mold were quantified using linear and non-linear regression (Hintze, 2001).

5.4. RESULTS

5.4.1. Field trials

5.4.1.1. Root visual ratings

ANOVA indicated that root visual ratings from all ten fungicide treatments and the control were not significantly different (Table 5.2), although a significant treatment x locality

interaction (P<0.05) was recorded indicating environmental influence on fungicide efficacy (Table 5.2). Azoxystrobin/difenoconazole sprayed 8 and 10 weeks after planting (wap) at Greytown and epoxyconazole/pyraclostrobin sprayed 8 + 10 wap at Potchefstroom resulted in higher root rot compared to the control. A significant cultivar x season x locality interaction was recorded (P<0.05) (Table 5.3). Mean root visual ratings of cultivars from Greytown were significantly (P<0.05) lower at 27.12% compared to 30.34% in Potchefstroom. NS 5511 was more susceptible to root rot in both localities, although higher root rot was recorded in season 13/14. PAN 8816 was the most resistant cultivar to root rot in Potchefstroom during 13/14 with similar resistance recorded in PAN 8911 during 13/14 and PAN 8906 during 14/15, indicating the potential of the environment to support or suppress root rot in the same cultivar. Based on sum of squares of ANOVA, cultivar contributed 9.62% to recorded root visual ratings, compared to 5.35% by locality and 2.08% by treatment.

5.4.1.2. Grain visual ratings

All the grain from the plots showed grain mold symptoms, including the grain from plots treated with fungicides.ANOVA indicated significant differences (P<0.05) in mean visual ratings over all localities associated with fungicide treatments that ranged from 2.64 in grain sprayed with azoxystrobin/difenoconazole at 8 wap to 2.89 in grain sprayed with azoxystrobin/difenoconazole at 6 and 8 wap (Table 5.4). Only visual ratings associated with the former treatment showed significantly lower (P<0.05) visual ratings than those recorded in the control plots, although its effectivity was not significantly different to azoxystrobin/difenoconazole sprayed at 6, 10 and 8 + 10 wap, as well as epoxyconazole/pyraclostrobin sprayed at 10 wap. A significant treatment x locality interaction (P<0.05) was recorded with the highest grain mold recorded in epoxyconazole/pyraclostrobin sprayed at 8 wap at Potchefstroom and the lowest grain mold in azoxystrobin/difenoconazole sprayed at 10 wap in Standerton (Table 5.4). A significant treatment x season interaction (P<0.05) was recorded with the highest grain mold recorded in azoxystrobin/difenoconazole sprayed at 6 + 8 wap during 14/15 and the lowest grain mold in azoxystrobin/difenoconazole sprayed at 10 wap during 13/14 (Table 5.5). A significant cultivar x season x locality interaction was recorded (P<0.05) indicating that cultivar susceptibility or resistance was influenced by environmental factors. The highest grain mold was recorded in PAN 8906 at Potchefstroom during 14/15 and the lowest grain mold visual rating in PAN 8816 and PAN 8911 at Standerton during 13/14 (Table 5.6), although NS 5511 proved to be the most resistant

cultivar throughout all environments. Sum of squares of ANOVA supported these observations, indicating that locality accounted for 47.80% of grain mold ratings, while cultivar and treatment accounted for 4.14% and 0.75% repspectively.

5.4.1.3. Root mass per plant

ANOVA indicated that root mass from plots treated with fungicides differed significantly (P<0.05) and ranged from a mean of 31.19 g in roots treated with azoxystrobin/difenoconazole sprayed at 8 and 10 wap to 38.88 g in plots sprayed with azoxystrobin/difenoconazole sprayed at 8 wap (Table 5.7). No significant differences (P<0.05) were recorded in root mass from roots treated with azoxystrobin/difenoconazole sprayed at 8 and 10 wap and roots from the control, suggesting no advantage in terms of root mass in double applications with this fungicide. Epoxyconazole/pyraclostrobin sprayed at 8 + 10 wap resulted in significantly higher root mass.

No significant differences (P<0.05) were recorded between roots treated with azoxystrobin/difenoconazole sprayed at 8 and 10 wap and epoxyconazole/pyraclostrobin sprayed at 10 and 8 + 10 wap, however the root masses of these treatments were all significantly higher than the control. Significant (P<0.05) cultivar x locality interaction (Table 5.8) and significant (P<0.05) cultivar x season interaction (Table 5.9) were recorded. Based on sum of squares of ANOVA, cultivar x locality interaction contributed 1.05% and cultivar x season interaction 1.22% to variation in root mass, hereby indicating the stable root growth response of cultivars over environments. A significant (P<0.05) season x locality interaction (Table 5.10) was also recorded.

5.4.1.4. Effective root mass

ANOVA indicated that effective root masses of cultivars treated with fungicides were not significantly different (P<0.05), although a tendency for increased effective root mass was observed as a result of fungicide treatment when compared to the control (Table 5.11). A significant treatment x season interaction (P<0.05) was recorded with the highest effective root mass of 35.53 g recorded in roots treated with epoxyconazole/pyraclostrobin sprayed at 8 and 10 wap in 13/14 and a lowest of 19.08 g in roots treated with epoxyconazole/pyraclostrobin sprayed at 8 and 10 wap in 14/15 (Table 5.11). A significant locality x season interaction

(P<0.05) was recorded (Table 5.12a). The effective root mass of the cultivars differed significantly (P<0.05) and ranged from 22.75 g in NS 5511 to 31.56 g in PAN 8816 (Table 5.12b).

5.4.1.5. Grain yield

ANOVA indicated no significant differences in grain yields from plots treated with fungicides (P<0.05). A significant (P<0.05) cultivar x season x locality interaction was recorded (Table 5.13). Yields of cultivars ranged from 1.85 t/ha in PAN 8911 at Greytown during 13/14 to 5.13 t/ha in NS 5511 at Potchefstroom during 14/15 (Table 5.13) with cultivar PAN 8906 showing significantly lower yields over all environments compared to the other cultivars. Yield potential of localities also differed significantly (P<0.05) during seasons with 2.57 t/ha in Potchefstroom 13/14 compared to 4.62 t/ha from the same locality during 14/15 (Table 5.13).

5.4.1.6. Weather variables

Temperature and rainfall contributed to disease severity and all of the significant interactions recorded in this study involved either season or locality as a variable. The mean minimum and maximum temperatures for Potchefstroom, Standerton and Greytown for the 13/14 growing season from October to May ranged from 12.78°C to 27.50°C, 10.29°C to 25.14°C and 14.46°C to 27.12°C respectively. The mean temperatures for the 14/15 growing season ranged from 12.71°C to 28.37°C, 9.89°C to 26.37°C and 14.50°C to 26.79°C respectively. The mean monthly rainfall in 13/14 was 3.16 mm for Potchefstroom, 3.12 mm for Standerton and 86.21 mm for Greytown. In 14/15 the amounts were 2.25 mm, 2.04 mm and 69.74 mm respectively (Figure 5.4).

5.4.2. Evaluation of ergosterol content to determine fungal biomass in grain and roots

5.4.2.1. Ergosterol levels in roots

Significant differences (P<0.05) as indicated by ANOVA were recorded in ergosterol levels in roots from fungicide treated sorghum (Table 5.14a) and ranged from 9.59 µg/g in epoxyconazole/pyraclostrobin sprayed at 6 + 8 wap to 44.92 µg/g in azoxystrobin/difenoconazole sprayed at 6 + 8 wap. Epoxyconazole/pyraclostrobin sprayed at

6 + 8, 10 and 8 + 10 wap had significantly lower (P<0.05) ergosterol levels compared to other time intervals with this fungicide. No significant differences (P<0.05) in ergosterol levels were recorded between azoxystrobin/difenoconazole treatments, nor epoxyconazole/pyraclostrobin application at 6 and 8 wap. Only epoxyconazole/pyraclostrobin treatments were able to suppress fungal growth when compared to the control. Significant differences were recorded between localities and ranged from 7.28 µg/g in irrigated plots to 75.50 µg/g in dryland plots in Potchefstroom (Table 5.14b). No significant interactions (P<0.05) were recorded (Table 5.14).

5.4.2.2. Ergosterol levels in grain

Significant differences (P<0.05), as indicated by ANOVA, were recorded in ergosterol levels in grain treated with fungicides, which ranged from 59.32 µg/g in azoxystrobin/difenoconazole sprayed at 8 wap to 459.24 µg/g in epoxyconazole/pyraclostrobin sprayed at 8 + 10 wap (Table 5.15a). No significant differences were recorded between ergosterol levels in any of the azoxystrobin/difenoconazole treatments compared with the control. Only epoxyconazole/pyraclostrobin sprayed at 6 wap had significantly lower ergosterol levels, similar to the control, than the significantly higher ergosterol levels in the other epoxyconazole/pyraclostrobin treatments. A significant treatment x locality interaction (P<0.05) was recorded (Table 5.15a). Efficacy of fungicides was dependent on environments as indicated by epoxyconazole/pyraclostrobin applied at 8 + 10 weeks with the highest ergosterol levels at Greytown and the lowest levels at Standerton. Seasons differed significantly (P<0.05) with the highest ergosterol levels recorded in 13/14 (Table 5.15b). Based on the sum of squares of ANOVA treatment x locality interaction contributed 14.75% to the fungal biomass recorded, followed by 12.04% by season and 7.40% by treatment.

5.4.3. qPCR to quantify Fusarium graminearum species complex in sorghum grain

No significant differences (P<0.05) were recorded in FGSC DNA levels in grain from fungicide treatments. Significant differences were recorded in FGSC DNA levels in grain from localities and ranged from 3.76 µg/g in Standerton to 181.90 µg/g in Greytown (Table 5.16a). No significant differences (P<0.05) were recorded in grain from Standerton and Potchefstroom. Significant differences (P<0.05) were recorded between FGSC levels from cultivars and ranged from 4.21 µg/g in NS 5511 to 111.10 µg/g in PAN 8906. No significant differences (P<0.05)

were recorded between NS 5511 and PAN 8816. A significant locality x cultivar interaction ($P<0.05$) was recorded (Table 5.16a). Seasons differed significantly ($P<0.05$) with the highest FGSC DNA levels recorded in 13/14 (Table 5.16b).

5.4.4. Mycotoxin accumulation in grain associated with F. graminearum

Fungicides made no significant difference ($P<0.05$) to any of the mycotoxin levels recorded in grain (Table 5.17; Table 5.18; Table 5.19). Significantly different levels ($P<0.05$) were recorded in grain from localities that ranged from 3.34 µg/kg and 4.40 µg/kg in Potchefstroom to 169.34 and 551.01 µg/kg in Greytown for DON (Table 5.17a) and ZEA levels (Table 5.19a) respectively. Significant differences ($P<0.05$) were recorded in NIV levels (Table 5.18a) and ranged from 4.94 µg/kg in Standerton to 207.79 µg/kg in Greytown. No significant differences ($P<0.05$) were recorded in DON, NIV and ZEA levels in grain from Potchefstroom and Standerton. Significant differences ($P<0.05$) were recorded between mycotoxin levels in cultivars, with DON and NIV levels that ranged from 4.80 µg/kg and 9.77 µg/kg in NS 5511 to 103.92 µg/kg and 135.23 µg/kg in PAN 8906 respectively (Table 5.17a; Table 5.18a). Significant differences ($P<0.05$) were recorded between ZEA levels in cultivars and ranged from 51.22 µg/kg in PAN 8816 to 383.72 µg/kg in PAN 8911/PAN 8625. A significant cultivar x locality interaction was recorded in DON, NIV and ZEA levels ($P<0.05$). Significant different ($P<0.05$) mycotoxin levels were recorded in seasons with the highest levels of DON (Table 5.17b), NIV (Table 5.18b) and ZEA (Table 5.19b) recorded in 13/14.

Significant positive relationships were recorded between FGSC DNA levels and mycotoxin levels (Figure 5.1) Increased FGSC DNA levels were associated with increased DON (R^2 = 0.56*), NIV (R^2 = 0.70*) and ZEA levels (R^2 = 0.45*) indicating production by FGSC pathogens and thus susceptibility of cultivars. Increased DON levels were associated with increased NIV (R^2 = 0.73*) and ZEA levels (R^2 = 0.37*) Increased NIV levels were associated with increased ZEA levels (R^2 = 0.47*).

5.5. DISCUSSION

The principle behind assessing root rots in relation to the application of fungicides for the control of sorghum leaf blight is the "photosynthetic stress-translocation balance concept" first introduced by Dodd in 1977 for maize and in 1978 for sorghum (Dodd, 1980). This principle is based on the assumption that that sorghum roots and stalks are more susceptible to rot fungi when a deficiency of photosynthetic carbohydrates persist as a result of damaged foliar tissues that restrict photosynthesis and subsequently photosynthate production and translocation to the roots and other plant organs. Insufficient carbohydrates in the plant tissues limits the natural metabolic processes that contributes to resistance and cell death is unavoidable following pathogenic attack. As more root tissue cells die, less water absorption occurs and soon transpiration tempo will pass absorption rate which will lead to wilting of the plant (Dodd, 1980). It is therefore necessary to prevent leaf blight damage to maintain sufficient photosynthesis.

Due to the limited sorghum production area and thus the limited number of cultivars on the South African market, the primary leaf blight control strategy is chemical control. Various products are available but with a limited number registered for use on sorghum, locally. A problem with chemical control is resistance build-up in pathogens due to fungicides being used continuously and excessively during the last decade. This can lead to a decrease in the efficacy or the total loss of classes of fungicides (Morton and Staub, 2008). Rotating dissimilar fungicides or using mixtures of diverse fungicides with different modes of action can enhance efficiency. Fungicides with a single active ingredient that work site-specifically, should not be considered (Ward *et al.,* 1997). Therefore fungicides with both triazole and strobilurin active ingredients were chosen in the current study with the primary focus on root rot and grain mold prevention.

Strobilurins, such as pyraclostrobin and azoxystrobin (Karadimos *et al.,* 2005), are antagonistic compounds derived from Basidiomycetes and can successfully control other Basidiomycetes, Ascomycetes, Oomycetes and Deuteromycetes (Sudisha *et al.,* 2005) by inhibiting mitochondrial respiration (Karadimos *et al.,* 2005). Triazoles are highly effective against a broad spectrum of fungi and work by interfering with ergosterol biosynthesis (Audilakshmi *et al.,* 2007), although they have difficulty inhibiting spore germination (Ramirez *et al.,* 2004).

Therefore, triazole and strobulurin combinations complement each other by initiating different metabolic responses in plants (Görtz, *et al.,* 2008).

According to manufacturer's labels Amistar®Top (azoxystrobin/difenoconazole) is the only fungicide registered for use on sorghum in South Africa (Croplife, 2018). Abacus® (epoxyconazole/pyraclostrobin) is registered for use on soybeans, sugarcane, maize and wheat. In the current study, azoxystrobin/difenoconazole appeared to be more efficient in controlling grain mold symptom development. This could probably be due to the specific plant properties that enhance the efficacy or the translocation of the fungicide through the plant or that the fungicide enhanced metabolic processes in the sorghum. Wu and Von Tiedemann (2002) indicated an increased enzyme activity in barley leaves in response to application of azoxystrobin and epoxyconazole. Elevated levels between 16%–144% of superoxide dismutase, catalase, ascorbate-peroxidase and glutathione reductase were recorded. Only guiacol-peroxidase was elevated between 50%-110% with azoxystrobin treatment. It could be that azoxystrobin/difenoconazole used on the sorghum in our study resulted in specific metabolic processes being enhanced, which is why it appeared more effective than epoxyconazole/pyraclostrobin in grain mold supression.

It remains possible that epoxyconazole/pyraclostrobin treatments did not translocate as effectively through aerial tissues and thus pathogens associated with grain molds may have escaped fungicides in the aerial floral structures which contributed to grain mold development. Higher ergosterol levels recorded with epoxyconazole/pyraclostrobin treatments in the grain compared with lower levels in roots, combined with increased root mass, support the assumption that this fungicide could have accumulated in lower plant tissues, possibly through the phloem (Hsu and Kleier, 1996), and support the notion of tissue specificity. Despite these increases, no significant yield increases were recorded and the treatments with this fungicide cannot be deemed economical.

According to Bandyopadhyay *et al.* (2000), spraying plants at pre-flowering growth stage, could limit colonisation by grain mold fungi. Grain mold fungi invade apical floral tissues, thus spraying fungicides before flowering could initiate the plant defence mechanism, as was recorded with, albeit limited, lower grain visual rating and ergosterol levels in grain sprayed with azoxystrobin/difenoconazole at 8 wap. However, Cromey *et al.* (2001) indicated that azoxystrobin applied early did not reduce *Fusarium* head blight of wheat and only the infection

rate was delayed. This indicated the need for re-applications throughout the growing season. Propiconazole sprayed three times, beginning with flowering and subsequent 10 day intervals, resulted in 30–50% less grain mold related discolouration during two consecutive field trials conducted by Audilakshmi *et al.,* (2007). However, repeated spraying could also have phytotoxicity effects on plants with induced stress levels and this is usually dependent on the sensitivity and recovery time of the cultivars in question. In the current study, no significant advantage with multiple sprays was recorded.

Phytotoxic effects of multiple sprays include the reduction of photosynthesis, chlorophyll pigments or carbohydrates (glucose and fructose), as reported in studies conducted by Saladin *et al.* (2003) in vineyards treated with fludioxonil and pyrimethanil. Sucrose accumulation on membranes or proteins was also recorded. The latter was observed in resistant cultivars which indicated a protective response that allows the plant to start its defense mechanisms and prevent pathogenic attack. High dosages of pyrimidinemethanol derivatives e.g. fenarimol and nuarimol, and triazole derivatives, triadimefon and etaconazole were phytotoxic and inhibited root growth, leaf formation and tiller development accompanied by chlorotic spots on the leaves of Kentucky Bluegrass applied in response to *Fusarium* blight (Kane and Smiley, 1983). These observed effects were cultivar dependent and possibly due to a disturbance of gibberellin biosynthesis (Kane and Smiley, 1983) which can lead to retarded growth (Rademacher, 2000). This could explain the differences in yield and root mass observed between the more resistant NS551 and PAN 8816 compared with the less resistant PAN 8906.

The tendency for differences in the concentrations of FGSC DNA recorded with fungicide applications could be attributed to the varying sensitivity levels of the FGSC pathogens to the fungicides (Spolti, 2014). This sensitivity is also dependent on the host defence mechanisms (Becher *et al.,* 2010) and could explain the differences observed in the resistant cultivars NS 5511 and PAN 8816 compared with the susceptible PAN 8906. Wegulo *et al.* (2011) indicated that fungicide application combined with host resistance mechanisms were effective in controlling *Fusarium* head blight of wheat and that efficacy was limited on susceptible cultivars compared to moderately resistant cultivars. This was in agreement with Mesterházy *et al.* (2003) who indicated a significant cultivar x fungicide interaction.

Effective root volume is a good indicator of the potential of a cultivar to withstand damage to root rot pathogens. Cultivars can compensate for the loss of tissue with an extensive root

system (Tarr, 1962). Thus, the effect of fungicides on root mass should not be seen in isolation from root rot. Multiple interactions between criteria remain critical considerations when evaluating plant responses to pathogens and control strategies (McLaren, 2000; van Rooyen, 2012). Therefore, a protective effect in the form of growth stimulation such as increased root mass, could theoretically be beneficial, although yield increases were not recorded in the current study. Triazoles, for example have been reported to improve environmental stress response in barley seedlings (Görtz, *et al.,* 2008). Therefore increased defense mechanisms combined with stress tolerance, could have led to the better growth observed.

Environments contributed to all the significant relationships observed in the current study and were also the main driving variable of many of the observed effects. Temperature and rainfall are the main factors influencing *Fusarium* spp. in grain cereals (Czembor *et al.,* 2015) and environmental conditions determine if *Fusarium culmorum* can reach wheat heads after penetrating the stem tissue (Covarelli *et al.,* 2012). Efficacy of fungicides to control *F. graminearum* is also dependent on environmental conditions (Ramirez *et al.,* 2004) and this supports the variation in response to fungicides at the different localities.

Greytown 13/14 had the highest rainfall compared to Potchefstroom and Standerton, particularly during the 13/14 season and this was associated with higher ergosterol, FGSC and subsequent mycotoxin levels. *F. graminearum* has specific temperature requirements for optimal germination and growth (Czembor *et al.,* 2015) and Greytown 13/14 had a mean maximum temperature that ranged from 26°C –28°C, the optimum FGSC growth (Reid *et al.,* 1999). Blandino *et al.* (2012) indicated that deoxynivalenol production was only reduced with triazole fungicides in areas with low *Fusarium* head blight potential, which can explain the low control observed in Greytown 13/14. In other environments there was a high visual grain mold rating compared with high ergosterol levels and low *F. graminearum* DNA levels, for example, the high temperatures at Potchefstroom contributed to the involvement of other grain mold pathogens that produced variation between evaluation criteria.

No significant differences were observed with all three mycotoxins as a result of fungicide treatments, although a tendency was observed for reduced DON and NIV accumulation with most of the fungicide applications. ZEA levels fluctuated. These fluctuations could be a last effort by the pathogen to defend itself by producing mycotoxins in a deadly environment (Ramirez *et al.,* 2004). Simpson *et al.* (2001) indicated higher deoxynivalenol levels after

fungicide application to wheat. Azoxystrobin increased deoxynivalenol in wheat in one of three growing seasons, but the exact mechanisms need further investigation (Mesterházy *et al.,* 2003).

5.6. CONCLUSION

Although fungicides effectively controlled some of the disease parameters investigated, most of the observed resistance was due to the inherent potential of the cultivars to withstand pathogen invasion in a particular environment. The application of a leaf blight chemical spray regime to limit sorghum root rots and grain mold would require re-examining using a wider range of active ingredients to minimize impracticality and cost in relation to growth and yield grains. In addition to the type of fungicide, application requirements need to be emphasized in relation to the two most important grain factors, i.e. quality and quantity. Since locality, season and cultivar are the main driving variables, further research is needed to examine the best climatic conditions under which fungicides could optimally improve host defense mechanisms.

5.7. REFERENCES

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Table 5.1 Eleven fungicide treatments used in sorghum leaf blight prophylactic spray regimes evaluated for their efficacy against root rots and grain mold.

Treatment no.	Fungicide	Concentration	Application times	Formulation	Application rate
1	Control				
2	Azoxystrobin/difenoconazole	200/125g/l	6 weeks	Suspension concentrate	500ml/ha
3	Azoxystrobin/difenoconazole	200/125g/l	8 weeks	Suspension concentrate	500ml/ha
4	Azoxystrobin/difenoconazole	200/125g/l	10 weeks	Suspension concentrate	500ml/ha
5	Azoxystrobin/difenoconazole	200/125g/l	6 + 8 weeks	Suspension concentrate	500ml/ha
6	Azoxystrobin/difenoconazole	200/125g/l	8 + 10 weeks	Suspension concentrate	500ml/ha
7	Epoxyconazole/pyraclostrobin	62.5/62.5g/l	6 weeks	Suspo-emulsion	1l/ha
8	Epoxyconazole/pyraclostrobin	62.5/62.5g/l	8 weeks	Suspo-emulsion	1l/ha
9	Epoxyconazole/pyraclostrobin	62.5/62.5g/l	10 weeks	Suspo-emulsion	1l/ha
10	Epoxyconazole/pyraclostrobin	62.5/62.5g/l	6 + 8 weeks	Suspo-emulsion	1l/ha
11	Epoxyconazole/pyraclostrobin	62.5/62.5g/l	8 + 10 weeks	Suspo-emulsion	1l/ha

Table 5.2. Analysis of variance and two-way interaction table of mean root visual ratings over four sorghum cultivars treated with eleven fungicide spray regimes at Potchefstroom and Greytown during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	528.42	52.84	1.46	0.15
Cultivar	3	2443.62	814.54	22.49	0.00*
Season	1	99.04	99.04	2.73	0.10
Cultivar x Season	3	955.68	318.56	8.79	0.00*
Locality	1	1359.42	1359.42	37.53	0.00*
Treatment x Locality	10	848.10	84.81	2.34	0.01*
Cultivar x Locality	3	852.52	284.14	7.85	0.00*
Cultivar x Season x Locality	3	697.46	232.49	6.42	0.00*
Rep	2	847.70	423.85	11.70	0.00*
Error	346	12532.80	36.22		
Total	523	25413.32			

Locality	**Control**	**Azoxystrobin/difenoconazole**					**Epoxyconazole/pyraclostrobin**					**Locality mean**
		200/125g/l	200/125g/l	200/125g/l	200/125g/l	200/125g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	
		6 weeks	8 weeks	10 weeks	6 + 8 weeks	8 + 10 weeks	6 weeks	8 weeks	10 weeks	6 + 8 weeks	8 + 10 weeks	
		500ml/ha	500ml/ha	500ml/ha	500ml/ha	500ml/ha	1l/ha	1l/ha	1l/ha	1l/ha	1l/ha	
Potchefstroom	32.71	28.71	27.00	26.57	31.54	29.04	31.17	31.13	31.75	30.39	33.79	**30.34[a]**
Greytown	27.88	27.67	27.96	28.04	27.46	25.67	26.33	27.00	26.63	27.21	26.46	**27.12[b]**
Treatment mean	**30.30**	**28.19**	**27.48**	**27.31**	**29.50**	**27.36**	**28.75**	**29.07**	**29.19**	**28.80**	**30.13**	**Interaction LSD (P<0.05) 3.41**

Table 5.3 Interaction table of root visual ratings of four sorghum cultivars at Potchefstroom and Greytown treated with fungicide spray regimes during 13/14 and 14/15.

Locality	Season 13/14					Season 14/15					Locality means over seasons
	Cultivars				Locality mean	Cultivars				Locality mean	
	PAN 8816	PAN 8906	PAN 8911	NS 5511		PAN 8816	PAN 8906	PAN 8625	NS 5511		
Potchefstroom	23.67	31.78	26.59	36.99	**29.76**	28.30	27.30	34.06	34.06	**30.93**	**30.34[a]**
Greytown	25.70	26.24	27.12	28.27	**26.83**	26.76	26.00	27.97	28.88	**27.40**	**27.12[b]**
Cultivar mean	**24.68[d]**	**29.01[bc]**	**26.86[d]**	**32.63[a]**		**27.53[cd]**	**26.65[d]**	**31.02[ab]**	**31.47[a]**		
Cultivar means over seasons	**26.11[c]**	**27.83[b]**	**28.94[b]**	**32.05[a]**							
Season mean	**28.30**					**29.17**				**Interaction LSD (P<0.05) 2.90**	

Table 5.4 Analysis of variance and two-way interaction table of mean visual grain ratings over four sorghum cultivars treated with eleven fungicide spray regimes at Potchefstroom and Greytown during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	4.47	0.45	1.98	0.03*
Cultivar	3	24.62	8.21	36.47	0.00*
Season	1	8.52	8.52	37.86	0.00*
Treatment x Season	10	10.44	1.04	4.64	0.00*
Cultivar x Season	3	2.62	0.87	3.89	0.00*
Locality	2	284.42	142.21	632.01	0.00*
Treatment x Locality	20	11.02	0.55	2.45	0.00*
Cultivar x Locality	6	22.79	3.80	16.88	0.00*
Season x Locality	2	40.34	20.17	89.64	0.00*
Cultivar x Season x Locality	6	16.55	2.76	12.26	0.00*
Rep	2	0.79	0.39	1.75	0.17
Error	526	118.35	0.23		
Total	791	595.18			

Locality	**Control**	**Azoxystrobin/difenoconazole**					**Epoxyconazole/pyraclostrobin**					**Locality mean**
		200/125g/l	200/125g/l	200/125g/l	200/125g/l	200/125g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	
		6 weeks	8 weeks	10 weeks	6 + 8 weeks	8 + 10 weeks	6 weeks	8 weeks	10 weeks	6 + 8 weeks	8 + 10 weeks	
		500ml/ha	500ml/ha	500ml/ha	500ml/ha	500ml/ha	1l/ha	1l/ha	1l/ha	1l/ha	1l/ha	
Potchefstroom	3.47	3.46	3.31	3.46	3.47	3.48	3.57	3.58	3.55	3.49	3.39	**3.48^{a}**
Greytown	3.00	2.62	2.79	3.10	2.94	2.77	2.93	2.73	2.75	2.97	2.63	**2.84^{b}**
Standerton	1.93	1.92	1.80	1.77	2.25	2.01	2.04	2.12	1.85	2.10	2.33	**2.01^{c}**
Treatment mean	**2.80^{abc}**	**2.67^{cd}**	**2.64^{d}**	**2.78^{abcd}**	**2.89^{a}**	**2.76^{abcd}**	**2.85^{ab}**	**2.81^{abc}**	**2.72^{bcd}**	**2.86^{ab}**	**2.79^{abc}**	**Interaction LSD (P<0.05) 0.27**

Table 5.5 Interaction table of mean grain visual ratings of sorghum cultivars treated with eleven fungicide spray regimes at three localities during 13/14 and 14/15.

Season	**Control**	**Azoxystrobin/difenoconazole**					**Epoxyconazole/pyraclostrobin**					**Season mean**
		200/125g/l	200/125g/l	200/125g/l	200/125g/l	200/125g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	
		6 weeks	8 weeks	10 weeks	6 + 8 weeks	8 + 10 weeks	6 weeks	8 weeks	10 weeks	6 + 8 weeks	8 + 10 weeks	
		500ml/ha	500ml/ha	500ml/ha	500ml/ha	500ml/ha	1l/ha	1l/ha	1l/ha	1l/ha	1l/ha	
13/14	2.82	2.65	2.63	2.54	2.63	2.71	2.68	2.57	2.78	2.81	2.57	**2.67^{b}**
14/15	2.78	2.68	2.64	3.01	3.15	2.80	3.01	3.05	2.65	2.90	3.00	**2.88^{a}**
Treatment mean	**2.80^{abc}**	**2.67^{cd}**	**2.64^{d}**	**2.78^{abcd}**	**2.89^{a}**	**2.76^{abcd}**	**2.85^{ab}**	**2.81^{abc}**	**2.72^{bcd}**	**2.86^{ab}**	**2.79^{abc}**	**Interaction LSD ($P<0.05$) 0.22**

Table 5.6 Interaction table of grain visual ratings of four sorghum cultivars at Greytown, Potchefstroom and Standerton treated with fungicide spray regimes during 13/14 and 14/15.

Locality	Season 13/14					Season 14/15					Locality means over seasons
	Cultivars				Locality mean	Cultivars				Locality mean	
	PAN 8816	PAN 8906	PAN 8911	NS 5511		PAN 8816	PAN 8906	PAN 8625	NS 5511		
Potchefstroom	3.09	3.45	3.40	2.82	**3.19^{b}**	3.90	4.10	3.52	3.51	**3.76^{a}**	**3.48^{a}**
Greytown	3.00	3.30	3.24	2.67	**3.05^{b}**	2.27	3.50	2.52	2.20	**2.62^{c}**	**2.84^{b}**
Standerton	1.62	1.85	1.62	1.98	**1.77^{e}**	2.37	2.07	2.40	2.17	**2.25^{d}**	**2.01^{c}**
Cultivar mean	**2.57^{de}**	**2.87^{b}**	**2.75^{bc}**	**2.49^{e}**		**2.85^{b}**	**3.22^{a}**	**2.81^{b}**	**2.63^{cd}**		
Cultivar means over seasons	**2.71^{b}**	**3.05^{a}**	**2.78^{b}**	**2.56^{c}**							
Season mean	**2.67^{b}**					**2.88^{a}**					**Interaction LSD ($P<0.05$) 0.23**

Table 5.7. Root system mass per plant of sorghum cultivars treated with 11 fungicide spray regimes.

Fungicide Treatment	Root mass per plant (g)
Control	32.35[cd]
Azoxystrobin/difenoconazole	
6 weeks	32.70[bcd]
8 weeks	38.88[a]
10 weeks	37.89[ab]
6 + 8 weeks	33.71[abcd]
8 + 10 weeks	31.19[d]
Mean	**34.87**
Epoxyconazole/pyraclostrobin	
6 weeks	37.01[abc]
8 weeks	35.65[abcd]
10 weeks	38.51[a]
6 + 8 weeks	36.27[abcd]
8 + 10 weeks	38.60[a]
Mean	**37.21**
Fungicide mean	**36.04**
Treatment mean	**35.71**

Table 5.8 Analysis of variance and interaction table of root mass (g) of four sorghum cultivars treated with fungicide spray regimes at Potchefstroom and Greytown during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	3702.35	370.23	2.04	0.03*
Cultivar	3	8679.62	2893.21	15.91	0.00*
Season	1	18066.84	18066.84	99.35	0.00*
Cultivar x Season	3	1792.23	597.41	3.29	0.02*
Locality	1	20686.91	20686.91	113.76	0.00*
Cultivar x Locality	3	1547.22	515.74	2.84	0.04*
Season x Locality	1	3331.84	3331.84	18.32	0.00*
Rep	2	93.23	46.61	0.26	0.77
Error	346	62917.96	181.84		
Total	523	146907.50			

Locality	**Cultivar**				**Locality mean**
	PAN 8816	PAN 8906	PAN8911/ PAN 8625	NS 5511	
Potchefstroom	33.57	27.82	27.87	28.40	**29.41[b]**
Greytown	51.70	36.66	40.15	39.50	**42.00[a]**
Cultivar mean	**42.64[a]**	**32.24[b]**	**34.01[b]**	**33.95[b]**	**Interaction LSD (P<0.05) 4.60**

Table 5.9 Interaction table of root mass (g) of four sorghum cultivars treated with different fungicide spray regimes at Potchefstroom and Greytown during 13/14 and 14/15.

Season	**Cultivar**				**Season mean**
	PAN 8816	PAN 8906	PAN8911/ PAN 8625	NS 5511	
13/14	46.73	38.08	38.70	42.86	**41.59[a]**
14/15	38.54	26.40	29.32	25.04	**29.82[b]**
Cultivar mean	**42.64[a]**	**32.24[b]**	**34.01[b]**	**33.95[b]**	**Interaction LSD ($P<0.05$) 4.60**

Table 5.10 Interaction table of root mass (g) of sorghum cultivars treated with fungicide spray regimes at Potchefstroom and Greytown during 13/14 and 14/15.

Locality	**Season**		**Locality mean**
	13/14	14/15	
Potchefstroom	37.82	21.00	**29.41**[b]
Greytown	45.35	38.65	**42.00**[a]
Season mean	**41.59**[a]	**29.82**[b]	**Interaction LSD ($P<0.05$) 3.25**

Table 5.11 Anaysis of variance and interaction table of effective root mass per plant (g) of sorghum cultivars treated with eleven fungicide spray regimes at Potchefstroom and Greytown during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	1750.57	175.06	1.77	0.07
Cultivar	3	6823.84	2274.62	22.98	0.00*
Season	1	8979.83	8979.83	90.71	0.00*
Treatment x Season	10	1857.87	185.79	1.88	0.05*
Locality	1	14726.87	14726.87	148.76	0.00*
Season x Locality	1	1233.11	1233.11	12.46	0.00*
Rep	2	62.13	31.07	0.31	0.73
Error	350	34649.37	99.00		
Total	527	83975.58			

Season	**Control**	**Azoxystrobin/difenoconazole**					**Epoxyconazole/pyraclostrobin**					**Season mean**
		200/125g/l	200/125g/l	200/125g/l	200/125g/l	200/125g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	
		6 weeks	8 weeks	10 weeks	6 + 8 weeks	8 + 10 weeks	6 weeks	8 weeks	10 weeks	6 + 8 weeks	8 + 10 weeks	
		500ml/ha	500ml/ha	500ml/ha	500ml/ha	500ml/ha	1l/ha	1l/ha	1l/ha	1l/ha	1l/ha	
13/14	24.75	27.50	33.87	30.33	25.88	24.48	32.54	28.96	30.43	30.39	35.53	**29.51[a]**
14/15	20.67	19.77	22.50	23.79	21.70	21.05	20.69	21.95	22.32	20.38	19.08	**21.26[b]**
Treatment mean	**22.71**	**23.64**	**28.19**	**27.06**	**23.79**	**22.77**	**26.62**	**25.46**	**26.38**	**25.39**	**27.31**	**Interaction LSD (P<0.05) 5.63**

Table 5.12 (a) Interaction table of effective root mass system per plant (g) from sorghum cultivars treated with fungicide spray regimes at Potchefstroom and Greytown during 13/14 and 14/15. (b) Effective root mass system per plant (g) from four cultivars treated with fungicide spray regimes during 13/14 and 14/15.

Locality	**Season**		**Locality mean**
	13/14	14/15	
Potchefstroom	25.75	14.46	**20.11[b]**
Greytown	33.27	28.06	**30.67[a]**
Season mean	**29.51[a]**	**21.26[b]**	**Interaction LSD (P<0.05) 2.40**

Cultivar	**Effective root mass (g)**
PAN 8816	31.56[a]
PAN 8906	23.13[b]
PAN 8911/8625	24.12[b]
NS 5511	22.75[b]
Season Mean	**25.39**

Table 5.13 Anaysis of variance and interaction table of yield (t/ha) of four sorghum cultivars at Greytown, Potchefstroom and Standerton treated with fungicide spray regimes during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	25.75	2.57	1.67	0.09
Cultivar	3	95.54	31.85	20.61	0.00*
Season	1	138.54	138.54	89.65	0.00*
Cultivar x Season	3	137.83	45.94	29.73	0.00*
Locality	2	65.88	32.94	21.31	0.00*
Cultivar x Locality	6	161.73	26.96	17.44	0.00*
Season x Locality	2	152.76	76.38	49.43	0.00*
Cultivar x Season x Locality	6	61.92	10.32	6.68	0.00*
Rep	2	3.66	1.83	1.18	0.31
Error	526	812.85	1.55		
Total	791	1995.74			

Locality	**Season 13/14**					**Season 14/15**					**Locality means over seasons**
	Cultivars				**Locality mean**	**Cultivars**				**Locality mean**	
	PAN 8816	PAN 8906	PAN 8911	NS 5511		PAN 8816	PAN 8906	PAN 8625	NS 5511		
Potchefstroom	2.73	2.75	2.04	2.76	**2.57[d]**	4.61	4.32	4.43	5.13	**4.62[a]**	**3.60[a]**
Greytown	3.61	2.17	1.85	3.23	**2.72[d]**	2.85	2.09	3.46	4.27	**3.17[c]**	**2.94[b]**
Standerton	4.08	3.10	3.37	3.45	**3.50[b]**	3.18	2.08	5.83	2.91	**3.50[b]**	**3.50[a]**
Cultivar mean	**3.47[cd]**	**2.67[fg]**	**2.42[g]**	**3.15[de]**		**3.55[c]**	**2.83[ef]**	**4.57[a]**	**4.10[b]**		
Cultivar means over seasons	**3.51[a]**	**2.75[b]**	**3.50[a]**	**3.63[a]**							
Season mean	**2.93[b]**					**3.76[a]**					**Interaction LSD (P<0.05) 0.60**

Table 5.14 (a) Anaysis of variance and root ergosterol levels (µg/g) from sorghum cultivars treated with fungicide spray regimes during 13/14 and 14/15. (b) Root ergosterol levels (µg/g) from sorghum cultivars treated with fungicide spray regimes at Potchefstroom and Greytown during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	22368.21	2236.82	3.18	0.00*
Cultivar	3	4818.14	1606.05	2.28	0.08
Locality	3	128488.20	42829.39	60.81	0.00*
Error	127	89445.35	704.29		
Total	173	252796.30			

Fungicide Treatment	Root ergosterol
Control	24.73[bcd]
Azoxystrobin/difenoconazole	
6 weeks	39.69[ab]
8 weeks	34.47[abc]
10 weeks	41.33[ab]
6 + 8 weeks	44.92[a]
8 + 10 weeks	32.53[abc]
Mean	**38.59**
Epoxyconazole/pyraclostrobin	
6 weeks	37.55[ab]
8 weeks	31.94[abc]
10 weeks	13.09[d]
6 + 8 weeks	9.59[d]
8 + 10 weeks	16.14[cd]
Mean	**21.66**
Fungicide mean	**30.13**
Treatment mean	**29.63**

Locality	Root ergosterol
Greytown 13/14	11.93[c]
Greytown 14/15	23.82[b]
Potchefstroom 14/15 (irrigated)	7.28[c]
Potchefstroom 14/15 (not irrigated)	75.50[a]
Season mean	**29.63**

Table 5.15. Anaysis of variance and (a) Interaction table of grain ergosterol levels (µg/g) from sorghum cultivars treated with eleven fungicide spray regimes at Potchefstroom, Greytown and Standerton during 13/14 and 14/15. (b) Mean grain ergosterol levels (µg/g) from sorghum cultivars treated with fungicide spray regimes during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	5160035.00	516003.50	2.11	0.03*
Cultivar	3	424464.30	141488.10	0.58	0.63
Season	1	8393337.00	8393337.00	34.25	0.00*
Locality	2	4304290.00	2152145.00	8.78	0.00*
Treatment x Locality	20	10288120.00	514406.00	2.10	0.00*
Error	127	31118950.00	245031.10		
Total	259	69737080.00			

Locality	**Control**	**Azoxystrobin/difenoconazole**					**Epoxyconazole/pyraclostrobin**					**Locality mean**
		200/125g/l	200/125g/l	200/125g/l	200/125g/l	200/125g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5g/l	62.5/62.5 g/l	
		6 weeks	8 weeks	10 weeks	6 + 8 weeks	8 + 10 weeks	6 weeks	8 weeks	10 weeks	6 + 8 weeks	8 + 10 weeks	
		500ml/ha	500ml/ha	500ml/ha	500ml/ha	500ml/ha	1l/ha	1l/ha	1l/ha	1l/ha	1l/ha	
Potchefstroom	317.67	267.38	133.86	180.96	185.99	218.39	179.97	211.39	218.68	159.96	395.04	**224.48^{a}**
Greytown	15.53	14.80	22.32	21.13	23.36	16.65	25.59	696.37	954.98	892.37	982.69	**333.25^{a}**
Standerton	44.12	44.18	21.78	24.10	15.47	21.35	58.49	3.56	33.47	40.04	0.00	**27.87^{b}**
Treatment mean	**125.77^{bcd}**	**108.79^{cd}**	**59.32^{d}**	**75.40^{d}**	**74.94^{d}**	**85.46^{cd}**	**88.02^{cd}**	**303.77^{abcd}**	**402.38^{ab}**	**364.12^{abc}**	**459.24^{a}**	**Interaction LSD (P<0.05) 485.11**

Season	**Grain ergosterol levels**
13/14	377.85^{a}
14/15	12.54^{b}
Season Mean	**195.20**

Table 5.16 (a) Anaysis of variance and interaction table of DNA levels (µg/g) in grain from four sorghum cultivars treated with fungicide spray regimes planted at Potchefstroom, Greytown and Standerton during 13/14 and 14/15. (b) DNA levels (µg/g) from sorghum cultivars treated with fungicide spray regimes during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	173852.20	17385.22	0.74	0.68
Cultivar	3	544875.70	181625.20	7.77	0.00*
Season	1	525278.90	525278.90	22.47	0.00*
Locality	2	1821842.00	910921.00	38.96	0.00*
Cultivar x Locality	6	1023096.00	170516.00	7.29	0.00*
Error	128	2992513.00	23379.00		
Total	260	7783280.00			

Locality	**Cultivar**				**Locality mean**
	PAN 8816	PAN 8906	PAN8911/ PAN 8625	NS 5511	
Potchefstroom	5.91	7.66	7.18	1.59	**5.59[b]**
Greytown	93.68	318.75	306.55	8.63	**181.90[a]**
Standerton	3.04	6.89	2.69	2.41	**3.76[b]**
Cultivar mean	**34.21[b]**	**111.10[a]**	**105.47[a]**	**4.21[b]**	**Interaction LSD ($P<0.05$) 90.36**

Season	**Grain DNA levels**
13/14	108.87[a]
14/15	18.63[b]
Season Mean	**63.75**

Table 5.17 Anaysis of variance and interaction table of DON (µg/kg) in grain from four sorghum cultivars treated with fungicide spray regimes at Potchefstroom, Greytown and Standerton during 13/14 and 14/15. (b) DON (µg/kg) in grain from sorghum cultivars treated with fungicide spray regimes during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	63635.34	6363.53	0.45	0.92
Cultivar	3	358729.70	119576.60	8.37	0.00*
Season	1	202681.00	202681.00	14.18	0.00*
Locality	2	1491412.00	745705.80	52.18	0.00*
Cultivar x Locality	6	629399.00	104899.80	7.34	0.00*
Error	127	1815120.00	14292.28		
Total	259	5150463.00			

Locality	**Cultivar**				**Locality mean**
	PAN 8816	PAN 8906	PAN8911/ PAN 8625	NS 5511	
Potchefstroom	3.92	1.82	2.83	4.77	**3.34[b]**
Greytown	150.37	278.62	243.56	4.80	**169.34[a]**
Standerton	20.06	31.32	4.80	4.84	**15.26[b]**
Cultivar mean	**58.12[b]**	**103.92[a]**	**83.73[ab]**	**4.80[c]**	**Interaction LSD (P<0.05) 70.65**

Season	**Deoxynivalenol levels**
13/14	90.76[a]
14/15	34.51[b]
Season Mean	**62.64**

Table 5.18 Anaysis of variance and interaction table of NIV (µg/kg) in grain from four sorghum cultivars treated with fungicide spray regimes at Potchefstroom, Greytown and Standerton during 13/14 and 14/15. (b) NIV (µg/kg) in grain from sorghum cultivars treated with fungicide spray regimes during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	29003.26	2900.33	0.22	0.99
Cultivar	3	555499.30	185166.40	13.90	0.00*
Season	1	234929.40	234929.40	17.64	0.00*
Locality	2	2310925.00	1155462.00	86.75	0.00*
Cultivar x Locality	6	1097470.00	182911.60	13.73	0.00*
Error	127	1691667.00	13320.21		
Total	259	6229594.00			

Locality	**Cultivar**				**Locality mean**
	PAN 8816	PAN 8906	PAN8911/ PAN 8625	NS 5511	
Potchefstroom	8.15	10.23	12.27	11.77	**10.61^{b}**
Greytown	165.19	385.91	268.79	11.26	**207.79^{a}**
Standerton	0.74	9.56	3.18	6.27	**4.94^{b}**
Cultivar mean	**58.03^{b}**	**135.23^{a}**	**94.75^{b}**	**9.77^{c}**	**Interaction LSD (P<0.05) 68.20**

Season	**Nivalenol levels**
13/14	104.74^{a}
14/15	44.15^{b}
Season Mean	**74.45**

Table 5.19 Analysis of variance and interaction table of ZEA (µg/kg) in grain from four sorghum cultivars treated with fungicide spray regimes at Potchefstroom, Greytown and Standerton during 13/14 and 14/15. (b) ZEA (µg/kg) in grain from sorghum cultivars treated with fungicide spray regimes during 13/14 and 14/15.

Source of variation	d.f.	s.s.	m.s.	F-ratio	Prop level
Treatment	10	653690.60	65369.06	0.49	0.89
Cultivar	3	5046248.00	1682083.00	12.61	0.00*
Season	1	1546652.00	1546652.00	11.59	0.00*
Locality	2	16758600.00	8379300.00	62.81	0.00*
Cultivar x Locality	6	10305680.00	1717613.00	12.87	0.00*
Error	127	16943560.00	133413.90		
Total	259	62463840.00			

Locality	**Cultivar**				**Locality mean**
	PAN 8816	PAN 8906	PAN8911/ PAN 8625	NS 5511	
Potchefstroom	1.78	2.85	11.97	1.01	**4.40[b]**
Greytown	117.55	771.09	1134.36	181.05	**551.01[a]**
Standerton	34.33	59.02	4.82	2.52	**25.17[b]**
Cultivar mean	**51.22[b]**	**277.65[a]**	**383.72[a]**	**61.53[b]**	**Interaction LSD ($P<0.05$) 215.85**

Season	**Zearalenone levels**
13/14	271.28[a]
14/15	115.77[b]
Season Mean	**193.53**

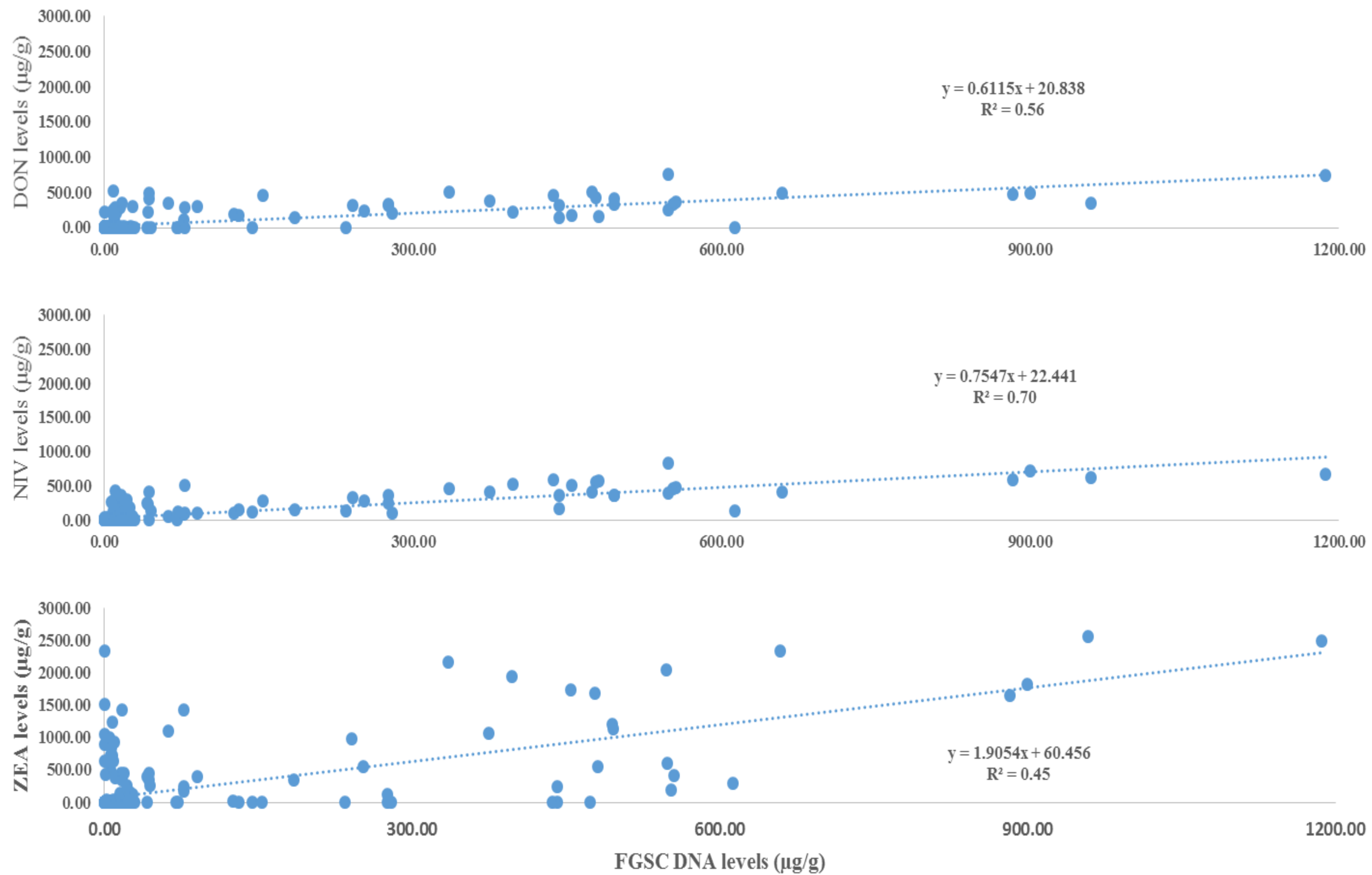

Figure 5.1 Relationships between *Fusarium graminearum* species complex DNA and (a) DON (b) NIV and (c) ZEA levels from four sorghum cultivars treated with eleven fungicide spray regimes during 13/14 and 14/15.

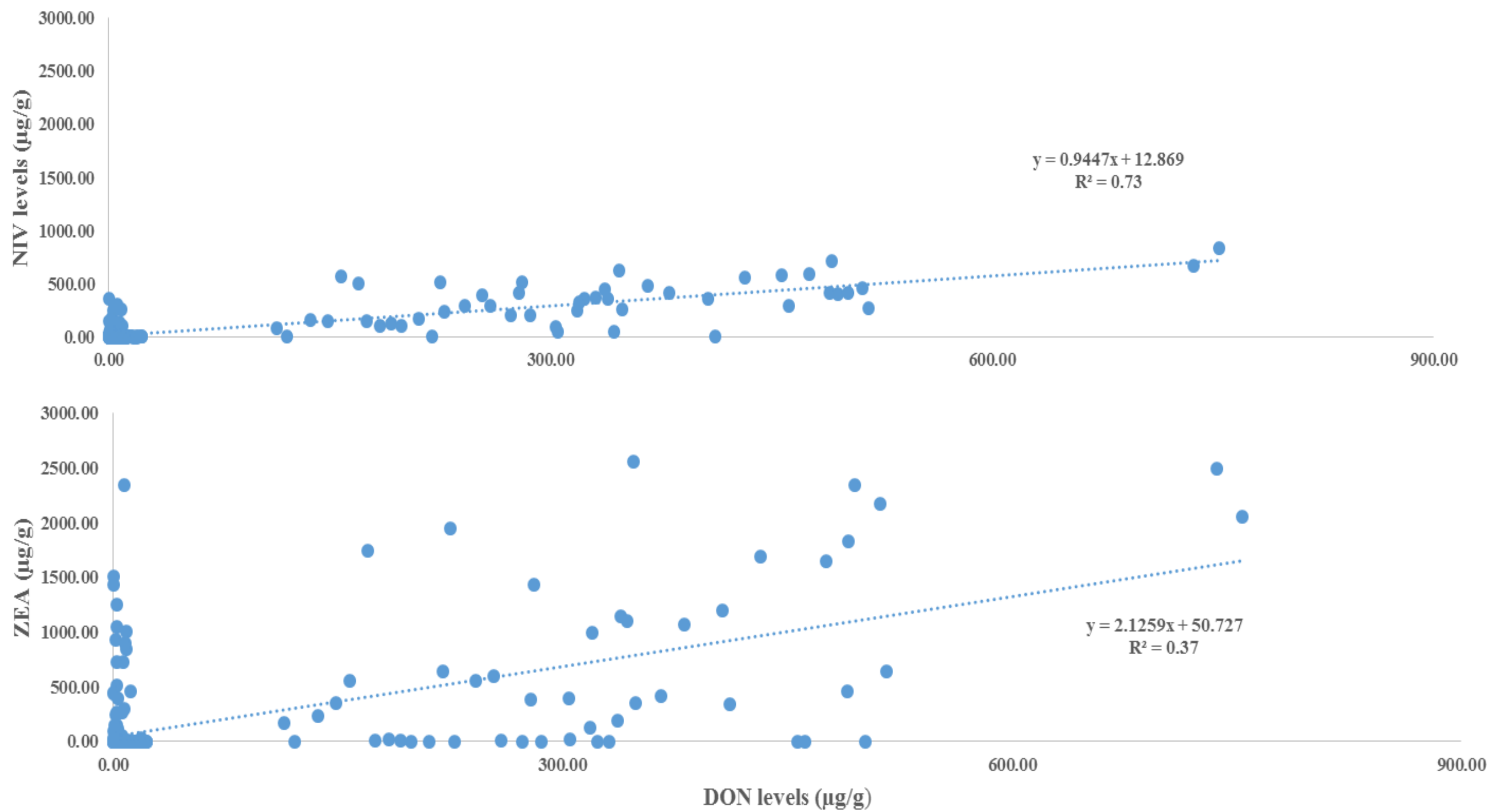

Figure 5.2 Relationships between DON and (a) NIV and (b) ZEA levels from four sorghum cultivars treated with eleven fungicide spray regimes at Potchefstroom, Greytown and Standerton during 13/14 and 14/15.

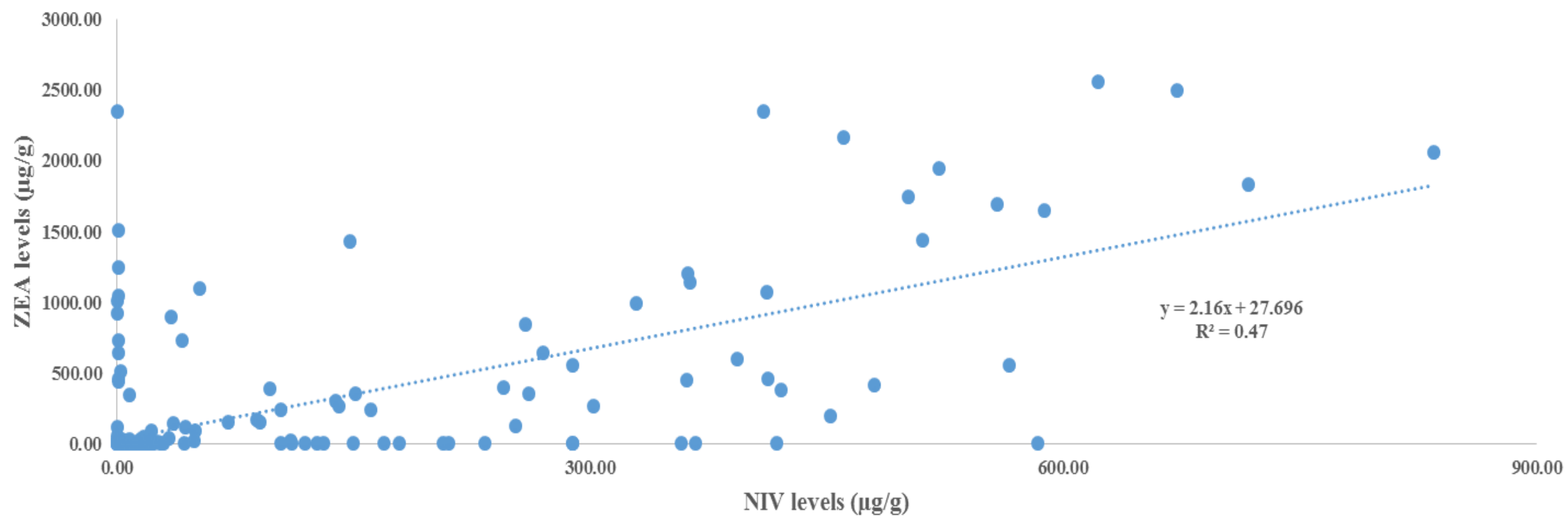

Figure 5.3 Relationship between Nivalenol levels and Zearalenone levels from four sorghum cultivars treated with eleven fungicide spray regimes at Potchefstroom, Greytown and Standerton during 13/14 and 14/15.

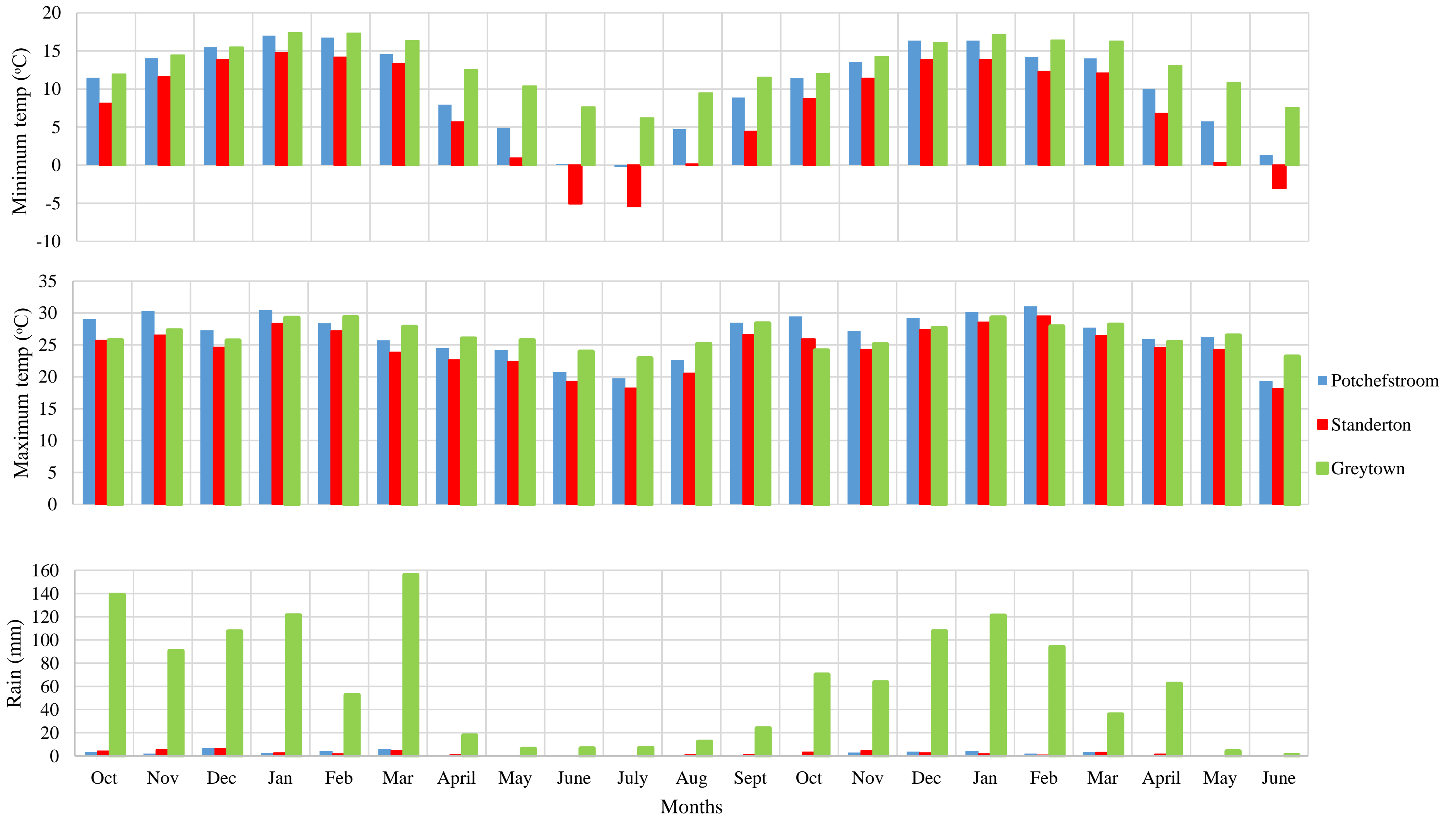

Figure 5.4 Weather data of Potchefstroom, Greytown and Standerton during October 2013 to June 2015. (a) Minimum temperatures. (b) Maximum tempearatures. (c) Rainfall.

SUMMARY

Sorghum grain mold is an economically destructive disease that can influence both the yield and quality of the grain. Causal fungi include *Fusarium* spp. that produce mycotoxins that affect the wellbeing of humans and animals. Ways to improve disease resistance have been explored and include exploitation of physical and chemical traits, and cultivation practices.

Field trials were conducted at Cedara and Alma to evaluate sorghum grain mold resistance in 22 sorghum lines representing the range of plant and grain characteristics in commercially planted cultivars. Grain mold visual ratings, fungal biomass through ergosterol analysis, FGSC (*F. graminearum* species complex) quantification by means of qPCR and subsequent mycotoxin concentrations were analysed. Only purple plant colour was associated with higher grain mold ratings. No other grain characteristic were associated with resistance to colonisation or mycotoxins produced, which led to the assumption that resistance appears to be the result of complex reactions. The wetter Cedara had higher grain visual ratings, FGSC colonisation and subsequent mycotoxin production. AMMI analysis revealed genetic stability to grain mold through changing environments when visual assessments were applied, while ergosterol levels indicated genotypic adaptions to different environments. A multi-variable approach is needed for evaluation of resistance.

Grain of the 22 sorghum lines planted in Cedara and Alma were evaluated for chemical traits contributing to grain mold resistance. The highest total phenolic acid levels were recorded in red pericarp grain compared to the lowest found in white pericarp grain. Total phenolic acids for half of the lines remained stable levels across environmental changes as indicated through AMMI analysis. HPLC analysis indicated inconsistent levels of specific phenolic compounds across genotypes, with the exception of gallic acid that correlated with resistance to grain mold in genotypes. Higher total protein levels were associated with coloured pericarp grain on a tan plant colour, but no relationship with disease severity

was recorded. Similarly no relationship was observed with glucanase and chitinase levels. Further in-depth studies are needed to understand the complex mechanisms involved in resistance.

Rotation systems were introduced into a field trial in Alma to evaluate the effect on grain mold and root rot severity in a high tannin and white tan genotype. Soil nutrient status, root and grain visual ratings, root mass and yield, ergosterol levels, FGSC and subsequent mycotoxin levels were measured. Throughout the trial, an increase in nutrients, root mass and yield were recorded in legume systems compared to fallow and monoculture sorghum systems. The effect of rotation systems on other parameters were variable. Cultivars also differed in their response with NS 5511, the high tannin cultivar, being more resistant to grain mold compared to PAN 8706W, the white tan cultivar, that was more resistant to root rot. Throughout the trial, beneficial effects of legume systems on root rot were observed, while responses to grain pathogens were variable.

Two fungicides, azoxystrobin/difenoconazole and epoxyconazole/pyraclostrobin were applied to four cultivars at different time periods (6, 8, 10, 6 + 8, 8 + 10 weeks after planting) in Potchefstroom, Greytown and Standerton. Only superficial discolourations on the roots and grain, general fungal colonisation and root growth were affected by fungicide treatments. Both fungicides reduced root rot, with only azoxystrobin/difenoconazole being effective against grain mold. Only root growth was stimulated with fungicide applications. Yield, FGSC colonisation and subsequent mycotoxins were influenced more by environment, i.e. locality and seasons, and choice of cultivars and not as a result of fungicide treatments.