Genetic Improvement of Zambian Maize (*Zea mays* L.) Populations for Resistance to Ear Rots and a Survey of Associated Mycotoxins

By

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GENERAL ABSTRACT

Maize ear rots are among the most important impediments to increased maize production in Africa. Besides yield loss, they produce mycotoxins in their host whose contamination has been linked to several human and animal mycoses. The main objectives of the studies reported on in this thesis were (i) to investigate farmer perceptions of maize ear rot disease and prospects for breeding for host plant resistance in Zambia; and (ii) to establish the levels of incidence and extent of maize ear rot infection as well as the level of mycotoxins in the maize crops of smallholder farms in central and southern Zambia; (iii) to appraise the field inoculation techniques and assess them for their suitability for the Zambian environmental conditions, (iv) to determine the combining ability of Zambian maize populations for resistance to ear rot and investigate the genetic basis of this resistance; and (v) to investigate both direct and indirect responses to full-sib selection for ear rot resistance in Zambian maize populations.

A participatory rural appraisal (PRA) was conducted in four communities, involving a total of 90 farmers. Participatory methods were used, such as focused group discussions, group interviews, participant scoring and ranking. Farmers ranked and scored the various constraints affecting their maize production in general and the maize ear rots in particular. Ear rots were ranked as the third most important biotic stress and it was evident that although farmers were aware of the disease, they were not aware of mycotoxins. This was reflected in the way they disposed of rotten maize: either by feeding livestock or eating it in periods of hunger.

The survey of ear rots and mycotoxins was carried out in the Southern and Central Provinces of Zambia. A total of 114 farms were covered in the survey: maize samples were collected and both ear rot fungi and mycotoxins were isolated. *Fusarium* and *Stenocarpella* were the most frequently isolated fungi from smallholder farms. The levels of fumonisins on these farms ranged from 0.05 to 192 ppm, while those of aflatoxins were between 1.5 and 10.6 ppb. In 50% of the farmsteads surveyed, the mycotoxins, i.e. fumonisins and aflatoxins, exceeded the recommended FAO/WHO [1]limits of 2 ppm and 2 ppb, respectively.

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[1] FAO, Food and Agriculture Organisation; WHO, World Health Organisation

Five field inoculation techniques namely, colonised toothpick, leaf whorl placement, ear top placement, spore suspension spray, and silk channel injection, were evaluated over three seasons in a series of experiments. It was found that the leaf whorl placement of inoculums, followed by colonized toothpick method, gave a constant ranking of genotypes across locations and years compared to the other three methods. In addition, the use of a mixture of ear rots as inoculum was as effective as its principal single species constituents.

In the population diallel analysis, five broad-based maize populations were crossed in a diallel and evaluated under artificial ear rot inoculation using an inoculum mixture of three ear rot fungi, *Aspergillus flavus, Fusarium verticilloides* and *Stenocarpella maydis* at four locations in Zambia. The purpose was to estimate general (GCA) and specific combining ability (SCA) and investigate genotype x environment interaction. GCA effects were found not to be significant for disease severity but were significant for grain yield across environments. Populations with a strong GCA effect for disease severity across sites included PRA783244c3, Pop25, MMV600, and ZUCASRc2. Across sites, the F_1 combinations, MMV600 x Pop25, ZUCASRc2 X Pop25, and Pop25 x PRA783244c2 had strong SCA effects for root lodging, ear drooping, husk cover and ear insect damage. In a related diallel analysis of 10 full-sib families derived from these populations, it was observed that resistant x susceptible families and their reciprocal crosses performed better than their resistant parents, suggesting an over dominant expression of resistance. Both maternal and non maternal effects were observed to be influencing resistance to ear rots. There was a preponderance influence of non-additive gene action.

A response to full-sib recurrent selection was conducted in four locations in Central Zambia. Out of the 343 families created in 2005/6 season, 10% were selected from each population and recombined to create five new populations. These, with the original populations, were evaluated in four sites during the 2007/8 season. There was a net reduction in ear rot incidence and rot severity in the new synthetic population. Pop10 had the largest reduction in disease severity. The predicted gain per cycle was -4.1% and realized gain was -2.5% for disease incidence, and 0.19% and 19.4% for grain yield. Genetic variability was maintained though with low heritability estimates. Negative but at times strong association between grain yield and ear rot disease severity was detected suggesting that in general selecting for ear rot resistance would enhance grain yield in the five populations.

Overall the importance of the ear rots and mycotoxins in compromising yield and health of the communities in Zambia, respectively, were confirmed and support the call to

improve maize varieties for resistance to ear rots. The results indicate that the five populations could be enhanced for ear rot resistance through population improvement procedures such reciprocal recurrent selection that exploit both additive and non-additive variation. Selection might be compromised by the large genotype x environment interaction effects, and large reciprocal effects and their interaction with the environments. To enhance repeatability genotypes should be artificially inoculated, by placing the inoculum in the leaf whorl followed by colonized toothpick inoculation, and screened in many environments to identify genotypes with stable resistance to ear rots.

Declaration

I, Mweshi Mukanga, declare that

1. The research reported in this thesis, except where otherwise indicated, and is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

    a. Their words have been re-written but the general information attributed to them has been referenced

    b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed

……..

Mweshi Mukanga

As the candidate's supervisors, we agree to the submission of this thesis:

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Dr. John Derera (Supervisor)

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Prof. Pangirayi Tongoona (Co-Supervisor)

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Prof. Mark D. Laing (Co-Supervisor)

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Dedication

This work is dedicated to the memories of my late father, MUKAYA J. MUKANGA

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GENERAL INTRODUCTION

1. Importance of maize in sub-Saharan Africa

Maize (*Zea mays* L.) is the second most important food crop after cassava on the African continent (De Vries and Toenniessen, 2001) and is the major cereal crop of sub-Saharan Africa. Farmers of this region produce over 28 million tons of maize grain every year, accounting for 35% of the world's maize production. The total maize production in Africa in 2007 was estimated at about 43.4 million tons (FAOSTAT, 2008).

Of this amount, 45% was produced in the SADC sub-region[2]. About 11 million hectares are under maize in Southern Africa, representing 71% of the area planted with cereals. Maize, in particular white maize, is the principal food crop in this region, with its importance equalling that of rice and wheat in Asia (Cutts and Hassan, 2003). The largest producer of the SADC sub-region is South Africa, followed by Tanzania. Zambia is a distant fifth in production (Table 1.1). The importance of maize to sub-Saharan Africa, and to the SADC sub-region in particular, is confirmed by maize consumption statistics. Sixteen countries with the highest maize grain consumption in the world are in sub-Saharan Africa, with their average per capita consumption over 60 kg per annum (FAOSTAT, 2008). The average per capita consumption within Southern Africa of between 100 to 120 kg per annum is about twice that for the rest of sub-Saharan Africa. On average, maize contributes 50% of the calories consumed in Southern Africa (Bänziger and Diallo, 2002).

2. Maize production and consumption in Zambia

Maize has dominated Zambian agriculture since pre-independence (Vickery, 1985). According to Sitkos (2008), the discovery of the copper ore deposits and the subsequent opening of mines in 1930s resulted in a mass migration of people from villages to the urban copper mining towns. The physical characteristics of maize made it the ideal crop to feed this growing industrial population. Its hard outer pericarp enables it to withstand long periods of storage without spoiling; its grain low water content at storage means it contains more calories per kilogram than many other starchy crops; and its small

[2] SADC is a Southern Africa grouping made up of 13 countries, Angola, Botswana, Lesotho, Namibia, Malawi, Mauritius, Madagascar, Mozambique, South Africa, Swaziland, Tanzania, Zambia, and Zimbabwe

kernel size makes it easy to transport large quantities of calories packed in a relatively small space.

The average consumption per capita of maize grain in Zambia has been estimated at 140 kg year^{-1} (Smale and Jayne, 2003). Between 1998 and 2007, the consumption rate was 148kg year^{-1} (Table 1.1). Maize and maize products account for 85% of diets for both urban and rural populations in Zambia. This account for 62% of the calories consumed by the poor, most of whom are women and children. In most SADC countries, maize is consumed mainly as a thick porridge.

Table 1.1 The average maize production (thousand tonnes) and per capita consumption (kg per annum) in selected SADC countries in 1995 - 2007

Country	Maize (Thousand Tonnes)	Per capita consumption (kg p.a.)
Angola	531	35
Malawi	1830	132
Mozambique	1250	57
South Africa	7533	195
Tanzania	2683	73
Zambia	1003	148
Zimbabwe	970	153
SADC	17 369	

Source: FAOSTAT (2008); p.a. = per annum.

Maize covers 35% of the cultivated area of Zambia, followed by pulses (23%), other cereals (5.4%), and oilseed crops (3.2%) (Central Statistics Office, 2003). The total maize production in Zambia is estimated at 1.2 million tonnes per annum (Central Statistics Office, 2003; FAO, 2003). Seventy percent of this is produced by small- and medium-scale farmers, farming between 0.5 to 5 hectares (Howard and Mungoma, 1996). Maize

production accounts for 4% of the Gross Domestic Product of Zambia and employs between 10-25% of its rural population.

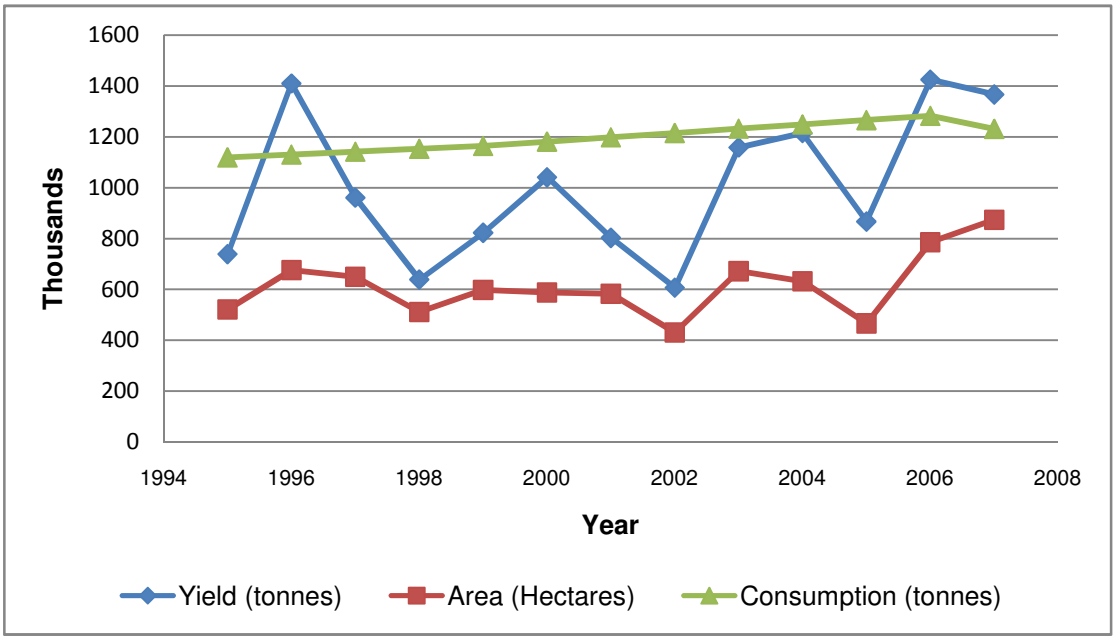

Figure 1. 1 Maize production and consumption trend in Zambia 1995 - 2007 (FAOSTAT, 2008)

Despite the importance of maize to the people of Zambia, the area under maize has largely remained the same during the period 1995 to 2007 (Figure 1.1). The average yield is 1.6 tons per hectare, which is far below the world average of 4.7 tons ha^{-1} (FAOSTAT, 2008). The annual increase in maize production has been very low, averaging 13.4% (Figure 1.1).The highest maize production during that period was in 2006 when the country produced 1.42 million tonnes and the lowest, 0.60 million tonnes was in 2002.

The annual increase in maize production is insufficient to meet the requirements of an annual 3.2% increase in population (Hakkert and Wieringa, 1986). With the population of Zambia estimated at 12.2 m, and increasing, and with internal food distribution problems (Sitkos, 2008) there is widespread food insecurity in Zambia. This situation is made worse by the occurrence of various production constraints on maize, among them diseases such as maize ear rots.

Zambia can thus be described as only a marginally self-sufficient country with irregular maize surpluses (Sitkos, 2008), always under threat of being unable to feed its population.

3. The maize ear rot problem and control measures

a) Ear rot fungi

Fungal species belonging to the genera *Fusarium, Aspergillus, Stenocarpella* and *Penicillium* are globally some of the most common pathogens of maize (Payne, 1999a; CIMMYT, 2004). These fungi are often ranked second to insect pests as the cause of deterioration in, and loss of, maize in tropical regions (Ominski et al., 1994; Olatinwo et al., 1999). They attack maize at all stages of plant growth and in all plant parts, causing poor seed germination, seedling blight, plant wilting, stalk rots and ear rots. Maize ear rot disease (or cob rot) may occur either as a pre-harvest infection or as storage moulds (causing kernel rots) after harvest. Of these fungi, *Stenocarpella* and *Fusarium* spp. have been reported to be the two most common causative agents of maize ear rots (Rheeder et al., 1990; Flett and Wehner, 1991). Grain losses due to these ear rots are usually in the form of reduced grain-fill and weight. Pre-harvest infection can cause significant rotting on as many as 50-75% of the ears in a field under epidemic conditions (Lipps and Mills, 2003).

Maize ear rot is a serious problem in sub-Saharan Africa in general and the SADC region in particular. Reports from surveys conducted in a few sub-Saharan African countries indicate the high prevalence of the *Stenocarpella maydis, S. macrospora, Fusarium graminearum* and *F. moniliforme* in pre-harvest and stored maize *(*MacDonald and Chapman, 1997; Kapindu et al., 1999; Bigriwa et al., 2007). Schjøth et al. (2008) identified *F. verticllioides* and *F. graminearum* as two of the most destructive diseases of hybrid maize in Zambia. Scientists in South Africa have reported a yield reduction of up to 15% (Gevers, 1988). In Zambia, though yield losses due to ear rots have not been quantified, Nawa (2005, unpubl.) reported a 10 - 50% yield loss in central Zambia following a severe epidemic of Fusarium ear rot.

b) Mycotoxin contamination

Even where no significant yield loss has occurred, the ear rot fungi often produce mycotoxins in their hosts, affecting the quality of yield (Bacon and Nelson, 1994; Payne, 1999b; Munkvold, 2003). Worldwide, mycotoxins have been isolated from maize and maize-based food products contaminated naturally with *Fusarium, Aspergillus, Penicillium, Stenocarpella* and other fungi. Of the several mycotoxins currently identified (Marasas, 1995; D'Mello and Macdonald, 1997), fumonisins B1 (FB1), B2 (FB2), and B3 (FB3), and aflatoxins are the most frequently detected in fungal cultures or in naturally

contaminated maize in many countries (Doko et al., 1995). The mycotoxins such as fumonisins and aflatoxins have been linked to livestock diseases, among them leukoencephalomalacia (ELEM) in equines (Sydenham et al., 1993), porcine pulmonary edema and diarrhoea, and reduced body weight in broiler chicks (D'Mello and Macdonald, 1997). Humans are also affected: epidemiological evidence suggests a correlation between the consumption of *F. verticillioides*-contaminated maize and a high incidence of human oesophageal carcinoma (Rheeder et al., 1992).

c) Current control measures

Several control measures have been suggested (Munkvold and Desjardins, 1997; Munkvold, 2003). The general strategy for all of them has been to alter the micro-environment under which maize is grown so that pre-harvest infection by the ear rot fungi is minimised. The methods used include improved tillage practices, fertilisation practices, crop rotation, adjustment in the planting date; improved irrigation to limit drought stress; and correct harvesting times. However, these methods have had little or no success, due to their effectiveness and cost hence the proposal for planting resistant varieties.

Genetic resistance has been proposed by many scientists since the 1950s (Hooker, 1956; Mesterhazy, 1982; Nankam and Pataky, 1996). Unfortunately few, if any, commercial varieties have adequate levels of resistance to be used for such a purpose. Inherent resistance to ear rot fungi has been shown to exist in maize, but its usual polygenic nature and the poor agronomic performance of resistance sources has lead to insufficient exploitation. More recently, the approach has been to use genetically modified maize or transgenic bt-maize hybrids (Bakan et al., 2002; Munkvold, 2003). However, due to the environmental and human health concerns associated with bt-maize, conventional breeding for resistance still remains the preferred option. However, it would be very useful to complement every possible source of resistance to ear rots and mycotoxins whether through transgenes or conventional methods.

The development of pre-harvest host resistance is probably the most effective and economical way of reducing ear rot infection and controlling mycotoxin contamination, especially in smallholder maize production. The majority of resource-poor farmers are not in a position to use other control methods such as improved irrigation, improved fertilizer application methods, and early use of fungicides because of the financial resources required to implement them.

4. Justification for a local breeding programme for resistance to ear rots

Maize ear rots are many and varied. Their occurrence is a complex expression of the interaction of evolutionary origins (old associations), seasonal origins (new associations), climatic suitability, and pathogen epidemic potential on one hand and susceptible host genotypes, pathogen populations and possible alternative hosts on the other. The reported high levels of maize ear rot infections with the occurrence of wet seasons suggest that this disease may be weather dependent in the tropics. Thus the challenge for maize breeding is to identify sources of resistance among adapted materials, and to design and develop varieties that will suffer fewer yield penalties during a favourable wet season when multiple types of maize ear rot occur. The threat posed by toxigenic fungi remains a complex and challenging problem despite years of progressive research worldwide. Identification of multiple resistance to ear rots is important if maize productivity has to be enhanced. To achieve this in a recurrent selection program, each cycle of the population has to be screened for all major ear rots. Superior disease resistant selections would then be crossed with typical variety development parents to produce progeny from which agronomically acceptable disease resistant varieties are selected.

In Zambia, though work on identifying the different maize ear rots has been done (Naik et al., 1982), very little has been done to elucidate the nature and level of resistance against these pathogens and how it could be enhanced (Schjøth et al., 2008). This is evidenced by the sporadic epidemic of ear rots that have been reported in some parts of the country, especially among smallholder farmers, the more recent being in the 2004/5 season (Nawa, 2005; unpubl.). Most of the commercially available hybrids continue to lack appreciable levels of resistance to ear rots and their associated mycotoxins. Currently the National Maize Breeding programme does not emphasise selection for disease and pests due to human resource limitations. Breeders have only been assessing for diseases as a secondary trait. The genetic improvement of both local and exotic populations for ear rot resistance would not only increase the frequency of genes for resistance but yield as well. Studies at the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico have shown that breeding for resistance to ear rots could increase yields by up to 2% (De Léon and Pandey, 1989). Given the low maize yields already reported as well as the occurrence of damaging epidemics, it is thus important for Zambia to develop disease resistance breeding research capacity and to design a programme that incorporates strategies that eliminate or reduce the impact of ear rots on the social and economic welfare of its people.

5. Research objectives and structure of thesis

The specific objectives achieved by this study are reported on in the various chapters of the thesis as follows:

1. To investigate farmer perceptions of maize ear rot disease and prospects for breeding for host plant resistance in Zambia;

2. To determine the incidence and severity of maize ear rots and the level of mycotoxins in central and southern Zambia ;

3. To appraise existing ear rot inoculation techniques that could be used to screen for sources of combined disease resistance against ear rot fungal pathogens;

4. To determine the combining ability of Zambian maize populations for combined resistance to ear rot, and investigate the type of gene action conditioning this resistance, and;

5. To investigate both direct and indirect responses to full-sib selection in Zambian maize populations for ear rot resistance.

This thesis is presented in a composite form, with discrete chapters. For this reason, there may be overlapping of content and references. The composite thesis is the standard format of the African Centre for Crop Improvement, and an accepted format of the Faculty of Science and Agriculture, University of KwaZulu-Natal.

6. Research hypotheses

The hypotheses tested in this study were as follows:

1. Smallholder farmers in Zambia recognise the key maize production constraints and have specific preferences for disease-resistant maize varieties;

2. *Fusarium* and *Stenocarpella* are the most abundant ear rots, and levels of mycotoxins on smallholder farms are high;

3. Existing inoculations techniques are able to consistently reproduce differences in the visual ear rot symptoms among the different classes of maize genotypes;

4. Adequate genetic variation, both additive and non-additive , exists in the Zambian open-pollinated maize populations for resistance to maize ear rots and could be exploited in a local breeding programme for the creation of resistant materials;

5. High levels of resistance to more than one ear rot fungal disease exists in the local maize populations of high yield potential and could be enhanced through recurrent full-sib selection;

6. A significant positive relationship exists between ear rot disease resistance, grain yield potential, and yield in Zambian maize populations.

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CHAPTER 1

A REVIEW OF LITERATURE

1.1 Introduction

Ear rots represent a major biotic constraint to increased maize production in sub-Saharan Africa. Maize production is constantly threatened by potential outbreaks of maize ear rots. Improvement of host plant resistance to these diseases provides the most feasible control option (Brown et al., 1999; Miller, 2001). Very little published information exists on ear rot resistance in the SADC sub-region, except South Africa where studies have produced maize breeding lines with suitable agronomic performance and resistance to maize ear rot complex mainly *Stenocarpella maydis* (McLennan, 1991; Rossouw et al., 2002b). However, many scientific papers have been published worldwide on the methods for assessing disease resistance to ear rots and mycotoxins.

1.2 Maize ear rots

1.2.1 Causal organism, symptoms and epidemiology

A number of anamorphic fungal species are known to invade maize grain before harvest in sub-Saharan Africa (Rheeder et al.,1993; Macdonald and Chapman, 1997; Seifert and Lévesque, 2004) however, the actual number is unknown. Williams and McDonald (1983) listed over fifteen ear and kernel rot fungi, including *Aspergillus flavus* Link ex Fries; *Fusarium verticillioides* Sacc. (Nirenberg) (Syn = *F. moniliforme* Sheld.); *F. graminearum* Schwabe; *Stenocarpella maydis* (Berk.) Sutton; *Nigrospora oryzae* Zimm. (Berk. & Br.) Petch; *Cladosporium sphaerospermum* Penz.; *Penicillium* spp.; *Botrydiplodia theobromae* Pat.; *Helminthosporium maydis* (Nisikado & Miyake); *Colletotrichum graminicola* (Ces.) Wilson, and *Botryosphaeria* spp. Bigirwa et al. (2006) identified more than 17 fungal species belonging to more than six fungal genera infecting ears and kernels in Uganda. Prominent among them were *F. verticillioides*, *A. flavus* spp., *F. graminearum,* and *S. maydis*. Naik et al. (1982) reported that *F. verticillioides, F. graminearum, S. macrospora, Cephalopsporium spp., Nigrospora spp.,* and *Helminthosporium spp.* were the most common maize ear rot fungi on Zambian maize. Schjøth (2002), isolated *F. subglutinans*, *F. nygamai*, *F. anthophilium, F. semitectum, F. proliferatum, F. compactum,* and *F. equiseti* from visibly diseased kernels of Zambian maize hybrids in addition to the *F. Verticillioides* and the *F. Graminearum species*. The

number of ear rot pathogens associated with maize may actually be higher than reported, especially in a tropical environment such as Zambia, because of seasonal and site variation in the ear rot disease spectrum and its occurrence with new associations. However, it is important to note that in these two reports, *Fusarium verticillioides* (Sacc.) Nirenberg was identified as the most frequently isolated fungi. A further point to note is that most of the fungi reported on have been isolated from the seed of a few commercial hybrids and none from open-pollinated maize populations.

Several writers have provided detailed descriptions of the symptoms associated with ear rot fungi (Marasas et al., 1984; Leslie et al. 1992; Payne, 1999a). *Fusarium verticillioides* infection is characterized by purplish-pink, cottony mycelium growth on a few scattered kernels, limited to certain parts of the ear. Infected kernels are salmon pink, lavender to reddish and often displaying white streaking ('starburst') on the pericarp, and also occasionally germinate whilst on the cob. *Fusarium graminearum* produces a pink-to reddish coloured mould (Reid et al., 2002). The mould growth of *F. subglutinans* is similar to *F. graminearum,* but with a slightly more orange than pink colouration (Payne, 1999a). Other common ear rots include Aspergillus ear rot, that is characterized by a yellow-green mould growth between grains and *S. maydis* (syn = *Diplodia maydis)* which includes light brown, bleached husks and light weight shrunken cobs with rotting starting from the base. The main symptoms of *Penicillium* rot are a bluish-green mould growth on and between grains, usually at the ear tip. The minor ear rots include *Cephalosporium acremonium, Nigrospora oryzae, Dreschslera maydis*, *F. semitectum and Curvularia lunata*. Multiple infections are common. Logrieco et al. (2002) reported that it is possible to isolate as many as nine ear rot fungi from one kernel. In some instances, the plant and the fungus can coexist without obvious disease symptoms for extended periods of time (Munkvold and Desjardins, 1997).

Three factors are believed to determine the occurrence of an ear rot epidemic: the presence of airborne or insect-borne spore inoculum at the correct time, appropriate moisture, and appropriate temperature (Miller, 2001). While two or more factors may be common to a number of ear rot fungal species, even within genera, there are some species specific requirements. For example, *F. graminearum* requires a period of warm temperatures with persistent wetness during silking and early kernel development, while *F. verticillioides* occurs during higher temperatures and drier conditions. Wet weather in combination with mild temperatures from late whorl development through early ear development favour Stenocarpella ear rot development. Aspergillus ear rot is favoured more by elevated temperatures, prolonged drought conditions (water stress) and insect

damage compared to most ear rot fungi (Wiatrak et al., 2005). The lack of similarity in the macro- and micro-environments of the ear rot species probably explains why cultural control measures are not highly successful. In addition, this diversity in species ecological niche makes these fungi among the most aggressive and widespread pathogens. The same fungal species may have several hosts, e.g. *F. moniliforme* have been recovered from maize, sorghum, rice, and field soil (Frederiksen, 1986).

1.2.2 Losses due to ear rots and mycotoxin contamination

Though actual losses have not been quantified in Zambia, the levels of ear rot damage are quite high. Vigier et al. (1997) reported that in periods of epidemics and environmental conditions favourable to ear rot in North America, losses of up to 48% can occur. Yield losses of up to 10% and 18% have been reported in Malawi (Kapindu et al., 1999) and Kenya (Ajanga and Hillocks, 2000), respectively. While Bigirwa et al. (2007) reported losses of up to 30% in Uganda. Generally, occurrence of ear rots can result in significant economic losses to the farmers, worldwide who may have to receive market discounts for contaminated maize produce or have to dispose of heavily infected maize. During the 1980s, the Stenocarpella epidemic was estimated to have cost the South African government almost USD 400 million (Rossouw et al., 2002a).

In addition to yield loss, these ear rot fungi produce mycotoxins that can harm animals and humans consuming mycotoxin-contaminated grain. The natural occurrence of kernel and ear rot infection with mycotoxins is a widespread phenomenon in most sub-Saharan Africa. The level of mycotoxin contamination of maize in Africa has been investigated by a number of scientists (Doko et al., 1995; Kedera et al., 1999; Bankole and Adebanjo, 2003; Fandohan et al. 2005). Kedera et al. (1994) found that high levels of mycotoxins were associated with maize lots with the high ear rot infection or visibly diseased grain. Shelby et al. (1994) found that conditions such as high humidity, hot weather, and drought at, or just before, flowering promoted high levels of ear rots and mycotoxins of maize. The detection of mycotoxins in symptomless grain has further compounded the problem (Munkvold, 2003) as farmers may unwittingly ingest grain and grain products that are heavily contaminated and feed it to their animals.

1.2.3 Effect of mycotoxins on humans and livestock

Between 20 000 and 300 000 mycotoxins have been identified (CAST[3], 2003); of these less than 15 have been associated with human and animal health. They include aflatoxins produced by various *Aspergillus* spp.; Deoxynivalenol (DON or vomitoxin); Fumonisins; and Zearlenone produced by *Fusarium* spp.; Ochratoxin A produced by both *Aspergillus* and *Penicillium* spp.; and diploidiatoxin and diploidiol produced by *Stenocarpella spp.*

The nature of mycotoxicosis in humans and animals is rather well established and includes a wide variety of toxic effects (carcinogenic, immunosuppressive, etc.) (CAST, 2003; Richard, 2007). According to Ajanga and Hillocks (2000), consumption of 2% rotten kernels is enough to cause harmful effects to humans. Yet there is widespread use of rotten maize for brewing beer and, in periods of hunger, it is milled into flour destined for human consumption. Rheeder et al. (1992) reported that human consumption of fumonisin contaminated maize grain may cause oesophageal cancer. Missmer et al. (2006) linked fumonsins to neural tube birth defects in humans. In livestock, Ross et al. (1992) reported that the ingestion of fumonsin contaminated maize in horses has led to equine leukoencephalomalacia.

As a result of these concerns and others, the United States' Food and Drug Agency (FDA) published *Guidelines for Industry* that set regulatory limits for fumonisins and aflatoxins between 2 to 4 ppm and 2ppb, respectively, for maize and maize products intended for human consumption (CFSAN, 2001). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended a Provisional Maximum Tolerable Daily Intake (PMTDI) for fumonisins of 2 µg/kg body weight per day (FAO, 2004); however, this is readily exceeded by individuals on a maize-based diet as is the case in SADC countries. The tolerable limit for fumonsins by livestock varies, depending on whether the animal is a ruminant or mono-gastric poultry destined for slaughter or breeding stock. The absence of regulatory measures in most sub-Saharan Africa countries including Zambia implies that the people in these countries are exposed to high levels of mycotoxins. Therefore, maize breeders have a task of developing maize varieties that are resistant to mycotoxin accumulation in order to lessen the exposure to mycotoxins and consequently improve the standard of living of people. In addition, there

[3] Council of Agricultural Science and Technology: American grouping of eminent experts.

is a need to create awareness on the dangers of consuming rotten maize. Continuous surveillance of maize grains for contamination by selected mycotoxins i.e. fumonisins, aflatoxins, zearalenone etc. and the monitoring of both human and livestock populations for diseases attributable to mycotoxins have to be carried out to ensure a supply of maize based food products free of mycotoxins.

1.3 Significance of maize ear rot in the small-scale farming sector and farmer management of ear rot and mycotoxin problems

In sub-Saharan Africa, farmers have recognised maize ear rots as a serious problem (Ajanga and Hillocks, 2000; Bigirwa et al., 2007). Payne (1999a) outlined some of the control measures that could be used by farmers to minimise ear rot infection. They included early harvest to avoid late season rains, planting adapted varieties, managing nutrient inputs, and optimizing planting dates. Use of fungicides is only suggested where systemic infection is suspected. Some of these control measures do not completely eliminate ear rot infection and mycotoxin accumulation either in the field or storage but minimise them only. Avantaggio et al. (2002) reported high fumonisin contamination levels in insect damaged maize ears and recommended control of insects as one option for reducing fumonisin contamination. Proper drying, hand sorting to separate rotten grain from good healthy grain, and burning rotten grain could also be used by farmers to minimise ear rot infection. However, most farmers are unable to implement most of the cultural control measures due to lack of financial resources and labour constraints. Many commercial maize varieties are affected by these mycotoxins mainly because the natural co-occurrence of ear rots and mycotoxins is common. Therefore breeding for a single ear rot may not necessarily confer resistance to other ear rots. It is therefore critical the research is initiated that would help small-scale farmers to meet internationally accepted maize quality standards.

1.4. Genetics of maize resistance to ear rot

Breeding for resistance has many hurdles to surmount. The breeder, in a bid to develop a farmer desired variety, must employ strategies that would combine sufficient resistance to ear rot with other traits equally important to farmers in a satisfactory manner. Information on the nature and the magnitude of genetic variability present in the available genetic material is thus important for the initiation of any effective selection programme. Genetic variation has been reported to exist for resistance to ear rots among both tropical and

temperate maize inbred lines and hybrids (Naidoo et al., 2002). Significant progress has been made in North America and Europe in understanding the genetics of resistance to maize ear rots (Munkvold, 2003). However, the amount of resistance realised has been limited due to complicated genetics and/or linkage to undesirable agronomic traits (Duvick, 2001) such as low yields, small hard kernels, and small stout ears with long husks. Resistance to ear rots has been reported to be quantitative and largely additive (Reid et al., 1992; Olatinwo et al., 1999; Naidoo et al., 2002; Brooks et al., 2005). The types and magnitudes of gene action and inheritance of resistance in a cross between resistant and susceptible maize genotypes have been studied by Walker and White (2001). They found that resistance to Aspergillus ear rot was mainly controlled by epistasis and additive gene action; they also found that heritabilities for the reduced aflatoxin production were higher in the F_3 generation than in the back-cross parent (BCP1)-selfed generation. Other studies have reported dominant gene action (McLennan, 1991; Dorrance et al., 1998; Maupin et al., 2003).

There are contradictory reports on the number of genes associated with resistance to ear rots. Boling and Grogan (1965) suggested that one dominant gene is involved in resistance to *Fusarium* ear and kernel rots while Nankam and Pataky (1996) suggested 3 to 12 minor gene pairs. Reid et al. (1994) provided some evidence of a single dominant gene, *fqs1,* which accounted for silk resistance to *F. graminearum* ear rot. Chromosomal regions that account for resistance to Aspergillus and Fusarium ear rots have been identified as well (Perez-Brito et al., 2001; Paul et al., 2003; Robertson-Hoyt et al., 2006).

Other scientists have argued that inheritance of resistance to Stenocarpella ear rot and other diseases by *Stenocarpella* fungal species such as Diplodia leaf spot and Diplodia stalk rot are probably independent of each other (Hooker, 1956; Thompson et al., 1971). Resistance to Fusarium ear rot has been reported to be polygenic with relatively low heritability.

Robertson-Hoyt et al. (2006) reported heritability values of low to moderate in two well known North American Populations, GEFR and NCB. There is no evidence for complete resistance to Aspergillus ear rot, Fusarium ear rot, and Stenocarpella ear rot, or for cross resistance to two or more ear rots. Although the reported heritability values are low for Aspergillus and Fusarium ear rots, in principle, progress to selection would still be made since the gene effect is largely additive.

1.5 Diallel analyses and combining abilities

According to Hayman (1954), a “diallel cross” is a set of all possible matings between several genotypes. These genotypes could be individuals, homozygous lines such as inbreds, or heterozygotes such as populations. Diallel mating designs permit the estimation of the magnitude of additive and non-additive components of heritable variance (Griffing, 1956; Mather and Jinks, 1982). Although data obtained from such cross combinations can be analysed in several ways, the most common analyses use the Hayman (1954) and Griffing (1956) models and most recent Gardner and Eberhart (1966) analysis (Murray et al., 2003). On this basis, a test for the validity of the additive dominance model has been suggested. Hayman (1960) and Mather and Jinks (1977) demonstrated that it was possible to obtain estimates of the additive and dominance component of the heritable genetic variation from the mean squares of these mating designs. Sprague and Tatum (1942) coined the term “general combining ability” (GCA) to define the average performance of a line in hybrid combinations, while specific combining ability (SCA) designated those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved.

Griffing (1956) proposed a general procedure for diallel analysis which makes provision for non-allelic interaction. According to this approach, the mean measurement of a cross is partitioned into four major components: GCA and SCA effects, the general mean (μ) and environmental variance: Falconer and Mackay (1996) defined GCA as the mean performance of the line in all crosses when expressed as a deviation from the mean of all crosses. The GCA consists of additive and additive epistatic variances. According to Sprague and Tatum (1942), SCA is the deviation to a greater or lesser extent from the sum of the GCA of the two parents involved in the cross. Specific combining ability effects consists of dominant genetic variation and all types of epistatic variances, thence regarded as an estimate of the effects of non-additive gene actions (Falconer and Mackay, 1996). Both GCA and SCA effects help locate parents and crosses that are responsible for bringing about a particular type of gene action (Baker, 1978). The GCA and SCA effects and variances have been used extensively in selecting parents for the construction of synthetics, the selection of suitable F_1s for a multiple crossing or composite breeding programme, and the possibility of employing an appropriate selection technique like recurrent selection and reciprocal selection (Dabholkar, 1992). Differences in GCA have been attributed to additive, additive x additive, and higher order interactions

of additive genetic effects in the base population, while differences in SCA have been attributed to non additive genetic variance (Baker, 1978).

Scientists have largely used the diallel mating scheme to estimate the potential value of genotypes *per se*, their combining ability, and heterotic effects for resistance to ear rots from a fixed or randomly chosen set of parental lines (Dorrance et al., 1998; Rossouw et al., 2002b; Naidoo et al., 2002). The diallel genetic design and its various modifications have been used to investigate the potential of maize inbred lines as sources of ear rot resistance and their potential for use in breeding programmes. Very few reports exist on genetically broad–based varieties or populations (Das et al., 1984; Olatinwo et al., 1999). Of the two main groups of the diallel analyses available for genetic analysis of resistance to ear rots in genetically broad-based populations, Gardner and Eberhart (1996) analysis II and III, unlike Griffing's methods are the more reliable. In general, there have been complications in the processing and interpretation of Gardner and Eberhart (1996) analysis II and III probably due to the hypotheses tested and the bulky calculations for the general and specific combining abilities, heterotic and varietal effects (Murray et al., 2003). More recently, computer programs have been developed for both Griffing's (1956) diallel analyses and Gardner and Eberhart's (1966) analyses (Zhang and Kang,1997; Zhang et al., 2005) that make the calculation of GCA and SCA much quicker and more accurate.

Both SCA and GCA have been reported to be significant in conditioning resistance to ear rots. Das et al. (1984) using open-pollinated maize varieties in a diallel cross found specific combining ability to be more important than general combining ability. Furthermore, McLennan (1991) reported that a South African public line, D940Y, exhibited high SCA for resistance to Stenocarpella ear rot. Using an eight-parent inbred line diallel cross, Dorrance et al. (1998) reported significant GCA effects for resistance to Diplodia ear rot for two years, with SCA effects being important in one of the two years. Rossouw et al. (2002b), in their work on 10-parent diallel crosses involving five South African, four USA and one Brazilian inbred lines of proven resistance, found that GCA effects were more important for inheritance of resistance to Stenocarpella ear rot. The GCA sum of squares accounted for 96.2% of total genotypic sums of squares. They also reported significant GCA and SCA effects for husk cover and ear declination. In both studies, the reciprocal effects were very small or insignificant. Naidoo et al. (2002) studied the genetics of resistance to aflatoxin and reported significant GCA effects and not SCA effects for ear rot rating and aflatoxin concentration; however, there were significant GCA x environment and SCA x environment interactions for aflatoxin

accumulation. After evaluating for aflatoxin accumulation in white and yellow maize inbreds, Betrán et al. (2002) reported significant differences among inbred GCA effects, among hybrid means, and the SCA effects for both white and yellow maize at two of the three locations used. GCA x environment and SCA x environment interaction effects were also significant.

Several researchers have tried to interpret the implications of GCA x environment or SCA by environment interaction effects (Rossouw et al. 2002b; Williams et al., 2008) and importance of reciprocal effects (Widstrom, 1972). While resistance to ear rots may largely be controlled by additive gene effects, the presence of non-additive gene effects in some resistant sources suggests that these breeding lines could serve as donor parents in hybrid oriented programmes. The occurrence of significant GCA x environment interaction effects in some experiments may indicate that it was possible to develop hybrids for a specific environment, while the SCA x environment interaction effects may enforce this in that some crosses are inclined to perform better only in highly specific certain environments. According to Widstrom (1972), while reciprocal effects are often ignored in most genetic studies, they need to be considered as potential sources of interference in the identification of sources of resistance because of their inconsistent interaction with the environment. Reciprocal effects are usually attributed to both maternal and non-maternal effects.

1.6 Recurrent selection for improvement of resistance to ear rot

Recurrent selection schemes are widely used in maize improvement. Since Hull (1945) first proposed the term “recurrent selection”, it has become the dominant selection method for the improvement of maize breeding populations for yield and other characters (Hallauer, 1985). It is a process of repeated cycles of selections that is used to increase the frequency of favourable alleles and the mean performance (Doersken et al., 2003). Several recurrent selection methods have been used for selecting for disease resistance breeding (Hallauer, 1992). Hallauer and Miranda (1988) have provided an adequate description of the two types of selection used in maize breeding. The two broad groupings are phenotypic evaluation and genotypic evaluation. The former group is based on performance of a group of varieties without determining the breeding value, while the latter involves progeny testing and therefore providing information on the breeding value. Genotypic evaluation has been very successful in identifying sources of resistance in both intrapopulation and interpopulation recurrent selection methods (De

Léon and Pandey, 1989). The intrapopulation methods include mass selection, full-sib, half sib, S1, and S2 selections, while the interpopulation recurrent selection methods involve progressive improvement of two populations from two diverse germplasm pools used reciprocally as testers, and the direct effects of selection estimated in the interpopulation cross.

1.6.1 Progress made using recurrent selection for ear rot resistance

Although recurrent selection methods have been reported to be effective in developing ear and stalk rot resistant maize breeding lines (Lal and Singh, 1984; McLennan, 1991), very few reports are available on the predicted responses to selection for disease resistance (Lambert and White, 1997; Abedon and Tracy, 1998). The rate of progress in developing resistance to ear rots could be influenced by the base level of resistance of the germplasm and intensity of selection (Nowell, 1998). At CIMMYT-Mexico, De Léon and Pandey (1989) used a modified ear to row (MER) selection scheme and reported a reduction in *F. verticillioides* ear rot infection of 1.46% to 1.52% per cycle, accompanied by gains in yield per cycle of about 1.38% when selections were made among half-sib family lines. Ramirez-Diaz et al. (2000) reported a 0.97% reduction in ear rot infection per cycle in the subtropical maize population PABGT-CE using full-sib families. In sub-Saharan Africa, full-sib recurrent selection has been used mostly for yield improvement and rarely for disease resistance (Sallah et al., 1998).

1.7 Non-conventional breeding strategies

Two main approaches are being employed in non-conventional breeding strategies. These includes molecular studies focusing on the identification of the chromosomal region associated with resistance to ear rots (Brown et al., 1999); and genetically engineering plants for resistance to either ear rot infection, or mycotoxin accumulation, or both (Munkvold, 2003). In the USA, chromosome regions associated with resistance to *A. flavus* and the inhibition of aflatoxin production in maize have been identified using restriction fragment length polymorphism (RFLP) analysis of three resistant lines (R001, LB31, and Tex6) (Paul et al., 2003). This has provided the basis for employing a successful strategy of pyramiding different types of resistance into commercially viable germplasm while avoiding the introduction of undesirable traits. Genetic engineering methods have been employed to develop bt-maize that can minimize not only maize stalk damage but ear rot infection and mycotoxin accumulation (Munkvold, 2003). Maize stemborer has been closely associated with *Aspergillus* and *Fusarium* ear rot infections. Munkvold et al. (1999) found in their study that the transgenic maize with Cry1Ab

expression had lower stemborer infestation and less *Fusarium* ear infection than their non-transgenic counterparts. Breeders in sub-Saharan Africa must take advantage of the genetic information from molecular studies and genes derived from various sources for developing resistant materials. However, in sub-Saharan Africa countries, lack of infrastructure and the socio-political concerns surrounding the use of molecular technology and genetic manipulation make the use of these methods unfeasible for most programmes.

1.8 Selection strategy for resistance to ear rots

1.8.1 Identification of sources of resistance

Several well-characterised sources of resistance to *Fusarium*, *Aspergillus* and *Stenocarpella* ear rots have been identified. However, most of these sources have poor genetic backgrounds (Munkvold, 2003). Clements et al. (2004) screened more than 1500 top crosses of potential sources of resistance to fumonisin accumulation in grain and to *Fusarium* ear and kernel rot and found significant genetic variation for both *Fusarium* ear rot and fumonisin concentration but none highly resistant. Windham and Williams (2002) found that less than 25% of the maize inbred lines and advanced breeding lines evaluated for aflatoxin accumulation resistance supported low levels of aflatoxins across seasons and location. Walker and White (2001) reported that previously used sources of resistance to *Aspergillus* ear rot and aflatoxin production were no longer acceptable sources due to lower heritabilities, disease, and lack of stability in multiple environments. Naidoo et al. (2002) evaluated eight North American inbreds associated with reduced ear rots and aflatoxin production and their F1-hybrids, and found high levels of resistance in resistant inbred x resistant inbred F1 hybrids.

In sub-Saharan Africa, excluding South Africa, very few breeding lines have been identified as sources of resistance (Brown et al., 2001; Fandohan et al., 2003), although tropical maize populations have been suggested as potential sources of resistance to ear rots (De Léon and Pandey, 1989). Most of the resistance studies, worldwide have been conducted on species specific resistance (Reid and Hamilton, 1996; Naidoo et al., 2002, Schjøth et al, 2008). Very little work has been done on developing resistance to multiple ear rot pathogens (or non-specific resistance) (Zummo and Scott. 1992; Clements et al., 2003; Abbas et al., 2006).

1.8.2 Important characteristics for ear rot resistance

Several studies have shown that resistance to ear rot can be developed through selection (Windham and Williams, 1998; Munkvold, 2003). The most common approach is to select for reduced ear rot infection components (Hoenish and Davis, 1994). According to Dent (1991), disease resistance does not necessarily have to be incorporated in homogenous elite material as a specific genetic addition, but rather selected for in unison with other favourable characteristics from a genetically broad-based source population. It is important that maize varieties with adequate ear rot resistance are stable and show the agronomic benefits of this resistance under field conditions (Munkvold and Desjardins, 1997). According to Kruger (1989), two main forms of resistance are suspected of being involved in the ear rot complex. These are static resistance, which involves morphological characters of the maize ear which prevent infection, and dynamic resistance, which represents the chemical/genetic defenses of the plant. These two forms have been used by both breeders and pathologists to screen varieties that are resistant. Successful breeding for maize ear rots depends on the identification and selection for traits that confer both resistances. Studies at CIMMYT (De Léon and Pandey, 1989) and elsewhere (Rossouw et al., 2002a) have shown that selecting for specific traits that are stable across environments and highly heritable has resulted in successful introgression of resistant materials in populations undergoing improvements. The selection for the enhanced expression of beneficial secondary traits such as husk cover, kernel endosperm, kernel pericarp, and ear declination has facilitated the identification of suitable sources of resistant (Bétran et al., 2002; Rossouw et al., 2002a).

There are several traits of importance that have been documented that a breeder may select for both directly and indirectly when improving a maize crop for resistance to ear rot. These are discussed below.

Grain yield: The overall objective for breeding for ear rot resistance is to produce the highest level of sustainable resistance that is compatible with optimizing crop yield and quality. Hence breeding for ear rot disease resistance may attract yield penalties (Brown, 2002). These may arise due to the negative effects associated with deployment of genes for resistance. Disease resistance is only quantifiable for the farmer if the crop being bred for resistance is also high yielding. However, disease resistance may be closely linked with undesirable characteristics such as increased susceptibility to other pathogens, inferior quality of the grain, and lower yielding capacity (Tarr, 1972). Therefore, the challenge for the breeder is to ensure that correlated response to selection, i.e. of two or

more traits that may have opposing effects on the yield, does not compromise the breeding strategies employed to achieve optimal response to selection for both ear rot resistance and improved maize quality.

Husk cover: Improvement in the ear husk cover has been used by several scientists improving maize inbred lines and their hybrids for resistance to ear rots. Rossouw et al. (2002a) reported a significant correlation between husk cover and ear rot infection and between ear declination and ear rot development. Maize genotypes with good husk cover extension and downward drooping had less ear rot infection. This led to Rossouw et al. (2002a) to argue that breeding for good husk cover could contribute to improved ear rot resistance.

Ear declination: Genotypes with increased ear declination, or drooped ears, have been reported to be less susceptible to ear rots (Rossouw et al., 2002a).

Endosperm traits: King and Scott (1981) observed that the site of action of resistance to kernel infection was the pericarp. Russin et al. (1997) reported that maize kernels from aflatoxin resistant GT-MAS: gk maize had more pericarp wax and rough surface than the susceptible hybrids.

Ear insect damage: Several insects have been implicated in the creation of wounds on either the maize stem or ear that act as entry points for ear rot fungi (Munkvold and Desjardins, 1997). Some of the insect pests associated with maize ear rots include maize stemborers, maize weevils, and other grain borers (Cardwell et al., 2000). Ako et al. (2003) reported that ear rot infected maize ears had higher insect damage than normal maize.

Root and stalk lodging: Several ear rot fungi are also responsible for stalk rot. A positive correlation appears to exist between root and stalk lodging and ear rot. Bottalico et al. (1985) isolated several species of *Fusarium* from stalk lodged maize.

Earliness: Though no documented evidence exists that associates increased ear rot fungal activity with late rains, Miller (2001) has linked late rains with increased ear rot infection. Earliness could thus be an important trait.

Biochemical traits: Besides morphological factors, there are chemical/genetic factors for ear rot resistance according to several publications. Assabgui et al. (1993) reported that high kernel concentrations of (E)-ferulic acid and other phenolic compounds at low levels had low ear rot infection. Reid et al. (1992) found the increased presence of phenolic

compounds, flavones, in the silk of resistant inbreds when compared to susceptible hybrids. The relationship of biochemical compounds in maize and resistance to aflatoxin accumulation was also explored by Tubajika and Damann (2001) in seven temperate maize varieties, using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). They found that resistant varieties had high amounts of a 14 KDa trypsin inhibitor and high amounts of pericarp wax that inhibited the penetration and severity of *Aspergillus* compared to the susceptible varieties. Guo et al. (1998) detected specific proteins (22 to 56 KDa in molecular mass) in higher concentration in aflatoxin field resistant maize genotypes compared to susceptible ones. According to Zeringue (2000), aflatoxin field-resistant maize genotypes possess a high concentration of antifungal aldehyde, furfural (2-furancarbo-aldehyde), compared to the susceptible genotypes. The presence of furfural in maize genotypes enhances disease resistance to *Aspergillus* ear rots.

The role of maternally inherited tissues such as pericarp and endosperm in conferring ear rot resistance has been reported (Headrick and Pataky, 1991; Bluhm and Woloshuk, 2005). The degree of silk senescence has been also reported to affect the rate of fungal colonisation of kernels (Headrick and Pataky,1990). Maternal influence on resistance to ear rots may be important if the characteristics of silks are maintained in the progeny or hybrid combination, and are not affected by the genotype of the endosperm. Bluhm and Woloshuk (2005) evaluated the role of kernel endosperm in regulating the fumonisin biosynthesis and observed that kernels lacking starch due to physiological immaturity accumulate less fumonisins.

In addition, enhanced expression of morphological and bio-chemical factors through recurrent selection would result in the accumulation of favourable alleles for resistance, and in the development of improved elite lines. Whilst phenotypic traits may be easily discernible in the field, bio-chemical related traits may require the development of suitable scales for morphological traits that may indirectly select for biochemical traits.

1.8.3 Selecting under artificial vs. natural conditions

Studies have shown that to make regular progress in selecting for ear rot resistance, artificial inoculation is indispensable (Simmonds, 1979; Clements et al., 2003; Kleinschmidt et al., 2005). An efficient inoculation technique must be developed to differentiate genotypes identified as resistant or susceptible under natural conditions. An artificial inoculation increases disease severity and decreases variability within and among genotypes (Clements et al., 2003). Schjøth et al. (2008) evaluated 20 Zambian

hybrids for resistance to *F. verticillioides* ear rot and reported that artificial inoculation provided a good estimate of ear rot resistance based on visual symptoms in a year of moderate disease pressure, but not in a year of high disease pressure. The high level of resistance identified from artificially inoculated trials would be the most likely to be resistant under farmer conditions. These materials enable the breeder to develop materials that are useful to the farming community during the normal growing seasons when disease development is favoured.

Brown et al. (1999) listed the labour-intensive, high cost preparation of inoculums and their inconsistency across environments as some of the challenges for screening for ear rot resistance. Ledenčan et al. (2003) suggested that tests with artificial infection should be followed by screening under natural conditions. The simulation of a maize ear rot epidemic in artificial experiments is provoked by administrating infectious material (fungal spore, mycelia and infested plant material) at the appropriate time and under appropriate environmental conditions.. Although artificial inoculation methods provide more uniformity in the evaluation process, and allow for the elimination of very susceptible genotypes from the breeding programme (Lal and Singh, 1984; Widstrom et al., 1984; Singh, 1986), these methods may not always result in symptoms typical of natural infection. However, artificial infection can generate large genetic variation, which is useful for selection (Ledenčan et al., 2003). The use of artificial inoculation does not exclude the use of locations with high levels of natural infection often referred to as “hotspots”.

Worldwide, two groups of artificial inoculation techniques are used (Silva et al., 2007). The Introduction of inoculum into the ears through the husks, or into the silk channel. However modifications of these two main methods have been used successfully and include the following methods: pinbar (Campbell and White, 1994); colonised toothpick (Reid et al., 1992; Silva et al., 2007); silk-channel (Reid et al., 1996); kernel stab (Chungu et al., 1996); leaf whorl placement (Bensch, 1995; Nowell, 1998; Rossouw et al., 2002a) and spore suspension (Headrick and Pataky, 1991).

Comparison studies of some of the inoculation techniques have been carried out (Reid et al., 1996). Chungu et al. (1996) compared six inoculation techniques and found silk channel and kernel stab techniques to be the most effective in measuring ear and kernel rot resistance. Clements et al. (2003) reported that injecting through the husks was more effective than the suspension spray and colonised toothpick methods in discriminated maize hybrids and maintained the ranking order over years. Silva et al. (2007) working in the Andeans region of South America, reported that the use of hypodermic needle was

not as effective as the colonised toothpick and stainless steel methods at those high altitudes.

Inoculation time and the point of entry of inoculation are critical for effective artificial inoculation. Using four maize hybrids, two resistant and two susceptible, Reid and Hamilton (1996) investigated the effects of time of inoculation on the resistance of maize to *F. graminearum* infection through the kernels. They found that disease severity decreased with kernel/silk age and recommended 15 days post silk emergence as the best time to conduct the inoculation. Reid et al. (2002) found that the highest disease severities for three *Fusarium* species (*F. verticillioides, F. graminearum,* and *F. Subglutinans*) occurred when the ears were inoculated in the early stages of silk development with the peak susceptibility at 50% silking date, and when the kernels were inoculated when immature or at milk stage. Clements et al. (2003) found that techniques that used non-wounding methods were most effective when applied within the first two weeks following pollination, while those that damage plant tissues were less dependent upon the time of inoculation for their success and resulted in severe infection. They also reported that techniques that apply inoculum to kernels or silks are more effective than techniques that emphasized systemic movement of ear rot fungi from other plant tissues to kernels. Several studies have found different optimum times for different pathogens or the same pathogens on different varieties. Naidoo et al. (2002) observed that 20 to 24 days after mid-silk was effective for the inoculation of *A. flavus*; Clements et al. (2003) and Silva et al. (2007) reported 14 days after mid-silk as being effective for both *F. verticillioides* and *F. subgluitinans;* while Balconni et al. (2004) indicated three days after pollination as being effective.

The reactions of maize varieties to mixtures of isolates of the same ear rot, or a mixture of pathogen, are less well documented and conflicting reports exist. One of the difficulties in breeding resistance to multiple ear rot fungal species into maize is the lack of knowledge of how these fungi interact when present in the maize ears and how damage is associated with each of ear rot fungus. Reid et al. (2002) cautioned against the use of a “cocktail”, or mixture of fungal species, when screening maize genotypes for resistance to multiple ear rots due to the possibility of interspecies competition. They reported that *F. verticillioides* tends to suppress the activity of *Aspergillus flavus* and *F. graminearum*. However, Robertson-Hoyt et al. (2006) reported a positive relationship between Aspergillus and Fusarium ear rot and reported that it may be possible to breed for resistance to both using only one, hence accelerating for resistance. In reality, no histopathology studies have shown the amount of damage associated with either when

present in the same ear. Hence indirectly breeding for resistance to one species would result in the breakdown of resistance when the crop is infected by new strains or different species of ear rot fungi.

Reid et al. (1999) investigated the interaction between two major ear-rot pathogens within the same fungal genera, that is, *F. graminearum* and *F. verticillioides,* in the same maize ear, and found that the mixture of these two fungi performed better than the weaker pathogen in the mixture when used alone or as good as the dominant pathogen in the mixture when used alone. Ledenčan et al. (2003) reported that a mixture of three *Fusarium* spp. (*F. graminearum*, *F. moniliforme* and *F. subglutinans*) was effective in discriminating resistance to stalk rot among more than 30 maize inbreds and their hybrids.

In contrast, Stewart et al. (2002), when developing a conceptual model of *Fusarium* growth in maize ears under field conditions, found that percent infection was lower with a *F. graminearum* and *F. verticillioides* mixture than when *F. graminearum* was used separately. This suggested a possible interference by *F. verticillioides*. This agrees with the observation by Zummo and Scott (1992) and Rheeder et al. (1990) that *F. verticillioides* may suppress the growth of other *Fusarium* species. *F. verticillioides* has been documented to exhibit a negative association with *F. subglutinans* and *F. graminearum* (Reid et al., 2002). This implies that while progress to selection would be accelerated through the use of ear rot fungal cocktails in the early stages of breeding, it must be followed by subjecting elite material to individual species of pathogens in order to be certain of resistance.

1.8.4 Ear rot rating schemes

One of the prerequisites of an efficient resistance screening system is the development of an effective and consistent disease assessment scheme. Stewart et al. (2002) found that the 7-class system, of rating kernels exhibiting visible disease symptoms, as illustrated in Reid et al. (1996), provided an adequate rating scale for ear rot infection. The rating scale is as follows:

1 = 0%

2 = 1 to 3%

3 = 4 to 10%

4 = 11 to 25%

5 = 26 to 50%

6 = 51 to 75%, and

7 = > 75% disease infection.

As a rating scheme, it is logarithmic in nature, and could easily be converted to percent visual infection, which is more closely related to the amount of ear rot fungal growth per ear. Reid et al. (1992) proposed a disease rating of 3 or less as being resistant. The scale also provided a good indicator of the mycotoxin contamination. Walker and White (2001), when assessing for resistance to *Aspergillus* ear rot, used a 1 to 10 scale, with 1 being no visible ear rot and 10 being fully rotted at the inoculation site.

In his studies at CIMMYT-Mexico, Jeffers (2002) rated *F. verticillioides* ear rot on a 1 to 6 scale, as follows:

1 = 0%

2 = 1 to 10%

3 = 11 to 25%

4 = 26 to 50%

5 = 51 to 75%, and

6 = >76% kernel or ear rot infection.

Trucker et al. (1986) used a similar scale in their work on *Aspergillus Flavus.* Jeffers (2002) rated *F. graminearum* and Stenocarpella ear rot on a 1 to 5 scale as follows:

1 = 0%

2 = 1 to 25%

3 = 26 to 50%

4 = 51 to 75%, and

5 = >75% disease infection.

In all these studies, only the primary ear was inoculated.

In Malawi, Kapindu et al. (1999) used a scale of 1 to 5 for the evaluation of the incidence and severity *F. verticillioides* and *S. maydis* in local and improved maize varieties from 29 smallholder farmers' fields in two communities in the central region.

1 = 1 to 25%

2 = 26 to 50%,

3 = 51 to 75%

4 = 76 to 99%, and

5 = 100% disease infection, completely rotten.

Besides expressing maize ear rot as percent disease severity (DS) (Kapindu et al., 1999; Silva et al., 2007), percent-kernel-infection (PKI) has been used to measure infection, especially when categorising into various groups of disease severity (Schaafsma et al., 1997). Expressing ear rot infection as percentage kernels colonised in a representative sample appears not to conceal the significance of the cob rot disease (Chungu et al., 1996). Rossouw et al. (2002a) found that the incidence of rotten ears (DI) was the most practical and reliable method. Most of the rating scales used so far has produced reliable results (Clements et al., 2003). Reid et al. (1992) concluded that the counting of infected ears per plot or genotype, a rapid disease assessment practice frequently used by breeders, was adequate. However, very few studies have been conducted to compare the different ear rot rating methods (Nowell, 1998). Rheeder et al. (1993) recommended the plating of a representative sample of maize kernels on agar, where symptomless kernel infection, such as in *F. verticillioides* ear rot infection is suspected. The other methods of assessment include image analysis (Chungu et al., 1997). Digitalized Image analysis is able to quantify or map in situ ear rot infection and allow for the discrimination of different levels of resistance to ear rots that may be indistinguishable using other methods. Whilst all these rating scales may provide an indication of the disease occurrence (incidence) or intensity (severity), visual assessment for each rating class requires technical skills that have to develop with time. Errors in rating would result in wrongly classifying a genotype as either resistant or susceptible. Assessment must not

be hurried and where less than 200 samples have to be assessed, it is best done by one individual.

1.9 Summary

Maize ear rots are caused by a variety of fungal species; prominent among them are *Fusarium, Aspergillus* and *Stenocarpella.* Worldwide, the accumulation of mycotoxins, the secondary metabolites they produce in the host plant, is a health concern. A congruence of favourable weather conditions, availability of inoculum, and susceptible genotypes is required for an epidemic to occur. Diallel mating designs and their modifications have been widely used to estimate genetic variances for resistance to ear rot in maize populations. The inheritance of genetic resistance is reported to be mainly additive, though there are also conflicting reports of dominance and epistatic gene effects.

Large genotype x environment interaction effects have been reported for inoculation techniques and genetic response to selection. Genotypes x Environment Interaction that result in a change in the rank order of genotypes tends to complicate the selection for materials for multiple or combined resistance to ear rots. Nevertheless, several sources of ear rot resistance have been identified, though no complete resistance is yet known. Some of inoculation techniques and ear rot assessment schemes developed have been quite effective in discriminating these sources of resistance but the challenge for the development of an efficient inoculation technique still remains. Lacks of consistency across environments and labour have been listed as some of the drawbacks of already developed techniques. An appraisal of inoculations at local environments maybe required if successful breeding is to be attained. While these screening tests may have been successful with genetically homogenous breeding material such as inbred lines and hybrids, they may not be that effective on genetically broad-based populations. Henceforth, sub-Saharan African countries like Zambia, where a large proportion of maize varieties are open-pollinated and are genetically diverse, there is a need for screening methods to be modified to address this local condition. Very few reports exist on the use of recurrent full-sib selection in improving maize populations for resistance to ear rot. However, where they have been applied, progress to selection has been made. Few molecular studies in highly specialized laboratories in Europe and North America have identified chromosomal regions that confer resistance to ear rots. However, once incorporated in those genotypes, they have not been re-isolated outside the

environmental conditions in which they were previously identified casting doubt on their transferability into other genotypes.

This review of the literature shows that though a lot of work might have been done on maize ear rot, breeding for ear rot resistance in sub-Saharan Africa still remains a challenge, and more genetic and applied breeding studies need to be conducted to avert crop losses and avoid problems associated with mycotoxin contamination.

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CHAPTER 2

SMALLHOLDER FARMERS' PERCEPTIONS OF MAIZE EAR ROT DISEASE AND THE PROSPECTS FOR BREEDING NEW VARIETIES WITH FARMER-PREFERRED TRAITS

2.0 Abstract

Despite an impressive array of maize varieties, maize production on Zambian smallholder farms is low due to several constraints. The objective of this study was to investigate Zambian smallholder farmers' perceptions of maize varieties, the constraints on their maize production, their preferred maize qualities, and their implications to breeding. A participatory rural appraisal (PRA) was therefore conducted across four districts in Zambia during the 2005/2006 season. The study included literature reviews, semi-structured interviews with key informants, focus group discussions, problem listing and analysis, and transect walks. Each PRA exercise involved more than 20 farmers. Maize ear rots, production constraints and farmers' preferences were scored and ranked. The farmers on all PRA sites grew maize, followed by beans, cotton, or vegetables. Principally, farmers grew hybrids but production and grain yield were low, with an average of about 1.5 t ha^{-1}. Maize ear rot disease was ranked as the third most important disease in maize after maize streak virus and northern corn leaf blight. Less than 6.7% of farmers were aware of mycotoxins. Other major constraints to maize production included the lack of fertiliser, the high cost of labour, poor soil fertility, and storage weevils. Production constraints differed among the districts and influenced farmers' preferences for varieties. In the most productive districts, farmers preferred drought-resistant, medium-maturing maize and maize weevil-resistant varieties.Those farmers close to urban areas in the cities preferred varieties with large cobs, multiple cobbing, superior milling and sweet taste properties. Farmers displayed strong preferences for the old hybrids and landraces like "Gankhata", due to their perceived superior drought tolerance, very white grain, large grains, and good taste. In general, farmers preferred varieties that combine some traits of this landrace with high yield potential, early maturity, low soil fertility tolerance, and grain weevil resistance. It is thus implied that breeders should aim at developing varieties that are not only ear rot resistant but combining farmers' preferred traits.

Keywords: Maize ear rot, mycotoxins, PRA, production constraints, variety preference

2.1 Introduction

Maize (*Zea mays* L.) is an important agricultural crop in Zambia (Howard and Mungoma, 1996). However, its productivity continues to be constrained by a myriad of biotic and abiotic stresses, among them diseases such as maize ear rot. Generally, maize diseases may cause huge yield losses of up to 30% in Zambia, and, in some years, and in localised areas, entire crops are destroyed during an epidemic (Rao and Ristanovic, 1986, MAWD, 1990). Besides maize streak virus, grey leaf spot, stalk rot, and maize ear rots, caused by several fungal pathogens, are amongst the most widespread of diseases in maize ears in Zambia (Rao and Ristanovic, 1986). Several fungal pathogens have been implicated, among them *Fusarium*, *Aspergillus* and *Stenocarpella* spp. The losses they cause include reduced grain yield and reduced grain quality due to discolouration and the mycotoxins they produce in their host. Over the years, several varieties have been developed by breeders that have been reported to possess some level of resistance to ear rots and other biotic stresses (Ristanovic et al., 1987) but this has not been proved. Smallholder farmers have continued to record huge losses due to these stresses. These farmers make up 60% of the Zambian farming community, thus their low maize production has a bearing on national food security. Understanding the production constraints that farmers face, ear rots in particular, could greatly assist in the design of an effective breeding programme that not only incorporates resistance traits to ear rots but improves other agronomic traits as well. Thus farmer's perceptions about maize ear rot varieties have to be established and included in the breeding programme for ear rots.

Worldwide, participatory rural appraisals (PRAs) have been used to solicit farmers' views on various agricultural resource management options necessary to ensure household food security and improvement in their welfare (Chambers, 1999.). Through the same forum, community-based action plans are drawn up and implemented for the farmers' benefit. The failure by formal breeding to achieve high adoption rates of improved varieties by farmers is well recognised (Singh and Morris, 1997). Acceptability of agricultural technologies in improved varieties by farmers depends on how well farmers' constraints and preferences have been identified (Kamara et al. 1996; Soleri et al., 2000). Thus, participatory approaches have been developed that tap on the extensive knowledge of the farmers, investigating the production constraints and the farmers' preferences in the varieties they grow.

Odendo et al. (2002) used PRA to solicit farmers' views on the selection of varieties they planted, and reported that earliness and high yield were the most important traits to farmers. Nkongolo et al. (2008) also used farmer participatory tools to access farmers' indigenous knowledge of the major characteristics of sorghum landraces and reported

that farmer characterisation of sorghum varieties had allowed for the selection of landraces that had outperformed already existing varieties.

The basic objective of the study was to determine the position and rank of ear rot disease in relation to other maize production constraints, to lay the ground for breeding ear rot resistant maize germplasm. Specifically, the study aimed to:

(i) assess farmers' perceptions of the maize ear rot problem in maize production;

(ii) establish baseline information about the maize varieties grown by farmers and the criteria, they used in choosing which variety to grow;

(iii) determine maize production constraints and farmers' coping strategies for the control of maize ear rots and other important constraints; and

(iv) investigate the opportunities for breeding new maize varieties with ear rot resistance and other important traits.

The hypothesis of the study was that farmers consider ear rot diseases among the most important maize production constraints, and have knowledge and varietal preferences which could contribute to ear rot resistance in a maize variety.

2.2 Materials and Methods

2.2.1 Study area

The participatory rural appraisal was carried out at four sites in central Zambia (Figure 2.1). These sites were chosen in consultation with the local district agricultural staff who knew the villages very well. The selected areas were representative of agriculture in the district. These villages were chosen according to the following criteria amongst others: accessibility by all-weather roads, the predominance of maize as a crop, and the diversity of the communities, i.e., what precipitated the establishment of those communities.

The four communities were Barlastone (10km NW of Lusaka), Kalimansenga, (50km NW of Chongwe), Kasaka (10km SE of Kafue), and Mulabo-kakunka (60km NW of Chibombo). They were all within a 150km radius of Lusaka District, as are most areas in central Zambia (Figure 2.1).

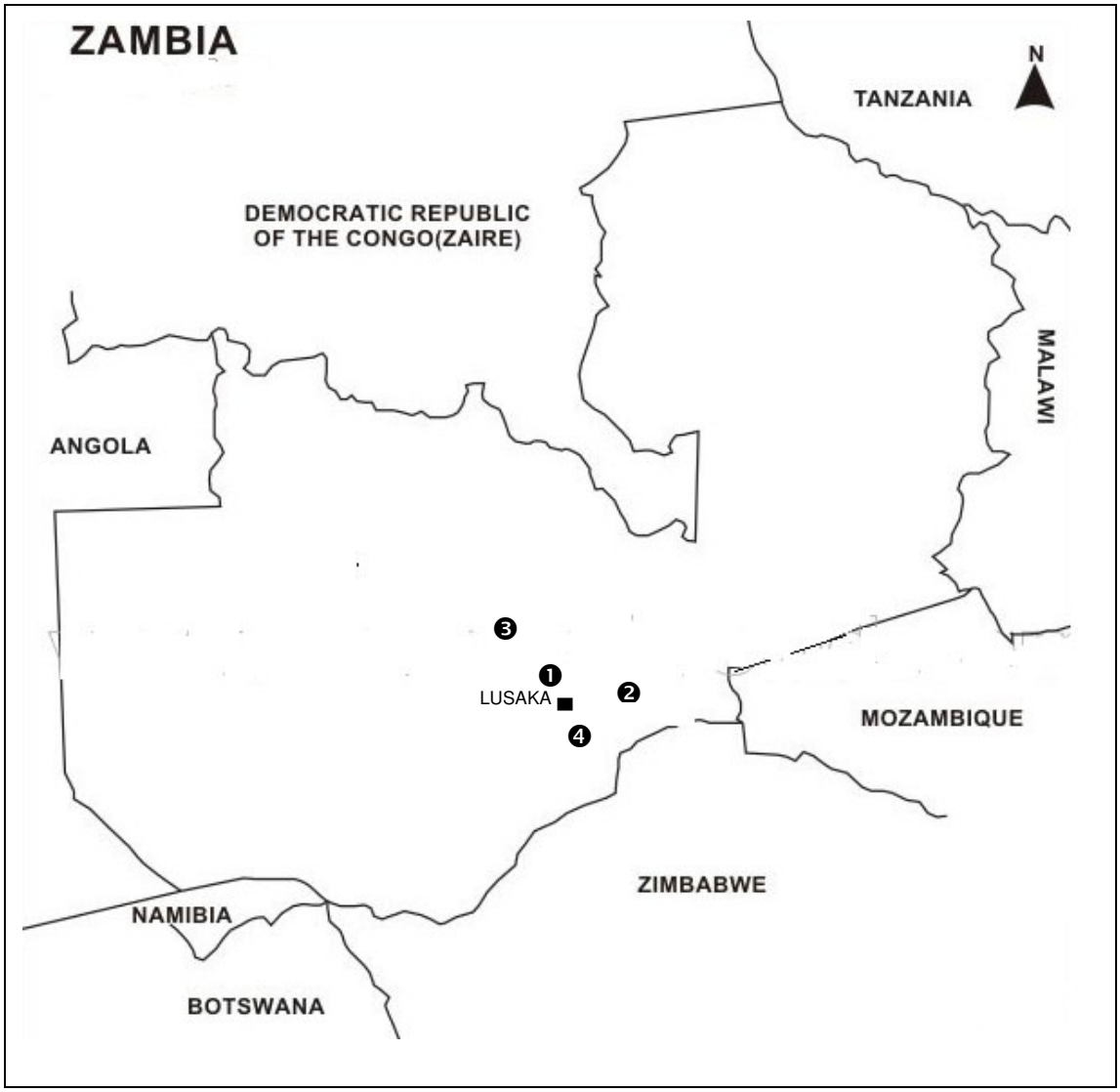

Figure 2.1 Participatory rural appraisal (PRA) sites location in central Zambia: ❶ = Barlastone, ❷ = Kalimansenga, ❸ = Mulabo-kakunka and ❹ = Kasaka

2.2.2 Selection of farmer respondents

Ninety small-scale farmer participants were involved in the PRA study (Table 2.1). They were identified through local agricultural officials. The participants were randomly selected without any bias towards age, gender, experience in farming, or status in the community. However, the key informants were usually traditional or community leaders; this was meant to tap into their wealth of knowledge of community history, organisation, and general welfare. Each PRA session involved a minimum of 20 farmers.

Table 2.1 Number of farmer participants by gender and age group in the four PRA sites during 2005/6

Sites	Number of participants				
	Male	Female	Adults	Young adults	Total
Barlastone	4	22	25	1	26
Kalimansenga	10	12	16	6	22
Kasaka	12	8	17	3	20
Mulabo-kakunka	14	8	16	6	22
Total	40	50	74	16	90

2.2.3 Study methods

Qualitative participatory research methods and tools used to generate information were adapted from those used by the PRA Programme at Egerton University in Kenya (PRA Programme, 1999). The techniques used involved a series of exercises in which the farmers played an active role.

An introductory visit was made to each of the four communities. (See Appendix 1 for places and dates of these meetings.) During the exploratory phase, secondary data were obtained about each community from the District Agricultural Office and field extension staff.

Semi-structured interview (SSI) and focus group discussion (FGD) methods were used to explore all the issues pertaining to maize production, with specific emphasis on ear rots and mycotoxins. The local extension officers assisted with the SSI and FGDs. The checklist of the topics for discussion and SSI were discussed among the investigating team prior to commencement of FGD so that facilitators for each group discussion were aware of what was expected. The topics for group discussions included community/village organisation, the importance of different crops, including maize, maize insect pests and diseases, and control practices for these pests and diseases (Appendix 1). Other topics were maize crop hectarage, constraints on maize production, criteria used in selecting maize varieties, current coping strategies, and farmer suggestions for overcoming the constraints. As far as possible, the farmers were not told that the focus of the study was maize ear rots in order to avoid, or at least, minimise any bias in the

responses. However, if maize ear rot was not mentioned as a major disease in the area, they were then asked about it.

Later, transect walks and triangulations were carried out, enabling the verification of the information given during the SSIs and FGDs. During the village transect walk, further information on the communities and their inhabitants was obtained. The outline of the transects and area maps, showing features encountered during the walk such as houses, storage bins, fields and wells, were drawn first on the ground and then transferred to an A2 paper.

After the transect walks, farmers were asked to draw daily activity charts to explore the differences and similarities in the workloads of men and women. In addition to these, seasonality charts depicted the various agricultural activities, month by month, and reflected the occurrence of pests and diseases in the fields.

The farmers used matrices to list, rank and score the different characteristics they preferred in maize varieties; the socio-economic production constraints affect maize production and the factors responsible for ear rot infection (Figure 2.2). Preference ranking exercises had two parts: pair-wise ranking and matrix ranking. The former showed why a particular subject was preferred, and each preferred subject listed minor reasons and one major reason for preference, while the latter provided the relative preferences without showing why these were preferred. The pest and disease affecting their maize crop was used to ranked and scored by counting the number of participants that were in agreement that a particular biotic constraint was important. The proportion of those in agreement was determined and percentage score used to rank the pest or disease. Thereafter, in a plenary session with farmers, corresponding plant-breeding interventions were listed against a list of selected biotic and abiotic constraints.

Figure 2.2 Women farmers at Kalimansenga, Chongwe, listing maize production constraints

2.2.4. Data collection

For the purpose of extracting information, farmers were grouped by gender and age-group for each PRA activity, except in Barlastone (Lusaka), where the participants were mostly women. The participants in Barlastone belonged to a multi-purpose cooperative club, whose membership was mainly female. At Kasaka (Kafue), the discussion groups were constituted according to the agricultural camps that make up the Kasaka Agricultural Block, namely, Nutrition Centre, Muchuto, Kasaka, and Julia Mpolamasaka, for activities such as the ranking of disease and pest scoring, For those activities requiring assessment in which gender or age might be influential, such as variety selection criteria, the groups were reconstituted according to gender and age.

Before each PRA exercise, the objectives and procedures of the exercise were explained to the farmers by the extension staff and the principal investigator, after which the farmers were allowed to ask any questions for clarification. The farmers were encouraged to use the local languages they understood best and a member of the research team most versed with the local languages facilitated the group discussion.

Key informants were identified and interviewed, and their household and fields were visited, where some topics were discussed in more detail. This triangulation was used to help verify some issues.

2.3. Results

2.3.1 Secondary Data

2.3.1.1 Social and economic issues of the study area

There were 231, 857, 3570, and 696 people in Barlastone, Kalimansenga, Kasaka and Mulabo-kakunka, respectively, in 768 agricultural households (MACO, 2006; unpublished). Between 13 to 17% of the households were female-headed households (Central Statistics Office, 2003). Rain-fed crop production was the main form of agriculture in all these areas. The average farm size varied considerably from 0.15 to 8 ha. In all the four sites, the family members were the main source of labour, although some farmers engaged extra labour, especially at critical times such as weeding and harvesting. The use of animal draught power for ploughing was prominent in Kalimansenga and Mulabo-kakunka.

. The road to Mulabo-kakunka was impassable during the wet season and at times the village was cut off. This affected the transportation of agricultural inputs to Kakunka and the surrounding areas. Irrigation facilities were available in Kasaka and Kalimansenga; Barlastone and Mulabo-kakunka farmers relied on rain-fed agriculture. However, even in communities with irrigation, very little off-season green maize production was done as farmers were pre-occupied with vegetable gardening. The farmers at the Kasaka agricultural settlement scheme (Kasaka) had access to communal pumps while in Kalimansenga, individuals owned small irrigation pumps.

2.3.1.2 Farming systems and crop production

The main livelihood of the farmers in all four communities was mixed farming, i.e., crops, poultry, and livestock. Crop production was dominated by rain-fed maize. The main maize cropping system practised was monocropping (and occasional intercropping). Farmers in Mulabo-kakunka and Barlastone had adopted conservation farming practices such as potholing (a conservation farming techniques that involves making a small hole is dug in ground. Little compost put in and half covered with soil. The seed is planted in the hole and when the rains come, it collects in there), fallowing, planting other soil-enriching crops such as sunhemp, and crop rotation. Fertiliser application was widespread. In addition, some farmers (10%) indicated that they also used kraal manure in fields nearer their homesteads. However, this was meant to supplement chemical fertilisers. There was little or no composting done. Most of the maize stovers were left to rot on the soil surface (in Barlastone), or fed to livestock (in Kasaka, Kalimansenga, and Mulabo-kakunka). Farmers also indicated that they conducted controlled burning of the fields

before ploughing. Intercropping was always with a leguminous crop, except where pumpkin or squash was planted. Farmers were aware that some of these leguminous crops improved soil fertility.

The most important food crops grown were maize, *Zea mays* L., sorghum, *Sorghum bicolor* L., cassava, *Manihot esculenta* Cranz., sweet potato, *Ipomoea batatas* L. (Lam.), banana *Musa* spp., bambara groundnut, *Vigna subterranean* (L.) Verdc. cowpeas, *Vigna unguiculata* and groundnut, *Arachis hypogaea* L. while the major cash crops were maize, sugarcane, *Saccharum officinarum* L. and cotton, *Gossypium hirsutum* L.(Table 2.2). Most of the other crops served a dual purpose as cash and subsistence crops, but were mainly for subsistence. There were marked differences in the combination of crops grown. Vegetable production was the second most important activity after maize in Kalimansenga and Kasaka, where a variety of vegetables were produced to supply the huge markets in Lusaka and Kafue, respectively.

Table 2.2 Major crops grown by location in the PRA sites in 2004/5

PRA sites			
Barlastone	**Mulabo-kakunka**	**Kalimansenga**	**Kasaka**
Maize	Maize	Maize	Maize
Bean	Bean	Bean	Bean
Groundnut	Groundnut	Groundnut	Groundnut
Sweet potato	Sweet potato	Sweet potato	Sweet potato
Sugarcane			Sugarcane
Cowpea	Cowpea	Cowpea	Cowpea
Pumpkin		Pumpkin	Pumpkin
Soybean	Soybean		
	Cotton	Cotton	Cotton
	Eggplant	Eggplant	Eggplant
Rape	Onion	Onion	Onion
	Cassava	Cassava	Cassava
Bambara groundnut			Banana
		Squash	
			Other fruit trees
	Sorghum		
	Pearl Millet		
	Okra		Okra
	Paprika		
	Tomato	Tomato	Tomato
	Cabbage	Cabbage	Cabbage
		Rape	
		Other vegetables	
			Green maize

Source: District Agricultural Offices, MACO.

2.3.1.3 Maize production

The size of maize field ranged from 0.25 to 8 ha^{-1} in all the four sites, with the largest fields found at Kalimansenga. On average, the maize yields were highest in Chongwe, 2.5 tonnes ha^{-1}, followed by Kasaka, 2 tonnes ha^{-1}. The lowest among the four sites were in Barlastone and Mulabo-kakunka (Table 2.3). The fields were planted continuously with maize. Except in Barlastone where the fields were rented, the size of fields planted to maize in the other three sites, i.e. Kalimansenga, Mulabo-kakunka and Kasaka were mostly determined by ability to pay for animal draught power (and/or external labour), fertilizer and seed. Availability and ownership of farm implements such as ploughs which is part of the physical capital endowment of the communities also to some extent influenced the size of the field that was ploughed and planted with maize.

Table 2.3 Maize production by location for 2004/5 season in the sites where participatory rural appraisal was conducted

Location	Agricultural block	District	Yield (tonnes ha^{-1})	Maize production (tonnes)
Barlastone	Barlastone	Lusaka	1.0	50
Kalimansenga	Kalimansenga	Chongwe	2.5	100
Kasaka	Kasaka	Kafue	2.0	87
Mulabo-kakunka	Shimukuni	Chibombo	1.0	325

Source: District Agricultural Offices, Ministry of Agriculture and Cooperatives

2.3.2 Primary Data

2.3.2.1 Maize ear rots and mycotoxins

Maize ear rots were ranked the third most important disease after the maize streak virus and leaf blight (Table 2.4), and the fourth most important biotic stress after stemborers and these two diseases. A wide range of colours associated with rotten cobs and grain were mentioned and reported during FGDs (Table 2.5). The most common colours and descriptions given by the farmers were brown, pink, or lavender grain; red and shrunken grain; lightweight, dull white-brown cobs; cobs with an enlarged white/grey/black head; and a whitish cottony mass over the ears, starting from the shank to the tip of the ear. Other descriptions included discoloured grain, a chaffy ear that easily broke when squeezed, and greenish-yellow, black and blue-green husks with tiny black spots on them adhering to rotting ears. No single colour or description was provided by each

farmer group during the PRA as they usually provided three or more. Some farmers even claimed to have six types of kernel and ear rot colours in their field. Dried maize kernels that appeared to be smeared with white paint was one description given by one farmer group at Kasaka. After close inspection of the grain, the farmers found that it white stripes. A characteristic "starburst" symptom of Fusarium ear rot. The majority of the farmers agreed that the whitish or dry brown shrunken kernels were most prominent during the high rainfall years in both the flint and dent maize varieties. The other types were observed during much drier or less favourable years. In all four sites, the younger participants argued that the rotting was largely due to early harvesting because of a fear of heavy late rains or thieves.

Table 2.4 The ranking of major diseases affecting maize at the four sites in Zambia

Diseases	Barlastone n=26		Kasaka n=22		Kalimansenga n=20		Mulabo-kakunka n=22	
	%	Rank	%	Rank	%	Rank	%	Rank
Grey leaf spot	69.2	4	72.7	4	65.0	4	50.0	5
Cob rot	92.3	2	68.2	5	75.0	3	86.4	2
Leaf blight	76.9	3	86.4	2	85.0	2	72.7	3
Head smut	34.6	5	-	-	85.0	2	68.2	4
Maize streak virus	96.2	1	77.3	3	95.0	1	90.9	1
Common rust	-	-	95.5	1	60.0	5	36.4	6
Downy mildew	-	-	27.3	6	-	-	18.2	7

* Classified as major if two or more sites ranked them 1–5; minor if a site ranked 6 or more

One women's farmer group at Mulabo-kakunka had observed an increase in ear and kernel rotting in some of the newer maize varieties compared to the old ones. Other farmers associated an increased occurrence of ear rots with increased insect and bird damage. When asked about the loss they incurred as a result of maize ear rots, farmers reported losses of between 1 tin (approximately 15 kg) to 2.5 bags (125 kg) per hectare during the 2004/5 season.

A large proportion of the farmers interviewed were not aware of mycotoxins and the threat these posed to both human and animal health. This lack of awareness of

mycotoxins was reflected in the manner of disposal of the rotten maize, which was either sold to illicit beer brewers or fed to livestock, especially free-range chickens and pigeons. About 10% of the farmers in Barlastone, Mulabo-kakunka, and Kalimansenga admitted selling rotten maize to people brewing illicit beer. They said that rotten maize increased the potency of the beer. Two farmer groups, one at Kasaka and the other at Mulabo-kakunka, indicated that during periods of extremely low yields, they did not throw away rotten maize but rather mixed it with healthy maize for consumption. There were some differences between men and women in the degree of awareness of ear rots and their associated mycotoxins (Table 2.6). More men than women considered maize ear rot as a serious disease.

Table 2.5 Ear rot colours and descriptions of damage reported by farmers at four PRA sites in Zambia in 2005/6

Colour of maize ear rots	Farmer-observed damage	Likely name of the disease
Yellow-green grain	Rotten grain accompanied by some insect mining between the grain	*Aspergillus* ear rot (caused by *A. flavus*)
Black powder on kernels		*Aspergillus* ear rot (caused by *A. Niger_*)
Whitish mould all over the grain	Bleached husks, sometimes with black spots Lightweight and rotten Rotting start from ear shank upwards	*Stenocarpella* ear rot
Grey	Chaffy kernels Sticky husks adhering to the cob	*Nigrospora oryzae*
Red Pink or pale pink Grain surface with whitish streak marks	Rotten grain, scattered all over the cobs Rotting from ear tip downwards	*Fusarium* ear rot
Powdery-blue green growth on and between grains		*Penicillium* ear rot
Brown grain Black grain	Brown, rotten grain, sometimes black	*Botrydiplodia* ear rot
Dark-brown grain Black mass	Black grain	*Physalospora* ear rot
Green-black with streak marks	Grain discoloured with black lines (streaks)	*Cladosporium* ear rot
Large grey-whitish black on the upper part of cob		Common smut (not an ear rot)

Table 2.6 Proportion of farmers aware of maize ear rot diseases, mycotoxins, disease-resistant and drought-tolerant varieties in all four sites during 2006

Sites	Gender	Percent awareness			
		Maize ear rots	Mycotoxins	Disease resistant varieties	Drought resistant varieties
Barlastone	Men	25	0.0	50.0	50.0
	Women	75	0.0	12.5	25.0
Kalimansenga	Men	60	12.5	25.0	50.0
	Women	40	0.0	0.0	0.0
Kasaka	Men	75	14.3	28.6	20.0
	Women	25	0.0	0.0	28.6
Mulabo-kakunka	Men	100	0.0	37.5	37.5
	Women		0.0	0.0	0.0
Mean	Men	65.0	6.7	35.3	39.4
	Women	35.0	0	3.1	13.4

2.3.2.2 Factors seen as responsible for ear rots

No gender or age influence was observed when listing and ranking the factors that farmers perceived as promoting ear rots on their maize. Too much rainfall and lack of disease-resistant varieties rather than poor agricultural practices were perceived as the most important factors (Table 2.7). Seventy-eight percent (78%) of the farmers mentioned too much rainfall as the major cause, followed by the planting of susceptible varieties. Other farmers indicated the lack of crop rotation and prolonged seed storage. None of the groups interviewed associated the presence of insect pests with an increased incidence of ear rots on their farms.

2.3.2.3 Other maize production constraints

The two most important problems affecting maize production in the four PRA sites were listed by farmers as low maize productivity and a lack of resources, that is, cash to pay for various farm inputs. After FGDs, besides maize ear rots which were grouped under insect pests and diseases, farmers identified several major constraints to maize production. These include lack of animal draught power, high cost of farm inputs, poor markets, scarcity of farm land, and low producer prices (Table 2.8). Others were low soil fertility, lack of information about improved drought-, pest- and disease-resistant varieties, and lack of storage facilities (Table 2.8).

Table 2.7 Factors influencing the occurrence of ear rot on farmers' fields. Proportion of participants (%) that agreed in all four PRA sites by gender

Site	Gender	Lack of resistant varieties	Too much rainfall	Lack of crop rotation	Presence of weeds	Drought	Late planting	Prolonged storage
Barlastone	Men	100	68	60	50	43	34	35
	Women	72	100	50	75	0	16	78
Kalimansenga	Men	100	100	76	49	47	39	0
	Women	72	33	51	78	25	12	25
Kasaka	Men	80	100	75	80	57	23	52
	Women	45	48	36	32	0	46	67
Mulabo-kakunka	Men	67	77	100	67	0	11	100
	Women	75	100	100	25	0	100	100
Mean	Men	86.8	86.4	77.7	61.5	36.8	26.8	46.8
	Women	66	70.3	59.3	52.5	6.3	43.5	67.5

Although the group interviews were held separately for men and women, there were no marked differences in the ranking of production constraints. Hence only the overall score for each production constraint was used in the final ranking at each site.There was a was a general appreciation of the socio-economic environment by participants as evidenced by the number of maize production constraints listed in Table 2.8. . Lack of farm inputs, especially fertiliser, was confirmed by the widespread yellowing of plants in maize fields during the transect walks. During these walks, farmers identified a number of soil types and knew their characteristics. For example, they considered sandy soils to be prone to erosion, gravelly (chromic luvisols) soils to have a high moisture-holding capacity and hence to be good for crops, and silty loam (sodosols) to have a high population of cutworms and other soil-borne pests that cause severe stress during dry spells or drought years.

Among insect pests and diseases, stem borers were considered the most important pests causing damage to maize crops, followed by storage weevils, *Sitophilus* spp. (Table 2.9). The least important were armoured crickets *Acathoplus discoidalis* Walker; Monkeys were also listed and were ranked at Kasaka as a major hindrance to increased yields. The maize streak virus was identified as the most important disease, followed by leaf blight. The least important was downy mildew. It was the combined influence and interactions of these factors that were observed to be responsible for low maize yields as opposed to individual constraints.

Table 2.8 Constraints affecting maize production in four PRA communities in central Zambia during 2005/6: a summary of the scoring matrix and rank

Constraint	Ranking[a]			
	Barlastone	**Kasaka**	**Kalimansenga**	**Mulabo-kakunka**
Lack of animal draught power	-	5	1	2
Lack of farm implements	8	-	-	-
High cost of farm inputs/fertilizer	2	2	3	-
High external labour costs	7	-	-	-
Inadequate irrigation equipment	-	1	-	-
Lack of improved seed	-	3	-	-
Lack of information about pest-, disease- and drought-resistant varieties	-	6	6	4
Lack of storage facilities	-	9	7	8
Low producer prices	5	7	5	-
Low soil fertility	-	8	2	7
Poor markets	5	8	4	5
Insect pests and diseases	3	10	8	6
Quelea birds	-	-	-	9
Poor roads	-	-	9	-
Scarcity of farm land	1	4	-	-
Lack of transport	4	10	-	-
Unreliable rain	6	12	-	4
Growth of weeds	-	11	-	8

[a] Constraint classified major if two or more sites rank it: 1 – 5; important: 6 – 9 in more than two sites and minor: when a site indicates 10 or more.

Table 2.9 Major pests affecting maize production at PRA four sites in Zambia

Pests	Barlastone n=26		Kasaka n=22		Kalimansenga n=20		Mulabo-kakunka n=22	
	%	**Rank**	**%**	**Rank**	**%**	**Rank**	**%**	**Rank**
Quelea birds	30.8	7	30.8	5	-	-	30.8	7
Stem borers	96.2	1	80.8	1	90.0	1	80.8	1
Termites	68.4	3	61.5	2	50.0	3	57.7	3
Cutworms	50.0	5	46.2	3	-	-	46.2	5
Rats and mice	-	-	23.1	7	-	-	15.4	8
Maize storage weevils	76.9	2	80.8	1	65.4	2	69.2	2
Grasshoppers	28.9	8	-	-	-	-	-	-
Larger grain borer	57.7	4	42.3	4	35.0	4	53.8	4
Army worm	46.2	6	-	-	-	-	-	-
Armoured bush cricket	-	-	7.7	8	-	-	7.7	9
Leaf aphids	-	-	-	-	-	-	42.3	6
Earworms	-	-	-	-	-	-	15.4	8
Monkeys	-	-	26.9	6	-	-	-	-

* Classified as major if two or more sites indicate 1–5; minor: a site indicates 6 or more.

2.3.2.4 Maize varieties grown

More than 20 different maize commercial varieties and landraces were grown by farmers during the 2004/5 season. Most of the farmers grew Seedco varieties, followed by Maize Research Institute (MRI) varieties (Table 2.10). Only 29.3% of the farmers grew traditional varieties or landraces. Prominent among these was "*Ghankata*" (Hickory King). A few farmers grew yellow maize hybrids. Among the hybrids, SC513 and MRI634 were the most popular, followed by MM604. The most productive areas, Chibombo and Chongwe, had the highest assortment of varieties, and Barlastone had the least.

Table 2.10 Proportion of farmers (%) in the four sites who planted different maize varieties during the 2005/6 season

Varieties	Percentage						Reasons provided
	Maturity	Barlastone	Mulabo-kakunka	Kalimansenga	Kasaka	Overall*	
Africa-1	Early	-	20	9.1	-	7.3	Familiar, given with the NGO seed pack
Landraces	Early	80	40	-	-	29.3	Whiteness, good cooking quality, and taste
MM603	Medium	-	10	-	70	19.5	Familiar, good milling flour
MM604	Medium	50	20	36.4	40	36.6	Big cob size, less weevil-infested
MRI455	Early	-	10	-	-	7.3	High yields, less weevil-infested
MRI514	Early	-	10	9.1	20	9.8	Large grain size
MRI634	Medium	-	20	-	-	4.9	Large and heavy grains
MRI534	Medium	-	-	-	30	7.3	Heavy grain, milling quality
MRI614	Medium	-	30	9.1	10	12.2	Good plant stand, high yields
MRI624	Medium	-	20	-	-	4.9	Big cobs, high yielding
MRI634	Medium	90	20	54.5	20	46.3	Earliness, drought tolerant
MRI734	Medium	20	10	9.1	-	9.8	Familiar, given in seed packs
PAN6363	Early	-	-	18.2	-	4.9	Disease tolerant
PAN67	Early	30	-	18.2	30	19.5	Drought tolerant, good texture
Pool16	Very early	-	30	-	-	7.3	More grains per ear
SC403	Very early	-	40	18.2	-	14.4	Earliness, drought tolerant
SC513	Early	30	80	54.5	40	51.2	Big cobs
SC621	Medium	30	10	18.2	20	19.5	Earliness
SC627	Medium	30	40	18.2	30	29.3	Earliness, drought tolerant
SC709	Late	-	30	27.3	-	14.6	Less weevil infested in storage

* Percentage of the total number of farmers growing certain varieties across all sites; majority of farmers grew more than one variety

The availability of seed, pricing, and the presence of a non-governmental organisation (NGO) seed project were also reported by farmers as some of the factors that determined which varieties were grown (Table 2.10). The high cost of seed forced some farmers to recycle their seed for at least 2 to 3 years before making a seed change. Although the national fertiliser support programme of the Zambian Government enabled some of the farmers to have access to seed, the majority of farmers felt the seed pack eliminated variety of choice and, at times, did not provide the right combination of varieties that would enable them to exploit different planting dates. When asked about the pest resistance of the varieties in the seed pack, some farmers indicated that most of the varieties were susceptible to major storage pests and diseases, including ear rots, although they gave good yields with the recommended fertiliser application. In addition, several farmers were not able to differentiate open-pollinated varieties (OPVs) from hybrids. They claimed that the two were the same. Farmers at Kalimansenga, Mulabo-kakunka, and Barlastone reported that landraces gave some yield during drought years and produced much whiter maize flour than some hybrids. However, they also indicated that OPVs yielded less than hybrids and did not respond well to fertiliser application.

Due to the vigorous advertising of disease-resistant varieties, most of the farmers at the four sites knew that these varieties existed. When asked whether they read the instructions on the brochures or fliers on some varieties, however, the response was overwhelmingly negative. Thus, although most of them were literate, they relied on traditional knowledge, extension officers, media (radio), and information from neighbours. More than 50% of farmers in the PRA sites claimed to have attended seed fairs and agricultural shows.

2.3.2.5 Farmers' criteria in choosing varieties

Although Farmers' criteria in choosing varieties were similar across the four PRA sites, there were marked differences in the variety characteristics farmers preferred. These differences varied from site to site. A summary of these varietal preferences and the reasons behind them are given in Table 2.11. High yield, drought tolerance, early maturity resistance to storage insect pests, and husk cover were considered to be the most important, with scores of 1 to 5. A second group of criteria, with a rank score of 6 to 10 were, in that order, double cobbing, cob size, resistance to field pests and diseases, large grain size, and good germination. Most of the major traits preferred were those connected to yield, followed by those that enabled the crop to escape drought (e.g., earliness), or produce yield, even when attacked by pests and

Table 2.11 Summary of the farmer preference scores and derived rank from four different villages in central Zambia

Common preferred characteristics	Total score	Rank*	Some of the reasons advanced by farmers
High yielding	127	1	Grain yield
Early maturity	118	3	Unreliable rainfall, early provision of food, room for socio-economic activities
Good brewing quality	3	19	Beer preparation
Drought tolerant	123	2	Unreliable rainfall
Resistant to storage insect pests	79	4	Weevils and lack of storage facilities
Good but loose husk cover extension	70	5	Avoid rotting in the field and minimal bird damage
Large grain size	59	8	Plant less seed, more grain yield
Heavy grain weight	30	12	Produce good plant stand, more mealie meal, yield component
Seed type	16	16	Ease of shelling, softness, taste, weevil resistance, flint vs. dent types
Big cob size	63	7	Higher price for big green cobs, more grain yield
Double cobbing	67	6	Less hectarage to plant but more yield
Whiteness of maize flour	29	13	White mealie meal desired
Higher s germination percentage	45	10	Guaranteed plant stand, no gap filling, less seed cost
Grain colour	11	17	Boiling, appealing to the consumer
Low seed price	10	18	Affordability
Resistant to field pests and diseases	52	9	Minimal loss to insects and pests
Tolerant to weeds	18	15	Minimises cost of weeding
Sweetness	10	18	Sweet taste when roasted or boiled
Soft grain texture	37	11	Easy to pound (softness), flour extraction
Resistant to rotting	22	14	Minimal crop loss due to rotting disease

* Rank score 1 - 5 = Very important, 6 – 10= moderate important, 11-15 = minor importance and 16 - 20 = rarely considered

(i.e., tolerant varieties). Early maturity ensured the early provision of food for households and hence the avoidance of hunger. The least-preferred traits were seed type, brewing quality, taste, low cost of seed, and grain colour, which were ranked, in order, from 15 to 19. Some gender-based differences were apparent in the way the farmers ranked the traits within and across the communities. For example, women farmers in Mulabo-kakunka ranked drought tolerance highly, followed by earliness, while their male counterparts ranked high yield as being the most important criterion. In Kalimansenga, women ranked grain whiteness above yield, while in Kasaka; good plant stand was highly ranked. Farmers from Mubu Co-operative, Barlastone were divided as to whether drought tolerance or high yield was the most important criterion, but they concluded after some lengthy discussions that a variety must be able to escape or withstand moisture stress, even if high yielding; as such drought tolerance was to be considered the most important criterion.

Although ear or cob rot resistance was ranked highly as a preferred trait only at Kasaka, pest and disease resistance in general were considered as major selection criteria. However, farmers were not sure whether to treat ear rot separately from other diseases or not. Some commercially available varieties, such as Pannar hybrid PAN6363, were less preferred because they became easily infested with weevils whilst in the field. Some farmers reported that they would only change to improved varieties if they were disease resistant and drought tolerant. When asked which ones they felt were drought tolerant, farmers in Kasaka, Barlastone, and Kalimansenga overwhelmingly picked Zamseed varieties MMV400 and Pool16. Some farmers grew the local varieties continuously because they were more familiar with them as they had seen these varieties being planted for many years by their parents, other relatives and neighbours.

Grain quality characteristics were also important criteria. Texture was a preferred trait for women farmers, especially those from Barlastone. According to farmers at this site, the major demerits of improved varieties were the poor milling properties, poor poundability, and poor taste. Conversely, farmers felt that traditional varieties such as “Ghankata” had good attributes, such as being tastier, less prone to weevils in storage, and producing whiter maize flour with an extended shelf life. The dictates of the markets were also reported to be important. Farmers who sold green maize preferred varieties with bigger cobs, as these fetched a higher price than small ones and required few to fill a bag. The hybrid SC627 was an example of such a variety. The low seed price was also listed as an important criterion.

Some farmers felt that the final choice between any two given varieties, in spite of all other good attributes such as resistance to ear rots, would be largely influenced by price of seed. Most farmers did not like most of the commercial OPVs currently on the market because they produced small grains and smaller cobs. They preferred larger cobs and heavier kernels. When asked about the practice of recycling seed, farmers reported less yield depression with landraces or OPVs such as MMV600, Pool16, and MMV400 than with the more common hybrids. It was apparent from FGDs that the recycling of seed did take place.

2.3.2.6 Willingness to use ear rot resistant maize varieties

Farmers were asked to give their opinions on their willingness to use maize varieties that could be resistant to ear rots; they gave affirmative responses but expressed reservations about the current seed price. Most of the participants were not willing to pay anything more than the existing market seed price. They indicated that they would purchase the new varieties only after observing their on-farm field performance

2.3.2.7 Farmers' coping strategies and breeding opportunities

Management of ear rots

Farmers' methods for managing ear rots in infected crops on their farms included burning rotten maize, allowing animals to consume crop debris after harvesting, hanging maize from tree branches to air dry, smoking maize selected as seed for planting, and thoroughly drying the grain on roof tops or the ground before shelling, hand sorting, etc. These practices were meant to reduce the amount of disease inoculum in the subsequent seasons. When faced with high levels of infection, some of the rotten maize was sold to illicit beer brewers. Farmers claimed that the use of rotten maize increased the potency of beer.

Other constraints

For other constraints, such as the lack of fertilizers, farmers used several strategies to ensure that their crops were healthy, and these included the application of animal manure, the use of crop rotation and intercropping, and other soil conservation practices as an alternative to inorganic fertilizers. A majority of the farmers were fully aware that maize hybrids responded better to inorganic fertilizers.

When seed was in short supply, or the cost was prohibitive, many participants admitted to the recycling of the seed of hybrids, Open-pollinated varieties, or landraces. Farmers generally viewed pests and diseases as a problem. Though no clear coping strategies emerged from the FGDs, crop rotation, mixed cropping, and clean fields were listed as some of the ways of overcoming these problems. Several maize breeding opportunities were identified during the plenary session (Table 2.12), among them the development of maize with drought tolerance, disease resistance, nitrogen use efficiency, and good storability. The farmers overwhelmingly agreed that improved varieties with such properties would be beneficial to them.

2.4 Discussion

2.4.1 Maize ear rots

The results of the PRA showed that maize ear rots were well recognised by the farmers in all the four communities. However, very few farmers were practising sound post-harvest control measures such as ensuring that the crop was dried to low moisture content before storage and was properly shelled and bagged to mitigate the effects of ear rots. Rather, the majority of farmers continued with their traditional ways of sorting the healthy grain from the rotten grain, drying grain on the roof top, or smoking it over fire. The early harvesting of ears was widely practised, and sometimes the maize was harvested fresh. Farmers claimed that thieves and monkeys were the reason of doing so, but this practice increased the rotting of the maize. It was observed that at the homesteads of nearly all the key informants, who were mainly traditional leaders, it was common to finds piles of maize cobs on the ground where moisture from the ground created an environment conducive to increased ear rot fungi activity. Hence some of the rotting actually took place at the homestead rather than in the field. It was clear from the number of ear rots that farmers identified, and the fact that more than one ear rot was identified correctly by symptoms, that the occurrence of ear rots, caused mainly by the *Stenocarpella* and *Fusarium* species, was widespread,. Some of the maize samples they brought to meetings had more than one visible symptom, suggesting that some infections were caused by more than one ear rot fungi. The results further suggested that most of the maize grown by the farmers was highly susceptible or may not have possessed adequate ear rot resistance.

Table 2.12 Constraints and maize breeding opportunities identified during the participatory rural appraisal at Barlastone, Kalimansenga, Mulabo-kakunka and Kasaka in central Zambia during 2006

Problem	Constraints *	Coping strategies by farmers	Opportunities for maize breeding*
Low maize productivity (yield)	Lack of irrigation equipment	Rain-fed crop production	Drought-tolerant varieties
	Low farm inputs	Intercropping, conservation farming	Development of varieties that more tolerant to low nutrient supply Low
	Unreliable rainfall	Rain-fed crop production; planting with onset of rains; conservation farming practices; planting of more than one variety	Improved drought-tolerant varieties
	Pest and diseases	Crop rotation; intercropping; burning of crop residues; uprooting or burning of diseased plants; field sanitation	Improved varieties with disease- and pest-resistant characteristics
	Storage weevils	Drying over smoke; use of traditional stores; sometimes, if air-dried, use ash	Better storage characteristics incorporated into improved maize varieties
	Ear rots	Burn rotten maize, feed to poultry	Improved varieties with ear rot-resistance
	Weeds	Manual weeding	varieties that more tolerant to weeds or suppress weed growth
	Poor soil fertility	Use kraal manure, farm conservation, fallowing	Low Nitrogen and acid-tolerant varieties; matching of crop with soil type
	Lack of access to fertilizer	Use kraal manure, Sometimes mix basal and top dressing fertiliser to reduce cost	Development of varieties that more tolerant low nutrient supply and those that may use bio-fertilizer inoculants
	High cost of improved seed	Recycle seed	Improved open-pollinated varieties
	Lack of production packages for pests, diseases and drought-resistant varieties	Rely on variety adverts and information from friends and neighbours	On-farm demonstrations of improved varieties

* Identified during plenary session with the farmers

The lack of knowledge of mycotoxins exhibited by the participants indicates that farmers and their households were being exposed to high levels of mycotoxins. There is, therefore, a need for extension work to educate the farmers on the dangers of consuming rotten maize and feeding it to livestock.

2.4.2 Maize varieties grown

There was a broad interest amongst the farmers in the new maize varieties and a willingness to adopt them if they incorporated farmers' preferences and were adapted to farming conditions. This agrees with the results of Nkonya and Featherstone (2001), who found that varieties with farmers' preferred traits were easily adopted. Farmers' personal experience influenced what varieties they grew. This was evidenced by the rejection of some commercial hybrids that were easily infested by weevils while in the field or produced smaller cobs and grains. Similar findings have been reported in studies conducted in Kenya and Tanzania (Wekesa et al., 2003). Though these improved hybrids were widely grown in all four communities, farmers were sometimes reluctant to use them because they were not familiar with them compared to the landraces. In addition, farmers' own experiences with the landraces were given more weight than any advertisements about new improved varieties. Landraces were considered to be tastier, with fewer weevils in storage, producing whiter mealie meal, and yielding better even in depleted soils (or in poor soils) and in drought years. However, due to the shortage of preferred varieties, i.e., traditional and improved varieties, farmers used whatever was on the market, and in some cases recycled the seed. The high price of the available certified seed was another reason given for recycling seed.

2.4.3 Criteria used in choosing maize varieties

The top three criteria used to choose which variety to grow were high yield potential, early maturity, and tolerance to drought. Studies conducted in southern Mali (Defoer et al., 1997) and Kenya (Odendo et al., 2002) reported similar findings, though earliness was the most preferred trait in those studies, which was not the case in this study. The differences in the criteria used across sites and among groups reflected the different uses of the varieties. For example, women farmers preferred the more drought-tolerant varieties, while men and youths favoured high-yielding varieties. Farmers used earliness interchangeably with drought tolerance as criteria and felt that early maturing varieties allowed the crop to escape the vagaries of the weather (drought and the unreliable rainy season), while ensuring the early provision of food to households to alleviate hunger. Other reasons were that early maturing varieties allowed them to engage in other farming

activities such as off-season vegetable farming, and early preparation of land. Yield potential and earliness seem to be universally important criteria for farmers. The desire to use more of the drought-tolerant varieties has been rekindled, probably as a result of the droughts in the early 1990s that affected the whole Southern Africa Development Community sub-region. Though the farmers wanted to plant pest- and disease-resistant varieties, they did not know whether these existed. However, they were aware that planting susceptible varieties would result in huge losses in maize grain yield in the field and storage, hence their preference for growing certain commercial varieties that they had heard of, or knew performed better against certain diseases and pests. From the breeder's viewpoint, if a particular characteristic is a desired criterion, it would be useful in practice to combine this with important farmer preferred traits, thus adding value to the varieties.

2.4.4 Other maize production constraints

The production constraints listed by farmers in this study as impeding maize productivity in the smallholder sector are common to the vast majority of small-scale farmers in sub-Saharan Africa (De Vries and Toenniessen, 2001; Odendo et al., 2002; Wekesa et al., 2003). However, as observed in this study, the ranking of these constraints varies significantly across smallholder farming systems, and estimated economic losses associated with these impediments may be difficult to predict due to the diversity of the sector. Bio-physical constraints such as unreliable rainfall, soil fertility, and disease resistance require long-term research interventions; while socio-economic constraints such as the lack of animal draught power, markets, and irrigation equipment require adjustments to specific community coping strategies. Lack of information (knowledge about disease resistance and drought tolerance) not only affects the rate of adoption of improved maize varieties by farmers but is evidence of the information gap in the agricultural extension delivery system. Pests and diseases did not rank highly on farmers' perceived constraints as they are not able to apportion the losses incurred to individual factors and some stresses rarely occur in isolation.

2.4.5 Farmers' coping strategies

Air or smoke drying of maize grain, sorting diseased or rotten grain and burning rotten grain were some of the methods employed to reduce the incidence and severity of ear rots in storage. The burning or throwing away of rotten grain eliminated the future sources of inoculum. The physical removal of visibly diseased ears and kernels at, or after, harvest has been previously mentioned elsewhere as an effective control option

(Munkvold and Desjardins, 1997). Feeding rotten grain to poultry and using rotten maize for beer brewing are at minimising the economic losses associated with the disease, but this has had the negative effect of increasing the incidence of mycotoxin-related illness in poultry and humans (Sydenham et al., 1990).

2.4.6 Maize breeding opportunities

Several opportunities exist for the development of a wide range of varieties including ear rot resistant in Zambia, among other traits. The occurrence of several ear rots as reported by farmers suggests the need to develop ear rot resistant maize. It was evident that while farmers may institute some ear rot management practices, such as harvesting at the right moisture content or sorting or smoke drying , these were not effective.. A snap assessment carried out of the economic levels of participants and communities where these PRAs were conducted revealed that many of the participants could not afford control measures that required the purchase of fungicides or those that demanded extra labour such as winnowing and sorting. The cultivation of varieties that are resistant to ear rots appears to be the most practical and cost-effective means of ear rot management. Increased farmer-breeder interaction would allow for the identification of other farmer-preferred traits besides ear rots and the prioritising of these during the selection process. Generally, farmers' perceptions are not included in the planning phase of a breeding programme. Breeders tend to design programmes to meet the policies of the government for ensuring household food security; hence high yield becomes the main focus. This has lead to the non-adoption of new varieties which lack the traits farmers prefer. Farmers in these four sites indicated the type of varieties they preferred as follows: drought tolerant, with good storability, disease and insect resistant and low input requiring. It is up to breeders to take stock of such requirements of farmers and to develop suitable varieties. This would increase the probability of adoption rates. Considering that farmers prefer recycling as a strategy for coping with the high cost of seed, an effort should be made to breed composites or open-pollinated varieties (OPVs) that are cheaper than hybrids. Furthermore, breeders must conduct vigorous demonstrations and testing to increase the adoption of improved open-pollinated varieties.

2.5 Conclusions and implications for maize breeding

Maize production was the main form of agriculture practiced in the PRA communities. While farmers were aware of ear rots and ranked them as the third most important biotic constraint after stemborers and maize streak virus, they were not aware of mycotoxins;

as a result, they were being exposed to high levels of mycotoxins through their disposal methods of rotten maize. High yield, drought tolerance, and earliness, as well as disease and pest resistance were the most important farmer-preferred traits that must be taken into account when designing an ear rot resistant maize variety. In order to increase farmers' utilisation of improved maize varieties, breeders must adopt two maize ideotype breeding approaches to address stress and market needs. The former must have drought tolerance, MSV tolerance, ear rot resistance, resistance to storage pests, tolerance of low fertility, and early maturity traits. The latter should possess the traits of high yield, good taste, flint texture, large cobs, and large grain size.

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APPENDIX 1

1. Location and dates of the meetings

1.1. Arrangement of Participatory Rural Appraisals

November 2005	District Agricultural Office (DACO) – Lusaka
December 2005	DACO – Chongwe
January/February 2006	DACO – Chibombo and DACO-Kafue

1.2 Participatory appraisal

Date	Place	PRA site/area
December 2005	Mrs Sakala's homestead	Barlastone
March 2006	Cooperative hall	Kalimansenga
March 2006	Nutrition Centre, Apostolic Church	Kasaka
March 2006	Mulabo Community School	Mulabo-kakunka

2. Checklist of topics and semi-structured questionnaire

2.1. Maize ear rot – is this a problem?

2.2. Extent? Yield loss ascribed to this disease?

2.3. Any farmer description of symptoms?

2.4. How does this disease fare in comparison to other constraints?

2.5. Awareness of mycotoxins?

2.6. What factors do farmers ascribe as cause of ear rots?

2.7. Other production constraints (list and rank – all including ear rots)?

2.8. Maize varieties grown and criteria for selection (list and rank)?

2.9. Traditional varieties? New varieties vs. local?

2.10. Any farmer selection practices? Seed storage?

2.11. Awareness of resistant varieties?

2.12. Farmer coping strategies?

2.13. Management of ear rots?

2.14. Other production constraints?

2.15. Opportunities available for overcoming these problems?

2.16. By farmers – coping strategies and best for improvement (list)?

2.17. Maize breeding opportunities (researchers)?

2.18. Any maize production problems that require attention?

CHAPTER 3

A SURVEY OF PRE-HARVEST EAR ROT DISEASE OF MAIZE AND ASSOCIATED MYCOTOXINS IN SOUTH AND CENTRAL ZAMBIA

3.0 Abstract

Maize ear rots reduce grain yield and quality, and some of the disease-causing fungi produce mycotoxins in maize grain, which pose a health risk to humans and livestock. Unfortunately, ear rot and mycotoxin infection levels in grain produced by the small-scale farming sector, especially in Zambia, are unknown. A survey of maize ear rot was thus conducted on 114 farmsteads randomly sampled from 11 districts in Lusaka and the southern provinces of Zambia during 2006. A total of 10 randomly selected cobs were examined per farmstead and the ear rot disease incidence and severity were estimated on-site. This was followed by standard seed health testing for percent disease incidence and severity. A visual assessment for ear rot was initially made on-site, followed by the standard seed health testing procedure for fungal isolation in the laboratory. Predominant among the ear rots observed were Fusarium and Stenocarpella (Diplodia). The incidence of *F. verticillioides* ranged from 2 – 21.2%, while that of *S. maydis* ranged from 3 – 37.1%. The mean rank of fungal species, from highest to lowest, was *F. verticillioides, S. maydis, Aspergillus significant flavus, F. graminearum, A. Niger, Penicillium* spp*., Botrydiplodia* spp*. and Cladosporium* spp.. Although not significant (P>0.05), Fusarium and Diplodia ear rot infections were positively correlated with some macro-climatic data in the study area. The direct competitive ELISA-test on 90 samples indicated higher levels of fumonisins than aflatoxins and there was no deoxynivalenol in pre-harvest maize grain samples. The concentration of fumonisins in the maize samples from six districts was 10-fold higher than the FAO/WHO daily maximum intake of 2ppm; while the aflatoxin concentration in samples from two districts was far higher than 2ppb which is recommended by FAO/WHO. The study therefore suggested that consumer sector might be exposed to mycotoxins as a result of the high incidence of ear rot infections in the maize grain.

Keywords: *Aspergillus flavus*, Aflatoxin, disease incidence, disease severity, *Fusarium vertcillioides*, Fumonisins, maize, *Stenocarpella maydis*, visual assessments

3.1 Introduction

Ear rot occurs worldwide wherever maize is grown, reducing yield and quality (Kommedahl and Windels, 1981). Approximately 19 genera of fungi species, among them *Fusarium* spp*., Aspergillus* spp., *Penicillum* spp*.* and *Stenocarpella* spp., affect maize (Williams and McDonald, 1983; Payne, 1999). Toxicological investigations of naturally or artificially infected maize have shown that some of these fungi produce mycotoxins in maize (Logrieco et al., 2002). The occurrence of mycotoxins in pre-harvest maize is of great concern, because they cause health disorders in both human and livestock grain consumers (Munkvold and Desjardins, 1997; Miller, 2001). Under certain environmental conditions, maize grain is infected by ear rot fungi which produce mycotoxins such as fumonisins (B1 and related fumonisins), deoxynivalenol (DON), and zearalenone, which are produced by *Fusarium* spp. (Edwards, 2004); aflatoxins and Ochratoxin A by the *Aspergillus* species (Campbell and White, 1995; Wagacha and Muthomi, 2008); Ochratoxin A by *Penicillium* spp. (CAST, 2003); and diplodiotoxin by *Stenocarpella spp.* (Olatinwo et al., 1998). The *Fusarium graminearum* species produces vomitoxins in the host (Bennet et al., 1988; Payne, 1999; Sutton, 1982).

High levels of ear rot infection and mycotoxin accumulation have been reported in pre-harvest maize in Europe, North and South America, and Asia (MacDonald and Chapman,1997; Vigier et al.,1997; Logrieco et al.,2002;), South Africa (Rheeder et al., 1992), East Africa (Kedera et al., 1994; Bigirwa et al., 2007) and Malawi (Kapindu et al., 1999). Mycotoxins, the toxic secondary metabolites produced by ear rot fungi in their host, are known to contaminate maize before harvest or under post-harvest conditions (CAST, 2003). According to Fandohan et al. (2005), the climatic conditions of most tropical countries such as high temperatures, high rainfall, late rains during harvest and flash floods may lead to increased fungal proliferation and the production of mycotoxins. The Food and Agricultural Organization of the United Nations (FAO) has estimated that up to 20 – 25% of the world's food crops are contaminated with both pre-harvest and post-harvest mycotoxins (Adams and Motarjemi, 1999). Lack of public awareness, scarcity of food and lack of regulatory mechanisms have been advanced as some of the reasons why in Africa, the problem of mycotoxins is not given adequate attention. Among the groups of mycotoxins, two that have received some attention by African scientists in the last two decades are Aflatoxins and fumonisins (Bankole and Adebanjo, 2003). Surveys conducted have revealed high levels of these two mycotoxins in maize (Kedera et al., 1994; Doko et al., 1995; Bigirwa et al., 2007). The joint FAO/WHO Expert Committee on Food Additives (JFECFA) has set the provisional maximum tolerable daily intake of 2 µg/kg of body weight/day while in the U.S.A, the Food and Drug

Administration (FDA) has set industry guidelines for levels of fumonisin acceptable in human food and animal feed, with a recommended total fumonisin of 2ppm (FAO, 2004).

In Zambia, very little information exists on the occurrence of ear rot and mycotoxins in maize (Doko et al., 1995; Schjøth, 2008), though the disease has previously been ranked among the top three important maize diseases and the second most important in Zambia after the maize streak virus (Rao et al., 1987). A survey conducted in 1981 by Naik et al. (1982) in the central, southern and eastern provinces of Zambia involving 15 commercial growers indicated *F. moniliforme* (syn.= *F. verticillioides*) to be the most prevalent maize seed-borne fungus, followed by *F. graminearum* and S*. macrospora* (syn=*Diplodia macrospora*). In 2005, Nawa (2005, unpubl.) reported a 10 – 50% yield loss due to ear rots among small-scale farmers' crops in Lusaka west and Mumbwa district in Central Province.

Apart from environmental factors (Miller, 2001), farmer practices/management of the crop may influence the occurrence of ear rot (Bilgrami and Choudhary, 1998). Though most small-scale farmers might recognise the disease, they may not have a clear understanding of it. Factors that influence the incidence and severity of the disease in this sector are not well understood. The overall objective of this study was to determine the relative importance of maize ear rot and mycotoxin contamination in maize grain in the smallholder farming sector in central and southern Zambia. The specific objectives were to:

i. identify the fungal microflora associated with maize ear rot infection on smallholder farms in south and central Zambian,

ii. assess the incidence and severity of maize ear rot infection on smallholder farms and,

iii. determine the mycotoxin levels in maize kernels from smallholder farms.

The study tested the hypothesis that there are high levels of fungal and mycotoxin contamination in maize grain from smallholder farms in Zambia.

3.2 Materials and methods

3.2.1 General features of the study area

The 11 districts covered in the survey (Figure 3.1) were spread out over two provinces. Four of these districts are in Lusaka Province: Chongwe, Kafue, Luangwa and Lusaka. The other seven districts are in Southern Province: Kazungula, Choma, Sinazongwe, Monze, Namwala, Itezhi tezhi and Mazabuka.

Three of the districts surveyed, Kazungula, Sinazongwe and Luangwa lie wholly in agroecological zone one (AEZ I). The southern parts of Kafue and Choma, and the eastern part of Chongwe, are also in this zone (Figure 3.2). AEZ I is generally a dry area with less than 800mm annual rainfall, and often has recurrent droughts. The soil conditions are dominated by the Zambezi-Luangwa rift valley, which has solonitzi soil with a steep slope, semi-arid plain and sandy soil (MAFF, 2001). The growing season is 80 to 120 days. Lusaka, Chongwe, Kafue, Mazabuka, Monze, Choma, Namwala and Itezhi tezhi are in agro-ecological zone II (AEZ II) (Figure 3.2). AEZ II is characterised by an annual rainfall in the range of 800 mm and 1000 mm with a growing season of 90 to 150 days. It includes the plateau areas with elevations between 1000 and 1520 m. The area is part of the most productive region in the country for both food and cash crops. In general, the climate of these two provinces is favourable for different forms of agriculture, with an abundance of arable land.

The selection of the districts was based on their location, i.e., in potentially high maize growing region therefore have a high level of distribution of both local varieties (landraces) and improved maize varieties due to increased maize production activity. In the sample area, all of the districts are accessible by all-weather roads. Individual farmsteads were randomly selected within the district and were spaced about 5 – 20 km apart.

A global positioning system (GPS) data recorder was used to determine altitude, longitude and latitude data for the areas surveyed. Rainfall, relative humidity and temperature data were collected from the Department of Meteorology, Ministry of Transport and Communications, Lusaka. The agro-climatic data were clustered and grouped together to define the environmental groupings for the occurrence of maize ear rot.

Figure 3. 1 Map of Zambia showing the districts surveyed for maize ear rots and mycotoxins during 2006 (shaded green).

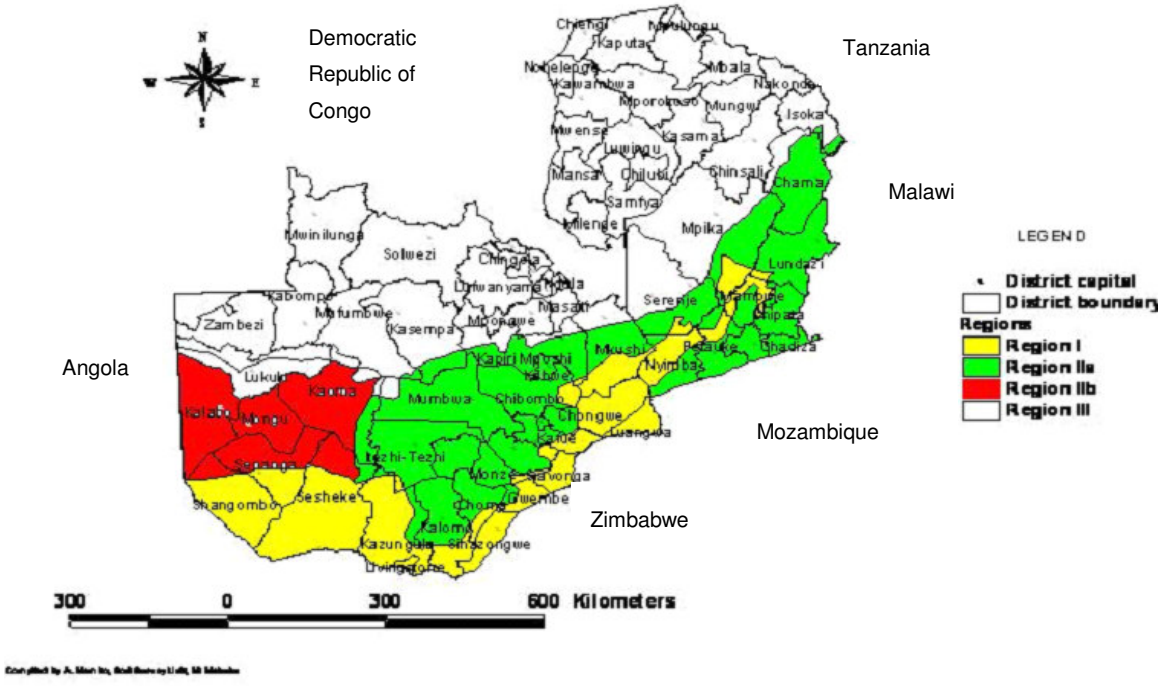

Figure 3. 2 Map of Zambia showing the agro-ecological zones. (Source: Soil Survey Unit, Mount Makulu)

Table 3.1 Macro-climatic information of the districts surveyed in 2006

District	Altitude (m)	Latitude (South)	Longitude (East)	Rainfall† (mm)	Relative humidity (%)	Mean temperature (°C)
Choma	1274	16° 46.67	27° 46.67	913.6	73.1	22.3
Chongwe	1095	15° 23.83	29° 29.60	946.1	90.2	22.5
Itezhi tezhi	1006	15° 43.53	26° 13.16	921.1	87.7	27.0
Kafue	1095	15° 41.93	28° 14.85	906.7	71.1	23.0
Kazungula	1024	17° 44.06	25° 44.17	865.8	57.3	24.5
Luangwa	341	15° 37.64	30° 17.25	445.2	51.3	31.0
Lusaka	1224	15° 21.69	28° 13.99	893.8	75.3	22.3
Mazabuka	1073	15° 55.35	27° 57.66	1114.0	92.6	23.4
Monze	1186	16° 24.25	27° 28.07	790.8	68.5	23.9
Namwala	993	15° 63.21	26° 36.44	895.0	68.1	23.2
Sinazongwe	507	17° 16.92	27° 27.26	505.5	51.3	29.5

Source: Department of Meteorology, Lusaka. †During the growing season.

Based on macro-climatic data, i.e. rainfall, temperature and relative humidity (RH), collected from the Department of Meteorology (Table 3.1), the 11 districts were clustered in four groupings, namely, group 1: Luangwa and Sinazongwe; group 2: Kazungula; group 3: Monze, Namwala, Kafue, Choma and Lusaka; and group 4: Chongwe, Itezhi tezhi and Mazabuka.

3.2.2 Maize sample collection

Grain samples were collected during April and May 2006. One hundred and fourteen (114) samples were collected from the 11 districts. A minimum of three villages per district was sampled. The farmsteads were spaced approximately 20km apart. Each sample consisted of 10 randomly selected cobs with five seemingly healthy (asymptomatic) and five infected (symptomatic) ears were of variable size and grain texture (semi-dent to dent) but mostly white.. The maize ears were picked from the farmers' fields when the crops were dry or from the freshly harvested unshelled maize crops heaped at the homestead before storage. To motivate and maintain farmers' cooperation during the study, they were offered a packet of salt in return, hence avoiding any reservations regarding the sampling of 'healthy' cobs.

The cobs were put in paper bags and taken to the Plant Protection Laboratory at Mount Makulu Central Research Station for evaluation of the fungal flora and a mycotoxin analysis. Each sample was accompanied by a collection form (Appendix 1) which was designed to capture information on some of the basic agronomic practices of the farmer in relation to maize production and ear rot infection. The samples were deep frozen to avoid any increase in fungal and mycotoxin accumulation until analysis.

3.2.3 On-farm visual assessment

Maize ear rot infections were evaluated on site based on the symptoms or nature of damage (Figure 3.4). The incidence of infected cobs per farmstead was calculated using the following formula:

Cob rot incidence = 100 (x / N)

where:

x = the number of infected cobs with a rating of 2 or more

N = total number of cobs in maize sample

Disease severity (DS) of cob rot (combined) in each ear in the sample was assessed using the disease rating of Reid et al. (1996) as follows:

1 = 0% infection

2 = 1 to 3%

3 = 4 to 10%

4 = 11 to25%

5 = 26 to50%

6 = 51 to75% and

7 = 76 to100%

A modified disease severity index (DS) of Silva et al. (2007) was used to express the severity of the cob rot diseases from each farmstead.

The mean scale values were retransformed into percentage DS by the equation:

$DS = (n_1 \times 0 + n_2 \times 2 + n_3 \times 7 + n_4 \times 18 + n_5 \times 38 + n_6 \times 63 + n_7 \times 88)/N$ (Silva et al., 2007)

Where: n_1 to n_7 are the number of ears with scale values 1 to 7, which are multiplied with the mean percentage of the rating class scale; and N is the total number of cobs in the sample.

Figure 3. 3 The rating scale used to score the visibly diseased maize on site

3.2.4 Maize kernel rot assessment

Each maize sample from a homestead was shelled, kernels mixed thoroughly and percent moisture content determined (using the Dickey John moisture meter).. From the homogenous mixture of grain, three sub samples of 100 kernels each were randomly picked and the percent kernel rot determined by counting the number that were seemingly healthy and those that were not (Figure 3.4).

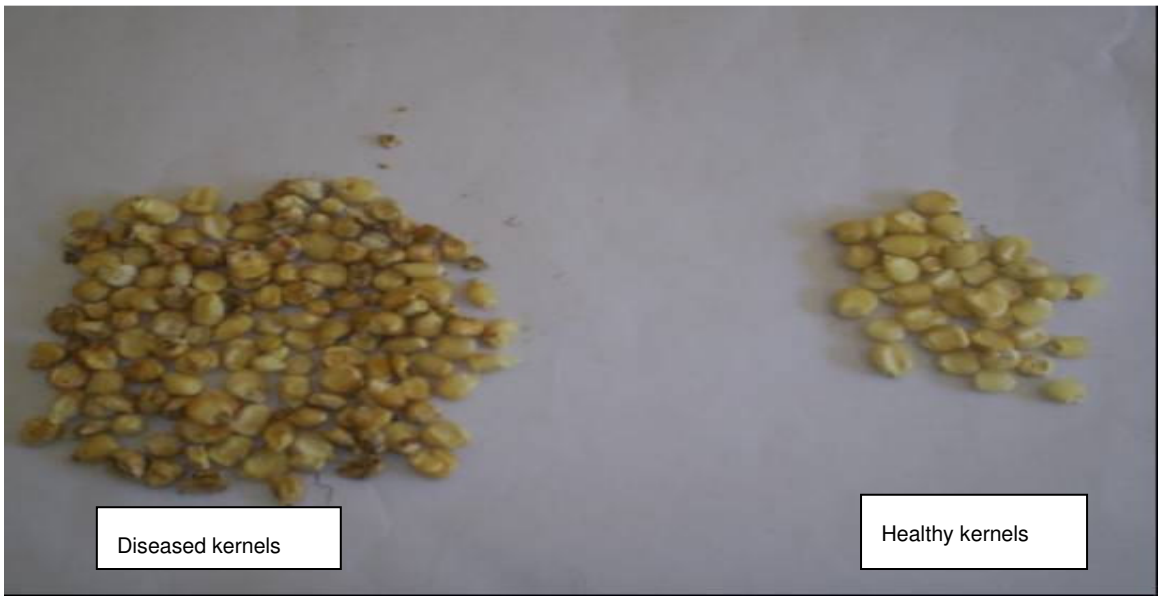

Figure 3. 4 Visibly diseased kernels and "seemingly" healthy kernels shelled from an ear rot infected maize cob

3.2.5 Maize ear rot identification and isolation

Hundred maize kernels were drawn out at random from the mixed shelled grain from previously identified mouldy and healthy ears from each farmstead. The maize kernels were examined by the blotter method for infection by fungal pathogens (Mathur and Kongsdal, 2003). The kernels were surface-disinfected for 1 min in 3.5% NaOCl, then rinsed twice in sterile, distilled water. Thirteen maize kernels were plated on moist, sterile filter paper in plastic Petri plates, 14 cm in diameter. The plated kernels were incubated at 25℃ $\pm$ 2℃ for 7 to 10 days. The shorter period ensured that the colonies did not overgrow, which could make it impossible to distinguish the kernels developing a fungal colony. All cultures that developed from the kernels were identified initially on the plates.

A stereo-microscope (16x to 40x) and, occasionally, the compound microscope were used to identify the maize ear rot fungi up to species level, with the help of the relevant taxonomic books (Barnett and Hunter, 1972; Toussoun and Nelson, 1976; Mathur and Kongsdal, 2003). For further identification, some of the cultures were transferred to a ¼-strength Potato Dextrose Agar (PDA) medium for single spore isolation and then incubated for 5 – 7 days at 25℃ ± 3℃. The morphological and cultural characters, i.e. the pigmentation of aerial mycelium and of the media, and the extent of mycelial growth, shape and size of macroconidia, nature of conidiogenous cells, the presence/absence of macroconidia, chlamydospores and perithecia, were used to identify the species.

The percent fungus abundance for each farmstead was calculated according to the formulae of Kapindu et al. (1999), as the number of maize kernels infected by a particular fungal species divided by the total number of seeds plated out and multiplied by 100, as follows:

Pathogen abundance (%) = Y x 100/N

where:

Y = number of plated grains from which a fungus is isolated/identified

N = total number of plated grains

The values were computed for each farmstead.

3.2.6 Mycotoxin analysis

A maize sample from each farmstead was thoroughly mixed and freeze-dried until analysis. A 50 g sub-sample was ground to fine powder in a Romer Series–mill (Romer Labs, Singapore). When 75% of the sub-sample had passed through a 20 mm mesh screen, it was judged to be thoroughly mixed. From each ground sub-sample, 20 g was taken for each mycotoxin analysis. Aflatoxins, fumonisins and deoxynivalenol (DON) were extracted in 5 ml of methanol/water (1:5) using end-over-end mixing for 1 h followed by centrifuging for 5 min at 2000 rpm in a table-top centrifuge, and filtered through no.1 Whatman filter paper. The concentration of aflatoxins, fumonisins and DON in the filtrates was determined by direct competitive enzyme-linked immunosorbent assay (CD-ELISA) procedure using the test kits provided by the manufacturer (AgraQuant Assay, Romer Labs). The concentration of aflatoxins in the maize samples was quantified by a test kit with the quantification range 1 – 20 ppb, while the concentration of fumonisins was 0.25 – 5 ppm. The former had a minimum detection limit of 1ppb and the latter of 0.2ppm. A total of 42 samples and 6 standards (0 – 20 ppb for aflatoxins and 0 – 5 ppm for fumonisins)

were run in any one experiment. The range and mean for aflatoxins (ppb), fumonisins and DON (ppm) for the samples were computed. For quantities higher than the detection range, extrapolation was done using an assay spreadsheet from the supplier, Romer Labs, Republic of South Africa.

3.2.7 Data analysis

The maize ear rot incidence and severity, grain moisture content, fungal species abundance and mycotoxin concentration were analysed separately using Genstat Statistical Programme 11th Ed (Payne et al., 2008). The means were separated using a Least Square Difference test for significance at P=0.05. Responses from the collection form were analysed by the Statistical Program for Social Sciences (SPSS) program version 15. Pearson's correlation coefficient was calculated to determine the relationship between maize ear rot infection and macro-climatic data.

3.3 Results

3.3.1 Maize varieties grown in the survey areas

Several maize varieties were grown in the study area during 2005/6. Hybrid was the most dominant type. (Table 3.2). Among the more common ones were the Zamseed, Seedco and Pannar varieties. The landraces were "Ghankata" and "ilisuba" while MMV 400, Pool 16 and MMV 600 were the only open-pollinated varieties grown.

Table 3.2. The variety class grown in the study areas during 2005/6 season

District	Percent used		
	Open-pollinated varieties	Hybrid maize varieties	Landrace (local)
Choma	4.2	70.7	25.1
Chongwe	0.0	66.7	33.3
Itezhi tezhi	15.3	65.4	19.2
Kafue	27.7	55.7	16.6
Kazungula	9.1	54.6	36.3
Luangwa	11.7	76.6	11.7
Lusaka	0.0	73.3	26.7
Mazabuka	0.0	100.0	0.0
Monze	12.6	62.4	25.0
Namwala	0.0	76.5	23.5
Sinazongwe	8.7	69.6	21.7
Mean	4.2	70.7	25.1

3.3.2 Ear rot infection ratings

Maize samples from the 11 districts differed significantly ($P<0.05$) for grain moisture, percent ear rot incidence and severity, and farmer-estimated grain loss per hectare (Table 3.3). The healthy maize samples had moisture content of between 10.6 and 29.6% while the mouldy maize had moisture content of between 13.6 and 32.3%. The mean grain moisture content was highest in the healthy maize from Lusaka (21.78%), followed by Mazabuka (20.05%), and the lowest was from Namwala (16.43%). Among the mouldy or visibly diseased maize, the highest moisture content was found in the maize samples from Mazabuka and the lowest was in the grain from Kazungula.

The average incidence of ear rot was 70.2% (Table 3.3). The highest incidence occurred in Monze (100%) and the lowest in Kafue (57.8%). Maize ear rot was most severe in Lusaka, at 32.1 %, followed by Sinazongwe, at 28.7%, and least severe in Luangwa, at 6.1%. There were no significant differences ($P>0.05$) observed for percent kernel infection among the districts. According to the farmers' estimates, the highest grain yield

loss due to ear rots was in Choma, followed by Itezhi tezhi, and the lowest was in Monze).

Table 3.3 Mean maize ear rot infection in 11 districts in Zambia

District	No. farmers	Maize cobs/ sample	Grain moisture		Percent ear rot		Percent kernel	Est. grain loss†
			Healthy maize	Mouldy Maize	Incidence	Severity		
Choma	15	9	20.5	22.5	74.8	15.7	32.7	13.33
Chongwe	16	10	19.0	22.3	61.7	22.6	27.6	7.29
Itezhi tezhi	10	10	17.1	19.3	83.1	22.1	27.0	8.69
Kafue	17	9	16.5	18.8	56.7	14.3	28.1	6.43
Kazungula	15	10	15.4	17.5	76.8	9.9	25.1	7.03
Luangwa	6	10	20.0	22.1	68.1	6.1	19.8	7.02
Lusaka	10	10	21.7	22.5	61.2	32.1	34.8	3.1
Mazabuka	10	9	20.1	23.2	71.0	11.8	28.6	5.0
Monze	3	10	18.9	21.9	100.0	10.5	22.8	2.2
Namwala	3	10	16.4	21.8	60.0	15.7	22.7	15.2
Sinazongwe	5	10	19.2	23.1	92.0	28.7	41.3	6.2
Mean			18.5	21.0	70.2	17.2	28.7	7.0
LSD (0.05)	114		4.2	3.9	26.1	15.7	19.2^{ns}	8.4
CV%			18.4	15.4	29.9	48.4*	15.*4	14.1*

† Farmers' estimates; *Angular transformed data.

3.3.3 Types of ear rot diseases

Eight different ear rot diseases were identified from 114 maize samples (Table 3.4). Significantly high incidences of *Fusarium* ear rot ($P<0.05$) were observed in different districts, followed by *Stenocarpella*, *Aspergillus*, black kernel and red ear rot. Other less abundant ear rot infections were *Cladosporium*, *Nigrospora* and *Penicillium*. *Fusarium* was the most common ear rot disease in all of the 11 districts, except for in Itezhi tezhi, Kafue, Lusaka and Luangwa, where *Stenocarpella* ear rot was the most dominant disease (Table 3.4). The highest incidence of *Fusarium* ear rot was in a sample collected from Monze (48%), followed by Mazabuka (41.6%). It was least prevalent in Luangwa (12.1%). The incidence of *Stenocarpella* ear rot was higher in Monze (39.5%) and the lowest was in Namwala (3.3%). While there were significant differences in the incidence of *Aspergillus* infection among the 11 districts, these were low compared to *Fusarium* and *Stenocarpella* ear rot. The incidence of *Aspergillus* ear rot was highest in Kazungula (14.4%) and lowest in Choma (0.5%).

There were also significant differences ($P<0.05$) in the severity of *Aspergillus*, *Stenocarpella*, black kernel and red ear rots among the different districts (Table 3.5). *Stenocarpella* ear rot was most severe in Lusaka (23.6%) followed by Itezhi tezhi (14.1%) and least severe in Namwala (1.3%). *Aspergillus* ear rot infection was most severe in Kazungula (6.93%), followed by Luangwa (1.86%). No *Aspergillus* ear rot was recorded in Mazabuka and Namwala districts. Red rot was significantly higher in Namwala (17.3%), followed by Sinazongwe (8.8%), and least severe in Kafue (0.3%). Although not significant, *Fusarium* ear rot was more severe in Mazabuka (16%), followed by Monze (9.6%) and lowest in Luangwa (3.7%). There were low but insignificant DS and DI for the other ear rot diseases of *Cladosporium*, *Nigrospora* and *Penicillium* in all the districts surveyed.

3.3.4 Correlations between disease incidence and climatic data

Significant correlations existed between the incidence of *Aspergillus* ear rot and rainfall, relative humidity and temperature; and between the incidence of black kernel rot (caused by *Botryodiplodia theobromae*) and rainfall and temperature (Table 3.6). Although not significant, positive correlations were observed between the incidence of *Fusarium* and *Diplodia* and high altitude, rainfall and humidity. However, unlike *Fusarium* and *Diplodia*, higher temperatures favoured a high incidence of *Aspergillus* ear rot and black kernel rot. A highly significant correlation ($P<0.05$) was observed between *Fusarium* ear rot severity

Table 3.4 The mean percentage incidence of maize ear rot diseases in 11 districts in southern and central Zambia during 2005/6 season

District	*Aspergillus*	Black kernel	*Cladosporium*	*Stenocarpella*	*Fusarium*	*Nigrospora*	*Penicillium*	Red rot
Choma	0.5	1.72	2.61	22.8	25.9	2.1	1.11	8.5
Chongwe	3.8	0.7	0.7	14.0	20.5			2.3
Itezhi tezhi	2.1	2.1		31.9	31.8	2.2	1.1	4.1
Kafue	1.1	0.5	1.1	23.1	21.2	1.1	1.6	1.1
Kazungula	14.4	1.3	1.3	12.1	24.7	4.5	1.7	0.6
Luangwa	5.5	6.6	1.1	18.6	12.6	1.4		3.3
Lusaka	2.9		0.0	32.9	20.4	1.0	1.1	2.8
Mazabuka		1.8	1.3	21.7	41.6	1.6		4.6
Monze	4.3	4.3		39.7	48.0			4.3
Namwala				3.3	13.3			16.7
Sinazongwe	12.4	6.4	6.4	4.0	19.8	2.2	3.8	8.2
Mean	4.1	1.8	1.3	20.6	25.0	1.7	0.9	4.0
$LSD_{0.05}$	8.8	4.5	4.6	19.9	20.3	6.7	5.3	7.4
CV%*	27.6	21.8	24.9	21.8	18.0	22.6	48.0	33.3

*Angular transformed data

Table 3.5 The mean percentage severity of maize ear rot diseases in 11 districts in southern and central Zambia during 2005/6 season

District	Aspergillus	Black kernel	Cladosporium	Stenocarpella	Fusarium	Nigrospora	Penicillium	Red ear rot
Choma	0.1	0.1	0.7	8.6	6.5		0.2	3.1
Chongwe	1.3	0.1	0.1	8.8	7.1			2.9
Itezhi Tezhi	0.9	0.4		14.1	5.9		0.7	1.6
Kafue	1.2	0.1	0.2	12.7	9.4		0.5	0.3
Kazungula	7.0	0.1	0.3	11.5	6.1	1.0	0.4	1.1
Luangwa	1.9	0.9	1.0	9.4	3.7		0.3	1.6
Lusaka	0.2			23.6	7.6		0.1	1.8
Mazabuka		0.1		8.4	16.0			1.0
Monze	0.3			10.7	9.7			1.7
Namwala				1.3	6.3			17.3
Sinazongwe	4.0	0.4	0.4	2.8	4.6			8.9
Mean	1.7	0.2	0.3	11.1	7.7	0.01	0.3	2.4
$LSD_{0.05}$	4.8	0.6	1.3	14.1	9.8	1.3	1.5	4.7
CV%*	54.1	29.1	27.1	57.6	47.9	25.6	29.4	59.3

* Angular Transformed data

Table 3.6 Correlation matrix for agro-climatic conditions and incidence of maize ear rot diseases

Parameter	*Aspergillus*	Black kernel	*Cladosporium*	*Stenocarpella*	*Fusarium*	*Nigrospora*	*Penicillium*	Red ear
Altitude	-0.49	-0.51	-0.41	0.49	0.43	0.00	-0.06	-0.05
Rainfall	-0.54	-0.59*	-0.45	0.26	0.40	0.03	-0.15	-0.04
Relative humidity	-0.67*	-0.29	-0.39	0.41	0.39	-0.05	-0.09	-0.12
Mean temperature	0.45	0.50	0.28	-0.18	-0.14	0.11	0.07	-0.06

**, * Correlation coefficient significant at *P≤0.05* and *P≤0.01,* respectively.

and rainfall and, though not significant, with relative humidity (Table 3.7). In contrast to other relatively minor ear rots, black kernel rot was highly correlated with temperature, rainfall and altitude while relative humidity was negatively correlated with severity of *Aspergillus* ear rot

3.3.5 Ear rot fungus analysis

Fifteen fungal species were isolated from the maize kernels taken from the 11 districts (Tables 3.8 and 3.9). There were significant differences ($p<0.05$) in a number of fungal species isolated from both the mouldy and healthy maize samples. *Stenocarpella maydis* and *F. verticillioides* were the most commonly isolated fungal species. The other fungi recorded all averaged less than 5% in mouldy kernels except for *F. graminearum, Penicillium spp.* and *Aspergillus spp.* (*A. flavus* and *A. niger*). The order of abundance in mouldy kernels was *S. maydis* > *F. verticillioides* > *F. graminearum* > *Penicillium* > *A. flavus* > *A. niger* > *C. sphaerospermum* > *Nigrospora spp.* > *Rhizopus* > *F. solani* > *B. theombrae* > *F. subglutinans*. The highest number of fungal species was isolated from maize samples from Luangwa and Sinazongwe (10 species, followed by Choma, 9) while the lowest number was recorded from Chongwe and Namwala (4). The highest number of *S. maydis* isolations from visibly diseased maize grain was found in samples from Monze (37.09%), followed by Kafue (30.0%).

With regard to asymptomatic or healthy kernels, the highest number of fungal species was in samples from Kazungula (8), followed by Choma and Namwala (6), while the lowest number was found in samples from Mazabuka (1) (Table 3.9). Among the fungal species, *F. verticillioides* (4.7%), followed by *A. flavus* (3.64 %), were the most abundant fungal species (Table 3.8). A significantly high number of *F. verticillioides* isolations were found in samples collected from Kazungula (7.12%), followed by Kafue (7%). The least number of isolations was found in samples from Monze. The order of abundance in the healthy kernels was *F. verticillloides* > *A. flavus* > *S. maydis* > *F. Subglutinans* > *A. strictum* > *F. graminearum* > *B. theobromae* > *Penicillium spp.* > *C. Sphaerospermum* > *A. niger*.

Table 3.7 Correlation matrix for agro-climatic conditions and severity of maize ear rot diseases

Parameter	*Aspergillus*	Black kernel	*Cladosporium*	*Diplodia*	*Fusarium*	*Nigrospora*	*Penicillium*	Red ear
Altitude	-0.42	-0.84**	-0.49	0.31	0.44	-0.18	-0.06	-0.20
Rainfall	-0.38	-0.72*	-0.59*	0.35	0.65*	-0.27	-0.01	-0.16
Relative humidity	-0.58*	-0.40	-0.54	0.34	0.54	-0.29	-0.07	-0.25
Mean temperature	0.34	0.87**	0.35	-0.11	-0.44	0.34	0.37	0.01

*, ** Correlation coefficient significant at *P≤0.05* and *P≤0.01,* respectively.

3.3.6 Mycotoxins analyses

High levels of fumonisin, compared to aflatoxin, were detected in all the maize samples collected from the eleven districts surveyed (Figure 3.5). No deoxynivalenol was detected in the maize samples. The highest amount of fumonisins was detected in maize samples from Namwala (73.3ppm; range: 3.7 to 192ppm), followed by Sinazongwe (54.34; range: 33.5 to 58.8ppm). The highest pre-harvest accumulation of aflatoxins occurred in Luangwa (5.4; range: 0.2 to 10 pbb), followed by Chongwe (2.5ppb; range: 0.8 to 7.8pbb) The lowest was in Namwala (0.02ppb; 0.01 – 4ppb).

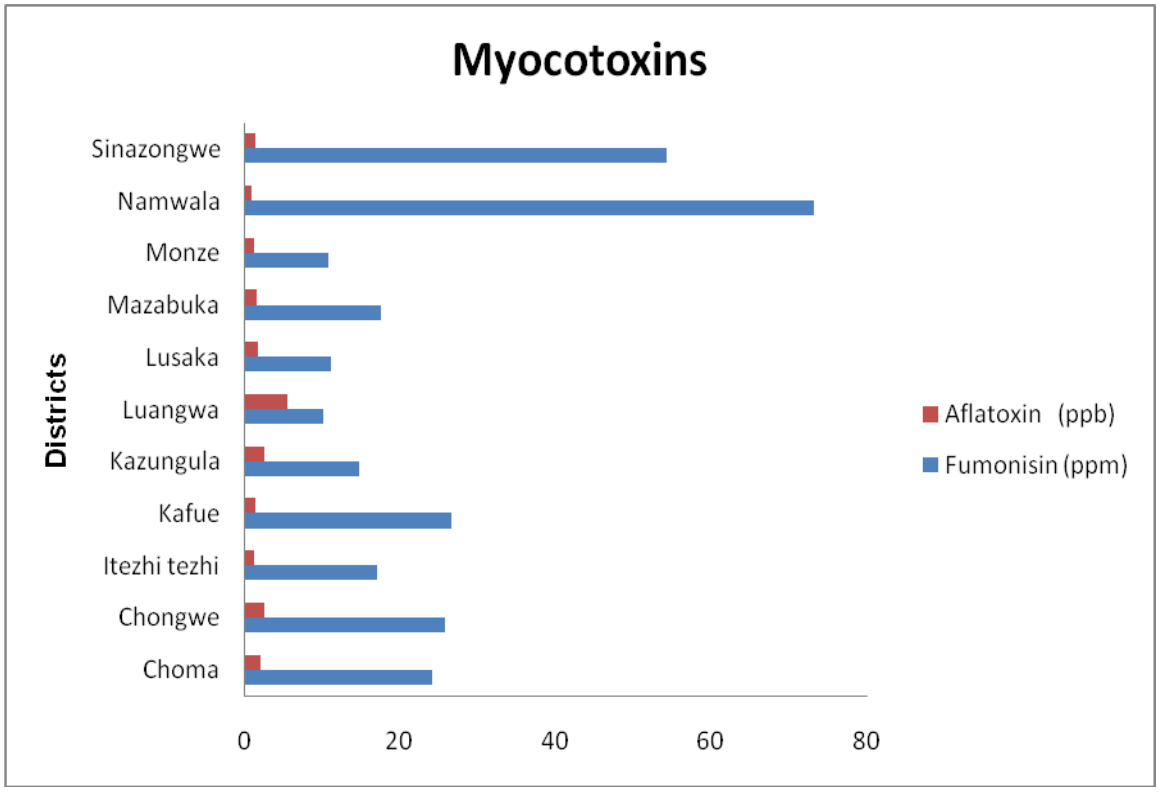

Figure 3.5 Level of mycotoxins in the survey of 11 maize-growing districts of Zambia in 2006 (*N=114*).

Table 3.8 Percent fungal species isolated in maize grain from the 11 districts during the 2005/6 season

Species	**Choma**	**Chongwe**	**Itezhi tezhi**	**Kafue**	**Kazungula**	**Luangwa**	**Lusaka**	**Mazabuka**	**Monze**	**Namwala**	**Sinazongwe**
Mouldy maize											
Acremonium strictium	4.0			2.0	4.0	8.0					
Aspergillus flavus	10.0		18.0	9.0	11.4	6.0	8.0	12.0		6.1	5.0
Aspergillus niger	7.5	6.0	24.0	6.0	12.0		6.0	8.5	6.0		
Bipolaris maydis	6.0		6.0						9.0		2.0
Botrydiplodia theobromae						6.0					12.0
Cladosporium Sphaerospermum				15.0	6.0	14.0					3.0
Fusarium graminearum	10.8	12.0		6.0	6.0	6.0	6.0				48.0
Fusarium solani	6.0		6.0						40.1		
Fusarium subglutinans						12.0					
Fusarium verticillioides	16.1	20.9	15.3	15.0	17.2	18.8	21.1	21.2	19.5	27.0	15.2
Nigrospora spp.						6.0					22.0
Penicillium spp.	12.0		8.0	8.0	6.0	11.0	15.0	12.0		18.0	6.0
Rhizopus spp.	4.5										24.0
Stenocarpella maydis	20.0	16.0	18.0	30.0	27.2	16.3	22.8	19.7	37.1	15.0	25.6
Trichoderma spp.							12.0				
Mean	6.9	8.85	7.4	9.1	9.4	6.8	9.2	9.2	12.9	10.3	8.7
LSD	1.2	2.4	1.6	1.8	1.8	2.2	2.7	1.2	4.5	3.0	2.0
CV%	39.2	37.7	35.6	41.9	39.3	41.4	37.2	48.5	47.0	47.1	40.2

[a] Evaluation used 100 kernels.

Table 3.9 Percent fungal species isolated from maize grain from the 11 districts during the 2005/6 season

Healthy maize	Choma	Chongwe	Itezhi tezhi	Kafue	Kazungula	Luangwa	Lusaka	Mazabuka	Monze	Namwala	Sinazongwe
Acremonium strictium	6.0					8.0				4.0	
Aspergillus flavus	3.3	4.7	2.7	2.0	6.0	4.0	18.0				3.0
Aspergillus niger							7.0				
Botrydiplodia Theobromae					8.0	6.0					-
Cladosporium Sphaerospermum					2.0					6/0	
Fusarium graminearum			4.7	6.0	2.0					2.0	
Fusarium subglutinans	10.0	2.0	4.0	8.0	5.5					6.7	
Fusarium verticillioides	5.6	4.3	4.7	7.0	7.1	4.0	5.4	4.0	2.0	6.0	2.0
Penicillium spp.	2.7	4.0			2.0	2.0					
Stenocarpella maydis	3.0		8.0	6.0	7.0				4.0	6.0	6.0
Mean	2.2	4.2	4.5	6.4	5.7	4.3	6.5	4.0	2.5	5.56	3.2
LSD	0.3	1.4	1.0	2.3	1.0	1.4	2.4	4.0	1.0	0.2	1.6
CV%	37.4	28.8	26.7	32.9	29.9	41.3	42.6	38.3	18.9	19.6	27.5

[a] Evaluation used 100 kernels.

3.4 Discussion

Several fungi were found to cause maize ear rot infections, the predominant ones belonging to the genera *Fusarium*, *Stenocarpella* and *Aspergillus. Fusarium verticillioides* followed by *S. maydis* were the most commonly isolated ear rot fungal species. Similar results have been reported elsewhere in tropical regions of Africa (MacDonald and Chapman, 1997; Kapindu et al., 1999; Bigirwa et al., 2006). Fewer fungal species were recovered from the symptomless or seemingly healthy maize kernels than the mouldy ones. However, this was an indication that seemingly healthy kernels were also infected but with fewer species. The main symptomless infecting fungi were *Fusarium* spp. (*F. verticillioides* and *F. Subglutinans*) and to a lesser extent *Stenocarpella* and some *Aspergillus* spp.

Symptomatic kernels were easy to differentiate, based on colour and damage to the ear with further confirmation using the blotter method, while with symptomless kernels, plating on potato dextrose agar (PDA) was needed. The mean incidence of ear rot fungi in mouldy maize samples (17.1%), was three times higher than in healthy maize (5.1%). This implied that on average there are more contaminants in diseased kernels than in healthy kernels. The presence of similar contaminants in both types of kernels indicated that with the right environmental conditions (Miller, 2001), ear rot epidemics could easily occur. The consistent recovery of *F. verticillioides* suggested that these fungi, compared to other maize ear rot fungi, had a high tendency of systemic colonization of the maize crop systematically. Similar observations were made by Naik et al (1982). Maize grain infected with this fungus with its characteristic brown or pinkish-red discolouration (MacDonald and Chapman, 1997) was found in significant amounts in samples from Kafue, Choma and Chongwe, showing a discrete preference for a more humid and wetter environment. Infection by *Aspergillus* spp., which results in blue-green or yellowish discolouration accompanied by ear insect damage (Payne, 1999), did not follow a clear pattern. This might be due to the study being conducted over – one season (2005/6) – and also the influence of ear insect damage was not considered in this study.

The results of the site-rating exercise indicated a predominance of *Fusarium* and *Stenocarpella* ear rot. These results agree with the findings of Naik et al. (1982) who reported a predominance of *Fusarium* ear rot in the maize from central, eastern and southern Zambia. The high incidence of *Fusarium* ear rots in this study also corroborate the previous findings of Schjøth (2002) who reported an incidence of 14 – 63% in the medium rainfall zone of Zambia, part of which was covered in this survey. These two ear rot fungal species are responsible for much of the grain loss experienced by farmers in southern and central Zambia. However, the incidence of these two diseases was not significantly correlated with the macro-climatic conditions observed during the 2005/6 season. The extensive geographical occurrence of these ear rots in Zambia raises serious concerns about crop loss and suggests that the local environmental conditions are

suitable for maize ear rot epidemics. Farmers interviewed in this study indicated yield losses of 10 – 40% due to ear rots.

The high grain moisture content found in the maize samples, attributed to continued rainfall towards the end of the harvest period, creates an optimal condition for increased ear rot infection and mycotoxin contamination in storage. Even though no clear relationship was established between the moisture content and maize ear rot infection in this study, it is apparent from the literature that once the kernels start to dry and harden, this creates a morphological barrier to the spread of infection from kernel to kernel (Reid and Sinha, 1998). It appears the difference in moisture content between the mouldy kernels and healthy kernels was due to increased fungal activity in the mouldy kernels.

It was observed that most of the farmers grew commercial dent-textured hybrid maize (69.1%) and very few open-pollinated varieties (4%). The high incidence of *Fusarium*, *Stenocarpella* and other ear rot diseases was an indication that the majority of commercial hybrid maize varieties do not possess the desired level of resistance to ear rots and mycotoxin accumulation. This reaffirms the suggestion made by Schjøth et al. (2008), who concluded that this vulnerability was due to the narrow genetic base of the main commercial varieties in the country.

High levels of fumonisins compared to aflatoxins, were detected in the maize samples from all 11 districts. The maize samples from 6 out of the 11 districts surveyed were found to be contaminated with more than 20ppm of fumonisins, while 3 districts had aflatoxin contamination of more than 2.5ppb. The high levels of these two mycotoxins were worrying as they are toxic to animals and are suspected human carcinogens (FAO, 2004). The FAO/WHO provisional daily maximum intake is 2ppb and 2ppm for aflatoxins and fumonisins, respectively. According to Wagacha and Muthomi (2008), once the maize crop is infected under field conditions, ear rot fungal growth and the associated mycotoxins continues un abated and with vigour during post-harvest and storage. This increases the chances that the farmers in these areas are exposed to even higher levels of both fumonisins and aflatoxins. Doko et al. (1995) found similarly high levels of fumonisins in their survey of a few commercial farms in Zambia. The mycotoxins analysis further suggests that the *Fusarium* strains isolated from the maize samples were high fumonisin producers. However, the occurrence of high levels of fumonisins and very low levels of aflatoxins in some districts was a consequence of the influence of macro climatic parameters had in creating particular ear rot epiphytotic conditions. It was evident that the more drier and drought conditions that characterise Sinazongwe, Luangwa and Kazungula favoured aflatoxin contamination that fumonisns. Betran and Isakiet (2004) found that the incidence and severity of pre-harvest aflatoxin is greater under such conditions. More humid areas are prone to fumonisins (Vigier et al., 1997). In addition, the occurrence of symptomless Fusarium kernel rot infection in most maize samples imply that people in these areas are consuming higher level of fumonisins.

Unless steps are taken to stem it, health problems associated with mycotoxins could be on the increase in these areas.

3.5 Conclusion

More than six ear rot diseases were identified in the maize samples with *Fusarium* and *Stenocarpella* ear rots being the most important in Lusaka and Southern provinces of Zambia. There was high prevalence of *F. verticillioides, S. Maydis* and *Aspergillus* spp. in maize samples collected from the 11 districts. These three together with *F. graminearum* were found to be the most common endophytes responsible for the maize ear rot observed in these areas.

High levels of fumonisins and aflatoxins were detected in the maize samples from the 11 districts. The concentration of fumonisins from the maize samples collected from 6 of the 11 districts was 10 times higher than the FAO/WHO daily maximum intake of 2ppm, while the concentration of aflatoxins from four districts was far higher than the 2ppb FAO/WHO recommended level.

The development of new maize varieties – both hybrid and open-pollinated varieties (OPVs) – with genetic resistance to *Aspergillus*, *Fusarium* and *Stenocarpella* ear rots is highly desirable, in order to minimise the risk of maize ear rot and mycotoxin contamination.

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APPENDIX 4.1

Maize sample collection form

Date……Respondent's full name……

Location/community/district……Agricultural block ……

Agricultural camp……

GPS coordinates……..

Enumerator ……..

Sex (M/F) 1. [ ] Male 2. [ ] Female

Status of the farmer 1. [ ] Head of household 2. [ ] Not head of household

What is the size of your farm? [ ] Hectares [ ] Acres (1 Acre = 0.4 ha)

What was the total area cultivated in 2005/6 season? [ ] hectares [ ] acres

Is maize the most important crop on your farm? 1. [ ] Yes 2. [ ] No

Do you grow maize every season? 1. [ ] No 2. [ ] Yes

What was the area under maize in 2005/6 season? Estimated size [ ]

What was the maize production in 2005/6?

(Yield/ha in 50 kg bags/ha) [ ]

What maize seed type do you use?

1. [ ] Hybrids 2. [ ] Open-pollinated varieties

3. [ ] Recycled 4. [ ] Local

Provide name(s) where possible_____

What varieties did you grow in 2005/6 season? (Give names and area planted)

Number of cobs collected:

Ear rot disease observed:

1. [...] Fusarium 2. [...] Stenocarpella 3. […] Aspergillus 4. [...] Penicillium

5. [...] Red rot 6. […] Black rot kernels 7. […] Others…….

Disease rating: (1-7); 1 = 0, 2 = 1-3%, 3 = 4-10%, 4 = 11-25%, 5 = 26-50%, 6 = 51-75%,

7 = completely rotten >75%

Number of cobs rated as 1……, 2……, 3……, 4……, 5……, 6……, 7…….

Thank you.

CHAPTER 4
FIELD EVALUATION OF INOCULATION TECHNIQUES FOR MULTIPLE EAR ROT RESISTANCE IN MAIZE

4.0 Abstract

Several inoculation methods exist worldwide; however, many are inconsistent over locations and years. The objective of this study was to evaluate existing ear rot inoculation techniques and modify them so that they would identify consistently sources of resistance to more than one ear rot disease affecting maize in Zambia. A series of field experiments were set up in the 2005/6 – 2007/8 seasons to compare the effectiveness of five inoculation techniques mainly colonised toothpick, ear top placement, leaf whorl placement, silk channel injection and suspension spray and their combinations in inducing ear rot disease conditions in three catergories of maize genotypes. These were inbreds, hybrids and Open-pollinated varieties (OPVs). These experiments used three fungal species, *Fusarium verticillioides*, *Stenocarpella maydis* and *Aspergillus flavus* and their mixture obtained from diseased maize grain. On average the disease severity ranged from 0.9 to 58%. The range being similar for all three fungal isolates and the mixture of fungi. The open-pollinated varieties (OPVs) had comparably lower disease severity and incidence than the hybrids and inbreds. The application of either individual ear rot fungal species or mixtures of ear rot fungi using colonised toothpick technique consistently led to higher infection; followed by leaf-whorl placement using colonised maize kernels or grit. High ear rot disease severity was observed during the off season compared to the main rain season. When these inoculation techniques were used in combination, colonized toothpick plus ear top placement did not differ significantly from leaf whorl-placement followed by either syringe injection or ear-top placement. Stability analysis indicated that colonized and leaf whorl placement were stable and consistent in at least in classifying three hybrids at two sites while correlation of the ranking order revealed only colonised toothpick plus leaf whorl placement ranked the genotypes consistently across two sites. This suggests that using of these two methods and mixture of ear rot fungi would effectively discriminate maize genotypes in early generation testing for combined resistance to major ear rots

***Keywords*:** *Aspergillus*, *Fusarium*, Varieties, Inoculation methods, resistance, *Stenocarpella*

4.1 Introduction

Worldwide, Aspergillus ear rot caused by *Aspergillus flavus* Link: Fr, Fusarium ear rot caused by *Fusarium verticillioides* (Sacc.) Nirenb. (Syn=*F.moniliforme*), and Stenocarpella ear rot caused by *Stenocarpella maydis* (Berk.) Sutton (=*Diplodia maydis* (Berk.) Sacc.) are serious diseases of maize (*Zea may* L.), causing extensive loss of grain yield and quality. Several inoculation techniques have been developed and used to evaluate maize germplasm for resistance to ear rots. These techniques include those that introduce the single fungal isolate inoculum through the husks and silks from a single inoculation (Silva et al., 2007) and those that use several isolates of ear rot fungi and inoculate twice or more (Clements et al., 2003) to select for resistance. The techniques are further sub-divided into non-wounding and wounding methods. The non-wounding methods involve the use of infested maize bran (Nowell, 1998; Rossouw et al., 2002) and suspension spray (Reid et al., 2002), while injurious methods include the use of a colonised toothpick, pinbar (Clements et al., 2003), and silk channel injection (Chungu et al., 1996). The availability of reliable methods for the screening and evaluation of maize genotypes for improved tolerance to ear rot infection is critical for maize breeding programmes that emphasise resistance to ear rots (Balconi et al., 2004). These methods must be able to induce sufficient disease pressure to discriminate repeatedly among the genotypes across locations and years, with different fungal species (Brown et al., 1999).

Several studies have been undertaken to compare some of these techniques (Tucker et al., 1986; Chungu et al., 1996; Clements et al., 2003). However, most of these studies have focused on methods that reproduce disease symptoms and permit the screening of germplasm for resistance to a single ear rot species only. Most of these, though initially effective in the environment in which they have been developed, have failed in other localities, resulting in increased variability in the results; some of the genotypes previously described as resistant have become susceptible.

Koehler (1960), working with *Fusarium* spp., found that inoculation methods involving wounding caused more ear rot than non-wounding methods. On the other hand, Boling et al. (1963) assessed many inoculation methods and found that pellets coated in *F. moniliforme* inoculum and injected into a maize cob produced remarkably high ear rot disease. Gulya et al. (1980) and Fajemisin (1987), both reported that the insertion of a colonised toothpick infected with *F. verticillioides* spores into the middle of the ear was successful in creating an epiphytotic condition that was etfective in differentiating resistant inbreds. Tucker et al. (1986) evaluated four inoculation techniques for *A. flavus* infection and found that the use of a pinbar was superior to a knife stab, while exposed kernel and silk inoculation permitted the discrimination of maize hybrids into resistance classes. Chungu et al. (1996) compared six inoculation techniques comprising wounding and non-wounding methods for resistance to *F. graminearum* and reported that the silk

channel and kernel-stab techniques were the best. Schaafsma et al. (1997) tested maize hybrids for resistance in the silk and kernels for *F. verticilloides* and found that the silk method resulted in better discrimination among hybrids than the kernel method. Rossouw et al. (2002) reported that placing 5 g of inoculum in the leaf whorl of the plant was effective in discriminating among 10 inbred lines for resistance to Stenocarpella. While all these methods have produced good results, the inconsistency associated with some of them has not fully been understood. Drepper and Renfro (1990) reported that environmental factors may affect the development of ear rot epiphytotic conditions even where artificial inoculation methods are used. They found that wound inoculation performed better in an environment that was unfavourable for natural disease development. This may imply relegating the use of wounding methods to unfavourable environments only.

Due to the multiplicity of ear rot pathogens, especially in a tropical environment and its unique set of environmental conditions, there is a need to appraise and modify existing inoculation methods if successful screening for resistance to ear rots is to be accomplished. Most inoculation evaluation experiments that have been conducted in the USA, Europe and South Africa have involved genetically uniform material such as inbred lines and hybrids (Silva et al., 2007) and the focus has been on screening for resistance to single ear rot fungi (Chungu et al., 1996; Reid et al., 1996). While this may be an ideal situation where a single species has been identified as the prime causal agent of an epidemic, as was the case in South Africa (Nowell, 1998), it may not be so in early generation testing, where resistance is sought in hundreds of breeding lines or where recurrent selection programmes involving genetically broad-based populations have been initiated. The cumbersome procedure of isolating a single pathogen maybe too costly and time consuming for a breeder at this stage. Hence a technique has to be found that could use a mixture of ear rot pathogens capable of discriminating effectively genotypes that are in the early stages of development is desirable. In Zambia, as in many sub-Saharan African countries, between 30 to 40% of the varieties are open-pollinated materials (Ristanovic et al., 1987; 1989; Howard and Mungoma, 1996; Zamseed, 2003) with variable responses to diseases. Therefore, the challenge is to identify inoculation techniques that would discriminate among inbreds, OPVs and hybrids, and at the same time avoid presenting a high number of escapees as resistant, hence compromising the genotypic response of the sources of resistance.

The objective of this study was to appraise the effectiveness of inoculation techniques at different locations for their ability to reproduce differences in visual ear rot symptoms of *F. verticilloiides*, *A. flavus,* and *S. maydis* applied singly and in mixtures in selected maize genotypes.

The two hypotheses tested in this the study were:

(i) At least two previously described artificial inoculation methods are reliable and consistent in their ability to induce ear rot epidemics in the test genotypes; and

(ii) the use of a mixture of ear rot fungi as the source of inoculum was as effective in inducing ear symptoms as the principal constituents (the individual ear fungus making up the mixture).

4.2 Materials and Methods

4.2.1 Location and environmental conditions

Three field experiments were conducted at the Mount Makulu Central Research Centre, Chilanga, from 2005 to 2008, Kafue in 2006/7, and Lusaka west in 2007/8. The climatic and soil conditions of the experimental sites are summarised in Table 4.1. During the growing season, dry spells were common.

Table 4.1 Environmental characteristics of three research sites used for evaluating the inoculation methods from 2006 – 2008

Environmental factors	Mount Makulu	Kafue	Lusaka west
Latitude (S)	15°32.87	15°48.14	15°23.683’
Longitude (E)	28°14.92	28°14.81	28°14.345’
Altitude (m)	1225	1108	1221
Annual rainfall (mm)	765	780	734
Maximum temperature (°C)	14	19	18
Minimum temperature (°C)	24	20	26
Soil type	Clay loam	Sandy loam	Clay loam
pH	5.7	4.6	5.4
N%	0.08	0.07	0.01
Org C %	1.5	1.3	1.4
P (ppm)	26.7	6	5
K (me%)	0.5	0.6	0.5
Ca (me %)	6.6	4.5	4.2
Mg (me %)	1.3	2.2	1.1

Source: ZARI soil advisory laboratory, Chilanga.

4.2.2 Evaluation of four inoculation techniques

4.2.2.1 Maize Varieties

The seed of 23 maize varieties, comprising seven inbred lines, eight open-pollinated varieties, and eight commercial hybrids were obtained from the local maize breeding programme, Zambia Seed Company, PANNAR, Seedco and the Maize Research Institute Company (Lusaka, Zambia); these were planted at Mount Makulu Central Research Centre on 10th December 2005. The main agronomic characteristics of the genotypes used are presented in Table 4.2.

Table 4.2. Agronomic characteristics of maize varieties used in the three experiments during 2005/6, 2006/7 and 2007/8 seasons

Variety	Type	Grain type	Maturity	Yield potential (t ha^{-1})
L12	Inbred	Dent	Medium	3 – 4
L1214	Inbred	Dent	Medium	2 – 4
L152	Inbred	Dent	Medium-	2 – 3
L5522	Inbred	Semi dent	Late-	2 – 3
L151	Inbred	Dent	Medium	2 – 4
L913	Inbred	Semi dent	Medium	2 – 3
L917	Inbred	Dent	Late	2 – 4
MMV 400	OPV	Flint	Very early	3 – 4.5
ZM 421	OPV	Flint	Early	4 – 5
ZM 521	OPV	Flint	Early	5 – 6
ZM 621	OPV	Semi flint	Medium	5 – 6
MMV 600	OPV	Semi flint	Medium	5 – 6
Pool 16	OPV	Semi dent	Very early	3 – 4
Pop 10	OPV	Dent	Medium	4 – 5
Pop 25	OPV	Flint	Medium	4 – 5
GV 659	Hybrid	Dent	Medium	6 – 7
MM 604[a]	Hybrid	Semi dent	Medium	6 – 7
MRI 514	Hybrid	Dent	Medium	6 – 7
MRI 634	Hybrid	Dent	Medium to late	6 – 7
PAN 6243	Hybrid	Dent	Medium to late	6 – 7
PAN 67	Hybrid	Dent	Late	6 – 7
SC 513	Hybrid	Dent	Early	6 – 7
SC 627	Hybrid	Semi flint	Medium	6 – 7
ZMS 737	Hybrid	Dent	Late	8 – 10

Information supplied by the Seed Companies in Zambia and CIMMYT.

[a] MM 604 was not planted in the 2005/6 experiment but in the other two that followed in 2006/7and 2007/8 .

4.2.2.2 Experimental design

The experimental design was a randomised complete block arranged as a split-split plot with three replicates. Ear rot fungal pathogens were applied as the main plots, inoculation techniques as sub-plots, and maize varieties as sub-sub-plots. The experimental units were single row plots with 32 plants per plot. The rows were 7.75 m long and spaced 0.75 m apart. Half of the plants per row were inoculated. The inoculated plants were marked with a swab of paint. A different colour of paint was used for each ear rot-causing pathogen. Tags were used to identify the inoculation treatments.

4.2.2.3 Inoculation techniques

The four inoculation techniques evaluated in the first season of the study were

(i) spore suspension spray using a 2 – 5 ml ear rot inoculum applied once to the silk of the primary ear at mid-silking stage;
(ii) leaf whorl placement of 3 – 4 gm of ear-infested maize grit or broken kernels, applied at 8 – 10 leaf stage (Figures 4.1 and 4.2);
(iii) injection of 2 – 5 ml of a spore suspension through the husk into the developing silk; and,
(iv) insertion of two to three colonised toothpicks through the husks into the developing ear at the milk stage (Figure 4.3).

The quantities used for different the inoculums were based on studies conducted else; spore suspension (Reid et al. 1992); leaf whorl placement (Nowell, 1998; Rossouw et al., 2002), silk injection (Chungu et al.,1996) and colonised toothpick (Clement et al., 2003)

4.2.2.4 Inoculum preparation

The inoculum for *Aspergillus flavus, Fusarium verticillioides* and *Stenocarpella maydis* was prepared following documented methods with some minor modifications (Chungu et al., 1996; Clements et al, 2003).

(i) Conidia spore suspension for spray and silk injection treatments was prepared by pouring sterilised distilled water on fungal culture on a petri dish. The resulting suspension was strained through a layer of cheesecloth. The concentration of conidia was quantified using a hemacytometer, adjusted to 10^6 condia ml^{-1}, with distilled water amended with 5% honey as a sticker. Spray inoculations were applied with a hand sprayer equipped with a cone-shaped nozzle. The silk channel injection method used hog or bovine syringes fitted with 2.5 by 0.2 cm hollow stainless steel needles.

(ii) Colonised toothpicks were prepared by placing round wooden toothpicks vertically in a 500 ml bottle filled with distilled water and allowing them to soak for 12 hours, then autoclaving them in deionised water for 1 hour, then sub-dividing them into smaller quantities of between 200 – 300 toothpicks, sealed in 1 litre flasks containing 250 ml potato dextrose broth, autoclaved at 121^0C for 20 min, and allowed to cool to room temperature. Each flask was inoculated with a spore suspension of any one of the three ear rot pathogens. The flasks were labelled to indicate the pathogen used to avoid duplication. The toothpicks were then incubated for up to 21 days before use at 26^0C to promote colonisation.

(iii) Infested maize grit or colonised kernels were prepared by placing three 2.5 cm^3 PDA culture blocks of each ear rot pathogen on sterilised, moistened maize grit or broken kernels in 500 ml bottles and then incubated for 21 – 28 days. The infested maize grit was later dried on newspapers or A1 white paper for another 2 to 3 weeks prior to inoculation, then ground into powder (Figure 4.1).

Figure 4.1 Ear rot-infested maize grit

Figure 4.2 Leaf whorl placement at eight leaf stage

Figure 4.3 Colonised toothpick method

Figure 4.4 Ear top placement technique

4.2.3 Evaluation of five inoculation techniques for ear rot infection of maize

4.2.3.1 Maize varieties

In this experiment, the number of varieties was reduced to eight, besides accommodating as much diversity in disease reaction from the initial screening of 23 cultivars, poor performing genotypes were discarded. The two criteria used to select these genotypes (i) representativeness of the whole spectrum of the disease reaction upon inoculation, based on the observation of the previous season, and (ii) availability of seed. The latter is the reason why no inbred lines were included in this second experiment. The eight maize varieties – four commercial hybrids (MM 604, PAN67, PAN6363, and SC513) and four open-pollinated varieties (MMV 600, Pool16, Pop10 and Pop25) – were tested. . The maize varieties were planted in a randomised complete block design arranged as a split plot with three replications, at Mount Makulu on 30 June 2006 (off the normal crop season) and 6 December 2006, and at Kafue on 11 December 2006, in 5-row plots that were 4 m long, with 17 plants per row. The inter- and intra-row placing was 75cm and 25cm, respectively. To avoid the confusion that arose in the 2006 experiment regarding in identifying treated and untreated plants at harvest when lodged, each row received only one type of inoculum. *F. verticillioides* was applied to the first row in the plot and to all 15 plants in the row; *S. maydis* was applied to the second row; *A. flavus* to the third row; mixed ear rot to the fourth row. As the control, the fifth row was not artificially inoculated.

4.2.3.2 Inoculation techniques

Five artificial inoculation techniques were used in this experiment, one more than the previous experiment. The fifth method was the ear top placement technique (Figure 4.4). An unpublished report by Mulenga (1996) had suggested that this technique might be suitable for breeding work, especially under Zambian weather conditions, because of its similarity to the hand pollination activity. In this technique, the ear was cut back 1 to 2cm at the ear tip at mid-silking, similar to the methods breeders use to expose fresh silks. The sliced-off part was smeared with 2g ear rot inoculum powder. The moisture from the sliced ear tip ensured that the powder stuck to the silks. The ear was thereafter covered with a waxy shoot bag or loose plastic bag for 24 to 48 hours to avoid desiccation (Figures 4.4). The bag provided the necessary humidity conditions for increased pathogenesis.

4.2.3.3 Mixed ear rot inoculum, preparation and application

In addition to the four inoculums in the previous experiment, a fifth source of ear rot propagules, mixed ear rot, was added. The mixed ear rot was made up of the three individual ear rot fungi, *A. Flavus*, *F. verticillioides,* and *S. maydis*. It was included after an observation made during the survey in Lusaka and Southern Provinces (see Chapter 3), that ear rots rarely occur in isolation and some maize ears had more than two types of ear rots (Fig 4.5).

Figure 4.5 Multiple infection of ear rots: *Aspergillus flavus*-infected maize ears with *Fusarium graminearum* and *F. verticilloides.*

The mixed ear rot fungi inoculum was made by flooding an equal number of pure cultures of *F. verticillioides*, *S. maydis,* and *A. flavus* with deionised water, then pouring the suspensions into one conical flask. The flask was then placed on a shaker for 30 minutes. Thereafter, the mixture was blended with a broth made from ordinary rotten maize, previously identified, infected with Aspergillus, Fusarium and Stenocarpella ear rots. The mixture was mixed thoroughly for another 30 minutes.

Regardless of how the inoculum was to be applied, the procedure for making the final inoculum to be used in the field was the same. The inoculum for spray and injection inoculations was prepared by sieving the mixed ear rot through two layers of cheese cloth. It was further diluted it with sterilised water to a concentration of 10^5 to 10^6 conidia per ml determined by using a haemacytometer, and amended with 10% pure honey. Mixed ear rot colonised toothpicks and colonised broken maize or grit were prepared by pouring the inoculum broth over moistened, sterilised toothpicks and maize grits.

4.2.4 Evaluation of three selected inoculation techniques and their combinations for ear rot infection of maize

4.2.4.1 Inoculum and Inoculation techniques

The three best performing inoculation techniques from the previous experiments and their combinations were further investigated. The inoculation techniques used were

(i) Leaf whorl placement
(ii) Leaf whorl placement plus colonised tooth pick (Lw+ct);
(iii) Leaf whorl placement plus ear top placement (Lw+ep);
(iv) Colonised toothpick;
(v) Colonised toothpick plus ear top placement (Ct+ep);
(vi) Ear top placement.

4.2.4.2 Maize varieties

Based on the disease reaction was observed in the two experiments in 2005/6 and 2006/7, four maize varieties – two moderately susceptible varieties, SC627 and MM604, and two moderately resistant varieties, MRI 514 and Pool 16 were selected as further evaluation of inoculation techniques. The varieties were planted at Lusaka West on 30 November 2007 and at Mount Makulu Research Centre on 11 December 2007.

4.2.4.3 Experimental design and inoculation methods

The experimental design was same as in the 2006/7 experiment; a randomised complete block arranged as a split plot with three replicates. Varieties were planted as main plots and inoculation techniques were applied as sub-plots. A subplot consisted of two rows with 17 plants per row. The rows were 4m long and spaced as in the other two experiments reported on in this chapter. However, there was a slight modification in the method of inoculation. The first row within the experimental plot was inoculated with an equal mixture of ear rot fungal pathogens while the second row was not (a natural infection). All the ears within first row were inoculated and marked with either a dot of paint or a tag.

4.2.5 Trial management

Standard field trial management applied to all trials. Before planting, 200kg of mineral fertiliser (10 N: 20 P: 10 K: 10 S) was broadcast per hectare, then incorporated into the soil by hoe. At planting, the insecticide Furadan (10% carbofuran, 90% inert) was added to the planting holes.

Top dressing with 100 kg ha^{-1} urea (46% N) was applied when the plants reached knee height. The plots were hand weeded twice.

Except for the winter crop at Mount Makulu in 2006, the field experiments were planted at the start of the rainy season. The first experiment at Mount Makulu was planted on 10 December 2005, the following year at the same site on 6 December, and in 2007, the screening trial was planted on 11 December 2007. In Kafue, the trial was planted on 18 December 2006, and in Lusaka West, on 30 November 2007. The crop was harvested at the end of April for normal crop growing season and between 20 and 30 November 2005 for the winter crop.

4.2.6 Data collection

At harvest, in all three field experiments, the primary ears in an experimental unit were harvested and rated for the incidence and severity of the ear rots using the 1 – 7 rating scale (Reid et al., 1996), based on a visual assessment of grain colour and development:

Where 1 = sound, unblemished kernels = 0%

2 = 1 to 3% of the kernels rotten

3 = 4 to 10% of the kernels on the cob (or ear) rotten

4 = 11 to 25% of the kernels on the cob rotten

5 = 26 to 50% of the kernels on the cob rotten

6 = 51 to 75% of the kernels on the cob rotten, and

7 = 76 to 100% of the kernels damaged, covered with fungus, or discoloured.

The mean scale values per plot were retransformed into percentage disease severity (DS), following the equation of Silva et al. (2007): $DS = (n_1 \times 0 + n_2 \times 2 + n_3 \times 7 + n_4 \times 18 + n_5 \times 38 + n_6 \times 63 + n_7 \times 88)/\Sigma N$, where n_1 to n_7 are the number of ears with the scale values 1 to 7, which are multiplied with the mean percentage of the scale. Pictorial diagrams developed by Reid et al. (1996) showing the different rating classes were also used.

4.2.7 Data analysis

An analysis of variance (ANOVA) using GENSTAT 11th edition software (Payne et al., 2008) was conducted to assess the significance of replicate, variety, pathogen and inoculation method on

mean disease severity and incidence ratings after verifying assumptions for normality of data and homogeneity of variances. Data were transformed to stabilise the variance. Analyses were made for individual environments and then across locations (for 2006/7 and 2007/8 seasons) after a Bartlett's (1937) test was completed and had revealed that the variances between locations were homogenous ($P<0.05$). The interaction effects, i.e., pathogen x inoculation, genotype x inoculation, and genotype x inoculation x environment interactions effects, were compared using least square differences (Steele and Torrie, 1980).

The stability of the inoculation techniques versus the hybrid response to ear rot infection was analysed by regressing severity means of the individual hybrid (or OPV) for each site for a particular inoculation technique against the overall mean of the site for an inoculation technique (Eberhart and Russell, 1966). Variety stability was analysed separately for each inoculation method. The dependent variable of disease severity and incidence were different from that of Eberhart and Russell (1966). According to Schaafsma et al. (1997), the hybrid stability being referred to here could be defined as whose regression line has a slope approaching zero indicating no change in the ranking order across sites. The purpose was to identify inoculation techniques that give constant ranking of genotypes across sites.

4.3. Results

4.3.1 Evaluation of four inoculation techniques

Genotype, genotype class, inoculation, and genotype x inoculation differed significantly ($P<0.05$) in the severity of ear rots (Table 4.3). The highest severity occurred when a colonised toothpick was applied. Spore suspension spray produced the least severe disease symptoms. On average, artificial inoculation methods produced higher severity than the natural infection or control and the lack of discrimination in the natural control was evident.

Among the hybrids, most of the methods used were able to differentiate between the varieties, with the leaf whorl placement, followed by the colonised toothpick technique being the most effective (Table 4.3). The least effective was the spore suspension method, the range of which was even significantly smaller than that of the natural infection. PAN6243 had the highest disease severity among all of the inoculation methods, including the natural control. This may suggest that this variety is generally susceptible to ear rots. On the other hand, GV659 had the lowest infection for at least two of the inoculation methods.

Among the inbreds, silk channel injection produced the best discrimination of genotype, with disease severity ranging from 3 to 20.7%, followed by colonised toothpick, for which disease severity ranged from 2.2 to 20.3%. The lowest was in the natural control (1.2 to 7.2%). For open-pollinated varieties (OPVs), the separation of genotypes was highest using the colonised

toothpick method, with disease severity ranging from 3.6 to 21.6%, followed by leaf whorl placement, ranging from 1.3 to 12.5%, and the least was in the spore suspension, ranging from 0.6 – 4.4%.

Among the inbreds, L917 had significantly higher ear rot infection compared to other lines, while among the OPVs, Pop10, followed by MMV600, had higher disease severity for two of the inoculation treatments, colonised toothpick and leaf whorl placement. However, when silk channel injection was used, ZM621 had the highest disease severity, followed by Pop10, and MMV600 was ranked third. It was clear that OPVs tended to react differently depending on the inoculation method used.

Table 4.3 Mean disease severity of twenty three maize genotypes inoculated with maize ear rot fungi using four artificial inoculation methods

Genotype	Class	Colonised toothpick	Leaf whorl placement	Spore suspension	Silk channel injection	Control
GV 659	Hybrid	1.5	1.9	0.8	1.6	0.8
MRI 514	Hybrid	3,9	2.8	1.3	13.2	3.7
MRI 634	Hybrid	4.4	6.4	2.3	6.7	2.7
PAN 67	Hybrid	7.5	4.4	1.6	6.7	2.1
PAN6243	Hybrid	20.3	18.4	5.2	15.7	9.6
SC 513	Hybrid	12.5	1.2	0.3	4.2	0.7
SC 627	Hybrid	2.9	1.9	0.5	5.1	0.7
ZMS 737	Hybrid	3.9	1.5	0.8	3.4	0
Mean		7.0	4.8	1.9	7.4	2.5
L 12	Inbred	12.4	4.9	2.0	5.9	1.2
L 1214	Inbred	3.9	5.6	1.6	5.3	1.9
L 151	Inbred	17.6	2.8	0.7	3.0	1.8
L 152	Inbred	10.0	6.0	4.2	3.6	7.2
L 5552	Inbred	8.9	6.0	0.8	7.3	2.7
L 913	Inbred	2.2	4.2	1.6	8.3	1.5
L 917	Inbred	20.3	16.7	10.7	20.7	2.9
Mean		10.7	6.6	3.1	7.7	2.7
MMV 400	OPV	3.6	1.3	0.9	4.3	0.6
MMV 600	OPV	13.9	3.6	2.7	6.3	2.0
Pool 16	OPV	3.9	2.6	1.5	2.1	1.6
Pop 10	OPV	21.6	12.5	5.8	13.9	0.6
Pop 25	OPV	8.6	3.1	0.6	2.5	5.3
ZM 421	OPV	7.7	2.8	1.4	5.6	4.5
ZM 521	OPV	4.3	1.6	1.0	6.1	0.6
ZM 621	OPV	7.3	3.2	4.4	13.4	2.3
Mean		8.8	3.8	2.3	6.8	2.2
Overall Mean		8.8	5.0	2.4	7.2	2.5
P-values		<.0001	<.0001	<.0001	<.0001	<.0001
LSD		1.50	0.99	0.64	1.38	0.60
CV%		25.77[a]	26.04[a]	23.52[a]	30.05[a]	21.37[a]

[a]Angular transformed data.

4.3.2 Evaluation of five inoculation techniques

Significant inoculation, location x inoculation, variety x ear rot x inoculation and location x variety x ear rot x location interaction effects (P<0.05) and not variety by inoculation method were observed for disease severity (Table 4.4).

Table 4.4 Mean square for disease severity in experiments conducted at Mount Makulu in 2006 off season, Mount Makulu and Kafue 2006/7 main season

Source of variation	Df	Severity[a]
Replication	2	36.00
Location	2	463.80
Location (rep)	6	112.33
Variety	7	105.73
Location x variety	14	101.70
Error	42	60.10
Inoculation	5	7227.88**
Location x inoculation	10	1411.87**
Variety x inoculation	35	100.69
Location x variety x inoculation	70	140.18**
Error	240	62.89
Ear rot	3	3493.24**
Location x ear rot	6	1529.97**
Variety x ear rot	21	108.02**
Inoculation x ear rot	15	651.99**
Location x variety x ear rot	42	74.36
Location x inoculation x ear rot	30	717.05**
Variety x inoculation x ear rot	105	136.79**
Location x variety x inoculation x ear rot	210	111.17**
Error	862	55.19

*, ** = significant at *P≤0.05* and *P≤0.01,* respectively; [a] Angular transformed data.

The results in Table 4.5 indicate that leaf whorl placement compared to the other methods consistently ranked five maize varieties across two sites. While in the third site, there was an

intrusion of a rating class that was one or two classes above or below that affect ranking order in that particular site.

Significant correlation coefficients were obtained between colonised toothpick and leaf whorl placement, colonised toothpick and silk channel injection, and ear top placement and leaf whorl placement (Table 4.6). No significant correlation was found between the ranking orders of the different inoculation methods. The ranking order of varieties by the different inoculation techniques was negative but not significant except for ear top and spore suspension.

Leaf whorl consistently ranked MM604, MMV600, PAN6363, PAN67 and Pool16 across two sites at same level, i.e. Mt. Makulu and Kafue, while colonised toothpick consistently ranked MM604 and PAN6363 across two seasons, i.e. Mt. Makulu in 2007 and 2008. Spore suspension ranked SC513 across two seasons and MMV600 across two sites. However, the level of infection was lower compared to the colonised toothpick and leaf whorl placement methods. The other two methods, ear top placement and silk channel injection, did not perform better than the natural control and rank only one genotype consistently across sites or seasons.

The stability analysis revealed that only colonised toothpick, ear top placement and silk infection methods had slopes approaching zero, implying that they might have been constant in the ranking of genotypes across the three sites (Table 4.7).

Table 4.5 Percent ear rot disease severity and rank order (in parenthesis) of the eight maize varieties inoculated using five inoculation techniques (plus the control) in three different environments in Zambia

Varieties	Colonised toothpick			Ear top placement			Leaf whorl placement		
	Kafue	Mt.Makulu'07	Mt.Makulu '08	Kafue	Mt.Makulu '07	Mt.Makulu '08	Kafue	Mt.Makulu '07	Mt. Makulu '08
MM604	9.83 (7)	11.77 (5)	22.47 (5)	1.53 (8)	8.43 (6)	5.59 (5)	7.82 (6)	10.01 (6)	2.40 (2)
MMV600	20.77 (2)	13.83 (3)	28.81 (1)	7.63 (5)	6.50 (8)	2.67 (8)	8.88 (5)	10.17 (5)	1.47 (6)
PAN6363	15.27 (4)	14.90 (2)	28.09 (2)	4.57 (7)	11.80 (1)	5.73 (3)	10.70 (4)	13.49 (2)	1.28 (8)
PAN67	8.92 (8)	15.45 (1)	22.31 (6)	9.57 (4)	8.04 (7)	4.26 (6)	1.42 (8)	7.11 (8)	1.32 (7)
Pool16	14.00 (5)	13.68 (4)	19.54 (7)	9.90 (2)	9.56 (4)	8.60 (1)	11.50 (3)	12.80 (3)	2.11 (4)
Pop10	16.60 (3)	11.24 (6)	15.02 (8)	9.65 (3)	8.93 (5)	2.99 (7)	6.18 (7)	14.06 (1)	2.20 (3)
Pop25	11.23 (6)	9.60 (7)	25.39 (4)	7.60 (6)	10.83 (2)	5.64 (4)	13.35 (1)	10.93 (4)	2.49 (1)
SC 513	21.00 (1)	11.24 (6)	25.57 (3)	11.43 (1)	10.59 (3)	6.43 (2)	12.68 (2)	7.51 (7)	1.53 (5)
Mean	14.7	12.72	23.4	7.74	9.33	5.24	9.07	10.76	1.85
	Natural infection			Silk-–channel injection			Spore suspension spray		
MM604	0.98 (5)	0.79 (7)	1.22 (6)	4.85 (7)	5.41 (8)	12.76 (2)	5.93 (2)	9.22 (7)	7.67 (4)
MMV600	0.65 (8)	1.16 (5)	1.75 (5)	3.63 (8)	14.46 (1)	2.36 (6)	4.53 (3)	11.63 (3)	9.19 (2)
PAN6363	1.07 (4)	2.39 (2)	0.32 (8)	13.57 (3)	9.17 (5)	2.72 (4)	2.48 (6)	7.69 (8)	8.44 (3)
PAN67	0.78 (7)	1.68 (3)	2.23 (1)	20.42 (2)	6.50 (7)	3.84 (3)	3.33 (5)	11.68 (2)	5.17 (6)
Pool16	1.33 (2)	1.64 (4)	2.07 (2)	7.75 (4)	11.97 (2)	1.57 (7)	8.43 (1)	9.58 (6)	4.20 (8)
Pop10	0.80 (6)	2.75 (1)	1.83 (4)	5.32 (6)	9.98 (4)	1.18 (8)	1.00 (8)	10.47 (5)	9.54 (1)
Pop25	2.12 (1)	0.59 (8)	2.00 (3)	21.03 (1)	8.39 (6)	2.59 (5)	3.50 (4)	13.97 (1)	6.04 (5)
SC 513	1.18 (3)	1.05 (6)	0.75 (7)	7.23 (5)	10.24 (3)	13.72 (1)	2.30 (7)	10.53 (4)	4.58 (7)
Mean	1.11	1.51	1.52	10.48	9.52	5.09	3.94	10.60	6.85
$LSD_{0.05}$	6.05								
CV%	58.63								

Table 4.6 Coefficient of association among the different inoculation techniques used to rank the eight maize genotypes and that of the ranking order of the eight maize genotypes (bold) within the inoculation techniques across the three environments , Mount Makulu (2006 and 2007) and Kafue (2007)

Inoculation technique	**CT**	**EP**	**LW**	**SI**	**SS[a]**
Colonised toothpick (CT)		-0.37	-0.59**	-0.44*	-0.21
		0.05	**-0.09**	**-0.04**	–
Ear top placement (EP)			0.52**	.0.28	0.05
			0.20	**0.06**	**-0.44***
Leaf whorl placement (LW)				0.32	0.44
				0.14	-0.10
Silk channel injection (SI)					-0.12
					-0.16

*, ** Correlation coefficients indicates significance at *P≤0.05* and *P≤0.01,* respectively.

[a] SS=Suspension spore spray.

Table 4.7 Comparison of the stability of the response of varieties to the inoculation techniques over different environments (Mt. Makulu in 2006 and 2007 and Kafue in 2007)

Varieties	Slope of disease severity curve				
	Colonised toothpick	Ear top placement	Leaf whorl placement	Silk channel injection	Spore suspension spray
MM 604	1.14	0.17	1.20	-1.53*	0.49
MMV 600	1.26	0.65	1.00	1.01*	1.05
PAN 6363	1.31	0.38	0.74	1.82*	0.74
PAN 67	0.91	0.58	0.94	2.31	1.28
Pool 16	0.57*	2.49*	0.80	1.52*	0.23
Pop 10	0.19**	0.49	0.67	1.15*	1.37
Pop 25	1.52*	0.76	0.77	2.77	1.60
SC 513	1.10	0.65	0.67	-1.10*	1.25

*, ** indicates significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

4.3.3 Evaluation of three inoculation methods and their combinations

Significant differences were observed for inoculation, variety and location by variety interaction effects and not inoculation x variety effects ($P<0.05$) for the severity of ear rots across the two sites, Lusaka west and Mount Makulu (Table 4.8).

Table 4.8 Mean square for disease severity and incidence in experiment conducted in 2007/8 at Mount Makulu and Lusaka west

Source of variation	Df	Severity[a]
REP	2	59.62
Location	1	87.20
Location (rep)	4	143.30
Inoculation	5	901.90**
Variety	3	1082.40**
Location x inoculation	5	288.30*
Location x variety	3	10.00
Inoculation x variety	15	223.30*
Location x variety x inoculation	15	108.30
Residual	90	109.84

[a] angular transformation data; *, ** indicates significance at *P≤0.05* and *P≤0.01,* respectively.

The highest disease severity was observed at Mount Makulu (15.5%) and in SC627 (45.3%) when the colonised toothpick method + leaf whorl placement of infested maize grit was used (Table 4.9). Ear rot was least severe at the same site when ear rot inoculum was applied using the combination of colonised toothpick and ear top placement in MRI514 (3.67%). Of the six inoculation methods, the colonised toothpick and ear top placement methods gave consistent ranking order results for the four varieties across the two sites (Table 4.9).

Table 4.9 Disease severity at the two sites and ranking of the varieties by the six inoculation methods at Mount Makulu and Lusaka West in 2008

Variety	% Severity					
	Colonised toothpick	Colonised toothpick + ear top placement	Colonised toothpick + leaf whorl placement	Ear top placement	Ear top placement + leaf whorl placement	Leaf whorl placement
Lusaka						
MM604	16.37 (2)	40.00 (1)	17.03 (2)	5.87 (3)	4.00 (4)	5.00 (4)
MRI514	5.00 (3)	16.33 (3)	13.42 (3)	2.20 (4)	5.00 (3)	11.33 (3)
Pool16	3.00 (4)	6.67 (4)	12.63 (4)	12.63 (1)	9.00 (2)	14.77 (1)
SC627	19.07 (1)	32.10 (2)	45.33 (1)	6.99 (2)	12.07 (1)	12.30 (2)
Mean	10.86	23.78	22.10	6.92	7.52	10.85
Mt. Makulu						
MM604	20.67 (2)	18.13 (1)	17.00 (4)	9.67 (3)	6.77 (3)	16.49 (2)
MRI514	18.14 (3)	3.67 (4)	22.33 (3)	3.97 (4)	10.89 (2)	8.93 (3)
Pool16	6.93 (4)	7.87 (3)	31.33 (2)	13.33 (1)	5.73 (4)	6.43 (4)
SC627	28.20 (1)	17.20 (2)	38.00 (1)	10.59 (2)	18.45 (1)	24.73 (1)
Mean	18.49	11.72	27.17	9.39	10.46	14.15
$LSD_{0.05}$	17.09					
CV%	32.09[a]					

[a] Angular transformed; Where 1 is the most diseased and 4 the least.

4.4 Discussion

The great majority of the breeding materials, i.e. hybrids, inbred and OPVs used in these studies were susceptible to maize ear rots. However, there was a high degree of variation in disease severity among the genotypes used in the different experiments. The inbreds, hybrids and OPVs differed significantly ($P<0.05$) for ear rot disease severity. In addition, some inoculation techniques were unable to discriminate maize genotypes with any consistency. Such techniques may be unreliable for the Zambian environment. Some of the commercial varieties previously described as resistant succumbed to disease pressure. In this study, no attempt was made to measure yield due to the lack of an established check.

The methods that employed wounding induced a higher level of infection than those that did not. Discrimination of the disease reaction was more effective with the wounding methods than the non-wounding methods due to the absence of escapes with the former rather than the latter methods. The colonised toothpick, ear top placement and leaf whorl placement methods were consistent in ranking of the genotypes in at least two sites and across seasons. However, leaf whorl placement may be more vulnerable to environmental conditions than the colonised toothpick method or ear top placement. Chungu et al. (1996) reported similar findings and concluded that wounding methods were superior in inducing ear rot epiphytotic conditions non-injurious techniques. The significantly negative rank correlation coefficient (-0.56**) between colonised toothpick and leaf whorl placement indicated that the methods ranked the genotypes differently, and hence, using them in the programme would help to verify the consistency of ranking. Thus, genotypes that may be ranked resistant by both would be truly “resistant” and not false. These two methods indirectly measured two types of resistance mechanisms, the toothpick method, through the pericap and endosperm of the seed, and the leaf whorl method, through the leaf and stem tissues.

In the stability analysis, only the ear top and leaf whorl methods produced on average slopes approaching zero across the genotypes, indicating that these two methods followed by the colonised toothpick technique were more consistent in the ranking of genotypes and hence these methods may be useful in the screening of breeding populations. However, less than 7% of the varieties had significantly stable slopes, i.e. less than 1.0, which illustrates the difficulty associated with ensuring the inoculation methods achieve consistency in their ranking of genotypes.

The control used in the tests was natural control (where no ear rot fungi was applied), though natural infection lacked the damage done to the ear when techniques like the colonised toothpick method are used, involving the introduction of inocula through the husks. However, it mimics

what occurs when evaluating maize genotypes under natural infection, as is the case in most breeding work in Zambia and in the region. Most breeding materials are scored as false resistant. It was acknowledged that creating a wound through the husk may increase the disease inoculum to which the genotype is subjected; it nevertheless ensures that no escapes would be scored as resistant.

There were no significant differences in the infection levels observed among the inocula used, the mixture of three ear rot fungi, and the two dominant species. This suggests that the use of a mixture of maize ear rots as the source of inoculum followed the trend of the most dominant species, in this case *F. verticillioides* and to some extent *S. maydis.* This suggests that in ear rot screening programmes, the inoculum mixture could be used to accelerate the screening of germplasm breeding materials in the early stages of screening. Breeders, together with the pathologists, could defer the tedious species-specific inoculum preparation in early generation testing to a later stage of their breeding work.

4.5 Conclusion

The following conclusions could be drawn from this study

1. The use of a mixture comprising different ear rot fungi was as effective as using its principal constituents in inducing ear rot epiphytotic conditions.
2. Colonised toothpick and leaf whorl placement techniques were superior to other methods in inducing ear rot disease conditions and in consistently discriminating genotypes for resistance across sites and seasons. However, in order to effectively use these methods in a breeding programme, they must be used in combination, especially for tropical and sub-tropical environments like Zambia, and where a large percentage of maize germplasm consists of open-pollinated varieties.

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CHAPTER 5
GENETIC ANALYSIS OF RESISTANCE TO EAR ROT IN FIVE TROPICAL MAIZE POPULATIONS

5.0 Abstract

Maize ear rots are a serious disease of maize, a staple crop for millions of people in sub-Saharan Africa. However, there are very few varieties with ear rot resistance. Two studies were conducted simultaneously to determine the combining ability and gene action conditioning ear rot resistance in five tropical maize populations. In the first study, five broad-based maize populations were crossed in a diallel mating to evaluate multiple ear rot resistance under artificial inoculation across four environments in Zambia in 2007 and 2008. The results indicated that both the populations and their crosses were highly significant for ear rot severity. The SCA effects were significant, while the GCA effects were not significant for ear rot severity. The most resistant cross, MMV600 x Pop25, demonstrated the largest negative SCA effects for ear rot severity, indicating a contribution to ear rot resistance. Conversely, only GCA effects were significant for grain yield. The environment main effects and their interaction with GCA and SCA effects were highly significant, implying that selecting hybrids for specific environments could maximize the use of these maize populations. In the second study, 10 breeding lines, comprising one resistant and one susceptible, selected from each of the five populations after one cycle of full-sib family selection, were crossed in a full diallel mating scheme to estimate gene action conditioning ear rot resistance.. The resulting 90 reciprocal F_1 hybrids and their parents were evaluated under artificial inoculation at two sites. There were significant differences among the crosses for ear rot severity. Both the GCA and SCA effects were significant for ear rot severity, indicating that both additive and non-additive effects, respectively, were significant for ear rot resistance. Reciprocal differences were also highly significant for ear rot severity and were attributable to both maternal and non-maternal effects, suggesting that cytoplasmic gene effects and their interaction with nuclear genes, respectively, played a significant role in the inheritance of ear rot resistance in this germplasm. Some F_1 crosses between the resistant and susceptible lines out-performed their resistant parents, suggesting over-dominance gene action for ear rot resistance. On the whole, the progeny performance could not be predicted based on their parents' performance *per se*, while significant interactions of the environments with GCA, SCA, maternal, and non-maternal effects, indicated the need to conduct multi-location trials to identify germplasm with stable ear rot resistance.

Keywords: Ear rot resistance, general combining ability, gene frequency, maize, maternal effects, non-maternal effects, specific combining ability

5.1 Introduction

Maize ear rot fungi have been reported to be responsible for significant amounts of economic loss due to reduced crop yield and reduced grain quality (Munkvold and Desjardins, 1997; CAST, 2003). In addition, several of the mycotoxins they produce in the host plant are associated with human and animal health risk concerns. In Zambia, on-farm losses, and in some cases, ear rot epidemics, have continued to be reported (MACO, 2002; Nawa, 2005 unpublished). Schjøth et al. (2008) indicated that due to the narrow genetic base of the existing Zambian varieties, there are increased chances of susceptibility to ear rots. Currently no direct fungal control measure has been developed (Munkvold and Desjardins, 1997; Munkvold, 2003). The most feasible option, therefore, is to develop ear rot resistant maize. In spite of the large genetic variability of the Zambian maize germplasm (Ristanovic et al., 1987), little is known about the genetic potential of different breeding materials as sources of resistance to ear rots and the nature of gene action controlling field resistance. Identification of sources of resistance to be used in the development of new commercial varieties and understanding the genetic mechanisms underlying ear rot resistance have become very important because of the increasing incidence of ear rots.

According to Sprague and Tatum (1942), the two main concepts that define the potential of a parent population in a breeding programme are general and specific combining ability. General combining ability (GCA) is linked with additive gene effects, and specific combining ability (SCA) with non-additive effects. Diallel mating designs have been used worldwide since the 1950s to estimate GCA and SCA effects and their implications in breeding (Griffing, 1956). Population diallel analyses have also been used to estimate GCA and SCA effects in maize populations (Gardner and Eberhart, 1966; Murray et al. 2003). The combining ability studies conducted so far for ear rot resistance have reported the preponderance of GCA over SCA effects, and have concluded that inheritance for resistance is largely controlled by additive gene effects (Dorrance et al., 1998; Naidoo et al., 2002). Other researchers have reported significant reciprocal effects which were less important than GCA and SCA effects (Rossouw et al., 2002). Other reports indicated significant GCA x environment interaction effects also to be important for ear rot resistance (Naidoo et al., 2002). Negative values of GCA and SCA of the parents and crosses, respectively, indicate a contribution towards ear rot resistance. Following two years of evaluation of seven inbreds and 1 synthetic line, Dorrance et al. (1998) reported that four of the inbred lines had significantly large negative GCA effects and therefore contributed to ear rot resistance. However, Das et al. (1984), using open-pollinated maize varieties, found SCA to be more important than GCA effects. McLennan (1991) found that combinations involving D940Y, a South African public inbred line, with six other inbred lines had high SCA effects for Stenocarpella ear rot resistance. Though all these studies have provided useful information to breeders, the genetic information is on average, relevant only to specific germplasm and the range of tested environments (Falconer and Mackay, 1996).

A survey of the literature indicated few genetic studies on the field resistance of maize germplasm to ear rot diseases, especially in sub-Saharan Africa. Even worldwide, very few reports exist on the type of gene action conditioning resistance to ear rots. Boling and Grogan (1965) suggested that dominant gene action could be involved in the resistance to Fusarium ear and kernel rots, while Reid et al. (1994) reported that resistance to *F. graminearum* was controlled by a partial dominant gene. Lunsford et al. (1976) tested a diallel cross of maize for *F. moniliforme* seedling blight in maize, and found that both additive gene action and maternal effects were more important than dominant gene action in the inheritance of resistance to the disease.

Previous studies (Willman et al., 1987; Rossouw et al., 2002) have shown lodging (root and stalk), ear declination, husk cover, insect ear injury, and grain type to be important secondary characteristics for ear rot resistant maize. These traits, root lodging in particular, may be caused by the same fungal species, while the other four traits impede fungal entry and colonisation. Improving these traits in the desired direction in the local population could increase performance against ear rot infection.

Most of the studies were conducted on temperate germplasm in temperate environments. The genetic information and the germplasm characterised might not have a direct application under tropical environmental conditions in sub-Saharan Africa. Therefore, it was found prudent to evaluate local germplasm for ear rot resistance under tropical conditions in Zambia. The five maize populations that were evaluated are adapted or adaptable to tropical conditions in Zambia but they would require some improvements for ear rot resistance to enhance both yield and end-user grain quality requirements.

The objectives of this study were:

(i) to estimate the combining ability among maize populations and early generation lines for resistance to ear rots, with a view to selecting superior hybrid combinations with a high degree of resistance to multiple maize ear rot infection, and

(ii) to investigate the type of gene action conditioning resistance to maize ear rots in five tropical populations.

The hypotheses of this study were:

(i) At least two local maize populations have large general combining ability effects for ear rot resistance such that they could be used as a source material in a breeding programme.

(ii) Additive gene effect was the predominant form of gene action conditioning resistance to ear rots in the five Zambian maize populations.

5.2 Materials and Methods

5.2.1 Combining ability study

5.2.1.1 Germplasm

Five maize populations, MMV600, PRA783244c3, and ZUCASRc2 from the local breeding programme, and Pop10 and Pop25 from International Maize and Wheat Improvement Centre (CIMMYT) were used for the study (Table 5.1). These populations were being used as source germplasm to extract lines to develop locally adapted hybrids and open pollinated varieties for deployment in tropical environments by the local maize breeding programme. Three of these populations, MMV600, Pop10, and Pop25 have been released as open pollinated varieties which are widely grown by small-scale farmers in Zambia

Table 5.1: Background information[1] of the maize population used in this study

Populations	Origin		Maturity
Pop 10	CIMMYT	Latin America	Medium to Late
PRA 783244 c3	Zambia	Latin America	Medium
MMV 600	Zambia	Latin America	Medium
Pop 25	CIMMYT	Latin America	Medium
ZUCA SRc2	Zambia	Tanzania/Kenya/CIMMYT	Late

[1] Information obtained from CIMMYT and Zambia Agriculture Research Institute Annual Reports

5.2.1.2 5 x 5 population diallel mating scheme

A full diallel mating scheme was used to generate 20 F_1 reciprocal crosses among the five populations. For each interpopulation cross, five sets of paired rows were used, planted with 15 plants per row. Each plant within the row was used only once as male (pollinator), and female. About 30 to 40 ears were pollinated with bulked pollen from the same number of plants from the other population. At harvest, pollinated ears from each cross were shelled and the seeds were bulked. The seeds of reciprocal crosses were also bulked.

5.2.1.3 Experimental design and management

The experimental design was a randomised complete block with three replications. The entries were grown in one-row plots, 3.2 m long with 17 plants per row. Inter-row spacing was 90 cm. The plants were initially overplanted with two seeds per hill, but were thinned at the six-leaf stage to 17 plants per row to give a population of 55555 plants ha^{-1} at all the three locations. The experiments were planted on the 5th and 12th December, 2006, at Mount Makulu and Zamseed, respectively. The trials for the 2007/8 rainy season were planted on 14th December, 2007, at Mount Makulu, and 7th January, 2008, at Nanga. The characteristics and the location of the experimental environments are given in Table 5.2. Standard cultural practices such as two hand weeding and application of both basal and top dressing fertilisers were followed. Fertiliser was applied at the rate of 80 kg N ha^{-1}, 40 kg P_20_5 ha^{-}, and 40 kg K_2O ha^{-1} at all the locations. No insect pest control measures were undertaken.

Table 5.2 The environmental and soil characteristics of three sites in Zambia where genotypes were evaluated for ear rot resistance

Environmental characteristics	Mount Makulu	Nanga NIRS	Zamseed
Latitude (South)	15°13.10'	15°32.87'	14°12.10'
Longitude (East)	28°14.93'	27 °10.93'	28°11.93'
Altitude (metres above sea level)	1206	1190	1235
Relative humidity (%)	69.4	54.8	75.7
Mean annual temperature (°C)	20.6	23.7	21
Annual rainfall (mm)	802	790	820
Soil type	Chromi-haplic lixisols	Vertisols	Chromi-haplic lixisols
Soil characteristics	Fine loam to clay	Sandy clay	Loamy clay
pH	5.8	5.2	5.3
N (%)	0.1	0.2	0.2
Organic C (%)	1.2	1.5	1.4
P (ppm)	21.0	27,0	13,0
K (me%)	0.2	0.5	0.6
Ca (me%)	4.5	6.1	5.2
Mg (me%)	0.3	0.9	0.5

Sources: Department of Meteorology, Lusaka; Soil Chemistry Laboratory, Mount Makulu

5.2.2 Gene action study

5.2.2.1 Germplasm and 10 x 10 diallel mating scheme

A full diallel mating of ten full-sib families, comprising five resistant and five susceptible families selected from an on-going full-sib recurrent selection programme for ear rot resistance, was used to generate crosses (Table 5.3). One resistant and one susceptible full-sib families were selected from each of the five populations under study. Seed for the evaluation trials was produced by planting selected full-sib families in 5 paired rows for each cross. Bulked pollen from 30 to 40 plants (males) was used to pollinate the same number of plants from the other full-sib family involved in the cross. At harvest, the ears representing each of the crosses were bulked.

5.2.3.3 Experimental design and management

The full-sib families and their 90 F_1 crosses were planted at two sites described in Table 5.2, Mount Makulu Central Research Station and Nanga NIRS, on 12th December, 2007 and 7th January, 2008, respectively, using a 10 x 10 simple lattice design. The environments are described in Table 5.2. The experimental units consisted of two-row plots, 3.2 m long. Spacing was 90 cm between rows and 20 cm within rows, with an expected stand of 17 plants per row after thinning. The trials were managed as described earlier.

Table 5.3 Features of the ten maize genotypes used to form crosses in a diallel mating scheme

Name	Parent population†	Reaction to ear rot
Pop10#7-8	Pop10	Resistant
MMV600#9-10	MMV600	Resistant
PRA783244c3#61-62	PRA783244c3	Resistant
Pop25#5-6	Pop25	Resistant
ZUCASRc2#55-56	ZUCASRc2	Resistant
ZUCASRc2#59-60	ZUCASRc2	Susceptible
Pop25#131-132	Pop25	Susceptible
Pop 10# 143-144	Pop10	Susceptible
MMV600#109-110	MMV600	Susceptible
PRA783244c3#135-136	PRA783244c3	Susceptible

† Populations are described in Table 5.1

5.2.4 Inoculum and inoculation methods

In both experiments, a mixture of isolates from three ear rot pathogens*, Aspergillus flavus* Link Fr., *Fusarium verticillioides* (Sacc.) Nirenberg (Syn. =*F. moniliforme* J.Sheld.), and *Stenocarpella maydis* (Berk.) Sutton, was used to inoculate the genotypes. The mixtures of ear rot fungi mimicked the predominant ear rot spectrum to which farmers' crops are subjected in Zambia. The inoculum was prepared by infesting maize grit and sterilised toothpicks with a mixture of these three ear rot fungi isolated from diseased maize kernels collected during the survey of 2006 in the Southern and Lusaka provinces of Zambia. Prior to infesting the maize grit and toothpicks, the *Fusarium* and *Aspergillus* fungi were grown on potato dextrose agar (PDA) at 24^{o}C and the *Stenocarpella* on malt extract agar at 26^{o}C for 7 to 10 days. Then a spore suspension mixture of an equal number of agar plates of *F. verticillioides*, *A. flavus,* and *S. maydis* was poured over agar moistened sterilised toothpicks and maize grit separately in 500 ml glass jars with lids. The jars with colonised toothpicks were stored on laboratory shelves away from light for at least one month. This ensured effective colonisation of the toothpicks. In the case of maize grit, after a month in storage, the infested maize grit was air dried on A4 white paper. Thereafter, it was finely ground using a mill and transferred back into the glass jars for later use.

Fifteen plants per plot were artificially inoculated using two inoculation methods: leaf whorl placement at the 10 to 12 leaf stage (Rossouw et al., 2002), followed by colonised toothpick at mid-silking stage (Campbell and White, 1994). In the leaf whorl placement method, 3 g of infested maize grit was placed in the leaf whorl, while in the colonised toothpick method; three colonised toothpicks were inserted in the primary ear. The combined method ensured that there were no genotypes that escaped the ear rot infections. The inoculated primary ears were marked with paint or tagged to differentiate them from uninoculated ears in the same row.

5.2.5 Data Collection

The severity of the multiple (or combined) ear rot infections was rated at harvest. The inoculated primary ears were hand-picked and dehusked.

Percent disease severity was rated using a seven-class rating scale (Reid et al., 1992), based on a visual assessment of grain colour and development, in which

1 = no infection, sound, unblemished kernels

2 = 1 to 3%, slight infection, 1 to15 kernels rotten per ear3 = 4 to 10%, moderate infection; between 10 - 40 kernels rotten per ear

4 = 11 to 25%, high scattered infection of between 40 - 100 kernels rotten per ear

5 = 26 to 50%, high coalescing infection; up to half the ear rotten

6 = 51 to 75%, high infection; 150 - 300 kernels rotten per ear, depending on the genotype

7 >75% of the kernels heavily infected; kernels damaged, covered with fungus, or discoloured

The mean scale values per plot were retransformed into percentage disease (DS) by the equation DS = ($n_1 \times 0 + n_2 \times 2 + n_3 \times 7 + n_4 \times 18 + n_5 \times 38 + n_6 \times 63 + n_7$ x 88)/∑N, where n_1 to n_7 are the number of ears with scale values 1 to 7 which are multiplied with the mean percentage of the scale (Silva et al., 2007).

The maize trait descriptions used at CIMMYT (Vivek et al., 2003) were used to collect data on the other agronomic traits. Grain yield kg ha^{-1} was recorded at harvest, calculated from the inoculated ears only, and adjusted to the 12.5% moisture using the formula:

Grain Yield (t/ha) = [Grain Weight (kg/plot) x (100-MC) x (Shelling percent/100) x 10000)/ (100-12.5) x (Plot Area)]

Where MC = grain moisture content.

The some maize traits previously reported to be associated with either ear rot infection and mycotoxin accumulation (Ramirez-Diaz et al., 2000;Betran et al., 2002; Rossouw et al., 2002) were also measured as follows:

Root and stalk lodging were rated separately on a plot basis by counting the number of plants that had inclined more than 45^o as root lodging, and those whose stalk had broken below the ear as stem lodging, multiplied by 100. For the purpose of statistical analysis these two were combined.

Ear declination was rated on a scale of 1 - 5, where 1 = drooping downwards and 5 = standing upright along the stalk.

Husk cover was measured by sampling five primary ears in the row, which were then rated using the scale of 1 to 5 proposed by Kossou et al. (1993), where the rating is done by placing the hand around the husk leaves as they extend beyond the ear tip and making a fist such that the base of the hand rests on the tip of the ear. If the husk leaves are longer than 4 fingers, the rating is 1; longer than 3 fingers, the rating is 2; longer than 2 fingers, the rating is 3; longer than 1 finger, the rating is four. When the husk leaves are not longer than 1 finger, the ear tip is exposed and the rating is 5, and then averaged for the plot.

Grain type was rated on a scale of 1 - 4, where 1 = flint; 2 = semi flint, more than 50% flint in the kernel row, or slight flint grain; 3 = more than 50% dent in the kernel row or slight dent grain; and 4 = dent.

Insect injuries to the cob were rated on a scale of 1 - 5, where 1 = clean or no damage and 5 = severe damage with visible holes. The percentage of grains damaged was calculated on per plot basis. Insect wounds are closely associated with ear rot infection (Setámou et al., 1997; Ajanga and Hillocks, 2000) and their damage creates pathways for ear fungal invasion (Munkvold and Desjardins, 1997).

5.2.3 Statistical Analyses

5.2.3.1 General analysis of variance

Data collected was initially subjected to analyses of variance (ANOVA), as per established methods (Steel and Torrie, 1980), for each location, using the Genstat 11th Ed. computer package (Payne et al., 2008). The populations were considered as fixed effects, and environments and replications as random effects. Percent ear rot disease severity values were transformed to arcsin of the square root to stabilise the variances.

Individual location analysis used the model:

$$Yij = \mu + r_k + \alpha g_i + e_{ij};$$

Where μ is the grand mean, r_k = block or replication effect, αg the genotype effect, and eij the experimental error

The linear mathematical model for the pooled analyses of variance across years was

$$Y_{ijk} = \mu + y_i + r(y)_{j(i)} + g_k + (gy)_{ik} + e_{jk(i)}$$

Where Y_{ijk} = observed ear infection rate or severity for the ith location,

jth replication within the ith location, and kth genotype,

μ = grand mean of the experiment,

y_i = effect of the ith location,

$r(y)_{j(i)}$ = effect of the jth replication within the ith location,

g_k = effect of the kth genotype,

$(gy)_{ik}$ = interaction of the kth genotype with the ith location, and

$e_{jk(i)}$ = residual effect or random error of the experiment.

Before combined analysis, a test of homogeneity of variance (Bartlett, 1937) was conducted using the Genstat 11th Ed. programme (Payne et al., 2008). Test of significance for genotype, environment, and their interaction was made using the least significant difference at 1% and 5 % probability levels (Steel and Torrie, 1980).

5.2.3.2 Combining ability effects

The genotypic effects that were statistically significant were subjected to diallel analysis using Gardner and Eberhart (1966) analysis III (GE3) (Murray et al., 2003). This method provides estimates of both general and specific combining abilities. The *n* parents and their *n* (*n*-1)*/2* crosses are evaluated, and variation among populations (entries) partitioned into varieties (parents), parents versus crosses, and crosses (Table 5.4). The analyses were performed using the Diallel SAS-05 program (Zhang et al., 2005) in the SAS computer package.

Table 5.4 Analysis of variance table for Gardner and Eberhart Analysis III (GE3): partitioning of the overall population sum of square (Murray et al., 2003)

Sources of variation	Degrees of freedom	Sum of squares
Populations	[*n*(*n*+1)/2] -1	-
Varieties (v_j)	n-1	S"1
Varieties vs. crosses(h)	1	S"2
Crosses (x_{ij})	[n(n-1)/2] -1	S"3
GCA (g_j)	n -1	S"31
SCA (s_{ij})	*n*(*n* -3)/2	S''_{32}

n is the number of parents or varieties; GCA denotes General Combining Ability; SCA denotes Specific Combining Ability

The mathematical model for combining ability for each cross at each location was

$X_{(jk)} = \mu = g_i + g_j + s_{ij} + bk + e_{ijk,}$ Where

$X_{jj'}$ = the performance of the cross between i^{th} and j^{th} genotypes in the k^{th} replicate,

μ is the population mean,

g_i (g_j) is the general combining ability (GCA) effect,

s_{ij} is the specific combining ability (SCA) effect, such that sij = sji,

e_{ijk} is the error associated with the $ijkl^{th}$ observation.

The mathematical model for combining ability analysis across the locations was

$X_{(jkl)} = \mu = L + b_k\ (L) + g_i + sij + L^* g_{jl} + L^* s_{ijl} + e_{ijkl,}$ Where:

$X_{jj'}$ = the performance of the cross between i^{th} and j^{th} genotypes in the k^{th} replicate at l^{th} location,

μ is the population mean,

b_k (L) is the replications with locations

L is the location main effects

g_i is the general combining ability (GCA) effect,

s_{ijl} is the specific combining ability (SCA) effect, such that sij = s_{ji},

L *g and L *s_{ij} is the interaction of locations with GCA and SCA effects, respectively

e_{ijk} is the error associated with the ijklth observation.

The relative importance of GCA and SCA was estimated according to Baker (1978) as the ratio $2\delta^2$ GCA / ($2\delta^2$ GCA+δ^2 SCA); where δ^2 GCA and δ^2SCA are the variance components for GCA and SCA, respectively.

5.2.3.3 Gene action conditioning ear rot resistance

The analyses were performed using Griffing's (1956) Method 1, using the Diallel SAS-05 program (Zhang et al., 2005) in the SAS computer package. The source of variation and expected mean squares are presented in Table. 5.5.

Table 5.5 Degrees of freedom and expected mean squares from Griffing (1956) Model 1, Method 1

Source	Df	Mean Square	Expected mean squares Fixed effects
Environment (E)	*l – 1*		
Reps (R)/E	*l(k - 1)*		
F1 hybrid (H)	*p(p - 1)*	*Mh*	$\delta^2_e + k\,\delta^2_{hy} + k\,l\,[1/(v-1)\Sigma hi$
GCA	*p – 1*	*Mg*	$\delta^2_e + 2k(p-2)\,\delta^2_{gy} + 2k\,l(p-2)/9p-1)\Sigma gi^2$
SCA	*p (p - 3)/2*	*Ms*	$\delta^2_e + 2k\,\delta^2_{sy} + 2k\,l[2/p(p-3)]\Sigma\Sigma\, s^2 ij,\ i<j$
REC	*p(p - 1)/2*	*Mr*	$\delta^2_e + 2k\,\delta^2_{ry} + 2k\,l[2/p(p-1)]\Sigma\Sigma\, r^2 ij,\ i<j$
M	*p – 1*	*Mm*	$\delta^2_e + 2k\,\delta^2_{my} + 2klp[1/p(p-1)]\Sigma\Sigma\, m^2 i$
N	*(p -1)(p - 2)/2*	*Mn*	$\delta^2_e + 2k\,\delta^2_{ny} + 2kl[2/(p-1)(p-2)]\Sigma\Sigma\, n^2 ij,\ i<j$
H x E	*p(p - 1)(l - 1)*	*Mhy*	$\delta^2_e + k\,\delta^2_{hy}$
GCA x E	*(p - 1)(l - 1)*	*Mgy*	$\delta^2_e + 2k(p-2)\,\delta^2_{gy}$
SCA x E	*p(p - 3)(p - 1)/2*	*Msy*	$\delta^2_e + 2k\,\delta^2_{sy}$
REC X E	*p(p - 1)(l - 1)/2*	*Mry*	$\delta^2_e + 2k\,\delta^2_{ry}$
M X E	*(p - 1)(l - 1)*	*Mmy*	$\delta^2_e + 2k\,\delta^2_{my}$
N X E	*(p - 1)(p -2)(i - 1)/2*	*Mny*	$\delta^2_e + 2k\,\delta^2_{ny}$
Error (H X R/E)	*[p(p - 1)][l (k - 1))]*	*Me*	δ^2_e

Where GCA = general combining ability; SCA = specific combining ability; REC = reciprocal; M = Maternal; N = Non-maternal; K = number of replications = 1… b; l = number of environments = 1…y; p = number of parents; δ^2g = genotypic variance; and δ^2e is the environmental variance. The error term is the interactions of F_1 hybrids and replications in each environment.

To make further inferences on the type of gene action conditioning resistance, the relative contribution of GCA to the total genotypic sums of squares and relative importance of GCA, i.e., $2\delta^2$gca/[$2\delta^2$gca+ δ^2sca] (Baker,1978) were calculated.

5.3 Results

5.3.1 Combining abilities among five maize populations

Disease severity

Combining ability analysis revealed no significant GCA but significant SCA mean squares ($P<0.05$) for disease severity data (Table 5.6). The mean squares due to parents versus crosses also differed significantly for disease severity. However GCA x environment interaction effects was significant, though disease severity data showed no significant GCA mean square ($P>0.05$), the GCA effects ranged from -1.98 to 1.24 for disease severity (Table 5.7). Only Pop25 had the largest negative GCA effects, followed by MMV600. The F_1 combination, MMV600 X Pop25, had consistently the best negative SCA effect. Other crosses, Pop10 x PRA783244c3, ZUCASRc2 x PRA783244c3, and ZUCASRc2 x Pop25 had negative SCA effects though not significant. In contrast, PRA783244c3 x Pop25 had the highest positive SCA effect (5.06). The relative importance and contribution of GCA to the total genotypic sums of squares was 0.41 and 23%, respectively.

Grain yield

Only significant GCA effects were detected from the analysis of the F_1 crosses and parents (Table 5.6.). The GCA effects ranged from -1.38 in Pop10 to 40 in Pop25. The non-additive effects were small and non-significant. The inter population cross, MMV600 x Pop25, exhibited significant large SCA effects for yield, while the crosses Pop10 x MMV600 and ZUCASRc2 x MMV600, had the least effect for yield (negative SCA). Other crosses with positive and higher SCA values were ZUCASRc2 x Pop25 (0.340) and PRA783244c3 x Pop10 (0.030). However, the absolute magnitude of SCA effects for grain yield was very small, with GCA accounting for 95% of the total genotypic sums of squares (Table 5.7).

Secondary traits associated with ear rots

General combining ability effects for the four secondary traits measured were non-significant except insect ear damage (Table 5.6). However significant GCA X environment interaction was observed for root lodging, drooped ears, and husk cover. The GCA effects accounted for 16%, 61%, 71%, 34%, and 43% for lodging, ear declination, husk cover, insect ear injury (damage) and grain type, respectively. Significant SCA was observed for root lodging in the crosses involving Pop10 and ZUCASRc2, MMV600 x ZUCASRc2 for drooped ears, PRA783244c3 x MMV600 for husk cover and grain type for the cross Pop10 x MMV600.(Table 5.8). Significant SCA effects for ear insect damage in the crosses involving Pop25 and other populations except ZUCASRc2 were also observed.

Table 5.6 Mean square for the 5 x 5 population diallel analysis of disease severity data, grain yield and secondary traits across four tropical environments in Zambia

Genotypes	Df	Disease Severity %	Grain yield tons ha^{-1}	Lodging (%)	Ear declination (1 – 5)	Husk cover (1 – 5)	Ear Insect damage %	Grain type (1 – 4)
Replication/E	8	32.14	0 79	53.16	4.26	0.28	2.47	0.29
Environment (E)	3	2383.11**	38.58**	5219.43**	2.11*	11.72**	2.97	4.89**
Populations	4	618.10**	0.61	570.48	1.68	2.28	129.44**	0.52
Variety vs. Cross	1	1.56	33.77*	297.03**	0.04	0.07	1.85	2.72
F1 diallel crosses	9	124.34**	9.74**	251.34	1.98	2.15**	30.15**	1.04
GCA	4	63.40	20.89**	91.36	2.73	3.46**	22.96	0.99
SCA	5	173.09*	0.82	379.32*	1.38	1.10	35.90*	1.07*
Varieties x E.	12	9.18*	0.621	601.49**	0.81	1.53**	18.59**	1.38**
Variety-Cross x E.	3	40.76**	2.67**	783.31**	1.67*	3.05**	5.41*	1.76**
F1 diallel crosses Vs, E.	27	56.15**	1.54**	179.33**	1.54**	0.76**	11.21**	0.55
GCA x E.	12	65.64**	0.55	243.54**	2.25**	0.75**	10.65**	0.78
SCA x E.	15	48.56**	2.34**	127.96**	0.97	0.77**	11.66**	0.36
Error	111	4.94	0.65	40.19	0.63	0.29	2.04	0.46
Ratio GCA/SCA		0.41	1.00	0.23	0.85	0.89	0.55	0.63
Relative contribution of GCA to Total Genotypic Sums of Squares	(%)	23	95	16	61	71	34	43

*, ** Indicates significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 5.7 GCA and SCA estimates for grain yield, severity and grain yield (tons ha^{-1}) of maize ear rots for five maize populations evaluated in four locations across Zambia during 2006 to 2008

Population	Pop10	PRA783244c3	MMV600	Pop25	ZUCASRc2
% Severity					
Pop10		-2.94	0.75	0.50	1.69
PRA783244c3			0.72	5.06**	-2.83
MMV600				-4.08**	2.61
Pop25					-1.47
GCA effects	**1.10**	**0.21**	**-0.57**	**-1.98**	**1.24**
Grain yield (ton ha^{-1})					
Pop10		-0.05	0.17	-0.07	-0.50
PRA783244c3			0.12	-0.25	0.18
MMV600				0.07	-0.37
Pop25					0.24
GCA effects	**-1.35****	**0.38**	**0.17**	**0.40****	**0.39***

*, ** Indicates significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 5.8 GCA and SCA estimates for lodging, ear declination, husk cover, insect ear injury and grain type declination in five maize populations at four locations in Zambia

Parameter	Population	Pop10	PRA783244c3	MMV600	Pop25	ZUCASRc2
% Root lodging	Pop10		**4.04**	**2.93**	-1.18	-5.79*
	PRA78344c3			**0.96**	-2.15	-2.85
	MMV 600				-4.60	0.71
	Pop25					7.93**
	GCA	**2.47**	**-1.97**	**-0.36**	**0.00**	**-0.14**
Drooped ears	Pop10		0.13	**0.03**	**0.10**	-0.27
(score 1 - 5)	PRA78344c3			**-0.18**	**0.24**	-0.99
	MMV 600				-0.33	0.48**
	Pop25					-0.01
	GCA	**-0.23**	**-0.18**	**0.27***	**-0.19**	**0.33**
Husk cover	Pop10		-0.28	0.10	0.22	-0.03
(score 1 -5)	PRA78344c3			0.38*	-0.16	0.06
	MMV 600				-0.25	-0.22
	Pop25					0.19
	GCA	**-0.33**	**0.33**	**-0.31***	**0.25**	**0.10**
Ear insect damage	Pop10		-1.36**	**1.75****	**-1.06***	0.68
%	PRA78344c3			**-0.85**	**2.53****	-0.30
	MMV 600				1.00*	0.10
	Pop25					-0.47
	GCA	**-0.01**	**0.76***	**0.4**	**0.17**	**0.05**
Grain type	Pop10		0.04	0.32*	-0.18	-0.18
(score 1 – 4)	PRA78344c3			0.07	-0.01	-0.10
	MMV 600				-0.24	-0.15
	Pop25					0.43**
	GCA	**0.14**	**-0.28****	**0.03**	**0.10**	**-0.01**

*, ** Indicates significance at P ≤ 0.05 and P≤0.01, respectively.

5.3.2 10 x 10 diallel analysis for gene action

There were significant differences among the crosses for ear rot severity data. Both GCA and SCA effects were significant for ear rot severity data (Table 5.9). The reciprocal differences were also highly significant for ear rot severity. Both maternal and non-maternal effects were highly significant for ear rot severity. The interactions of environments with GCA, SCA, and maternal and non-maternal effects were also highly insignificant for disease severity. However, with respect to the cross sum of squares, the SCA effects accounted for 45.4% and the GCA 10.1%. The reciprocal differences accounted for 44.5% which is partitioned into 7.8% for maternal and 36.6% for non-maternal effects.

Only Pop10#7-8 expressed significant GCA effects though positive (3.40) for disease severity, followed by ZUCASRc2 # 55-56 (1.66); both full-sib families had been selected as resistant genotypes (Table 5.10). On the other hand, MMV600#109-110, which was susceptible, had the largest negative GCA (-2.22) effect though not significant. The relative importance of GCA effects on disease severity was 0.47.

The maternal effects ranged from -2.19 (PRA783244c3#61-62) to 2.59 (Pop25#5-6). Apart from MMV600#9-10, which had both negative GCA effect and negative maternal effect, all the full-sib families had either a positive GCA and negative maternal effect or vice versa. Four resistant full-sib families had negative GCA effects compared to three susceptible families (Table 5.11). When compared for maternal effects, three resistant full-sib families had negative maternal effects compared to two from the susceptible group.

Table 5.9 Mean square of the 10 x 10 diallel analysis for ear rot disease severity across two sites in Zambia

Source of variation	Df	Mean Square
Environment	1	11321.91
GCA	9	207.92**
SCA	45	186.89**
Reciprocal	45	183.39**
Maternal	9	162.38**
Non Maternal	36	188.65**
GCA x Environment	9	189.96**
SCA x Environment	45	155.10**
Reciprocal x Environment	45	182.90*
Maternal x Environment	9	207.52**
Non maternal x environment	36	176.75**
Error	197	38.15
Relative contribution of GCA to total genotypic SS (%)		18
Importance of GCA		0.47

Where GCA = General combining ability; SCA = Specific combining ability.

*, ** indicates significant at *P≤0.05* and *P≤0.01,* respectively.

Table 5.10 Mean percent ear rot disease severity, maternal effects, and GCA effects for ear rot severity of diallel parents across two sites in Zambia

Full -sib families	Class response	Severity (%)	GCA effects (%)	Maternal effects (%)
Pop 10# 7-8	Resistant	5.52	3.40**	-0.21
Pop 10# 143-144	Susceptible	14.45	-0.73	0.14
PRA783244c3#135-136	Susceptible	11.51	0.98	-0.01
MMV600#109-110	Susceptible	12.08	-2.22	0.24
Pop25#131-132	Susceptible	15.96	-0.28	1.73
PRA783244c3#61-62	Resistant	4.46	-1.27	-2.19*
MMV600#9-10	Resistant	5.50	-0.69	-0.84
Pop25#5-6	Resistant	7.58	-0.08	2.59**
ZUCASRc2#55-56	Resistant	4.94	-0.78	0.26
ZUCASRc2#59-60	Susceptible	24.36	1.66	-1.70
Mean		10.60		
$LSD_{0.05}$		6.499		
CV%		41.41		
SE (gi-gj)			1.46	2.27

*, ** significant at *P≤0.05* and *P≤0.01,* respectively. SE = Standard error

Five out of nine crosses involving Pop10#7-8 had positive SCA effects (Table 5.11). The highest positive SCA effect was in the cross, Pop10#7-8 (resistant) x ZUCASRc2#59-60 (susceptible), followed by Pop25#5-6 (resistant) x PRA783244c3#135-136 (susceptible). The cross, MMV600#109-110 (susceptible) x ZUCASRc2#59-60 (susceptible), did not have any SCA effect (-10.27).

More than 50% of F_1 crosses had negative reciprocal effects on ear rot disease severity (Table 5.12). Significant negative reciprocal effects were observed in the cross, Pop10#7-8 x Pop10#143-144(-12.19), followed by MMV600#109-110 x Pop25#5-6(-10.13). However, the largest but positive reciprocal effect on disease severity was observed in the cross PRA783244c3#135-136 x MMv600#109-110 (12.88).

The non-maternal effects ranged from -.11.84% .to 11.80%, the largest negative effects being in the cross, Pop 10# 143-144 x Pop10#7-8. This was followed by Pop25#5-6 x MMV600#109-110 (-7.98). The highest positive effects were in the cross, MMV600#9-10X PRA783244c3#135-136 (Table 5.12).

Table 5.11: Specific combining ability effects of 45 crosses for disease severity across two sites in Zambia

Parental Full Sib lines		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Pop 10# 7-8	P1		6.60**	0.00	-3.12	0.58	4.16	-4.31	-3.21	0.38	23.45**
Pop 10# 143-144	P2			-4.06	-0.21	-0.68	-1.98	2.54	-3.32	1.39	-10.16
PRA783244c3#135-136	P3				0.97	-5.35	-3.41	6.24	8.80**	2.90	-3.25
MMV600#109-110	P4					0.92	2.60	-4.33	4.91	-2.59	-10.27
Pop25#131-132	P5						3.54	-1.51	-1.06	-3.26	-4.31
PRA783244c3#61-62	P6							6.88**	2.08	-1.95	-3.96
MMV600#9-10	P7								-2.14	2.51	2.30
Pop25#5-6	P8									4.05	-3.64
ZUCASRc2#55-56	P9										5.51**
ZUCASRc2#59-60	P10										

*,** indicates significant *P≤ 0.05* and *P≤0.01* respectively

Table 5.12 Reciprocal (above diagonal) and non maternal (below diagonal) effects of 45 crosses for disease severity recorded in 45 crosses grown across two sites in Zambia

Parental Full Sib lines		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Pop 10# 7-8	P1		-12.19**	0.92	-1.23	-0.63	4.62	0.58	-0.29	6.24	-0.13
Pop 10# 143-144	P2	-11.84**		1.89	-1.46	-1.63	1.84	-4.45	1.16	-7.32*	-0.86
PRA783244c3#135-136	P3	1.12	1.74		-4.81	-0.84	2.29	12.68**	-9.32*	-0.35	3.06**
MMV600#109-110	P4	-0.78	-1.35	-4.56**		-5.08	5.37	0.88	-10.13**	1.76	2.12**
Pop25#131-132	P5	1.32	-0.04	0.90	-3.59		6.04	-2.63	2.32	0.53	2.86**
PRA783244c3#61-62	P6	2.65	-0.49	0.11	2.94	2.12		7.16	-4.76	-3.92	-0.21
MMV600#9-10	P7	-0.05	-5.43	11.80**	-0.21	-5.20	8.51**		2.49	-1.43	4.74**
Pop25#5-6	P8	2.50	3.61	-6.73	-7.78	3.18	0.02	5.91**		8.06**	-0.73
ZUCASRc2#55-56	P9	6.71	-7.19	-0.08	1.78	-0.94	-1.47*	-0.33	5.73		6.17**
ZUCASRc2#59-60	P10	-1.62	-2.70	1.37	0.17	-0.57	0.28	3.88**	-5.01	4.21	

*,** indicates significant *P≤ 0.05* and *P≤0.01* respectively .

Analysis of cross combinations

The results of the combined analysis across the two sites revealed that there were significant differences (P≤0.05) among the four disease response classes. Susceptible x susceptible crosses had the highest ear rot infection (12.02%), followed by resistant x resistant crosses (11.22%) (Figure 5.1). The lowest infection was in susceptible x resistant crosses (9.50%); however its reciprocal i.e. resistant x susceptible, was 1.2% more severe (10.78%) (Figure 5.1).

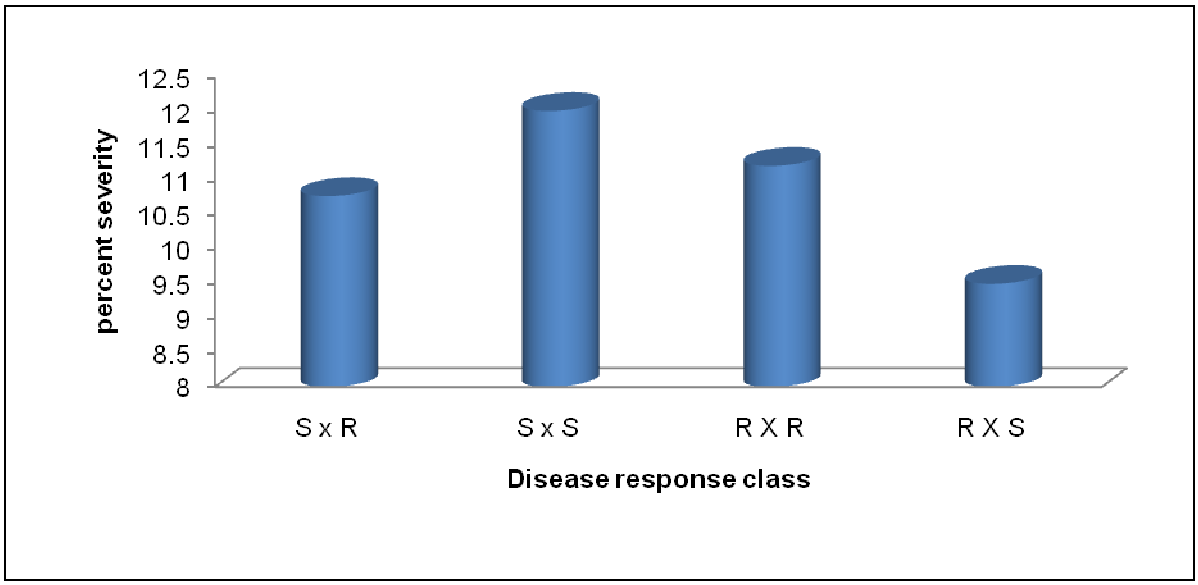

Figure 5. 1 Mean percent ear disease severity in the four response classes.

Environment and cross mean performance

Among the locations, Nanga was the best environment for screening genotypes for resistance to ear rots. The mean disease severity at Nanga (16.22%) was 3 times more than at Mt. Makulu (5.54%). Across environments, ear rot infection was 5 to 8 times lower in the top 10 crosses than the bottom 10 crosses. In addition most of the crosses in the top 10 displayed lower ear rot infection than better parents (Table 5.13).

Table 5.13: Percent disease severity of the five resistant parents and the top 10 and bottom 10 of the 90 maize hybrids at Mount Makulu and Nanga NIRS during 2008 season

Genotype	Response Class	Nanga	Mt. Makulu	Across Sites	Better Parent
Top 10					
MMV600#9-10 x MMV600#109-110	R x S	3.25	2.43	2.84	5.50
PRA783244c3#61-62 x ZUCASRc2#55-56	R x R	3.40	2.69	3.04	4.46
PRA783244c3#61-62 x ZUCASRc2#59-60	R x S	2.55	3.87	3.21	4.46
MMV600#109-110 x Pop25#5-6	S x R	3.62	3.25	3.44	7.58
Pop10#143-144 x ZUCASRc2#55-56	S x R	5.15	1.90	3.53	4.94
ZUCASRc2#59-60 x MMV600#109-110	S x S	3.48	3.67	3.57	12.07
ZUCASRc2#55-56 x MMV600#109-110	R x S	2.87	4.34	3.61	4.94
ZUCASRc2#59-60 x PRA783244c3#61-62	S x R	4.00	3.25	3.63	4.46
MMV600#109-110 x Pop25#131-132	S x S	6.20	2.40	4.30	12.07
MMV600#109-110 x MMV600#9-10	S x R	6.20	2.98	4.59	5.50
Bottom 10					
Pop10#7-8 x ZUCASRc2#55-56	R X R	38.50	1.90	20.20	4.94
Pop10#7-8 x PRA783244c3#61-62	R X R	33.00	10.77	21.89	4.46
Pop25#5-6 x ZUCASRc2#55-56	R X R	28.60	15.82	22.21	7.58
PRA783244c3#61-62 x MMV600#9-10	R X R	38.50	7.57	23.04	5.50
Pop25#5-6 x MMV600#109-110	R X S	46.50	0.90	23.70	7.58
Pop10#7-8 x ZUCASRc2#59-60	R X S	49.50	4.67	27.80	5.52
ZUCASRc2#59-60 x Pop10#7-8	S X R	41.60	13.10	27.35	5.52
Pop25#5-6 x PRA783244c3#135-136	R X S	52.38	7.60	29.99	7.58
PRA783244c3#135-136 x MMV600#9-10	S X R	59.50	0.85	30.18	5.50
Pop10#143-144 x Pop10#7-8	S X R	57.25	7.60	32.43	5.52
Resistant Parents					
PRA783244c3#61-62	R	3.79	5.12	4.46	
ZUCASRc2#55-56	R	8.05	1.82	4.94	
MMV600#9-10	R	8.80	2.20	5.50	
Pop10#7-8	R	7.52	3.52	5.52	
Pop25#5-6	R	9.55	5.60	7.58	
Overall mean		16.22	5.54	10.89	
$LSD_{0.05}$		16.10	6.40	12.13	
CV%		23.4	32.8	29.6	

5.4 Discussion

5.4.1 Combining abilities of the five maize populations for resistance to ear rot incidence and severity, and grain yield

Specific combining ability effects were more pronounced than GCA effects for disease severity in the 5 x 5 population diallel analysis, suggesting that non-additive factors were the major source of genetic variation for resistance to ear rots. However, studies involving mostly inbred lines have reported more additive gene effects (Dorrance et al., 1998; Naidoo et al., 2002). Only, Das et al. (1984) reported similar findings with open-pollinated varieties for Stenocarpella ear rot. The results, therefore suggests that population improvement methods such as recurrent selection for SCA may be emphasized to improve ear rot resistance in these populations.

An estimation of the GCA effects of populations revealed that Pop25 and MMV600 were good combiners for disease severity, whereas PRA783244c3, Pop25, and ZUCASRc2 were better combiners for grain yield. For grain yield, positive and high GCA effects were desirable as this suggested that these genotypes had superior additive gene effects for higher yields. In contrast, Pop10 posted a negative GCA effect for yield. Hence, it may be desirable not to use Pop10 for the extracting superior progenies to form high yielding open-pollinated experimental varieties. Because GCA was predominant for yield in the other populations, recurrent selection procedures that emphasize GCA would be recommended to enhance yield.

For the other agronomic traits, PRA783244c3 had a high GCA effect for lodging, while Pop10, Pop25, and ZUCASRc2 were observed to be better combiners for ear declination (or drooping). Pop 10 and MMV 600, on the other hand, appeared to be better combiners for husk covers. PRA783244c3 posted a large negative and significant GCA effect implying that it contributed more to flinty grain type.

The significant SCA effects obtained for ear rot disease severity in this study identified two important F_1 crosses, PRA783244c3 x Pop25 (5.06**) and MMV600 x Pop25 (-4.08**); the former had a positive SCA effect, while the latter a significant negative SCA effect. Only negative GCA and SCA effects indicated contributions towards resistance, while positive significant values suggested contributions towards susceptibility because the orientation of genetic effects for resistance are negative, as resistant plants present lower severity values (Reid et al., 1992). Only 40% of the parents and F_1 crosses had negative GCA and SCA effects and these (parents) contributed to increased ear rot resistance.

Three parents, PRA783244c3, Pop25, and ZUCASRc2 had significant positive GCA effects for yield; hence they would be useful parents in breeding varieties for high grain yield potential. The higher expression of non-additive genetic effects for disease severity, lodging, and insect ear damage could be attributed to the presence of deleterious genetic factors that cause endogamic depression. Hallauer and Miranda (1988) indicated that, in specific hybrid combinations, these non-additive effects may be of paramount importance.

It is well-known that in many hybrid breeding programmes, the overall objective is to choose hybrids adapted to all environments, hence mean GCA effects are more important. However, the significant GCA x environment mean square effects obtained in this study suggest the following (i) that it was possible to select parental lines to obtain hybrids for specific environments and (ii) development of variety crosses between ear rot resistant populations could increase maize yields even under ear rot epidemic conditions. Furthermore, the observed high GCA for yield variation of the crosses suggest that parental populations of varietal crosses could be efficiently screened on the basis of per se performance under artificial ear rot epidemic conditions.

The results indicate that selection for ear rot resistance is affected by high genotype by environment interaction (G x E) effects. Studies aimed at resistance to ear rots conducted elsewhere have also reported significant GCA x environment and SCA x environments interaction effects (Olatinwo et al., 1999; Naidoo et al., 2002; Rossouw et al., 2002). Perhaps the large G X E interaction for ear rot disease severity might explain why there are very few hybrids or varieties that have been reported in literature to be resistant to ear rots. The high G x E, especially of that of crossover type involving changes in the rank of genotypes in different environments makes selection for ear rots difficult. A different set of genotypes is elected in each environment making it difficult for breeders to decide on which set to advance in their breeding. This suggests the need to conduct multi-location trials under artificial inoculation to identify stable genotypes.

5.4.2 Gene action for resistance to ear rot incidence and severity, and grain yield

The significance of both the GCA and SCA effects for ear rot severity, indicating that both additive and non-additive effects, respectively, were important for ear rot resistance in these populations. Reciprocal differences were also highly significant for ear rot severity and were attributable to both maternal and non-maternal effects, suggesting that cytoplasmic genes and their interaction with nuclear genes played a significant role in the inheritance of ear rot resistance in this germplasm set. The detection of additive, non-additive, and reciprocal effects indicates that all these effects could be important to ear rot resistance. The findings of this study disagree with William et al. (2008) (though they worked on inbreds rather than

open pollinated populations) who reported that reciprocal, maternal and non-maternal effects were insignificant and therefore played a negligible role in the inheritance of resistance to ear rots and mycotoxins. The observation of significant differences between the classes R x S and S x R crosses confirmed the significance of a possible role of both cytoplasmic factors and cytoplasmic x nuclear gene interaction effects in conditioning ear rot resistance in the five populations under study. According to Evans and Kemicle (2001), maternal effects are attributable to cytoplasmic genetic factors, while non-maternal effects are explained by interaction effect between nuclear genes and cytoplasmic gene effects. The results agree with those of Lunsford et al. (1976) who reported that maternal effects in some genotypes influence their reaction to ear rots. The significant maternal and non- maternal X environment interaction effects observed in this study indicate that there was a lack of stability in some crosses in resisting ear rot infection, underscoring the need to conduct multi-location trials in screening genotypes for ear rot resistance.

Some F_1 crosses between the resistant and susceptible lines out-performed their resistant parents (Table 5.13), suggesting over-dominance gene action for ear rot resistance. This has implication on the design of single cross hybrids. It appears that resistance should be emphasized in one parent while the other parent can be selected on the basis of other selection criteria such as high GCA for yield.

The partitioning of the full-sib family effects revealed that SCA, unlike GCA, was the main contributor to genetic variation in resistance, confirming the significance of non-additive gene effects for ear rot resistance in the five populations under study. In general, the average magnitude of GCA or SCA effects reported in this study are comparable with those reported by other authors. William et al. (2008) reported average rates of 0.50 and -0.22 for GCA and SCA effects, respectively, for resistance to *A. Flavus* infection and mycotoxin accumulation in ten inbred lines. Rossouw et al. (2002) reported SCA effects of between -22 and 16.3 for resistance to Stenocarpella ear rot.

Although the maternal mean squares were significant for the trait measured, the proportion of the entry sum of squares attributable to maternal effects was less than for GCA and SCA. Cruiso (1987) suggested that in the presence of high dominant gene effects, as suggested in this study, maternal effects are unable to reinforce the additive-genetic effects effectively, therefore progeny performance may not be based on their parents' per se performance. Widstrom (1972) reported that the presence of reciprocal effects (both maternal and non-maternal) tends to inflate the effects of additive gene action, thereby compromising the combining ability of genotypes due to their inconsistent interaction with the environment. On the other hand, maternal effects tend to inflate genetic variance and this consequently slows

response to selection especially where the trait of interest is under complete maternal influence (Roach and Wulff, 1987).

Therefore, as a breeding strategy, there is a need to consider the effect of both nuclear and non–nuclear genetic factors in the selection of parents of the next generation. Desirable maternal effects would be those that orient the genetic make-up of the progeny for resistance to ear rots and increased crop yield. The presence of limited additive genetic variation available for selection for ear rot resistance could be improved by bi-parental mating of the early segregating generations to break linkages. The hybridisation, or crossing of superior lines, would result in superior progeny. Both additive and non-additive variation could be exploited in reciprocal recurrent selection schemes to enhance ear rot resistance in these populations.

5.5 Conclusion

The following conclusions were drawn from these two diallel analyses:

1. Additive, non-additive gene effects and reciprocal effects were important in conditioning resistance to ear rots.

2. Two genetically broad based populations, Pop25 and MMV 600, had large negative GCA effects that suggested they may be useful parents in a breeding programme for improving resistance to maize ear rots. The cross combination of these populations also displayed large negative SCA effects for disease severity.

3. Non-additive gene effects appeared to be more important in some specific crosses. These non additive components of gene action could be optimised in upgrading the genetic potential of the crop by adopting reciprocal recurrent selection and hybrid technology, which is already a reality in maize breeding.

4. The reciprocal effects were due to both maternal and non-maternal factors, suggesting significance of both cytoplasmic genes and their interaction with nuclear genes in influencing ear rot resistance in the five tropical populations.

5. While selection for disease severity and other agronomic traits would be more desirable, selection based on disease severity alone could result in progress toward more resistant genotypes.

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CHAPTER 6
RESPONSE TO RECURRENT FULL-SIB SELECTION IN FIVE MAIZE POPULATIONS FOR EAR ROT RESISTANCE

6.0 Abstract

Maize ear rots are an important disease of maize (*Zea mays* L.) in the tropics, causing serious losses for many resource-poor farmers. This study evaluated whether the ear rot resistance of five maize populations could be improved by using a full-sib recurrent selection scheme. Synthetics were formed by recombining the most resistant 10% of at least 70 full-sib families screened for ear rot resistance in each of the five maize populations involved in the study. An evaluation experiment, consisting of a randomised complete block design with split-plot arrangement, was used to evaluate the two cycles of each population, C_0 and C_1, across four sites in the 2007/8 rainy season. Cycle response indicated reduction in the incidence of *Aspergillus* and *Stenocarpella* ear rots in Pop10 and PRA783244c3, *Stenocarpella* disease severity in all the five populations, and *Aspergillus* in Pop10 and Pop25. Estimate of progress per cycle of selection across the three ear rots were 16.8, 15.9, 26.5, 10.7 and 2.5% for Pop10, PRA783244c3, MMV600, Pop25 and ZUCASRc2, respectively. This yield increase may have resulted from average reduction in the disease incidence by -2.3 % gains per cycle' averaged over populations and environments. There were differences in the magnitude of genetic variance after selection. In 30 to 40% of population cycles, genetic variability shrunk, while in the others it increased by 2 to 6 times, indicating that recurrent selection was still feasible in these populations. While some reduction in genetic variation may be attributed to random genetic drift, this did not seem to seriously impede any response to selection. Broad sense heritability values were low to medium for resistance to ear rots, and medium to high for grain yield. Significant negative correlations were also found between ear rot disease severity and grain yield. Though our results were from one selection cycle, they indicated that full-sib recurrent selection was effective for improving these tropical maize populations for ear rot resistance and yield.

Keywords: Ear rots, full-sibs, genetic gain, maize, recurrent selection

6.1 Introduction

Maize (*Zea mays* L.) is the most important food crop in Zambia and is the principal crop in most farming systems in the country. However its production is affected by many factors, among them the susceptibility of the crop to ear rots. Several species, belonging to more than 10 ear rot genera, are known to infect maize, resulting in huge grain losses every year. Though yield losses have not been quantified under favourable weather conditions, losses could be high (Rao et al., 1987). Nawa (2005, unpubl.) reported a 10 to100% yield loss in central Zambia following a severe epidemic of *Fusarium verticillioides*. Schjoth et al. (2008) reported yield reduction due to artificial F*. verticillioides* epidemics of about 532 $kgha^{-1}$. Elsewhere in the world, crop losses of up to 18% have been reported (Ajanga and Hillocks, 2000). Vigier et al. (1997) estimated crop loss could be as high as 48% under weather conditions favourable for an ear rot epidemic.

Several control measures have been proposed for managing ear rots but none has been met with great success (Munkvold, 2003). Breeding ear rot resistant maize remains the most feasible option. The availability of resistant varieties could improve not only the crop yield levels and incomes of maize producers, but would result in a reduction in the amount of mycotoxins in the maize kernels, thereby improving the health standards of the local people and increasing the productivity of domestic animals fed with maize.

Since the 1900s when the disease was first noticed in farmers' fields, several breeding schemes, including recurrent selections, have been used to improve maize resistance to ear rots. However, few reports exist on the use of recurrent full-sib selection in improving maize for resistance to ear rots. Recurrent full-sib selection has been used largely to improve general population performance (Smith, 1979), as well as the performance of the hybrids that are developed for several traits, including grain yield, from succeeding cycles of selection in maize (Betrán and Hallauer, 1996). The agronomic value of the succeeding cycles of maize populations is improved through an increase in the frequency of favourable alleles within the population while managing the genetic variability (Hallauer and Miranda, 1988). Theoretically, recurrent full-sib selection is far superior to most recurrent selection methods due to the increased parental control because predicted gains due to selection are higher. Viana (2007) demonstrated a genetic gain of 2.47 through selection using full-sib families after a single cycle of selection for yield and reported further that the higher the degree of dominance of the favourable genes, the greater the efficiency of full-sib selection.

Most recurrent selections have been used for yield improvement, protein content improvement, and general adaptability. However, few studies that have been conducted at

the International Maize and Wheat Improvement Centre (CIMMYT), have shown that full-sib recurrent selection could improve resistance in maize to most diseases (De Leon and Pandey, 1989). Abendon and Tracy (1998) reported a significant linear reduction in percent leaf area infected by common rust after three cycles of recurrent full-sib selection. Ramirez-Diaz et al. (2000) reported a reduction in ear rot infection of 0.97% per cycle in the maize population, PABGT-CE.

The five maize populations used in this study have diverse origins (Table 6.1) and are widely used by the local breeding programme to extract open- pollinated varieties (OPV) and hybrids. The original MMV600, ZUCA, and PRA783244 populations formed part of the initial maize germplasm collection of the local breeding programme and are still represented in the pedigrees of the experimental varieties in the programme. In the latter part of the seventies, MMV600, in particular, was subjected to several cycles of recurrent full-sib and half-sib selection for yield and adaptability with a single cross, ZPL12, as tester. Significant improvement in the performance for yield per se was found and an improved version of MMV600 was released as an OPV in 1984 and is widely grown by the farmers throughout the country. The other two populations are from CIMMYT and were initially pre-released in Zambia for drought mitigation and not disease resistance. Hence there is a need to improve them for resistance to ear rots, among other diseases.

The objectives of the study were to:

(i) determine the effectiveness of full-sib recurrent selection in developing ear rot resistant maize varieties;

(ii) evaluate progress from using full-sib selection for resistance to ear rots and grain yield in five broad-based tropical maize populations.

The hypothesis of the study was that improved cycles of the five maize populations would perform better than the unimproved version after one cycle of full-sib selection for ear rot resistance.

6.2 Materials and Methods

6.2.1 Choice of populations

Five tropical maize populations, namely, Pop10, Pop25, PRA 78-32-44c3, MMV600, and ZUCA-SR-c2 were used in this study (Table 6.1).

Table 6.1 Description and source of five tropical maize populations used in this study

Population	Characteristic	Derived from
Pop 10	Intermediate maturity, white dent	CIMMYT Population 10
PRA783244c3	Intermediate maturity, white	Poza RICA7 832 and Across7844
MMV 600	Intermediate maturity, white semi flint	Locally developed populations
Pop 25	Intermediate maturity, white flint	CIMMYT Population 25
ZUCASR-C2	Late maturity, white semi-flint grain	Ukiriguru Composite A

6.2.2 Formation of advanced cycle

Three hundred and forty-three full-sib families were created from the five maize populations described above (Table 6.1) in 2005/6. These were evaluated for percent ear rot incidence, and severity, grain yield ($kgha^{-1}$), days to 50% silking, root and stalk lodging, husk cover, ear declination, ear insect damage and approximately 67 full-sib families (10% selection intensity) were selected from each population. The overall set means of these families and their source populations are presented in Appendix 1. These families were then recombined in a partial diallel mating scheme during the 2007 winter nursery at Mount Makulu Central Research Station, Chilanga, in Zambia, to create a new population (C_1) for initiating a new selection cycle. The mating scheme ensured that each of the selected families was crossed with each other. At harvest, all successfully pollinated ears were collected for the crosses involving the seven selected full-sibs, and an equal amount of seed from each cross was used to form a new, balanced, synthetic population, i.e., five new synthetic populations were created.

6.2.3 Evaluation of original populations and the new synthetics

6.2.2.1 Experimental arrangement

The original populations (Cycle-0, C_0) and new synthetic populations (Cycle-1, C_1) were evaluated in a split plot experiment replicated three times at Mt Makulu, Kafue, and Chongwe, and twice at Nanga (Table 6.2). The main plots were three ear rot causing pathogens, *A. flavus*, *F. verticillioides,* and *S. maydis,* while the population cycles were the sub-plot factor. Each plot consisted of two, 5 m long rows, including an approximately 1 m alley at the end of each plot marked with a peg. Inter-row spacing was 0.90 m. The plots were sown with 2 kernels per hill on 11th December, 2007. The first row was inoculated with *F.verticillioides*, the second row *S.maydis,* and the third row *A. flavus*. Data were collected on incidence and severity of the ear rots, and grain yield (t ha^{-1})

Table 6.2 Environmental characteristics of the five experimental sites in Zambia

Environmental characteristics	Mount Makulu	Nanga NIRS	Kafue	Chongwe
Latitude (South)	15°13.10'	15°32.87'	15 °41.93	15 ° 23.83
Longitude (East)	28°14.93'	27 °10.93'	28 ° 14.85	29 ° 29.60
Altitude (metres above sea level)	1206	1190	1095	1095
Relative humidity (%)	69.36	54.76	60.4	75.6
Mean annual temperature (°C)	20.6	23.7	23	22.3
Annual rainfall (mm)	802	790	906.7	946.1
Soil type	Chromi-haplic lixisols	Vertisols	Acrisols	Acrisols
Soil texture	Fine loam to clay	Sandy clay	Sandy loam	Sandy loam

Source: Meteorological Department, Lusaka

Plots were initially over planted, and then thinned to 17 plants per plot, two to three weeks after seedling emergence, to provide a uniform population of about 55555 plants ha^{-1}. On the basis of soil tests, NPK fertiliser was applied at the rate of 80:40:40 kg ha^{-1} for optimum plant growth. Two to three hand weeding were carried out to keep the fields clean. Additional nitrogen was applied at the V5 stage as a top dressing at the rate of 92 kg ha^{-1}.

6.2.3.2 Ear rot inoculation methods

The ear rot inoculum was prepared by infesting maize grit and sterilised toothpicks with one of three ear rot fungi*, Aspergillus flavus* Link Fr., *Fusarium verticillioides* (Sacc.) Nirenberg (Syn. =*F. moniliforme* J.Sheld.), and *Stenocarpella maydis* (Berk.) Sutton. The fungi were isolated from the diseased maize kernels collected during the ear rot survey of 2006 in the Southern and Lusaka provinces of Zambia (See Chapter 3). Prior to infesting the maize grit and toothpicks, the Fusarium and *Aspergillus* fungi were grown on potato dextrose agar (PDA) at 24^{o}C and *Stenocarpella* on malt extract agar at 26^{o}C for 7 to 10 days. Then a spore suspension of *F. verticillioides*, *A. flavus,* or *S. maydis* was poured over agar moistened sterilised toothpicks and maize grit separately in 500 ml glass jars with lids. The jars with colonised toothpicks were stored on laboratory shelves away from light for at least one month. This ensured effective colonisation of the toothpicks. In the case of maize grit, the infested maize grit was air dried on A4 white paper after a month in storage. Thereafter, it was finely ground using a mill and transferred back into the glass jars for later use.

The two cycles, C_0 and C_1 of each of the five populations, were subjected to three inoculums, namely, the three individual ear rot species, *Aspergillus flavus*, *Fusarium verticillioides* and *Stenocarpella maydis* that constituted the mixture used in full-sib family evaluations.

Fifteen plants from each row were artificially inoculated by one of the three ear rot causing fungi. A combination of two techniques, leaf whorl placement of an ear rot fungus infested maize grit at 8 -10 leaf stage, followed by colonised toothpicks inserted in the ear at mid-milk stage, was used. Inoculated plants and ears were marked by a spot of paint.

6.2.3.3 Data collected

Data were collected on the incidence and severity of ear rots, grain yield, ear declination, ear insect damage, husk cover, root and stalk lodging..

Incidence and severity of ear rots

The incidence and severity of maize ear rots were recorded at harvest. The number of harvested cobs that exhibited the disease were counted and expressed as percent incidence.

Using, the 7-class rating scale developed at the Canadian Research Station in Ottawa (Reid et al., 1996), percent disease severity was determined by visual assessment of grain colour and development, where:

1 = 0%, sound, unblemished kernels, no symptoms, 0%

2 = 1 to 3%, slight infection,

3 = 4 to 10%, moderate infection, 4 = 11 to 25%, high scattered infection,

5 = 26 to 50%, high coalescing infection,

6 = 51 to 75%, high infection, and

7 = >75% heavily infected, kernels damaged, covered with fungus, or discoloured

Grain yield (tonnes ha^{-1}) was determined at harvest and adjusted for grain moisture at 12.5% moisture after calculating shelling percentage. Grain moisture determinations, using a grain moisture tester (Dickey John, USA), were made on five shelled cobs randomly picked within the treatment row. Grain yield (kg ha^{-1}) was calculated using the formula:

Grain yield (kg) = (Field weight x (100-MC) x Shelling percent X 10000)/[100-12.5)*Plot area, where MC = moisture percent. It was later converted to tons per during data analysis.

Other agronomic traits

Data on a whole plot basis were also collected for the following traits: root lodging (%) of plants leaning more than 45^{o} or more from the vertical, stalk lodging (broken stem below the primary ear), ear insect damage on a scale of 1 to 5, where 1 = no damage and 5 = heavily infested. Ear declination or drooped ears were rated on five primary ears per plot for ears either upright or bending downwards, using a 1 to 5 rating scale (where 1 = hung completely downwards and 5 = upright). Husk cover was rated using the rating method of Kossou et al. (1993) on a scale of 1 – 5; the rating was done by placing a hand around the husk leaves as they extended beyond the ear tip, making a fist such that the base of the hand rested on the tip of the ear. If the husk leaves were longer than four fingers, the rating was one, longer than three fingers, the rating is two, more than two fingers, the rating was three, more than one finger, the rating was four, and when the husk leaves were not more than one finger long, ear tip exposed, the rating was five. The husk rating was reduced by a value of one when, by squeezing the husk leaves in the fist, they appeared to be loose or easily compressed - loose husks would allow water to get into the cob easily and this water might subsequently accelerate the rotting of the drying kernels.

6.2.4 Statistical analysis

The data for each population from each site were analysed separately, then pooled together after conducting a Bartlett test for homogeneity of error variance (Payne et al. 2008). The analysis of variances (ANOVA) was based on a randomised complete block design (Steel and Torrie, 1980) using Genstat 11th edition (Payne et al., 2008)

The linear mathematical model for the pooled analyses of variance across location was analysed according to a split-plot analysis.

Variance components were estimated using REML in Genstat 11th Ed. (Payne et al. 2008). Each population was analysed for each pathogen, locations being considered as random.

(i) Broad sense heritability was obtained using the formula σ^2_g / σ^2_p (Dabholkar, 1992). Where δ^2_g is the genotypic variance and σ^2_p, the phenotypic variance.

(ii) Standard error of broad sense heritability was calculated as suggested by Dickerson (1969) where SE (H^2) = $2SE(\sigma^2_g)/(\sigma^2_g + \sigma^2_p + \sigma^2_{we})$, $SE(\sigma^2_g)$ is the square root of the genetic variance , and σ^2_g,σ^2_p and σ^2_{we} refer to the genetic, between plot, and within plot variance, respectively. The latter two are due to common environmental variance or (σ^2_e).

(iii) Selection differential ($S=\mu_{fs}-\mu$) was calculated by subtracting the population mean (comprising all full-sibs) from the mean of 10% selected full-sibs based on the selection criteria above.

(iv) Predicted gain ($Rc = Sh^2$) was determined as the product of selection differential and heritability observed in the cycle-1 of each population.

(v) Observed response to selection was calculated as follows: $Rso = \mu_{C1} - \mu_{C0}$; Rso is observed response to selection, C_1 = mean of the advanced cycle (C_1) and C_0 = mean of original population.

(vi) Percent gain cycle^{-1} was calculated by the formula as:

Percent gain cycle^{-1} = $[(C_1 - C_0)/C_0] \times 100$ (Keeling, 1982)

Phenotypic correlations were calculated to determine the associations between the secondary traits with grain yield.

6.3 Results

6.3.1 Evaluation of realised gain and expected genetic gain for ear rot incidence and severity, and grain yield

The analysis of variance showed significant differences ($P<0.05$) among the two cycles for disease incidence (Table 6.3). The percent incidence of Aspergillus ear rot was significantly different ($P<0.05$) for the two cycles of Pop 10 and PRA 783244c3, and not for MMV600, Pop25, and ZUCASRc2. No significant differences ($P>0.05$) were observed among the populations for percent incidence of Fusarium ear rot. However, there were significant differences among the two cycles for the incidence of Stenocarpella ear rot in MMV600 and Pop10.

The two cycles of Pop10 differed significantly ($P<0.05$) for the severity of Stenocarpella ear rot (Table 6.4). The degree of infection in Cycle-1 was about half of that observed in the Cycle-0. The two cycles of PRA7344c3 differed considerably for Aspergillus ear rot severity and not Stenocarpella. Fusarium ear rot was most severe in Cycle-1 compared to Cycle-0 of Pop25 and ZUCASRc2. A net reduction in the severity of Stenocarpella infection was recorded in Cycle -1 of ZUCASRc2.

Significantly high yields were observed in Cycle-1 than in Cycle-0 of Pop10 and ZUCASRc2 under Fusarium ear rot disease pressure and with MMV600 and Pop25 under Stenocarpella ear rot disease pressure (Table 6.5). The largest increase in grain yield from cycle-0 to cycle-1 was in MMV600 under Stenocarpella infection (1.21 tons ha^{-1}), followed by MMV600 (1.08 tons ha^{-1}). The lowest was in ZUCASRc2 under Aspergillus ear rot condition.

Table 6.3 Percent disease incidence of Aspergillus, Fusarium and Stenocarpella ear rots in the original (C_0) and advanced (C_1) cycles of five maize populations evaluated across four sites in Zambia during 2007/8 season

Population	Cycle	*Aspergillus*	Fusarium	*Stenocarpella*
			% Incidence	
Pop10	C_0	71.60	55.20	67.20
	C_1	54.00	60.40	46.30
	$C_1 - C_0$	-17.60	5.20	-20.90
	$LSD_{0.05}$	8.65	9.82	17.79
	P-value	0.05	0.61	0.03
PRA783244c3	C_0	70.50	67.30	68.50
	C_1	59.70	63.20	53.30
	$C_1 - C_0$	-10.80	-4.10	-15.20
	$LSD_{0.05}$	6.76	27.58	17.30
	P-value	0.01	0.75	0.39
MMV600	C_o	66.88	64.86	58.39
	C_1	74.35	69.02	73.14
	$C_1 - C_0$	7.47	4.16	14.75
	$LSD_{0.05}$	15.66	18.90	12.80
	P-value	0.17	0.26	0.48
Pop 25	C_0	67.10	67.30	68.50
	C_1	71.80	63.20	53.30
	$C_1 - C_0$	4.70	-4.10	-15.20
	$LSD_{0.05}$	19.69	27.58	17.30
	P-value	0.75	0.17	0.07
ZUCASRc2	C_0	60.90	59.90	69.90
	C_1	73.20	61.10	73.50
	$C_1 - C_0$	12.30	1.20	3.60
	$LSD_{0.05}$	16.65	11.50	7.09
	P-value	0.30	0.85	0.59

Table 6.4 Percent disease severity of Aspergillus, Fusarium and Stenocarpella ear rots in the original (C_0) and advanced (C_1) cycles of five maize populations evaluated across four sites in Zambia during 2007/8 season

Population	Cycle	*Aspergillus*	*Fusarium*	*Stenocarpella*
			% Severity	
	C_0	5.00	12.00	8.00
	C_1	4.00	12.00	4.00
Pop10	$C_1 - C_0$	-1.00	0.00	-4.00
	$LSD_{0.05}$	1.70	2.20	2.60
	P-value	0.08	0.86	0.02
	C_o	6.00	11.00	6.00
	C_1	8.00	10.00	5.00
PRA783244c3	$C_1 - C_0$	2.00	1.00	-1.00
	$LSD_{0.05}$	1.30	1.40	2.30
	P-value	0.00	0.67	0.39
	Co	7.93	7.11	12.06
	C1	9.79	7.84	10.95
MMV600	$C_1 - C_0$	1.87	0.74	-1.11
	$LSD_{0.05}$	2.88	3.52	3.41
	P-value	0.17	0.26	0.48
	Co	12.00	7.00	6.00
	C1	11.00	10.00	5.00
Pop 25	$C_1 - C_0$	-1.00	3.00	-1.00
	$LSD_{0.05}$	3.70	2.10	2.30
	P-value	0.75	0.01	0.77
	Co	7.00	7.00	8.00
	C1	7.00	11.00	5.00
ZUCASRc2	$C_1 - C_0$	0.00	4.00	-3.00
	$LSD_{0.05}$	3.80	3.20	1.60
	P-value	0.17	0.00	0.01

Table 6.5 Mean grain yield of the original (Co) and advanced (C1) cycles of the five maize populations under artificial *Asergillus flavus, Fusarium verticillioides* and *Stenocarpella maydis* epiphytotic conditions across four sites in Zambia during 2007/8 season

Populations	Cycles	*Aspergillus*	Fusarium	*Stenocarpella*
		Grain yield (tha^{-1})		
Pop10	Co	3.32	2.88	2.77
	C1	2.91	3.96	3.47
	$C_1 - C_0$	-0.41	1.08	0.69
	$LSD_{0.05}$	0.54	0.34	0.87
	P-value	0.12	0.05	0.08
PRA783244c3	Co	3.53	2.89	3.65
	C1	3.55	3.01	3.42
	$C_1 - C_0$	0.02	0.12	-0.23
	$LSD_{0.05}$	0.23	0.27	0.59
	P-value	0.98	0.75	0.16
MMV600	Co	2.98	2.92	2.36
	C1	2.74	2.79	3.56
	$C_1 - C_0$	-0.24	-0.13	1.21
	$LSD_{0.05}$	0.20	0.34	0.56
	P-value	0.28	0.42	0.00
Pop 25	Co	3.24	2.67	2.89
	C1	3.56	3.28	3.75
	$C_1 - C_0$	0.32	0.59	0.86
	$LSD_{0.05}$	0.86	0.70	0.23
	P-value	0.42	0.09	<.001
ZUCASRc2	Co	2.93	2.46	2.47
	C1	3.01	3.23	2.39
	$C_1 - C_0$	0.08	0.76	-0.08
	$LSD_{0.05}$	0.39	0.14	0.40
	P-value	0.44	<.001	0.65

The expected genetic gain ranged from -0.14 in PRA783244c3 for Stenocarpella ear rot incidence to -7.53 in Pop25 for the Aspergillus incidence (Table 6.6) For disease severity the highest predicted gain was recorded in MMV600 for Fusarium ear rot and the lowest in PRA783244c3 under Stenocarpella ear rot. The highest percent gain per cycle for ear disease incidence was recorded for Pop10 (-31.10%), followed by the same population for

Aspergillus ear rot (-24.60%). Negative genetic gain and percent gain are desirable attributes of resistance. For grain yield, positive genetic gain and percent gain are desirable. The expected genetic gain was from 17.03 to 42, while that of percent gain per cycle was between -12.38 and 25.02 for Pop10 under Aspergillus and Stenocarpella ear rots.

6.3.2 Genetic variability and heritability

The genetic variances (δ^2g), phenotypic variances (δ2ph), and broad sense heritability (H^2) estimates for resistance to ear rot disease incidence are presented in Table 6.7. The genetic variances increased more than three times in PRA783244c3, MMV600, and Pop25 for all three ear rots, except in PRA783244c3 and Pop25 under Stenocarpella disease pressure. The biggest increase in genetic variation, in MMV600, occurred under Fusarium ear rot conditions. There was a reduction in genetic variance in ZUCASRc2 for the incidence of all three ear rots. The reduction in genetic variation in Cycle-1 of ZUCASRc2 was the largest under Fusarium ear rot conditions and the least under Stenocarpella ear rot disease conditions.

Generally, the magnitude of genetic variance for grain yield decreased in all five populations, except ZUCASRc2. The largest reduction in genetic variance occurred under Fusarium ear rot conditions. With ZUCASRc2, the largest increase in genetic variance for grain yield occurred under Aspergillus ear rot conditions and the least under Stenocarpella ear rot conditions. As indicated by the magnitude of their standard errors, these estimates of variances were subject to considerable sample size and sampling error confounded by the sample size and/or probability of the genotype x interaction biases (Williams et al,. 1965).

Table 6.6 Effect of selection on the four traits estimated in the five populations

Population	Parameter	Incidence (%)			Severity (%)			Grain yield (tons ha^{-1})		
		Aspergillus	***Fusarium***	***Stenocarpella***	***Aspergillus***	***Fusarium***	***Stenocarpella***	***Aspergillus***	***Fusarium***	***Stenocarpella***
Pop10	Observed response	-17.63	5.20	-20.90	-1.00	0.00	-4.00	-0.41	1.08	0.69
	Predicted gain	-1.96	-13.16	-6.56	-5.40	-2.51	-1.43	0.03	0.02	0.02
	Percent gain/cycle	-24.60	9.40	-31.10	-25.0	0.00	-50.0	-12.38	37.62	25.02
PRA783244c3	Observed response	-10.80	-4.10	-15.20	2.0	3.00	-1.00	0.02	0.12	-0.23
	Predicted gain,	-0.51	-0.18	-0.14	-0.31	-0.34	0.00	0.46	0.41	0.42
	Percent gain/cycle	-15.30	-6.10	-22.28	33.3	42.9	-16.9	22.19	3.33	22.26
MMV600	Observed response	7.47	4.16	14.75	1.87	0.74	-1.11	-0.24	-0.13	1.21
	Predicted gain	-6.45	-5.62	-5.44	-12.04	-15.80	-12.51	0.03	0.03	0.02
	Percent gain/cycle	11.20	6.40	25.3	23.6	10.40	-9.20	20.27	9.41	49.79
Pop25	Observed response	4.70	-4.10	-15.20	-1.00	3.00	-1.00	0.32	0.59	0.86
	Predicted gain	-7.53	-1.28	-0.48	-1.07	-1.23	-0.13	0.16	0.27	0.21
	Percent gain/cycle	7.00	-6.10	-22.20	-8.30	42.90	-16.70	9.70	19.66	2.78
ZUCASRc2	Observed response	12.30	1.20	3.60	0.00	4.00	-3.00	0.08	0.76	-0.08
	Predicted gain	-1.74	-9.54	-0.56	-0.24	-0.13	-0.69	0.33	0.28	0.30
	Percent gain/cycle	20.10	4.40	5.60	0.0	57.10	-37.50	8.00	2.91	-3.24

The broad sense heritability values for ear rot disease incidence ranged from 0.00 to 0.79, the highest being in cycle 0 for Aspergillus ear rot incidence, the lowest under the same ear rot conditions in PRA783244c3 (Table 6.7). Generally low heritability values were obtained for Stenocarpella ear rot incidence compared to the other ear rots in all five populations. High heritability values were recorded in Pop10 and ZUCASRc2 under Fusarium ear rot disease pressure.

The broad sense heritability values for resistance to ear rot disease severity ranged from 0.01 to 0.91. The highest heritability values were recorded under Aspergillus ear rot conditions, followed by Fusarium. The heritabilities were low to medium under Stenocarpella. Among the populations, low to moderate heritabilities were recorded in Pop10 and ZUCASRc2, while moderate to high heritability values were recorded in MMV600 and Pop25 for ear rot disease severity. High heritability values were recorded in all five populations for grain yield (Table 6.8). They ranged from 0.43 to 0.93 in Cycle-1 of Pop10 under Fusarium ear rot conditions and Cycle-0 in Pop25 under Fusarium ear rot conditions, respectively. On average, the highest heritability values for grain yield were recorded in ZUCASRc2 (Table 6.9).

Table 6.7 Estimates of genetic variance, phenotypic variance and heritability (H^2) for disease incidence in five populations

Population	Parameter	*Aspergillus*		*Fusarium*		*Stenocarpella*	
		Cycle-0	Cycle-1	Cycle-0	Cycle-1	Cycle-0	Cycle-1
Pop10	$\delta^2 g$	430.50	61.80	470.50	181.43	292.00	239.00
	$\delta^2 ph$	546.70	630.40	741.20	275.71	953.40	728.50
	H^2	0.79	0.10	0.63	0.66	0.31	0.33
	$SE(H^2)$	0.04	0.04	0.03	0.05	0.02	0.03
	SE ($\delta^2 g$)	10.37	3.93	10.85	6.73	8.54	7.73
PRA783244c3	$\delta^2 g$	0.72	249.38	8.02	35.38	109.48	57.70
	$\delta^2 ph$	315.30	265.75	69.74	107.94	171.78	223.10
	H^2	0.00	0.94	0.11	0.33	0.64	0.26
	$SE(H^2)$	0.01	0.06	0.07	0.08	0.07	0.05
	SE ($\delta^2 g$)	0.42	7.90	1.42	2.97	5.23	3.80
MMV600	$\delta^2 g$	136.30	759.50	35.00	1178.10	64.53	524.00
	$\delta^2 ph$	412.70	976.70	316.00	1741.10	102.00	799.00
	H^2	0.33	0.78	0.11	0.68	0.63	0.66
	$SE(H^2)$	0.04	0.03	0.03	0.03	0.09	0.03
	SE ($\delta^2 g$)	5.84	13.78	2.96	17.16	4.02	11.45
Pop25	$\delta^2 g$	72.90	360.80	23.00	99.10	323.30	13.90
	$\delta^2 ph$	513.40	587.50	278.50	950.40	961.70	353.40
	H^2	0.14	0.61	0.08	0.10	0.34	0.04
	$SE(H^2)$	0.02	0.02	0.03	0.03	0.03	0.02
	SE ($\delta^2 g$)	4.27	9.50	2.40	4.98	8.99	1.86
ZUCASRc2	$\delta^2 g$	368.80	3.20	819.10	332.80	86.40	8.10
	$\delta^2 ph$	718.10	26.14	1055.90	496.50	207.00	205.00
	H^2	0.51	0.12	0.78	0.67	0.42	0.04
	$SE(H^2)$	0.04	0.12	0.03	0.04	0.06	0.03
	SE ($\delta^2 g$)	9.60	0.89	14.31	9.12	4.65	1.42

S.E = Standard error

Table 6.8 Estimates of genetic variance, phenotypic variance and broad sense heritability for disease severity in five populations

Population	Parameter	*Aspergillus*		*Fusarium*		*Stenocarpella*	
		Cycle-0	Cycle-1	Cycle-0	Cycle-1	Cycle-0	Cycle-1
Pop10	δ^2g	0.91	11.79	133.69	17.06	12.74	1.70
	δ^2ph	9.24	12.90	164.08	40.16	25.50	6.98
	H^2	0.10	0.91	0.81	0.42	0.50	0.24
	$SE(H^2)$	0.19	0.21	0.07	0.11	0.14	0.30
	SE (δ^2g)	0.48	1.72	5.78	2.07	1.78	0.65
PRA783244c3	δ^2g	5.45	10.27	37.24	98.50	5.13	0.15
	δ^2ph	7.06	13.44	88.47	120.43	19.44	15.58
	H^2	0.77	0.76	0.42	0.82	0.26	0.01
	$SE(H^2)$	0.28	0.37	0.09	0.09	0.18	0.05
	SE (δ^2g)	1.17	1.60	3.05	4.96	1.13	0.19
MMV600	δ^2g	62.96	28.29	32.65	39.88	189.90	98.56
	δ^2ph	69.94	52.86	39.73	56.79	343.00	177.20
	H^2	0.90	0.54	0.82	0.70	0.55	0.56
	$SE(H^2)$	0.12	0.13	0.15	0.07	0.05	0.07
	SE (δ^2g)	3.97	2.66	2.86	3.16	6.89	4.96
Pop25	δ^2g	93.40	23.62	16.22	84.66	6.45	1.07
	δ^2ph	107.47	36.67	28.83	114.71	9.50	13.70
	H^2	0.87	0.64	0.56	0.74	0.68	0.08
	$SE(H^2)$	0.09	0.09	0.18	0.17	0.13	0.31
	SE (δ^2g)	4.83	2.43	2.01	4.60	1.27	0.52
ZUCASRc2	δ^2g	12.34	8.52	48.84	5.01	9.13	2.35
	δ^2ph	32.65	16.74	59.76	11.04	29.27	5.68
	H^2	0.38	0.51	0.82	0.45	0.31	0.41
	$SE(H^2)$	0.18	0.23	0.13	0.06	0.16	0.38
	SE (δ^2g)	1.76	1.46	3.49	1.12	1.51	0.77

S.E = Standard error

Table 6.9 Estimates of genetic variance, phenotypic variance and broad sense heritability for grain yield (tonnes ha^{-1}) in five populations

Population	Parameter	*Aspergillus*		*Fusarium*		*Stenocarpella*	
		Cycle-0	Cycle-1	Cycle-0	Cycle-1	Cycle-0	Cycle-1
Pop10	δ^2g	0.90	0.77	0.87	0.31	1.00	1.80
	δ^2ph	1.01	1.05	1.19	0.73	1.14	3.54
	H^2	0.90	0.74	0.73	0.43	0.88	0.51
	SE(H^2)	0.01	0.01	0.02	0.01	0.01	0.01
	SE (δ^2g)	0.47	0.44	0.47	0.28	0.50	0.67
PRA783244c3	δ^2g	0.88	0.21	0.16	0.48	1.35	0.50
	δ^2ph	1.00	0.36	0.22	0.95	1.51	0.96
	H^2	0.88	0.57	0.69	0.51	0.89	0.52
	SE(H^2)	0.01	0.02	0.02	0.01	0.01	0.01
	SE (δ^2g)	0.47	0.23	0.20	0.35	0.58	0.35
MMV600	δ^2g	1.46	0.73	1.38	0.87	1.08	0.44
	δ^2ph	1.56	0.91	1.80	1.24	1.63	0.80
	H^2	0.91	0.80	0.77	0.70	0.66	0.56
	SE(H^2)	0.01	0.01	0.01	0.02	0.01	0.01
	SE (δ^2g)	0.60	0.43	0.59	0.47	0.52	0.33
Pop25	δ^2g	0.92	0.17	1.81	1.16	1.30	0.36
	δ^2ph	1.46	0.44	1.93	1.78	1.82	0.70
	H^2	0.63	0.39	0.93	0.65	0.71	0.52
	SE(H^2)	0.01	0.01	0.01	0.01	0.01	0.01
	SE (δ^2g)	0.48	0.21	0.67	0.54	0.57	0.30
ZUCASRc2	δ^2g	0.38	1.13	0.90	1.02	0.81	0.89
	δ^2ph	0.64	1.29	1.04	1.37	1.06	1.13
	H^2	0.59	0.88	0.87	0.75	0.76	0.79
	SE(H^2)	0.02	0.01	0.01	0.01	0.01	0.04
	SE (δ^2g)	0.31	0.53	0.47	0.50	0.45	0.47

S.E = Standard error

6.3.3 Phenotypic correlation between grain yield and other agronomic traits in the original populations (C0)

The phenotypic correlation between ear rot incidence and severity with ear insect damage, ear drooping and grain yield, disease severity of the three ear rots and other agronomic traits are presented in Table 6.10. Generally, disease incidence and the severity of Aspergillus, Fusarium, and Stenocarpella ear rots were negatively or poorly correlated with grain yield.

The phenotypic correlation between the Aspergillus disease severities was positive, and significant with stalk lodging, Stenocarpella disease severity and husk cover (Table 6.10).

The association of ear insect damage and Fusarium disease incidence was strong and significant ($P<0.05$). The correlation of stalk lodging with husk cover and Stenocarpella were significant and positive ($P<0.05$). The other significant ($P<0.05$) estimates of phenotypic correlations were negative. There was a negative correlation between Aspergillus severity and Fusarium severity. Grain yield was negatively correlated with husk cover and Stenocarpella ear rot incidence, while ear insect damage was negatively correlated with root lodging. (Table 6.10). The negative correlation between grain yield and husk cover was due probably to differential allocation of carbohydrates to husk cover extension at the expense of increased grain filling. While the feeding or damage by insects may result in weakening the plants thereby making them more susceptible to root lodging. However, the results of the study indicated to the contrary mainly because insect damage to ear may have lighten ear carrying capacity of the plant thus did precipitate any lodging compared to stalk boring which was not studied..

Table 6.10 Phenotypic correlation of grain yield and other agronomic traits across the five populations (C_0)

ADS	-0.02										
DE	-0.17	-0.88**									
EID	-0.06	0.02	-0.04								
FDI	-0.18	0.44	-0.38	0.76**							
FDS	0.10	-0.60	0.52	-0.40	-0.48						
GY	-0.20	-0.59	0.51	0.11	0.14	0.65					
Husk cover	0.01	0.61	-0.34	-0.30	-0.09	-0.61	**-0.77****				
RLOD	0.32	-0.08	-0.05	-0.76**	-0.72**	0.66	0.03	-0.06			
SDI	0.33	0.41	-0.51	**0.36**	0.20	-0.43	-0.67*	0.21	-0.15		
SDS	-0.01	0.99**	-0.89*	0.00	0.43	-0.57	-0.56	0.60	-0.05	0.40	
SLOD	0.05	0.86**	-0.63	-0.08	0.35	-0.39	-0.47	0.64*	-0.05	0.32	0.87**
	ADI	ADS	Drooped	Ear insect	FDI	FDS	GY	Husk	Root	SDI	SDS

ADS = Aspergillus ear rot severity; FDS = Fusarium ear rot severity; FDI = Fusarium ear rot incidence; SDS = Stenocarpella ear rot severity; SDI = Stenocarpella ear rot incidence; GY = Grain yield; RLOD = Root lodging; SLOD = Stalk lodging; EID = Ear Insect damage; DE = Ear declination (or drooped ears)

*,** Correlation coefficients significant at *P≤0.05* and *P≤0.01,* respectively.

6.4 Discussion

6.4.1 Realised Gain

Selection reduced the incidence and disease severity of ear rots. However, there were differences among the populations. On the whole, all five populations exhibited a reduction in Stenocarpella ear rot disease severity. The findings of this study agree with those of Ramirez-Diaz et al. (2000) who reported a 0.97% reduction in ear rot infection per cycle in the subtropical maize population PABGT-CE using full-sib family selection.

There was a general increase in grain yield from C_0 to C_1 in all the populations under study, except for Pop10, when infected with Aspergillus ear rot. The same pattern was observed in MMV600 infected with Fusarium ear rot, and PRA783244c3 and ZUCASRc2 under Stenocarpella ear rot disease pressure. The increase in grain yield could be attributed to the reduction in the incidence and severity of ear rot in some populations under different ear rot conditions. This suggests that some populations harbour favourable alleles for resistance and high yields that could be exploited in a breeding programme through interpopulation crosses. The average rate of gain in grain yield observed in this study was comparable with those reported elsewhere. Pandey et al. (1987) reported average rates of gain of 2.11% across four selection cycles in tropical maize populations. Stromberg and Compton (1989), on the other hand, reported an average increase of 4.4% over 10 selection cycles in Nebraska krug maize for several plant traits including maize grain yield.

The observed rates of gain in reduced ear rot infection per cycle of selection appeared to be greater for Pop10 than for the other populations. The higher selection pressure that was exerted (10%) could have lead to a greater than expected progress with selection. However, at times, high selection pressure in a small population render drastic changes in the population structures due to population size causing an inbreeding effect. The favourable responses to selection obtained in some populations for reduced disease incidence and severity could open up opportunities for selection in those populations.

6.4.2 Genetic advance

The negative values for predicted gain and observed gain are desirable disease resistance attributes. This suggests that, in these populations, there could be alleles that may gradually be concentrated over time to confer resistance to the three ear rots studied. In general, one cycle may not be enough to ascertain whether any successful gain in improving resistance to ear rots and grain

yield has been achieved. However, the percent gain per cycle and expected genetic gain obtained in this study are comparable with those reported by other researchers (Pandey et al., 1986; De Léon and Pandey, 1989; Abedon and Tracy, 1998).

The percent gains, though variable and small, show that breeding for resistance to one ear rot does not necessarily ensure a similar reaction to another. Early generation testing could accelerate the screening process, as the study has shown, by using mixed ear rots; then the advanced materials could be subjected to specific ear rots. Several authors have reported that full-sib selection is effective for selecting for diseases, grain yield, and other agronomic traits (Hallauer, 1992). Of the five populations, Pop10, Pop25, and ZUCASRc2 appear to have responded well to full-sib recurrent selection, although the performance of Pop10 was slightly superior to Pop25.

6.4.3 Genetic variance

There were differences in the response to selection for the magnitude of genetic variance in the five populations. In 3 out of the 5 populations experienced a reduction in genetic variance. The biggest reduction across the five populations occurred under Aspergillus ear rot disease pressure for incidence, and under Stenocarpella for disease severity. Among the populations, MMV600 and PRA783244c3 experienced a more general increase in genetic variance for disease incidence and disease severity respectively. The decrease in genetic variance after one cycle of selection was probably due to genetic drift. However, Hallauer and Miranda (1988) attributed the reduction in genetic variance in advanced cycles of breeding to the fixation of favourable alleles or inbreeding depression. Helms et al. (1989) defined genetic drift as the change in gene frequency due to sampling in a finite population. Two major processes are believed to be operating to change the mean over two cycles of recurrent selection in these populations of finite size, selection acting to increase the mean and inbreeding depression due to genetic drift acting to decrease the mean. The magnitude of genetic drift for the population depends on the effective population size. The unselected population sizes were between 64 and 67 full-sib families across the population and 10% selection intensity was applied. So, effectively, in Cycle-one, genetic variance was a function of an independent assortment of alleles within seven selected full-sib families. It has been shown that with small population sizes (<25) the effects of drift may be large relative to the effects of selection (Helms et al., 1989), and significant effects of random genetic drift have been reported in some experiments (Smith, 1979; Helms et al., 1989). Genetic drift has the tendency to mask the progress achieved by selection. However, Flachenecker et al. (2006) reported a small to insignificant

reduction in response to selection in modified F2 Full-Sib recurrent selection due to the effects of random genetic drift.

6.4.4 Heritability

The results of the study indicate that Cycle 1 exhibited lower to medium heritability values after the selection for disease incidence and severity of Aspergillus, Fusarium, and Stenocarpella but higher heritability for grain yield. Similar trends in heritability values have been reported elsewhere. Robertson-Hoyt et al. (2008) reported heritability values of moderate low to high for Fusarium ear rots (h^2 = 0.13 to 0.54) in GEFR population and NCB populations. While Walker and White (2001) reported broad sense heritability values for resistance to Aspergillus ear rot of between 0.26 and 0.48. For grain yield, Welyrich et al. (1998) reported heritability of 0.54 and 0.8 for grain yield in Cycle-0 and Cycle-1, respectively. The implications are that low heritability may result in a low accumulation of favoured alleles for genetic improvement for the trait under study. The potential of a crop to respond favourably to selection depends upon the nature and magnitude of genetic variability. With complex traits like the ones studied, the knowledge of the existing genetic variation and their heritability assumes importance. However, in this study, there was a reduction in genetic variation in more than 30% of the population. Reduction in the genetic variation would inevitably slowdown genetic progress in those population cycles

6.4.5 Phenotypic correlation coefficients

The estimates of phenotypic correlation coefficients between four agronomic traits, root lodging, drooping ear, husk cover, and ear insect damage with disease incidence, disease severity of three ear rots, and grain yield, revealed moderate to large correlation coefficients in all five populations. Similar trends for correlations have been reported. Robertson-Hoyt et al. (2007) reported significantly high phenotypic correlations between Fusarium and Aspergillus ear rot, r = 0.81 (SE = 0.03). They concluded that making selections against Fusarium ear rot also would result in reduced susceptibility to Aspergillus ear rot.

Generally, the correlations were reliable in indicating whether the relationship between two traits was either positive or negative, except for ear drooping and husk cover with grain yield. The negative correlation of root lodging with grain yield in all the populations indicated that selection for resistance to root lodging could increase grain yield. Ear insect damage also had significant negative influence on yield, but positive direct effects on the incidence and severity of Fusarium and Aspergillus ear rots. Thus, selection for a reduction in ear insect damage would result in reduced incidence and

severity of Fusarium and Aspergillus ear rots. A strong relationship between ear insect infestation and ear rot severity has been also reported by other researchers (Sétamou et al., 1997; Cardwell et al., 2000).

6.5. Conclusion

On the basis of the results of this study, full-sib recurrent selection could be effective in reducing the incidence and severity of Aspergillus, Fusarium, and Stenocarpella ear rots in open-pollinated maize populations after one cycle of selection. However, in order to make progress, the genetic variances must be sufficient to allow for the gradual accumulation of favourable alleles. For long-term response, a larger population may be needed to maintain genetic variability and relaxed (>10) selection intensity, but for a short-term response selection programme, use of a smaller effective population size would not compromise genetic progress. The mean performance and estimated genetic variability for ear rot incidence, severity, and grain yield generally showed a favourable response to selection. An increase in the grain yield and reduced ear rot infection were more pronounced in Pop10, Pop25 and ZUCASRc2. However, further work may be required to ensure the gains made in this study were stable over generations.

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APPENDIX 1

Percent Incidence and severity of ear rots, and grain yield (kgha^{-1}) and other agronomic traits for the seven full-sibs and their source populations during 2006/7 rainy season.

Populations	Grain Yield	Ear rot		Root Lodging	Drooping Ears	Husk Cover	Plant Height cm)	Ear	Days to 50% silk	Grain Type
	(tons ha^{-1})	Incidence %	Severity %	%	(1- 5)	(1- 5)				
Pop10										
Selected Full-Sib	3.22	47.47	5.80	30.52	2.45	2.48	167.83	82.55	69.26	1.60
Population mean	3.18	67.42	11.71	33.4	2.56	2.41	172.8	84.91	69.34	1.68
PRA783244c3										
Selected Full-Sib	3.87	69.53	12.8	22.07	2.98	2.76	174.99	80.82	68.43	2.57
Population mean	3.06	70.07	13.21	21.63	2.43	2.42	165.93	82.21	68.84	2.11
MMV600										
Selected Full-Sib	3.44	51.31	11.21	16.51	2.79	2.52	180.04	86.98	69.5	1.93
Population mean	3,40	59.61	33.17	15.13	2.81	2.41	180.6	86.7	68.76	1.99
Pop25										
Selected Full-Sib	3.96	44.27	8.28	11.51	3	2.43	159.1	73.02	70.55	1.86
Population mean	3.54	56.53	9.94	9.7	2.82	2.57	164	77.18	70.21	1.78
ZUCASRc2										
Selected Full-Sib	3.54	47.1	6.39	12.96	2.83	2.52	148.49	66.06	68.14	2.02
Population mean	3.16	61.34	11.22	14.22	2.91	2.7	151.18	70.45	68.57	1.95

CHAPTER 7
GENERAL OVERVIEW

7.1 Introduction

Maize ear rots continue to pose a serious threat to food supply to both urban and rural populations, through reducing yield and reduced quality. While several efforts have been made to limit their impact on maize productivity, the magnitude of the problem is huge. This chapter provides an overview of the study, re-stating the main research thrust in achieving ear rot-resistant maize, its objectives and a summary of the main findings. The precincts of the research, challenges and implications of the findings and the directions for future research (recommendations) are outlined.

Five interrelated objectives formed the focus of this thesis. These were:

(i) to investigate and understand farmers' perceptions of maize ear rots and mycotoxins occurring among them
(ii) to conduct a survey to sample maize ear rots and mycotoxins occurring in the target region
(iii) to identify the most suitable screening technique that could be used for screening maize genotypes for ear rot resistance in a tropical environment like Zambia
(iv) to carry out genetic studies on the five tropical maize populations to be used in the full-sib recurrent selection and
(v) to investigate the response to selection using full-sib recurrent selection for the improvement of resistance to ear rots in five tropical maize populations.

7.2 Summary of the major findings

7.2.1 Smallholder farmers' perceptions of ear rots and mycotoxins

Farmers ranked ear rots as the third most important disease of maize after maize streak (Maize streak virus) and leaf blight (*Exserohilum turcium*), and the fourth most important biotic stress after these two diseases and stemborers. They were able to differentiate six ear and kernel rots based on visual symptoms (colour of the infected grain). From these symptoms, it was evident that Fusarium and Stenocarpella ear rot were the most prevalent ear rot fungal diseases of maize.

Among the factors farmers felt were responsible for the increased prevalence of ear rots were late planting, too much rainfall and lack of resistant maize varieties.

Farmers never linked maize ear rots to any human or animal diseases, this was evident in the manner of disposal of diseased maize, which was burnt, fed to livestock, sold to illicit brewers or consumed during periods of hunger.

Farmers preferred maize varieties that were drought tolerant, better tasting, good processing qualities and ear rot free. Local landraces were preferred to new and improved maize varieties on the basis of their excellent taste and yield stability, even though improved varieties have high yield advantages, which farmers appreciated. Farmers had a white kernel colour preference because it produces white flour.

Farmers associated the increased occurrence of ear rot infection with more varieties on the market, which suggests that the varieties on the market do not possess adequate resistance to ear rots.

7.2.2 Survey of ear rots and mycotoxins

A survey conducted in Central and South Zambia revealed high levels of ear rot infection in pre-harvest maize. More than 15 ear rot fungal species were isolated from both visibly diseased maize and 'seemingly healthy" maize. The most commonly isolated fungi were *Fusarium* and *Stenocarpella* species. Though no geographical distribution was discernible, *Stenocarpella* was more abundant in humid, higher rainfall areas; *Fusarium* was more prevalent in medium humid areas and *Aspergillus* in drier areas.

High levels of fumonisins and aflatoxins were also prevalent/observed. Fumonisin contamination was observed to be widespread. It ranged from 0.13 ppm to 192.2 ppm, with high levels found in the more humid areas of Southern and Lusaka provinces. Aflatoxin contamination had a much narrower range of 0.05 ppb to 10.2 ppb. Aflatoxin contamination was also widespread, with most amounts exceeding the recommended WHO/ FAO Provisional Daily Maximum Intake of 2µg/person.

7.2.3 Appraisal of screening techniques for sources of ear rot resistance

Five screening techniques: colonized toothpick, leaf whorl placement, syringe silk channel injection, ear top placement and spore suspension spray and their combinations were appraised. Colonised toothpick consistently produced a higher infection rate, followed by leaf-whorl placement of infested maize kernels or grit. When used in combination, they were not any different from using leaf-whorl

placement plus either syringe silk channel injection or ear top placement. At the final disease assessment, though the disease was much more severe in the wounded ears, the relative susceptibility of the maize varieties was not affected by wounding method.

The range of variety discrimination was found to be higher for colonised toothpick, followed by leaf whorl placement.

The results of the study showed that only techniques sensitive and stable in a wide range of discrimination environments could be relied upon in classifying the genotypes for resistance to ear rots.

7.2.4 Genetic analysis of resistance to ear rots in maize germplasm

Both specific combining ability (SCA) and general combining ability (GCA) effects were found to be important for genotypic variation in resistance to maize ear rots. However, there was a preponderance of the non-additive gene effects in determining resistance to maize ear rots. General combining ability effects were important for grain yield while SCA was not significant.

Reciprocal effects were significant in the crosses involving susceptible and resistant full sib families but partitioning of the reciprocal effects shows that it was due to both maternal and non-maternal effects.

Significant GCA x environment and maternal x environment effects were observed in the crosses involving resistant and susceptible full-sib families – the former suggests there is a possibility of developing hybrids for specific environments while the later indicates that there were inconsistencies in the ability of the progeny to resist infection, hence performance of the progeny could not be predicted using the parents.

7.2.5 Response to selection

There was a net reduction in the incidence but not severity for the three ear rots among the populations. Though no general reduction was observed for disease severity, Pop10 displayed the largest reduction in ear rot disease severity of 25%. , The mean predicted gain from selection among the populations for ear rot disease incidence, disease severity and grain yield was -4, -3 and 0.19.

There was a reduction in genetic variability in some populations after selection. This may have been due to random genetic drift resulting from the small population size and high selection intensity

(10%). In this study, four different situations were observed as follows: (i) Population cycles where both phenotypic and genotypic variance were large and by implication, the corresponding environmental variance was small, hence heritability was high. This was a favourable situation as it could accelerate population improvement; (ii) Population cycles where the phenotypic variance was large and genotypic variance small and by implication, the corresponding environmental variance was very large, hence heritability was low. Consequently, in these populations, response to selection could be poor. (iii) In those populations where all three variances were large, i.e. phenotypic variance, genotypic and environmental variance, heritability had a wider range and response to selection was slower, but over time favourable alleles could be accumulated. These populations could be improved but at a much slower rate; (iv) In populations where both phenotypic and genetic variance was small and heritability low to moderate, the only long term accumulation of favourable alleles could result in population improvement.

The results of the study, however, indicated there was enough genetic variability to justify the continuity of recurrent selection procedures with corresponding gains in grain yield and potential reduction in disease incidence and severity.

Phenotypic correlations revealed poor to negative correlations between grain yield and ear rots, root lodging and stalk lodging.

7.3 Breeding implications

The participatory appraisal study demonstrated the importance of breeder-farmer interactions, which could result in the designing of two maize ideotypes – one that incorporates the farmer preferences (i.e. market ideotype) for high yield, milling quality, good taste, drought tolerance and storability, and the other the attributes of resistance to ear rots, mycotoxins and other biotic stresses (i.e. stress tolerant ideotype). With these two ideotypes, the breeder may then develop varieties that have good potential for adoption by farmers. The high prevalence of Fusarium and Stenocarpella ear rots compared to the other ear rots in the major maize growing areas of the country suggests that improvement of host plant resistance can provide an important component of the integrated disease management of maize ear rots. In addition, while the development of ear rot-resistant maize must target these two fungal genera pathogens, breeders must not forget that the two ear rots do not occur in isolation. The occurrence of high levels of mycotoxins suggests the urgent need to explore ways of developing maize varieties that are resistant to mycotoxin accumulation.

Because most inoculation techniques are laborious and at times costly, the study has demonstrated that early generation materials could be screened using ear rot fungal mixtures, then subjecting the elite material to individual fungal species. The use of a combination of techniques is advocated as this minimizes the number of genotypes that escape disease infection (escapees) which could otherwise be termed resistant when they are not. A large number of escapees slow down progress in breeding for ear rot resistance because it reduces the repeatability.

Different inoculation techniques employed would measure different resistance mechanisms. For example, the toothpick method entails that fungi must overcome resistance in the pericap and endosperm. This method estimates the levels of actual resistance once the primary defense mechanism has been overcome. On the other hand, the leaf whorl placement indirectly measures the resistance mechanism associated with leaf and stem morphology, which the fungi has to overcome in order to create a disease condition in the susceptible genotype. Sometimes the first line of defense can be broken by other pests such as stemborers and facilitate ear rot infection. Therefore a combination would identify genotypes with both mechanisms of ear rot resistance.

The presence of significant GCA x environment interaction effects suggests that selecting hybrids for a specific environment could maximise the use of these maize populations. Selection in multi-location trials under artificial inoculation would be useful in identifying the genotypes with stable resistance to ear rot.

The preponderance of non-additive genetic effects for ear rot disease severity indicates hybridisation may be the best path to follow in breeding new germplasm with high levels of ear rot resistance. Reciprocal recurrent selection procedures that exploit both additive and non-additive genetic variation would be emphasized to improve ear rot resistance in these populations. The presence of significant reciprocal effects has confounding effects as to which single F_1hybrid can be advanced as an inbred.

7.4 Challenges in breeding for resistance to maize ear rots

There are a number of challenges that affect the effective breeding and subsequent delivery of maize ear rot-resistant varieties for use by small-scale farmers. These are outlined briefly as follows:

1. Very few varieties that are immune to most diseases, especially maize ear rots, have been developed. This is partly due to the nature of resistance to ear rots and the relatively late interest shown in developing maize ear rot-resistant varieties. Currently, there is no variety on the market that could be termed truly resistant to multiple maize ear rots. Rather, in most studies the focus has been on developing resistance to one or two ear rots only. In addition, multiple resistance has not been exploited fully.

2. The lack of resistant local checks, consistency in response and ranking of genotypes, and the potential occurrence of maize genotypes that are symptomless. These and many other factors hinder progress in selection and have discouraged scientists from breeding for resistance to maize ear rots. In addition, further discouragement may have arisen from laborious, time-consuming inoculation techniques associated with experiments and the practical difficulties of evaluating the large number of entries a breeding programme would require. Thus, screening for resistance has been delayed and was conducted on late generations after much of the variability has been lost.

3. Complications presented by low heritability values in the five populations, due in part to the preponderance of Genotype x Environment Interaction (GE) as heritability values (H^2), tend to increase with increasing replication within and across trials. However, the cost associated with multi-location trials is high and when coupled with the inconsistency of screen tests, this may further discourage breeders from engaging in breeding for maize ear rots. There is, therefore, a need to develop sound evaluation techniques that are highly repeatable so as to increase heritability for resistance in breeding populations. Some non-destructive methods for evaluating resistance in early generations should also be developed.

7.5 Conclusion and the Way forward

The maize populations that have been studied will be advanced through two to three more selection cycles, with the view of identifying superior progenies that will be source material for development of an ear rot-resistant experimental OPV. Selection methods that put emphasis on both additive and dominance gene effects such as full-sib recurrent method will be used. Promising diallelic single crosses will form base populations for the development of inbred lines. Artificial inoculation through placement of the inoculum in the leaf whorls followed by injection with the colonized toothpicks at a later stage would be used to ensure that adequate selection pressure is applied in screening many genotypes in many locations and seasons that represent the target environments in Zambia..

Selecting for reduced mycotoxin contamination will be one of the major goals of future research. Whilst in this study mycotoxins were only assessed in terms of their occurrence in different parts of the country, future research will explore the possibility of developing mycotoxin-resistant maize, in addition to conducting a survey for mycotoxins in other maize-growing parts of the country. The development of mycotoxin-resistant maize that will lessen the exposure of farmers and their households to the high levels of mycotoxins reported in this study.

Other traits, such as reduced ear insect damage, both in the field (mainly stemborer) and storage (maize weevil and the larger grain borer), improved husk cover, and tolerance to MSV (a result from farmers) will also be emphasized. This will add value to varieties to be developed from this programme.

Landraces will be brought into the ear rot resistant breeding programme. They will be screened for ear rots and other traits mentioned above. Though landraces were not part of this study, there is a high possibility that some of them harbour an inherent resistance to ear rots and possibly for other diseases, but have not yet been fully exploited. During the PRA, some farmers mentioned that improved varieties tend to develop more ear rots than landraces. This was an indication that some of these landraces may have desirable traits for resistance.

Future studies will also explore the possibility of using molecular markers to aid the selection process.. Research in North America (Paul et al., 2003) has shown that it is possible to identify quickly and effectively which early-generation plants carry the desired gene for resistance. Use of molecular markers would accelerate the evaluation process and save on many generations of field testing. The use of marker-assisted selection (MAS) is non-destructive of seed, as leaf samples are

taken at the seedling stage. However, the challenge will be the cost involved, especially in establishing laboratory facilities, identifying potential markers, and for a developing country such as Zambia there could be some other challenges in accessing foreign currency to import the research consumables. It is envisaged that a sub-regional approach to invest in laboratory facilities would be desirable to spread the cost of running the laboratories.

Generally the study undertaken here has shown that, in spite of the challenges cited, there are ample opportunities to make further improvements to the five tropical populations for ear rot resistance and resistance to mycotoxins. This would be achieved by maximizing repeatability through employing the optimum inoculation methods that were identified in the study, screening many genotypes across several environments, and applying selection methods that exploit both additive and non-additive variation in the five tropical populations.

References

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