Screening of sorghum (Sorghum bicolor) genotypes for resistance to covered kernel smut (Sporisoriumsorghi) disease

By

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A research project submitted in partial fulfilment of the requirements for the degree of Bachelor of Science (Honours)Degree in Agronomy

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June 2016

DECLARATION

I hereby declare that the research described in this thesis is the result of my own efforts and all the additional sources have been acknowledged by means of reference.

.....

Lindsay Phiri

Date

.....

CERTIFICATION OF THESIS

I declare and certify that I have supervised Lindsay Phiri R124147N thesis with the topic Screening sorghum genotypes for resistance to covered kernel smut disease under artificial field inoculation.

Name of supervisor
Signature
Date

ABSTRACT

Covered kernel smut of sorghum caused by Sporisoriumsorghi is a major threat to sorghum production globally. This pathogen has been reported to attack sorghum resulting in yield losses as high as 75% on susceptible sorghum genotypes in Zimbabwe. Twelve sorghum genotypes were screened to determine the incidence and severity of covered kernel smut during the 2014/15 growing season. The trial was done under field conditions, where two artificial inoculation techniques namely seed dressing with dry teliospores and stem injection were used to observe the reaction of the sorghum genotypes. The experiment was set up using Randomised Complete Block Design with three replicates in the field. The disease incidence was highly distributed and it varied significantly (p < 0.001) between the sorghum genotypes. A range of 0 - 40% incidence of the disease was recorded with SV-1 and ICSR 93034 having 0% and 40% respectively. Similarly, severity also followed the same trend as that of incidence with SV-1 having a mean score of 1 whilst ICSR 93034 had 4.667. NL 2015 produced the highest mean grain yield of 1.7467 tonnes/ha whilst NL 2014 had the lowest mean grain yield of 0.1903 tonnes/ha. The screening study suggests that farmers should grow sorghum genotypes SV-1, NL 2015 and ICSR 93024 which exhibited high levels of resistance to covered kernel smut disease.

DEDICATION

I dedicate this project to the Almighty God and to my wonderful mother Priscillah Phiri, who taught me that the best kind of knowledge to have is that which is learned and put into practise. To my late sister Daphne Phiri, who taught me that even the largest task can be accomplished if it done one step at a time. You have been with me every step of the way, through good and bad times. Thank you for all the unconditional love, guidance and support that you have always given me, helping me to succeed and instilling in me the confidence that I am capable of doing anything I put my mind to. Thank you for everything, I love you more than I can express. Also, this project is dedicated to all my friends who have been a great source of motivation and inspiration. Finally, this project is dedicated to all those who believe in the richness of learning.

ACKNOWLEDGEMENTS

From the formative stages of this project to the final draft, I owe an immense debt of gratitude to Mrs B. TMakaure and Mr Marco Mare who have been the ideal supervisors. Their sage advice, insightful criticisms and patient encouragement aided the research and writing of this project in innumerable ways.

I would like to formally thank Crop Breeding Institute: Sorghum and Millets Unitfor allowing me to conduct the research at their research station and for providing me with all the materials that I utilized in my research work. My fellow graduate colleagues MudzingiriThembinkosi, Chimbadzwa Collins, Mungombe Kenny and MbamboRumbidzai. I wish express gratitude to Ms Olivia Mukondwa (Agriculture Technician) and Mr Raymond Mashinya (Agriculture Assistant) for their friendships and support. Not forgetting Mr Enoch Chigamba (Agriculture Field Foreman) whose commitment made the experiment successful.

I wish to say that the past year have been quite an experience and you have all made it a memorable time of my life. Good luck to each of you in all your future endeavours. On a personal side, I wish to thank my mother, for her never-ending love and support in all my efforts, and for giving me the foundation to be whom I am today. My late sister Daphne and brother Rungano for their love and support when I needed it the most throughout the year of research.

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CHAPTER 1

Introduction and Justification

1.1 Introduction and justification

Sorghum (*Sorghum bicolor* (L) Moench) is ranked fifth in importance among cereals, and it is one of the world's major food cereal crops (FA0, 2012). Global production of the small grain is estimated to be 40 million hectares (FAO, 2012). Sorghum is particularly important in areas of high temperatures and low rainfall as the crop is drought tolerant (Hayden, 2002). In Zimbabwe the crop is specifically important in the Southern African Agro-ecological Zones (SAAZ) III, IV and with a production trend of 186 000 - 230 000 tonnes per annum (UN Relief and Recovery Unit, 2004). The small grain crop can be used for syrup production, making of leavened and unleavened bread, bio-energy and bio-ethanol production, preparation of alcoholic beverages, fence and broom making and floral arrangements (USDA, 2013).

Over at least 30 diseases have been recognized on sorghum in the southern region (Leslie, 2008), of these covered kernel smut caused by *Sporisoriumsorghi* is a major constraint in sorghum production (Mtisi and McLaren, 2008). The fungus is seed-borne and develops systematically as the sorghum crop grows. According to Howard *et al* (2005) maturing fruiting bodies of the fungus called sori ruptures and releases teliospores that infects seeds on other plants. The teliospores of the fungus replace the grain in the panicle causing direct crop losses in proportion to the area of the panicle infected (Howard *et al.*, 2005).

According to Sisay*et al* (2012) annual yield losses due to covered kernel smut in Africa reaches 10% with localised losses of 60% or more. The incidence of covered kernel smut varies from place to place, in Eastern African countries namely Ethiopia, the incidence was estimated to be about 50% (Sisay et al., 2012). In West Africa, covered kernel smut is more

significant to countries such as Sudan, Nigeria and Guinea Savannah.Gwary et al (2007) showed that at least 24.8%, 25% and 29.5% reduction of yield by covered kernel smut was recorded respectively. In Southern Africa, Zimbabwe has several smut hotspots recognized by the National Pest Survey conducted in 1985 which indicated the widespread of covered kernel smut in communal areas (Mtisi and McLaren, 2008). During the 2012-13 growing season of the crop, areas namely Binga, Hwange and Chiredzi had crop losses of 30-70% which were caused by covered kernel smut (CBI, 2013). According to Louis *et al.*, (2007) covered kernel smut is a major biotic limiting factor or constraint in sorghum production, resulting in a threat to food security at both household and national levels in Africa.

Macia, SV-2 and SV-4 genotypes were reported to be susceptible to covered kernel smut in Binga, Hwange and Chiredzi with yield losses of below 0.5 tonnes per hectare (CBI, 2013). Due to lack of smut resistant genotypes the farmers in these areas continue to share and exchange the retained old untreated seed within the communities (Gwary*et al.*, 2007). Therefore using the pedigree record of the advanced sorghum genotypes, only eight sorghum genotypes were chosen for the trial with outstanding phenotypic characteristics as compared to the other advanced sorghum genotypes.

The use of resistant genotypes is and always remains the best strategy for the control of smut (Kutama*et al.*, 2013). Given that cereals like sorghum in general have a low return to investment, the introduction of resistance remains the most cost-effective option for covered kernel smut (Wilson, 2011). It is thus necessary for stable sources of resistance to be screened, sought and be recommended for breeding programmes for the improvement of other sorghum genotypes in Zimbabwe. In Zimbabwe, the disease has continued to perpetuate regardless of other chemical and biological control methods which are considered to be effective in the developed countries. These methods are very costly to small-scale holder

farmers. Therefore, this study seeks to improve production of the crop through releasing resistant genotypes.

1.2.1 Main objective

To screen sorghum genotypes for resistance to covered kernel smut under artificial field inoculation.

1.2.2 Specific objectives

- 1. To determine the incidence of covered kernel smut disease on the artificially inoculated twelve sorghum genotypes.
- 2. To assess the severity of covered kernel disease on the artificially inoculated twelve sorghum genotypes.
- To evaluate the effect of covered kernel smut disease on grain yield of the artificially inoculated twelve sorghum genotypes.

1.2.3 Hypotheses

- There are significant differences on the incidence of covered kernel smut disease of the twelve sorghum genotypes which are artificially inoculated.
- 2. There are significant differences in covered kernel smut disease severity on the inoculated sorghum genotypes.
- There are significant differences in grain yield among the twelve sorghum genotypes inoculated with covered kernel smut.

CHAPTER 2

Literature Review

2.1 Sorghum production and uses in Zimbabwe

Sorghum (*Sorghum bicolor* (L) Moench) referred to as the "poor man's crop is ranked the fifth most important cereal crop in the world with a recorded annual production of over 60 million tonnes (FAO, 2012). As a result Africa produces 20 million tonnes over an exceeding area of 40 million hectares, accounting for 14% of the total area of cereal production (Taylor, 2003). This makes the sorghum crop the second most important cereal crop after maize in Africa (FAO, 2012). In the semi-arid tropics of the developing countries, sorghum accounts for 80% of the total world area sown although most of the area the crop is grown on a relatively small scale by small-holder farmers where it serves as a risk-reducing crop (Jere, 2004).

In Zimbabwe, sorghum is a crop of both the small holder mostly residing in the Southern Agro-ecological zones III, IV and IV, and commercial farmers in higher rainfall areas (Mtisi and McLaren, 2008). According to the United Nations relief and Recovery Unit (2004), it is reported that the area under sorghum production during the 2003/4 season was 207 000 hectares, which is an increase of close to 300% from 2002/3 season in Zimbabwe. According to Friis-Hansen (1995) he quotes that sorghum is a high yield potential crop and this was evident when Zimbabwe produced 20.1 tonnes per hectare. In 2004, UN Relief and Recovery Unit recorded an average yield varied from 0.9-1.1 tonnes per hectare giving an estimated total production in the range of 186 000 – 230 000 tonnes per annum.

According to FAO (2012), like many grains sorghum has a diversity of uses including human consumption and animal feed. *Human food*. The sorghum grains are used as human nutrition all over the world, it is rich in carbohydrates, zinc and iron in which other cereals like maize do not supply in human food. Grain sorghum is used for flour production, preparation of side

dishes and porridges, malted and distilled beverages production, preparation of special dishes such as popped grain and syrup production from sweet sorghums (FAO. 2012).

Sorghum is also considered to be a significant crop for animal feeds as it can be as fodder. High tannin grains are less palatable to cattle due to their hard and waxy covering, the grains need to be processed by cracking, rolling or grinding, when processed the nutritional value of sorghum is comparable (but not equal) to maize so it requires supplementation of Vitamin A (Wilson, 2011).Grain sorghum is also used for silage for example the sweet sorghums have a higher silage yield. Some other uses are as follows, sorghum fibres are used in making of wallboards, fences, biodegradable packaging materials, solvents, broom making and thatching house roofs. Sorghum plants are used also for bio-ethanol and bio-energy production in industries. Dried stalks can be used for cooking fuel and dye can be extracted from the plant to colour leather (FAO, 2012).

2.2 Sorghum production constraints in Zimbabwe

Sorghum production in most parts of the world is relatively low, estimated at 0.925 tonnes per hectare compared to 5 tonnes per hectare reported from experimental stations (ICRISAT, 2004). In Zimbabwe, low yields averagely 0.6 – 0.8 tonnes per hectareon the majority of farms growing sorghum has been recorded (Mtisi and McLaren, 2008). Over the years, sorghum production increase in Zimbabwe has been attributed to expansion of crop area rather than increased tonnage per unit area. The low yields are attributed to a number of abiotic factors like erratic and delayed rainfall, low soil fertility/ poor soils, insufficient seeds; and biotic factors such as low yielding varieties, poormanagement systems, weeds, insect pests for examples termites and black ants and diseases (Esele, 2013).

2.2.1 Abiotic and socio-economic factors

Erratic rainfall is a major problem for farmers since their agriculture is typically rain-fed. Farmers hold the view that the amount of rainfall has decreased and is insufficient. However, it appears that the problem stems from the lack of appropriate sorghum varieties that fit the current rainfall regime (Kudadjie*et al.*, 2004). Poor soils are a source of worry to all farmers. Sorghum production typically takes place in marginal areas that are prone to infertility and water stress conditions (Kudadjie*et al.*, 2004). The majority of smallholder farmers, especially in the semi-arid tropical regions of Africa, do not produce enough sorghum to meet family requirements. Furthermore sorghum is a semi-subsistence enterprise that offers smaller returns than other investments such as livestock. As a result, less attention is paid to invest in the use of seeds from improved varieties to boost production (FAO, 2012). These and other abiotic factors hamper sorghum production leading to low yields and incomes.

2.2.2 Biotic factors

Striga is a major constraint of sorghum production especially in some parts of Zimbabwe (Mtisi and McLaren, 2008). There are at least two species of striga known to affect sorghum production in the country namely: *Strigahermonthica Strigaasiatica*. Some *Striga*-resistant sorghum varieties have been developed, but these generally offer lower yields than traditional cultivars and improved (but *Striga*-susceptible) varieties (FAO, 2012). The effect of *Striga*has been found to decrease when crops are grown in conjunction with legumes (Carsky*et al.*, 2009). The most important arthropod pests of sorghum include sorghum shoot fly (*Atherigonavariasoccata*), sorghum midge (*Contariniasorghicola*) and sorghum stem borer (*Busseolafusca*) (Sharma, 2012). Sorghum shoot fly causes substantial losses in late and off-season sorghum (Davies and Reddy, 2001) and stem borers are also endemic in most parts of Zimbabwe (Gono*etal.*, 2004). The most important species of include *Chilopartellus*, *Sesamiacalamitis Buseolasorghida*. *Chilopartellus* is mainly found in distributed

throughout sorghum growing areas (Wilson, 2011) Birds are perhaps one of the most important pests of sorghum worldwide. They are capable of inflicting heavy losses and causing economic damage. In Zimbabwe the most notorious species is *Queleaquelea*.

2.2.3 Sorghum diseases

Like all crops, grain sorghum is subject to various infectious diseases which limit grain crop production in the agriculture sector (Vinceli and Hershman, 2011). Sorghum is plagued by an unlimited number of seed-borne diseases of which Covered kernel smut disease is of major importance in the study. These unusual diseases and the wide range of environments in which the crop is exposed to challenges of up to three smuts which are commonly namely, the covered kernel smut, loose kernel smut and head smut (Friederisken*et al.*, 2000). A different species of fungus *Sphacelotheca* or *Sporisorium*causes the mentioned smuts above. Smuts are one of the most significant diseases in sorghum production especially where untreated seed is planted. ICRISAT carried out surveys in Southern Africa and identified that the sorghum crop in most countries was suffering from the similar smut diseases with some variation due to ecological zones and the level of improvement in the sorghum lines (Wilson, 2011). The panicle disease, covered kernel smut will be concentrated more in this review, because this is most significant disease identified by farmers in the southern agro-ecological zones of Zimbabwe in which the study was conducted.

2.3 Covered kernel smut disease

Covered kernel smut is a seed borne panicle disease caused by the fungus *Sporisoriumsorghi* which is classified within the Ustilaginales, class Basidiomycetes (Perez, 2002). The disease occurs in every sorghum growing region globally and causes greater grain loss in yield than any other disease in the tropical regions (Frederisken and Odvody, 2000). According to Marley*et al.*, (2002) although the pathogen was suggested to be specific to the genus *Sorghum*, the weed CynodonDactylon was recognized as an alternative host of the pathogen.

All sorghum types are attacked by the pathogen for example Johnson grass. The disease is only apparent after heading. Individual ovules are replaced by smut fruiting bodies that vary in size, generally the smut sori are smooth, oval, conical or cylindrical in shape and vary in size from those small enough to be concealed by the glumes to those one cm long, but vary in colour from white to grey or brown (Howard *et al.*, 2005).

2.3.1 Distribution and significance of Covered kernel smut in Southern Africa

Covered kernel smut disease is widely distributed in all sorghum growing areas in Southern Africa. The most eastern reports have been made in Ethiopia and Congo and the most western reports were from Sudan and Nigeria and Guinea Savanna (Tarr, 2010). Published data on the actual incidences and severity of covered kernel smut disease in Southern Africa are limited. Tarr (2010) reported incidences of covered kernel smut in Africa of the range 8-43%, while Selveraj (2013) estimated losses up to 50% in Africa. Wilson (2011) quoted Doggett review of sorghum diseases in East Africa in 1980 who wrote that covered kernel smut disease was conspicuous and it was worth utilising seed dressings. However, Doggett was unaware of any estimates of yield loss, except for Ngugi*et al.*, (2002), who reported incidences ranging from 8-100% and losses greater than 30% in Tanzania.

ICRISAT's southern Africa surveys of 2012 reported that covered kernel smut was an important disease in the region. In Zimbabwe, Mtisi and McLaren (2008) wrote that in 1985 the National Pest Survey indicated that covered kernel smut indicated an incidence of up to at least 40% in the communal areas where sorghum is grown. Gono*et al.*, (2004) reported an average 85% disease incidence of covered kernel smut disease in sorghum growing areas namely Gwanda, Marange and Chiredzi. The sorghum crop grown in these areas were greatly affected by the covered kernel smut which almost wiped out the whole crop such that poor quality yields were obtained of below 1.5 tons per hectare in each of the areas listed above (Gono*et al.*, 2004). Crop losses in the semi-arid zones of Hwange and Binga were reported to

be between the ranges of 30-70% influenced by the causal pathogen *Sporisoriumsorghi* damage on the sorghum crop (CBI, 2013).

2.3.2 Biology of Sporisoriumsorghi pathogen

According to Frowd (2014) the fungus *Sporisoriumsorghi* produces diploid teliospores that are accurately spherical with a diameter of 4-7µm. the diplodteliospores when they germinate tend to produce a four celled basidium, which bears monosporodia that fuse together to produce the pathogenic dikaryon (Wilson, 2011). Munkacsi*et al.*, (2007) recent work indicates that*Sporisoriumsorghi* diverged from other crop smuts,*Ustilagomaydis*, *Ustilagoscitaminea* and *Sporisoriumreilanum*, and this occurred prior to domestication and modern agriculture and in the ecological context of the fungal population and host plant.

2.3.3 Conditions favouring the development of covered kernel smut disease

According to Silaev (2005) the fungus *Sporisoriumsorghi* can grow and develop at 10-32°C. The soil optimum temperature conducive for covered kernel smut disease development is 18-25°C and infection is established in warmer, wet soils with a humidity of 15-20%, during the period of delayed seed germination are optimal for the contamination of plants. The infection decreases at temperatures between 35-40°C. According to Sisayet al., (2012) spore germination varies morphologically under the optimum temperature from 20-30°C, and the spores retain viability for four years when kept in dry conditions. Covered kernel smut overwinters in the soil and the crop residues. Ashok *et al.*, (2011) stipulated that since infection takes place before the seedlings emerge out, the conditions suited for delayed germination of seeds favour infection. Host variety, soil temperature, soil moisture content and depth of sowing are known to affect the degree of infection. High temperatures after sowing have been reported to reduce smut incidence on the seedlings. Sisayet al., (2011) stipulated that high temperature and low soil moisture encourage seed germination and discourage smut mycelium invasion of the germinated coleoptiles of the host plant.

Meanwhile low temperature, moisture content of the soil and deeper planting of sorghum initiate high infection level (Sisay*et al.*, 2011).

2.3.4 Effects of covered kernel smut disease on the sorghum crop

2.3.4.1 Effect of the smut disease on sorghum growth

When smutted sorghum seed is planted, the spores germinate along with the seed. The growing fungus then invades the developing seedling and continues to grow systematically and undetected inside the plant before heading (Howard *et al.*, 2005). The fruiting bodies or the smut galls which have formed in place of the kernel become evident. The infected plants appear to be normal growing plants until the emergence of the panicle or the head, the diseased panicles are individually replaced by the dark brown powdery masses of teliospores (sorus) covered by a greyish brown membrane (Ashok *et al.*, 2011).Therefore to a lesser extent growth of the sorghum plant by covered kernel smut is not affected, as compared to loose kernel smut which stunts the infected plants and frequently induces the development of abundant side branches (Sisay*et al.*, 2012).

Individual grains of sorghum becomes misshapen as they develop and are filled with dark smut spores on maturity. The smut sori (cone-shaped gall) is inside the seed coat. The panicle may be reduced to a few twisted, distorted branches covered with large, superficial smut sori (Ashok *et al.*, 2011). In some cases, the panicle branches may be completely destroyed, leaving only the distorted central rachis covered with sori. Infected plants are generally the same height and size as healthy plants (Wilson,2011). Loose kernel smut causes a reduction in plant height, stalk diameter and leaf width with infected plants heading prematurely as a result of the fungus accelerating the growth cycle of the host. Further, infected plants show increased tillering, with tillers often short and slender (Kutama *et al.*, 2011).

2.3.4.2 Effect of smut disease on sorghum yield and quality

Covered kernel smut destroys all kernels in the head and replaces them with a cone-shaped gall or may affect only portions of the panicle. At harvest time, yield is reduced when the galls are broken, the spores disseminate and contaminates the outer surface of the kernels (Howard *et al.*, 2005). Damage is confined almost entirely to the head or panicles, thus the reduction in yield is conspicuous and direct (Jere, 2004). The kernel smuts of sorghum may reduce seed production seriously with probably much less effect on forage yields. The head itself retains the shape and these spore masses look somewhat elongated seeds (Ashok *et al.*, 2011)). The quality of the remaining yield is drastically reduced by the presence of the black smut spores on the surface of the healthy kernels (Jere, 2004). According to Thakur et al., (2007) when the infected kernels break open, the microscopic spores adhere to the surface of the healthy seeds where they over-winter thus reducing crop quality.

2.3.5 Control of Covered kernel smut disease of sorghum

To minimise sorghum yield losses due covered kernel smut, it is important to correctly identify the best suitable management methods to control the disease. Covered kernel smut can be controlled by the practise of two methods namely the chemical and cultural methods. No biological control strategies have been developed yet for covered kernel smut worldwide (Wilson, 2011).

Chemical method includes the use of fungicides which assist in reducing the incidence and severity of the disease on the sorghum but does not completely control the disease. Economical and complete protection from covered kernel smut can be achieved with proper seed treatment (Howard *et al.*, 2005). Covered and loose kernel smuts are easily and effectively controlled by treating the seed with a protectant fungicide. Seed treatment prevents introducing the kernel smut fungus into uninfected fields (Silaev, 2005).

Fungicide seed treatment also improves and stabilizes the stand when soil insects are not a problem. In addition, it provides protection against seedling smut fungi in the soil. The pathogen can be controlled by avoiding seeds from endemic areas and discarding smut sori during visual examination (Thakur *et al.*, 2007). Apparently healthy-looking seed should be treated with carboxin (Vitavax) at about 2 g active ingredient per kg of seed or elemental sulphur at about 5 g per kg of seed. The seeds can also be treated with fungicides such as Captan or Thiram at 0.3% per kg of seed (Jere, 2004).

If systemic seed dressings cannot be obtained cultural methods come into play by soaking the seeds in water for 4 hours, then dry the seeds, first in the shade and then in the sun. This procedure kills germinating smut spores without impairing seed viability (IPM, 2008)). Risk of pathogen infection on the sorghum seeds is reduced by minimizing mechanical and herbicide injury, while maintaining a balanced fertility program on sorghum growth. The practise of crop rotations and cultivation have little effect on controlling the disease, since the smut teliospores can survive and persist by overwintering in the soil for long periods of time and years (Perez, 2002).

The use of disease free sorghum cultivars is recommended which are highly resistant or immune to the sorghum kernel smut from certified seed producers in the country. Sorghum ratooning is practically not advisable to practise as most of the ratooned crops exhibit higher incidences of covered kernel smut disease (Wilson, 2011). One can also collect the smutted ear-heads of sorghum in cloth bags and destroy the pathogen by dipping in boiling water for at least thirty minutes. Where feasible, incineration of infested samples in the field should be done by promptly removing and burning the heads infested with the smut galls before the spores are scattered (IPM, 2008). Since the covered kernel smut fungus may live in the soil for several years therefore the farmer can grow sorghum in the same field only once in four years (Howard *et al.*, 2005).

CHAPTER 3

Materials and Methods

3.1 Site description

The study was conducted at Matopos Research Farm approximately 28,5km south west of Bulawayo along Kezi road during the 2014/2015 farming season. The farm is located in the agro-ecological region IV in Zimbabwe on the co-ordinates $28^{\circ}30$ 'S and $20^{\circ}23$ 'E at an elevation of 1340m above sea level. Land classification of the site is chromic-lepticcambisol (FAO, 2012) and it consists of 45% clay, 36% sand and 19% silt to (Moyo 2011). During the wet season the soil is susceptible to water logging conditions when a variable rainfall of 240 – 1400mm is received. The agro-ecological zone experiences low and erratic rainfall with higher temperatures. According to Moyo*et al.*, (2011) they tabulated the climatic characteristics and features of the agro-ecological zone IV (Table 3.1).

Table 5.1: Characteristics of the experimental site			
CHARACTERISTIC	RANGE		
Rainfall	500 – 600 mm per annum		
Mean temperature	25.15°C		
Soil pH	6.0 - 6.8		
Upper soil layer	0.15m		

 Table 3.1: Characteristics of the experimental site

3.2 Experimental procedure

3.2.1 Collection of sorghum genotypes

Twelve advanced sorghum genotypes including the three released genotypes collected from Crop Breeding Institute were used in the study (Table 3.2).the experiment was laid using Randomized Complete Block Design with three replications.

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ICSR 93024	ICSR 93034					
NL 2012	NL 2015					
SV-1	NL 2014					
SV- 2	ICSV 93046					
SV-4	MACIA					
SDSL 90004	SEREDO					

 Table 3.2: Sorghum genotypes used in the experiment

3.2.2 First inoculum collection and preparation

Collection of smut sori from mature panicle smut infected sorghum genotypes was done bythreshing affected individual panicles which were collected from on-farm trials in Chiredzi and Chipinge. The smut sori was collected at the physiological maturity stage of sorghum. Normally in an infected panicle, individual ovules are replaced by conical to oval smut soriteliospores or chlamydospores that are covered by persistent peridia that are larger than the normal grain (Thakur *et al.*, 2007). Smut is observable after ejection of inflorescence, when smut starts to develop instead of flower elements reaching 0.5-1.0cm in size (Silaev, 2005). Threshing was done by lightly pounding the affected sorghum heads in selfing bags. Teliospores refers to a thick walled or overwintering spore which germinates to form the basidium (Wilson, 2011). A sieve tube was used to collect the smut galls by removing plant material and other debris. The affected panicles remained in the selfing bags to prevent the dissemination of the teliospores by storing them at a temperature of less than 21°C and relative humidity of less than 12% to prevent teliospore desiccation and germination respectively. Sorghum seeds of the twelve different genotypes were inoculated using spores of covered kernel smut at a ratio of 100 seeds to 0.15g of teliospores in small envelopes. The inoculation was done by shaking the envelopes for at least a minute to facilitate proper seed coating prior to planting.

3.2.2 Second inoculum preparation

Second inoculation was done at boot stage when the plants had reached 50% of heading. Sporodial suspension was administered to fill the space between flag leaf sheath and panicle. 0.5g of the previously collected dried and stored teliospores (*Sporisoriumsorghi*) was made to germinate in a 1L of distilled water for 28 hours and blended for 1 minute using an electric blender. The sporodial suspension was collected using a 5ml pediatric syringe. This suspension was introduced into the gap between the flag leaf and the panicle by inserting the needle gently on the plant whilst carefully holding and supporting the whole plant with a hand to prevent damage. Panicle heads were covered just after inoculation using parchment selfing bags. Water was sprinkled using a 15L knapsack sprayer to enhance infectionand maintaining of high humidity during the period from inoculation to symptom expression.

3.2.3 Agronomic practices

Land preparation was done by practicing convectional tillage by using a tractor drawn plough. Ploughing was done to break the soil cap and pans in-order to facilitate good crop growth and root expansion, also improving aeration and drainage by lowering the soil bulk density. A disc harrow was used for discing the land so as to create a fine tilth. Row marking was done, rows were marked using a row marker at a spacing of 0.75m apart. The practice was done to facilitate easy drilling of seeds and basal application. Each plot had four rows. Basal fertilizer was applied using Compound D (N P K) (7:14:7)as a basal dressing at a rate of 100kg/ha on the rows by hand drilling. The inoculated seeds were then sown on the same day on the rows within the 3 blocks at a spacing of 0.75m by 0.2m by hand drilling. Although the trial was to be rain-fed, dry spells were encountered in the month of January at Matopos, we then practiced overhead irrigation of the trial. Irrigation was done at weekly intervals to provide adequate moisture for germination and emergence, and also to reduce heat stress on the seedlings.

After two weeks of planting a selective herbicide Basagran with sodium alt of bentazon as the active ingredient was applied to control only broad leaved weeds namely black jack and Mexican marigold at a ratio of 150ml per 15L of water, leaving only the narrow leaved weeds and the crop. Manual hand-hoeing was done to remove narrow leaved weeds in the trial at two weeks interval. Three weeks after seedling emergence thinning was done to achieve a spacing of 20cm. As a result ten plants were left per row in each plot.

Two weeks after crop emergence top dressing with ammonium nitrate (34.5% N)at a rate of 200kg/ha was applied at two equal split doses, 100kg after the first weeding at the vegetative stage after 2 weeks of crop establishment. The remaining 100kg was applied at boot stage of plant growth at day 63 after crop emergence. Maize stalk borer was controlled using Diptrex 2.5% Granules. The granules were applied in the funnel of the sorghum plants at a rate of 2.5kg/ha.

Second inoculation was done at boot stage when the sorghum genotypes had reached their 50% to heading. Parchment khakhi bags were used to cover the heads after inoculation. Water was sprinkled on the heads using a 15L knapsack sprayer to enhance infection and maintain high humidity during the period from inoculation to symptom expression. Twenty

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days after second inoculation the khakhi bags were opened. This was done to allow the heads to sun dry for two days. Harvesting was done when the heads had developed a black dot at the base of the grain by cutting of the heads using a machete to separate them from the mother plant and allowed the sorghum heads to adequately sun dry before weighing and threshing.

3.3 Data collection

All the data was collected on all sorghum genotypes on the net plots which comprised of the two inner rows.

3.3.1 Days to 50% flowering

Days to 50% flowering recording was done when sorghum genotypes began to flower by the appearance of yellow anthers at the tip.

3.3.2 Days to physiological maturity

Days to physiological maturity was recorded by counting the number of days from planting until when the sorghum genotypes head showed signs of being ready for harvest by the development of a black dot at the base of the grain.

3.3.3 Assessment of Covered kernel smut disease

Ten plants from each row of the two inner rows that is a net plot of twenty plants were randomly selected in the assessments. The incidence and severity of covered kernel smut disease on the selected sorghum genotypes was done when they had reached the maturity stage.

3.3.3.1 Covered kernel smut disease incidence

Incidence was recorded by establishing the proportion of sorghum plants showing the symptoms of covered kernel smut over the total number of the sorghum plants in the selected rows and the result was expressed as a percentage in each plot. The formula used is as follows:

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$\frac{\textit{Total number of infected plants in a plot}}{\textit{Total number of plants in a plot}} \times 100\%$

3.3.3.2 Covered kernel smut disease severity

Covered kernel smut disease severity was scored on the smutted plants using the disease resistance classification scale used by Gwary*et al.*, (2001) and Marley *et al.*, (2002) (Table 3.3).

SEVERITY RESISTANCE		INCIDENCE RATE (%)	DESCRIPTION	
RATING				
1		0 incidence	Immune	
2		1 -10 incidence	Very resistant	
3		11 - 25 incidence	Moderately susceptible	
4		26 - 50 incidence	Susceptible	
5		51 - 100 incidence	Very susceptible	

 Table 3.3: Disease resistance classification scale

3.3.4 Assessment of grain yield of the inoculated sorghum genotypes

The grain yield was assessed in each net plot of the selected sorghum genotypes. Threshing was done to separate the grain from the heads. The grain was then combined per net plot, weighed, oven dried for 48 hours and yield data was recorded.

3.4Data analysis

Analysis of variance was done using the 17th Edition of Genstat. Disease incidence and severity data was transformed using Square Root Transformation method.

CHAPTER 4

Results

4.1 Covered kernel smut disease incidence on the artificially inoculated sorghum genotypes

There were significant differences (p<0.001) on incidence amongst the twelve sorghum genotypes screened for covered kernel smut disease (Figure 4.1). ICSR 93034 had the highest mean incidence of 40% which was statistically different from SV-1 which had the lowest mean incidence of 2%. MACIA and NL 2014 are statistically the same but significantly different from SV-4, NL 2012 and ICSV 93046. SV-1 with the lowest mean incidence of below 5% was found to be statistically different from all other sorghum genotypes.

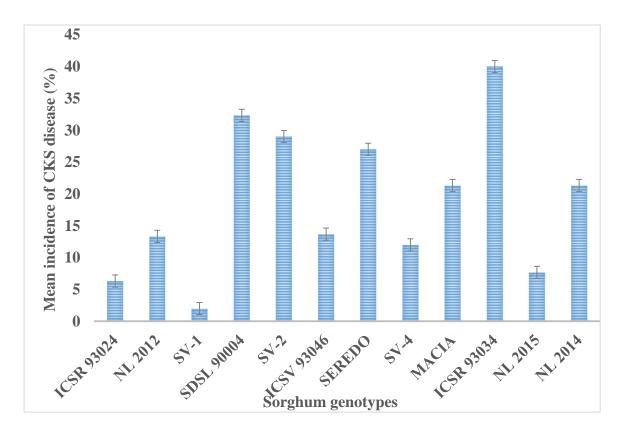


Figure 4.1: Covered kernel smut incidence of the inoculated sorghum genotypes

4.2 Severity score of covered kernel smut disease on the inoculated sorghum genotypes The differences on the severity of covered kernel smut disease were significant (p< 0.001) between the twelve sorghum genotypes (Figure 4.2). SV-1, NL 2015 and ICSR 93024 were statistically the same with the lowest score of 1 therefore these genotypes are resistant to covered kernel smut. Amongst all the sorghum genotypes ICSR 93034 had the highest severity score of 4.667which was statistically different from SDSL 90004, SV-2, SEREDO, NL 2014 and MACIA.

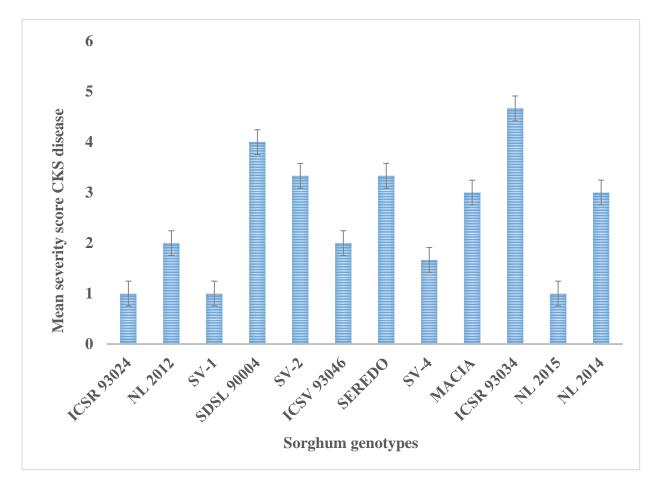


Figure 4.2: Severity of covered kernel smut on the inoculated sorghum genotypes.

4.3 Effect of covered kernel smut disease on the grain yield of the inoculated sorghum genotypes

There were significant differences in the grain yield (p<0.001) between the inoculated sorghum genotypes with covered kernel smut spores after harvesting, threshing, drying and weighing. NL 2015 yielded the highest with a mean grain yield of 1.7467 tonnes/ha which

was statistically different from the remaining sorghum genotypes(Figure 4.3). Statistically the mean grain yield of SV-1, SV-4, NL 2014, MACIA and SEREDO was the same. Statistically this was different from SDSL 90004, ICSV 93046 and ICSR 93034(Figure 4.3). The least significant grain yield was obtained from NL 2012 and NL2014 with a total yield of 4%.

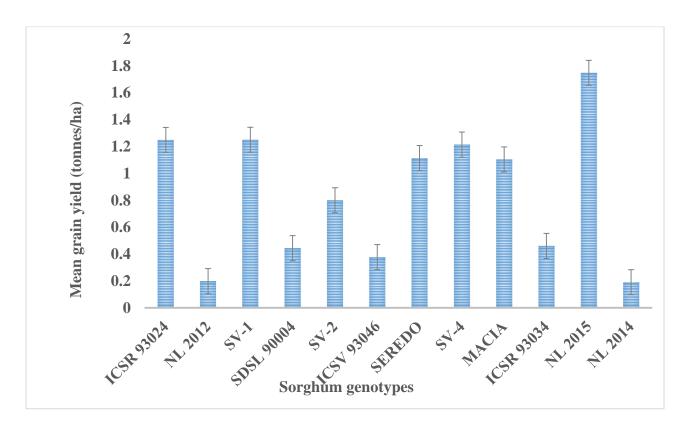


Figure 4.3: The effect of covered kernel smut on the grain yield of the inoculated sorghum genotypes.

CHAPTER 5

Discussion

5.1 Covered kernel smut disease incidence on the artificially inoculated sorghum genotypes

The disease incidence of covered kernel smut amongst the sorghum genotypes was significantly different. Disease mean incidence of ICSR 93034 was the highest with 40% and lowest for SV-1 with 2% (Fig 4.1). A possible explanation for this observed data could mean that the host species specificity of *Sporisoriumsorghi*pathogenic on sorghum may vary according to different genotypes of sorghum (Wilson, 2011).

Gwary*et al.*, (2007) stipulated that the variations obtained on disease incidence in the different sorghum genotypes screened for covered kernel smut may be due to the differences in the individual inherent reaction to the smut pathogen. This result agreed with a report by Nzioki*et al.*, (2000) that most studies to smut disease is controlled by a single gene, therefore whether resistant or susceptible the sorghum genotype is, it depends on the parents used. In this study, the reactions of various sorghum genotypes have been tested for *Sporisoriumsorghi*. The prevalent occurrence of covered kernel smut might be attributed to by the pathogen's biology. The fungal pathogen*Sporisoriumsorghi*, which transmit covered kernel smut over winters primarily as teliospores germinating with the seeds, which is systematic (Gwary *et al.*, 2007).

5.2 Severity score of covered kernel smut disease on the inoculated sorghum genotypes

There were significant differences in the severity scores of covered kernel smut disease on the sorghum genotypes. According to Marley *et al.*, (2002) the low severity score of 1 indicated that SV-1, NL 2015 and ICSR 93024 appeared to be very resistant to covered kernel smut disease. However in this study the level of covered kernel smut severity was not high on the first trial of screening the advanced sorghum genotypes for disease resistance, this was unexpected. The high severity value suggests that a number of sorghum genotypes appear to be susceptible to the disease. The general consensus in literature is that sorghum genotypes still undergoing evaluation should express lower severity levels of covered kernel smut than the released sorghum genotypes (Wilson, 2011). A ratio of 3:9 resistant to susceptible plants were obtained in the study. It seemed that the three sorghum genotypes exhibited some form of polygenic kind of resistance which is highly needed in breeding for covered kernel smut resistance in sorghum (Marley *et al.*, 2001). Studies in West Africa have earlier indicated that the existence of horizontal resistance in most sorghum genotypes is characterized by slow disease development (Thomas and Smart, 2010).Similar results were also reported by Fredresken*et al.*, (2000). Conditions available for disease development existed as exemplified by the severe infections observed on the sorghum genotypes. The fact that only a quarter of the sorghum genotypes screened had a high level of resistance to covered kernel smut means that further sorghum breeding work should make use of these rich sources of resistance to improve elite sorghum genotypes.

Due to the low rainfall received during the trial management, the rate of growth of the sorghum genotypes was slow. Wilson (2011) stipulated that due to environmental factor influencing the growth of the sorghum genotypes, the systemic covered kernel smut fungus has the ability to maintain its presence in the meristem easier than in fast growing sorghum genotypes receiving adequate moisture for growth. Therefore the sorghum genotypes shows a higher level of covered kernel smut severity.

5.3 Effect of covered kernel smut disease on the grain yield of the inoculated sorghum genotypes

Covered kernel smut significantly reduced grain yield among the artificially inoculated sorghum genotypes. In the large measure, the cultivating sorghum genotypes responded differently to covered kernel smut susceptibility thus yielding differently. The survey results showed covered kernel smut caused by *Sporisoriumsorghi* to be an important disease and caused heavy reduction in grain yield of sorghum in the surveyed areas (Merkuz*et al.*, 2011)

Sorghum plant have several methods to compensate for damage. Yield compensation in the panicle can compensate for up to 20% of the floret lost (Wilson, 2011). This is achieved by increases in grain number and size. However, this compensation does not occur when the apex florets are affected by the disease. Hamilton *et al.*, (2012) study was undertaken by completely removing the florets, but infected florets continue to draw nutrient and photosynthetic assimilates from the plant to develop. Infected florets also reduce photosynthetic capability of the head and Fisher and Wilson (2011) have reported that approximately 18% of grain yield are derived from the photosynthesis in the head.

Fisher and Wilson (2011) established that 10-12% of the grain yield is derived from carbon assimilated before anthesis. These assimilates are stored in the stem, which acts as a storage vessel for carbohydrates in grain filling. The level of the stem dry matter increases and decreases, as the need for carbohydrate in the panicle is either less or more than that available from photosynthesis in the leaves. Photosynthesis in the leaves can continue under dry conditions up to grain maturity. However, stored capacity can only be utilised when enough water is available to support transpiration to enable assimilates to be transported to the heads (Rattkanta, 2010).

In the Southern agro-ecological zones of Zimbabwe namely III, IV and V, the high yielding sorghum genotypes SV-2, SV-4 and MACIA showed susceptibility to covered kernel smut thus resulting in low yields per household level (CBI, Report 2013). After the trial was conducted SV-1, NL 2015 and ICSR 93024 showed resistance to the disease.

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CHAPTER 6

Conclusion and recommendations

The following conclusions were drawn from the study:

- a) Covered kernel smut does affect all sorghum genotypes. Incidence of the disease was comparatively lower on sorghum genotypes namely SV-1, NL 2015 and ICSR 93024 although some susceptible sorghum genotypes SV-2, SEREDO and ICSR 93034 exhibited high disease incidence levels.
- b) Sources of resistance to covered kernel smut possibly do exist, SV-1, NL 2015 and ICSR 93024 out of the 12 sorghum genotypes showed some level of resistance, however the hypothesis that there are significant differences in covered kernel smut severity level is accepted.
- c) Sorghum genotypes responded differently to covered kernel smut susceptibility thus yielding differently. SV-1, NL 2015 and ICSR 93024 proved to be resistant with high grain yields recorded.

The following recommendations do require address in order to fully understand sorghum covered kernel smut disease in Zimbabwe.

- a) The researcher recommends the Sorghum Breeders to further evaluate all other sorghum genotypes for resistance to covered kernel smut disease to be:
 - replicated across several agro-ecological zones in order to be sensitive to the differences in the complex constituents of the pathogen.
 - done for more than one cropping cycle to confirm out the findings under more disease pressure on the sorghum genotypes.

- used in the breeding programmesin-order to improve susceptible sorghum genotypes to covered kernel smut by incorporating a resistant ability to covered kernel smut infection.
- b) The researcher also recommends farmers in
 - high smut prevalent areas to adopt resistant genotypes namely SV-1, NL 2015 and ICSR 93024 as a long term disease strategy management to covered kernel smut disease.
 - non-hot spot areas of covered kernel smut disease to grow genotypes namely SV-2, SV-4 and MACIA where the pathogen is not problematic.

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APPENDICES

Appendix 1: ANOVA of Covered kernel smut disease incidence for the artificially inoculated sorghum genotypes

Source	D.F	S.S	M.S.	V.R F-Prob	
Replications	2	20.667	10.333	1.70	
Treatments	11	4442.333	403.848	66.30	<.001
Error	22	134.000	6.091		
Total	35 45	97.000			

Coefficient of Variation: 13.1%

Appendix 2: ANOVA of Covered kernel smut disease severity scoring for the artificially

inoculated sorghum genotypes

Source	D.F	S.S	M.S.	V.R	F-Prob
Replications	2	0.66667	0.33333	3.67	
Treatments	11	50.33333	4.57576	50.33	<.001
Error	22	2.0000	0 0.09091		
Total	35	53.0000			

Coefficient of Variation: 12.1%

Source	D.F	S.S	M.S.	V.R F-Prob	
Replications	2	0.01433	0.00717	0.55	
Treatments	11	8.37540	0.76140	58.69	<.001
Error	22	0.28542	0.01297		
Total	35	8.67516			

Appendix 3: ANOVA for the effect of Covered kernel smut disease on the grain yield of the artificially inoculated sorghum genotypes

Coefficient of Variation: 13.5%