CHAPTER ONE

1.0: INTRODUCTION

Maize (*Zea mays* L.) is one of the principal cereal food crop in the tropics and sub tropics (Makate, 2010) and forms an essential component of the global food security as a major part of the diet of millions of people including Zambia (Kamanula *et al*., 2011). It grows under a wider range of ecological conditions depending on the variety (ASARECA-TUUSI, 2009). The crop is versatile in its use, environmental adaptation and it is also consumed all over the world by both human being and animals (Keba and Sori, 2013).

Increasing production and productivity of maize has been achieved through the development of high yielding stress tolerant varieties. Despite this intervention at production level, there is evidence of food insecurity arising from food storage losses. Storage losses due to pests threaten livelihoods of farmers across Africa (Kamanula *et al*., 2011). The maize weevil (*Sitophilus zeamais* Motschulsky) is the most serious storage pest of maize in the tropics (Bosque-perez and Buddenhagen, 1992). The maize weevil affects the crop before harvest and multiplies further after storage (Caswell, 1962). Giga and Mazarura (1991) reported maize loss of 20 - 90 % worldwide due to the` maize weevil, *S. zeamais*.

Stored crop insect management technologies among rural communities include the application of chemical pesticides that are expensive to buy, unreliable in terms of time availability and inadequate handling practices (Rugumamu, 2011). In Zambia 50% of farmers use synthetic pesticides during storage of maize after harvest (Kamanula *et al*., 2011). The widespread use of pesticides has caused environmental hazards, resistance development, residue accumulation in food and feed and has also caused the negative effects on the non-target organisms (Dhuyo and Ahmed, 2007).

This problem is acute for subsistence farmers who produce and store their harvested maize grains locally often under conditions favorable for insect colonization (Dobie *et al*., 1984; Abebe *et al*., 2009). Rationally it makes more sense and is economical to safeguard the crop that has been harvested instead of trying to make up for the losses through increased production. Therefore, development of the Host-plant resistance as a pest control method is environmentally safe, economically cheaper to farmers and most compatible with other components in the Integrated Pest Management (IPM) initiatives (Chapman, 2000).

Most studies that have been conducted have been directed to growing of crops in the field with limited attention paid to protect the crop in storage. Little information has been gathered concerning host plant resistance (Derera *et al*., 1999). It was for this reason that this study was carried out to establish the mechanisms of weevil resistance in maize so as to generate information which would be useful in the breeding programmes.

The overall objective of this study was to establish mechanisms of weevil resistance in maize, whose specific objectives were;

1. To characterize genotypes for traits related to weevil resistance in maize

2. To estimate the genetic basis of the mechanisms of weevil resistance in maize

This study was carried out on the premise that there is enough genetic variability among maize genotypes which can be used in breeding programmes to develop weevil resistant varieties.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Maize crop overview

2.1.1 Origin of maize

Maize (*Zea mays* L), also known as corn, is a cereal grain that was originally domesticated in Mesoamerica (OECD, 2003). Though there are many speculations about the progenitor of maize, it is however generally accepted that maize originated from teosinte (*Zea mexicana* L) which is the closest known wild relative of maize (Aylor *et al*., 2005). Mexico and Guattemala are the native countries of teosinte and it grows wild in cultivated maize fields in its natural habitat (Hallauer and Miranda 1988: Aylor *et al*., 2005). This plant is similar to maize by having a monoecious flowering habit, same number of chromosomes and is readily crossed with maize (Poehlman and Sleper, 1995).

Maize spread to the rest of the world after European contact with the Americas in the late 15^{th} and early 16^{th} century. It has now become a principal cereal crop in the tropics and in the subtropical regions throughout the world. It is unknown what

precipitated its domestication, because the edible portion of the wild variety is too small and hard to obtain to be eaten directly, as each kernel is enclosed in a very hard bi-valve shell (Hampl and Hampl, 1997).

2.1.2 Importance of maize

The importance of any crop can be judged by its area of production, utilization and share in trade (IITA, 2013). The same criteria or standards can be applied to maize to judge its importance as a cereal crop. Maize is the most important cereal crop in Sub-Saharan Africa (SSA) and an important staple food for more than 1.2 billion people in SSA and Latin America (OECD, 2003). All parts of the crop can be used for food and non-food products. It is also widely used for animal feed and industrial raw material in the developed countries where as the developing countries use it in general for feed (IITA, 2013).

Recently, there has been interest in using maize for production of ethanol as a substitute for petroleum based fuels. Maize accounts for 30–50% of low-income household expenditures in Eastern and Southern Africa (IITA, 2013).

2.1.3 Composition of maize kernel

The major chemical component of the maize kernel is starch which provides up to 72 – 73 % of the kernel weight (FAO, 1993). Protein is the next largest chemical component of the kernel. Protein content varies in common maize varieties from about 8 to 11 percent of the kernel weight most of which is found in the endosperm. In some genotypes the protein content may even be lower than 8 percent (Keba and Sori, 2013). Oil content mainly from the germ ranges from 3 to 18 %. Others include the dietary fiber and some vitamins. As the kernel matures the sugars decline and the starch increases.

2.2 Biology of the maize weevil

A fundamental knowledge of the biology of *S. zeamais* is a prerequisite for devising methods of efficient control. The maize weevil belongs to the insect beetle family, Curculionidae, with a total of 60,000 species described world wide of which 30 species are pests of stored product, three of which are *Sitophilus* species and are of extreme importance (Siwale *et al*., 2007).

It varies in colour from dull red brown to nearly black and is usually marked on the back with four light reddish or yellow spots. The maize weevil has fully developed wings beneath its wing covers and can fly readily. The thorax is densely pitted with somewhat irregularly shaped punctures, except for a smooth narrow strip extending down the middle of the dorsal (top) side.

Adults bear much similarity to the rice weevils but maize weevils are essentially larger, stronger versions with sturdier, more efficient wings. Weevils are well adapted to darkness and to movements in confined spaces and amongst stored grain (Derera *et al*., 2001).

2.2.1 Life cycle

The maize weevil *Sitophilus zeamais* exhibit holometabolous type of postembryonic development of 36 days period; small, single oval white eggs is laid and go through cleavage and differentiation inside a tiny hole bored on the grain by the female parent and covered by a waxy secretion that creates a plug, Murata and Imanura (2008). A single female may lay 300 to 400 eggs during her lifetime and adults can live for five to eight months. Eggs hatch into larvae which voraciously feed within the grain, growing and molting before pupation, larvae are responsible for most of the damage on maize as they eat it away from the inside out until mature (Dobie *et al*., 1984; Abebe *et al*., 2009).

Adults then eat their way out of the grain kernel, females move to the high surface and release sex pheromone which attracts males (Chapman 1998). The adults immediately start aggressive feeding, reproducing, releasing more crop damaging larvae and hence increased destruction of the grains (Dobie 1974; Dobie *et al*. 1984; Abebe *et al*. 2009). Given that both larvae and adults feed on grains, they create much dust unfit for human consumption and consequently, great maize weight losses causing economic damage to maize, especially in traditional storage systems where control strategies are limited.

2.2.2 Host range

These insects are known for attacking most crops and food items pertaining to grain and while they usually breed within the grain, they can breed within other types of crops as well. Although the maize weevil cannot readily breed in finely processed grains, it can easily breed in products such as macaroni and noodles and milled cereals that have been exposed to excessive moisture (Alter, 2013).

The method or form of maize storage may either cause more infestation by weevils or not. Maize may be stored as loose grain, on cob without husks or on cobs with husks (Savidan, 2002). Other factors include moisture content of the grain and the environmental factors such as temperature and relative humidity. Husk cover protection is however limited to initial infestation (Savidan, 2002).

2.3 Economic importance of weevils

Maize weevil is an important pest especially on maize stored at the field for both food and seed (Thanda and Kevin, 2003). Normally weight loss of about 20 to 90 % of the untreated stored maize grain occurs (Pingali and Pindey, 2000). The maize weevil is of importance in this study because it is the most significant pest of stored maize grain in Eastern and Southern Africa (Giga and Mazarura, 1991).

2.3.1 Damage of seed by the weevils during storage

The direct damage made by weevils is that they may cause the reduction in seed viability, loss in weight; the seed whose germ once attacked will not germinate. Therefore this may reduce the future maize production for farmers who use saved grain as seed, a common practice in Eastern and Southern Africa (Dhliwayo and Pixley, 2003; Siwale *et al*., 2007). The indirect damage includes loss of quality of maize which may include nutrient loss, heating and spoilage, production of off flavors, discoloration and predisposition to diseases.

2.3.2 Influence of storage form on development of maize weevil

The extent of damage during storage depends on the number of emerging adults during each generation and duration of each life cycle and varieties. The more the number of adults that emerge the more rapid and serious the damage will be (Tefera *et al*., 2011). It is therefore important that special consideration is given to storage of maize. Food and Agriculture Organization (FAO, 1993) recommends that before storage of maize is done, the bin must be clean, watertight and preferably made out of steel. Application of the protective powder or fumigated promptly after binning must be done. Finally inspection of the bins is necessary to monitor if at all there is any infestation from weevils.

2.4 Mechanisms of resistance in maize grain to the maize weevil

Resistance in stored maize to weevil attack have been investigated by many researchers over a long period of time (Singh and McCain, 1963; Dobie, 1974; Dobie 1977; Tipping *et al*., 1988; Arnason *et al*., 1997; Dhliwayo and Pixley, 2003). They have found to include grain hardness, grain size, storage form (grain cobs with husks on or unshelled cobs), nutritional factors (such as proteins, lipids, and sugar content) and biochemical compounds.

2.4.1 Kernel hardness

This has been suggested as one of the weevil-resistant mechanisms in maize genotypes. When the pericarp is hard or the endosperm is hard, the female weevil will find it difficult to puncture a hole to oviposit (Jansen, 1977; Dick, 1988). Equally the hatched larva will find it difficult to feed on the hard endosperm resulting in a retarded rate of development and hence reduce the rate of weevil multiplication in the grain lot.

Despite this, Singh and McCain (1963) found that some varieties with hard kernels were susceptible to maize weevil attack. This prompted researchers to look for other mechanisms of resistance especially the chemical constituents in conferring weevil resistance. It has also been further suggested that there is no relationship between grain hardness and susceptibility parameters (Gudrups *et al*., 2001; Munjoma, 2004; Siwale *et al*., 2007). Chemical constituents were suggested to be more important in conferring weevil resistance than hardness since relative hardness had no influence on the progeny numbers in softer, high lysine endosperm maize and their normal endosperm counterparts.

2.4.2 Grain size and texture

Although Widstrom (1989) reported that grain size was not an important factor, researchers have reported a relationship between grain size and the susceptibility parameter (Gudrups *et al*., 2001). Smaller grain was more susceptible to weevil attack than larger grain in selected and shelled maize varieties. It is however not clear how kernel size could contribute to resistance, but Kossou *et al*., (1992) reported an increased mean development period for the weevils in improved varieties screened as ears. They suggested that when kernel size is larger, then the grain embryo will be further away from the crown and thus could result in a greater kernel area the emerging F_1 adult weevils would search before finding the emergence site, hence more time before emergence.

There are broadly two types of maize kernels in terms of texture, namely; the flint type with a higher proportion of hard endosperm and the dent type with the higher proportions of soft endosperm. Generally dent types tend to be more susceptible to insect attack on account of its softness than flint types (Kossou and Kim, 2003).

2.4.3 Biochemical compounds

It is believed that the biochemical factors are more important than morphological and physiological factors in conferring non-preference resistance and antibiosis (Singh, 2009). Many of these biochemical factors involved in resistance are synthesized independent of the pest as phytoanticipines (Micheal *et al*., 2009). The genetic differences in host plant response of maize varieties to insects are almost exclusively quantitative in nature. In the chemical defense of plants, they produce hundreds of thousands of unique low mass products, known as secondary metabolites. These are generally non-essential for basic metabolic processes of the plant, but improve defense against microbial attack, herbivore predation and control allelopathic interactions.

Biochemical compounds function through mechanical resistance and antibiosis (Derera *et al*., 2001; Dhliwayo and Pixley, 2003; Garcia–Lara *et al*., 2004). Maize genotypes that have higher levels of protein content tend to be more resistant to maize weevils than those with lower levels of protein (Siwale *et al*., 2007). In agriculture, the presence of the phenolic compounds particularly hydroxycinnamic acids is associated with the diminished pre-harvest losses due to bird predation and post-harvest losses due to storage pests. Cereal phenolics are primarily located in the grain outer layers. The chemical constituents of the pericarp have been found to retard development of the insects (Munjoma, 2004).

Dimboa (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) a naturally occurring hydroxycinnamic acid, is a powerful antibiotic present in maize and related grasses

particularly wheat. Dimboa serves as a natural defense against a wide range of pests including insects, pathogenic fungi and bacteria. In maize, Dimboa functions as a natural defense mechanism against European corn borer larvae and many damaging pests. The exact level of Dimboa varies between individual plants but higher concentrations are typically found in young seedlings and concentration decreases as the plant ages.

The role of Phenolic in the resistance of maize to maize weevils have been extensively studied (Serratos *et al*., 1987; Classen *et al*., 1990; Arnason *et al*., 1992; Arnason *et al*., 1994; Arnason *et al*., 1997; Munjoma, 2004; Siwale *et al*., 2007). The results from these studies have shown that populations of maize with high Phenolic acid content are more resistant to attack by the *S. zeamais* than those with the low Phenolic acid content (Classen *et al*., 1990). The levels of the Phenolic compounds are different in different maize genotypes (Sen *et al*., 1994).

This tendency of Phenolic conferring resistance has been observed not only in the maize weevil but also to even larger grain borers. Phenolics are not restricted to maize only but also to other crops as well such as sorghum, rice, bulrush millet. The principal Phenolic acid associated with resistance is (E) – Ferulic. P-coumaric, (Z)– Ferulic and synapic acids made a minor contribution (Classen *et al*., 1990). E-Ferulic the hydroxycinnamic acid is most abundant in maize grain constituting 90 percent, seconded by P-coumaric acid which is about 10 percent of all the phenolic acids in the maize kernel (Classen *et al*., 1990 as cited by Serratos *et al*., 1993; Siwale *et al*., 2007).

Ferulic acid makes the kernel harder through the formation of the cross linkages with carbohydrates in the pericarp tissue. It is found in the cell walls of the pericarp and the aleurone layer. The endosperm is very poor in ferulic acid content. It is an

antifeedant. This acid is also reported to confer resistance to feeding by the stalk borers in the field, depending on its relative abundance in the maize silk (Warnock *et al*., 2001). The ferulic acid also protects maize against Fusarium ear rot, a fungal disease caused by *Fusarium graminearum* (Schwabe) (Billy *et al*., 2003).

The levels of phenolics are negatively correlated with Dobie (1974) index of susceptibility, grain weight loss and developmental parameter (Classen *et al*., 1990; Arnason *et al*., 1992; Arnason *et al.*, 1993). Classen *et al*., (1990) also reported that correlations between (E)–Ferulic acid and susceptibility parameters were of similar magnitude as the correlations between susceptibility and the leading parameter, protein content and grain hardness (Eden, 1952; Singh and McCain, 1963; Dobie, 1977). Thus it was concluded that grain hardness play a great role in creating resistance of maize to weevils.

2.5 Measuring maize weevil resistance

Several methods have been proposed and are used for the assessment of weevil resistance in maize (Gudrups *et al*., 2001).

2.5.1 Grain weight loss

The degree of weight loss has been found to be a reasonable measure of maize grain resistance or susceptibility to the maize weevil (Adams, 1976; Serratos *et al*., 1997; Derera *et al*., 2001). Higher weight loss will occur in the susceptible varieties than in the resistant varieties (Siwale *et al*., 2007).

2.5.2 Adult mortality

This is also used to give an assessment of the maize weevil resistance as the number of adult insects alive and those that died are counted at about 10 days after

introducing weevils by counting (Tefera *et al*., 2011). However weevil's adult mortality is not a good indicator of susceptibility and or resistance of the varieties. Dobie (1974) and Abebe *et al.*, (2009) stated that there is no evidence for variation among varieties in their effect upon the mortality of *S. zeamais* and further states that there are significant differences between varieties when tested against *S. zeamais*.

Adult weevils can survive without food for more than ten days in a laboratory test (Abraham and Firdissa, 1991). This further indicates that weevil's mortality is not a good indicator of resistance in maize varieties. Nevertheless, less adult weevil mortality in storage is an indication of more number of eggs layed from the surviving adult weevils leading to more number of F_1 progeny emergency.

2.5.3 Nutritional factors

Lipids, protein and sugar content have been reported to be involved in conferring resistance of maize to weevils (Arnason *et al*., 1992). Many researchers have reported highly significant and negative correlations between protein content and Dobie index of susceptibility parameters (Classen *et al*., 1990; Arnason *et al*., 1994; Arnason *et al*., 1997).

2.5.4 Density of infestation

When the density of grain infestation is very high, larval competition leads to mortality of some of them (Ali and Smith, 2001). Widstrom (1989) determined the minimum number of unsexed parent weevils needed to infest each grain sample in order to get reliable data and consistent progeny numbers. Twenty unsexed adult weevils are needed to infest samples containing at least 1g of seed per weevil. A simplified and rapid protocol for screening maize genotypes encourages the infestation of the grain with 32 – 50 weevils which will result in progeny emergence and kernel damage similar to the standard of about 50g grain sample (Derera *et al*.,

2001; Mugo *et al*., 2005). The modified procedures take about 45 – 56 days to complete.

2.5.5 Susceptibility indices

The susceptibility indices are used as additional measures of the susceptibility of maize varieties to infestation by the *S. zeamais*. The higher the index, the greater the susceptibility of maize to weevils (Siwale *et al*., 2007). Two susceptibility indices have been developed. These are the Dobie's and Urrelo's Susceptibility indices which when compared have been found to give similar results.

2.5.6 Dobie's Susceptibility index

The Dobie's index of Susceptibility has been used as a criterion to separate genotypes into different resistance groups (Dobie, 1974; Derera *et al*., 2001; Gudrups *et al*., 2001; Dhliwayo and Pixley, 2003). The index of susceptibility is given by the formula:

$IS = (\text{Log}_e X / MDP)\ 100$

Where:

IS = Dobie's susceptibility index

$\text{Log}_e X$ = is the natural logarithm of the total number of the F_1 progeny emerged

MDP = Median development period

2.5.7 Urrelo's Susceptibility index

This Susceptibility index was developed by Urrelo and his colleagues (Urrelo *et al*., 1990; Gudrups *et al*., 2001). It is given by the following formula:

$I = \text{Ln } E \times 100 / DFE$

Where:

Ln = natural logarithm

I = Susceptibility index

E = the total number of egg plugs on the grain

DFE = the date of first emergence of F_1 (days)

Urrelo's method is not commonly used due to the intensive requirements of labour in the initial stages of an experiment, when numbers of eggs have to be counted. However, it has the advantage that the assessment may be terminated upon the emergence of the first F_1 adult (Gudrups *et al*., 2001).

2.6 inheritance of weevil resistance in maize

In order for the resistance mechanism to be useful to the breeder, they must be heritable. Studies have been conducted on his subject and the mechanisms involved have been found to be heritable (Derera *et al*., 1999). Several research workers have reported on the heritability of maize weevil resistance (Widstrom *et al*., 1975; Tipping *et al*., 1989; Widstrom *et al*., 1983; Kang *et al*., 1996; Derera and Pixley, 1998). Both additive and dominance components were important although the larger estimates of maternal associated intra-locus interaction effects indicated that dominance or non additive genetic effects were more important. Cytoplasmic effects were not important.

It was suggested that for a selection procedure to be efficient, it must utilize both additive and dominance variation of maternal endoplasperm genotypes (Widstrom *et al*., 1975; Tipping *et al*., 1989; Widstrom *et al*., 1983; Kang *et al*., 1996). Recurrent selection within populations and reciprocal recurrent selection between populations was suggested. Two populations were suggested; population A consisting of crosses among inbreeds common to most resistant single crosses and population B consisting of crosses which involved those inbreeds which performed well when crossed with those in A but were not themselves in A.

Kang *et al*., (1996) found the estimates of the General combining ability (GCA), Specific Combining Ability (SCA) and the reciprocal effects to account for 44.5%, 20.8% and 34.6% respectively in resistance of maize weevil among F1 seed. Combining ability has a prime importance in plant breeding since it provides information for the selection of parents and also provides information regarding the nature and magnitude of involved gene action. Combining ability estimates are important genetic attributes to maize breeders in anticipating improvement via hybridization and selection.

The knowledge of genetic structure and mode of inheritance of different characters helps breeders to employ suitable breeding methodology for their improvement (Kiani *et al.,* 2007). Kang *et al*., 1996 further concluded that GCA was more important than the SCA and therefore recurrent selection was suggested as an appropriate breeding approach. Tipping *et al*., (1989) also reported that GCA and to a lesser extent SCA effects were important in the inheritance of resistance to oviposition by maize weevil. However, Derera *et al*., (1998) reported that both GCA and SCA were of equal importance in contrast to Kang *et al*., (1996).

Li-Ruming *et al*., 1998 reported that genetic variability for weevil resistance does exist in maize but they found the broad sense heritability of 0.21 suggesting a very slow progress from selection. While it is true that considerable work has been done in this area, the existing variation observed from one place to another points to the need of careful study of this character as it applies to a given population before a breeding approach can be selected. Inheritance of weevil resistance is complex and it presents an intriguing challenge in the area of research (Derera *et al*., 1998).

2.6.1 Gene action

Genes, located on chromosomes, represent the basic unit of inheritance, and control the expression of characters, individually or in combinations. Gene action is the way genes express themselves.

In quantitative genetics, genetic components are divided into additive, dominance and epistasis gene action (Falconer, 1981). In the presence of additive gene action, characters of heterozygotes in the F2 generations are the intermediate of the two parents, because additive variation is associated with the average effects of the particular alleles (Falconer, 1981). The additive portion reflects the degree to which progenies are likely to resemble their parents, which is reflected in the narrow sense heritability. Non-additive gene action is observed when the additive model cannot adequately explain the variation (Falconer, 1981).

According to Mather and Jinks (1971), the size of dominance relative to the additive variance indicates the degree of dominance. Thus, levels of dominance in the progeny display a range from partial to over – dominance in relation to the mean of their parents. Negative variance components are not common and are often found for dominance variance components (Hallauer and Miranda, 1988). When estimates of maternal components of variances are greater than paternal components of variances, it indicates the possible presence of maternal effects on the traits of interest (Mather and Jinks, 1971).

2.6.2 Heritability

Heritability is defined as the proportion of the total variance that is attributed to the average effects of the genes and this determines the degree of resemblance between relatives (Falconer, 1989). It is the ratio of the additive genetic variance to the phenotypic variance. Heritability estimate is important in the successful breeding programme as the breeding value helps to predict the highest productivity that will

express the reliability of phenotypic value, (Nigussie and Saleh, 2007; Hefyny, 2010; Rashnaw, 2010; Wannows *et al*., 2010). Only the phenotypic values of individuals can be directly measured, but it is the breeding value that determines their influence on the next generation. Success in changing the characteristics of a population can be predicted from the knowledge of the degree of correspondence between phenotypic values and breeding values, which is measured by the heritability (Falconer, 1989). The heritability estimates of > 70% is considered very high; 50 – 70 % high; 30 – 50 % moderate and < 30 % low (Hallauer and Miranda, 1988).

Heritability can be defined in the broad sense or in the narrow sense. Heritability in the broad sense describes the proportion of the total variation due to differences among genotypes of individuals in the population. Narrow sense heritability refers to the proportion of total variance that is due to the differences among breeding values of individuals in the population (Van *et al*., 1987). Heritability (h^2) estimates in the narrow sense is estimated as follows (Derera, 2013).

$$h^2 = 2(\sigma_f^{2+} \sigma_m^2)/ \sigma_T^2$$

$$\sigma_T^2 = \sigma_{Am}^2 + \sigma_{Af}^2 + \sigma_D^2$$

Estimation and the interpretation of the average degree of the dominance (d) are estimated as follows:

$$d = \sqrt{} (2 \sigma_{fm}^2 / \sigma_m^2)$$

$$d = \sqrt{} (2 \sigma_{fm}^2 / \sigma_f^2)$$

This may therefore be interpreted as follows:

When: d = 0 no dominance of genes

d < 1 but >0 there is partial dominance of genes

d = 1 then dominance is complete

d > 1 there is over dominance of genes

Additive, non-additive and maternal effects are important in determining maize weevil resistance in hybrids (Derera *et al*., 1998; Dhliwayo and Pixley, 2003; Munjoma, 2004). Additive and non-additive gene actions are of similar importance in determining inheritance of antibiosis effects to weevils in hybrids. However, the previous studies have shown that additive gene action was more important than the non-additive gene action in determining resistance to the maize weevil (Derera *et al*., 1999). Heritability is estimated from the degree of resemblance between relatives.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials used

3.1.1 Maize and weevils

A total of 17 inbred lines were used in this study comprising of nine (9) (Appendix 1) from the International Centre for Maize and Wheat Improvement (CIMMYT) in Kenya used as males and eight (8) (Appendix II) from the Zambia Agricultural Research Institute (ZARI) as females. After hybridization and selfing a total of twenty seven genotypes were used in the evaluation. The maize weevils used in the study were collected from the bags of weevil infested maize harvested from field trials at ZARI during the previous growing season. Unsexed weevils of mixed age, in a no choice experiment (Serratos *et al*., 1997) were used.

3.2 Methods

Three phases were followed in the execution of the study. This included the development of crosses via the North Carolina Design two (NCDII) in phase I. Advancing the F1 crosses to F2 was done in phase II and Phase III involved the evaluation of the F2 population via insect bioassay and biochemical bioassay.

3.2.1 Phase I: Generation of crosses

Crossing was done at Golden Valley Agricultural Research Trust (GART) 80km north of Lusaka, Zambia during the 2011/2012 growing season. The station is located at latitude $14^{o}40'$ south, latitude $25^{o}01'$east and at an altitude of 1140m above sea level. The soil is described as Makeni Series which is fine, mixed, isohyperthermic ultic Paleustalf.

Land preparation was done on 20th December; 2011. Planting of maize seed was done on 30th December, 2011 at GART. Compound D fertilizer (10:20:10:6) was applied

prior to planting (pre-plant) at the rate of 400 kg/ha and urea (46% N) was equally applied at the rate of 400 kg/ha on 3rd February, 2012. Furadan was applied prior to planting in holes for control of cutworms and stem borers at the rate of 10 l/ha. The North Carolina mating design II (Hallauer and Miranda,1988) was used in which eight female susceptible inbred lines from ZARI and nine resistant male lines from CIMMYT were planted so that the main effects due to male, female and interaction was determined on the progeny. Each female variety was planted in 8 row plots with 4m row length. Each male variety was planted in a 2 row plot, 2m long with 2 seeds planted per station. The inter and intra-row spacing used was 75cm and 25cm, respectively. Standard management practices of the crop were followed with regard to weeding and pest control.

3.2.2 Phase two – Seed multiplication

Forty eight lines were advanced to F_2 by selfing under irrigation at Nanga Research. Due to the breakdown of the pump at Nanga during critical periods of production, some lines did not do well. Therefore only 27 lines were selected to the next phase for evaluation.

3.2.3 Phase three - Laboratory analysis

The laboratory analysis was done according to the procedures of Dobie (1977) and also Makkar 2003.

3.2.3.1 Weevil bioassay

Laboratory analysis based on the weevil bioassay was done using the modified Dobie's method (Dobie 1977; Serratos *et al*., 1993). The freshly harvested seed of each variety were first sun dried to about 12-13 % moisture content. Five cobs of each

genotype were hand shelled and the grain was packed into 5 x 8 polythene bags and then they were closed by using the rubber bands and were then stored in a deep freezer at the temperature of -16^{o}C for one week to kill any previous infestation by the insects which included the adults, larva or eggs (Kossou *et al*., 1993).

Fifty (50) grams of the grains, from each plot was placed into new 350 ml plastic jars, measuring 11.7 cm in height and about 5.2 cm in diameter at the mouth. The tops of the lids of these jars were cut out leaving only the screw top rings. The polythene screen lids allowing ventilation and preventing the escape of insects were placed on jars. Forty unsexed weevils of mixed age, in no choice experiments (Serratos *et al*., 1997) were initially counted into vials with the help of pairs of tweezers and a denominator Multiple - Tally counter were put into each jar.

Weevils were kept for ten (10) days for the *S. zeamais* to oviposit as described by Derera *et al*., (2001). After 10 days sieving was done to remove adult weevils. Live and dead weevils were counted. Tweezers were used to probe immobile weevils to establish whether they were dead or alive. Progeny emergence counts were made every two days beginning 25 days after the removal of the parent insects and ending when all progeny (F_1) had emerged. Emerged progenies were removed from the jars at each count (Siwale *et al*., 2007).

Seeds of each variety without the *S. zeamais* were kept under similar conditions and served as controls. The treatments were arranged in a Completely Randomized Design replicated three times. The jars were placed in the controlled temperature and the relative humidity room at 27- 30^{o}C temperature and 43 to 60% relative humidity. The relative humidity was provided by water placed in four troughs (Bekele and Hassanali, 2001). The genotypes were kept undisturbed (except times of sieving the F_1 emergency) in the controlled temperature and relative humidity room for ninety

days (90) days then assessment was done on physical parameters of seed as shown below.

3.2.3.1.1 Grain hardness

Fifty grammes of each sample were weighed. Each sample was ground in a laboratory mill under the brand name of Retsh, Type ZM 1000 (GmbH & Co. KG 5657 HAAN 1, Germany) at 10000 revolutions per minute. The collected meal was put back in labelled plastic bags. The meal was then hand sifted. The collected flour and retained grit were emptied in separate labelled 5 x 8 cm white plastics and these were subsequently weighed.

The weight of the grit and flour were added together for each genotype to get the total weight, which was about the same weight as the original weight of grain from where flour and grit samples were derived. Grain hardness was expressed as percent grit of total weight of sample (grit plus flour after sieving a 50g ground maize sample). Therefore grit percentage was used as a proxy for grain hardness.

3.2.3.1.2 Seed damage and weight loss

Ninety (90) days after infestation with insects, the glass jars were opened. The content in each jar was separated into grains, insects and dust using 4.7 and 1.0mm sieves to assess each genotype's seed damage. The weight of dust produced was recorded. The damaged seeds (holed seeds) and the undamaged seed by the *S.zeamais* was counted. Seed damage was expressed as a proportion of the total number of seed sampled (Abebe *et al*., 2009). Therefore, grain weight loss was measured as the difference in weight of grain samples before and after infestation of weevils in no

choice experiments (Serratos *et al*., 1997). Seed weight loss was determined using the count and weight method of Gwinner *et al*., (1996).

Weight loss (%) = (Wu x Nd) – (Wd x Nu) x 100 / Wu x (Nd + Nu);
Where Wu = Weight of undamaged seed
Nu = Number of undamaged seed
Wd = Weight of damaged seed
Nd = Number of damaged seed

3.2.3.1.3 Dobie's Susceptibility index

The Dobie index was used as a criterion to separate varieties into different resistance groups. The Dobie Index of susceptibility was calculated as described in section 2.5.6 Genotypes were separated into resistance or susceptibility groups following the scales used by CIMMYT (Pixley, 1997) which is as follows:

Dobie relative index of less than or equal to 4 was classified as resistant.
Dobie relative index of 4.1 to 6.0 classified as moderately resistant.
Dobie relative index of 6.1 to 8.0 classified as moderately susceptible.
Dobie relative index of 8.1 to 10 classified as susceptible.
Dobie relative index of more than 10 was classified as highly susceptible.

The parental materials, male and female inbred lines that were used in the study were also verified as to whether they were indeed susceptible or resistant using the Dobie's index of susceptibility following the same scale used by CIMMYT.

Figure 1. Seed damage due to weevil attack from some genotypes evaluated.

3.2.3.2 Biochemical bioassay

3.2.3.2.1 Protein content

The Kjeldahl procedure (Barbano *et al*., 1991) was used to determine the crude protein content. Twenty grams sample of the whole maize kernels were ground in a laboratory mill for each genotype. Three replications for each sample were used.

3.2.3.2.2 Phenolic acid content determination

This was determined by using the Folin – Ciocalteu method (Makkar, 2003) using the UV- Spectrophotometer. Tannic acid was used as a standard and this was done in three replications.

3.3 Statistical analysis

The analysis of variance (ANOVA) for all the measured parameters was done using the GENSTAT Thirteen Edition and SPSS 16.0. The simple linear correlation analyses were conducted among various components measured to determine the strength of association of these traits. In order to study the cause and effect relationship between the susceptibility index and other variables stepwise multiple regression was used.

Heritability was estimated using the North Carolina design II (Hallauer and Miranda, 1988).

Figure 2 . Sieving session at ZARI during the insect bioassay

CHAPTER 4
4.0 RESULTS

4.1 General observation

The summary of the analysis of variance of all the measured traits is shown in Table 1. Results indicate that there were highly significant differences ($p < 0.05$) among the twenty seven genotypes (27).

4.2 Performance of genotypes for weevil resistance

Twenty seven genotypes were evaluated for weevil resistance. They were significant differences among genotypes for all the traits measured ($p < 0.05$) as is summarized in Table 1. The summary of means of genotypes for measured traits is indicated in Table 4. Results for each trait measured are outlined below.

4.2.1 Protein content

Genotypes were significantly different ($p < 0.05$) from each other for crude protein with an overall mean content of 8.8% as shown in Table 2. Among genotypes crude protein was in the range of 7.2 % to 11.4%. Genotypes 60N and 24N had high significant crude protein whose means were 11.4% and 10.6% respectively (Table 2). Statistically, it was also found that genotypes 1N (9.2%), 77N (9.3%), 8N (9.3%), 74N (9.2%), 56N (9.2%) were not significantly different from each other in terms of crude protein content. Lower levels of crude protein were found in genotypes 95U (7.8%), 38N (7.7%) and with the lowest in 78N (7.4%) when compared to other genotypes (Table 2).

4.2.2 Phenolic content

Genotypes were highly significantly different ($p < 0.05$) for Phenolic content (Table 1). The Phenolic content among genotypes was found to be between 24 and 80.7 mg per 100g of maize grain. The mean Phenolic content among genotypes was 55.8mg/100g of maize grain. Genotype 60N had the highest Phenolic content of 80.7

mg per every 100g of grain. The lowest level of phenolics was found in genotype 78N (24mg per 100g of grain).

4.2.3 Parental survival

Parental survival of *S. zeamais* among the genotypes was significantly different ($p < 0.05$). Table 2 presents the number of live adult weevils obtained after the ten days oviposition period. The overall mean survival number for parent weevils at the end of the oviposition period was 12.6. The number of parental survival among the entries was in the range of 3.0 to 32.7. The highest parental survival was recorded from genotype 78 N (32.7) which was not statistically different from 67N (28.0). Least parental survival numbers were also found among the 31N (3.0), 60N (3.0) and 74N (3.0) genotypes.

4.2.4 Progeny emergence

The F_1 progeny emergency among the genotypes was significantly different ($p < 0.05$) among genotypes (Table 1). The means of all F_1 progeny emergency for all genotypes is presented in table 2. The grand mean for progeny emergence was 15.5 and the range of emergence among entries was 2.3 to 98 weevils. Among all the maize genotypes evaluated, significantly higher numbers of F_1 progenies (98) emerged from genotype 78N. Lowest numbers of F_1 progenies emergence were found in genotypes 60N (2.3), 74N (3.0), 4N (3.0) and 31N (4.0).

4.2.5 Median development period

The median development period among the genotypes was significantly different among the genotypes ($p < 0.05$). The median development period ranged from 26 to 79.8 days with the grand mean median development period of 50.3 days (Table 2).

Genotype 1N had a statistically higher median development period of 79.8 days. Genotype 78N had the least development period of 26.0 days.

The general observation was that the genotypes with high F_1 progeny emergency tended to have a shorter median development period and in turn also had a very minimum percentage of adult mortality.

Table 1: Summary of the combined analysis of the analysis of variance of measured traits in maize genotypes

Source	DF	SW	GH	PS	F_1E	MDP	MC	GWL	PR	PH	SI
Entry	26	0.006***	21.82***	21.2***	1080.9***	806.155***	1.0635**	25.43***	6.323***	6979.6***	8.98***
CV (%)		2.4	6.3	7.4	5.5	1.6	4.7	3.5	3.1	4.2	8.4
s.e.d		0.0073	2.171	0.748	0.6789	0.754	0.6719	0.1592	0.2167	2.187	0.1414

Key:

SW = 100seed weight, GH = grain hardness, PS = Parental survival, F_1E = progeny emergency, MDP = median development period, MC = moisture content, GWL = grain weight loss, PR = protein, PH = Phenolic, SI = susceptibility index. *** = significant at 5% level, DF = degrees of freedom

Table 2: Summary of means for the genotypes

Genotype	MDP	PS	F_1	PH	GWL	MC	PR	SI	GH	100SW
1N	79.84	4.00	4.00	80.00	4.53	11.60	9.23	0.75	47.83	35.24
4N	78.10	8.67	3.00	78.67	4.60	12.57	9.57	0.61	45.84	35.44
77N	63.10	10.00	9.00	58.00	4.67	13.23	9.30	1.65	39.71	30.25
60N	59.45	3.00	2.33	80.67	4.27	12.40	11.37	0.37	51.67	35.44
8N	52.30	18.33	29.00	68.33	6.93	11.77	9.33	2.14	39.24	36.02
6N	56.41	11.00	15.00	73.00	4.80	12.60	10.23	1.61	43.29	36.27
74N	78.14	3.00	3.00	58.00	2.47	13.43	9.20	0.82	43.07	36.66
24N	62.72	4.33	4.00	75.33	4.93	12.83	10.60	0.80	47.49	33.83
26N	31.00	29.00	27.00	31.00	6.73	12.20	7.57	4.62	34.50	30.87
73U	52.45	21.00	10.33	66.33	4.67	12.70	9.60	1.53	38.04	38.30

31N	69.72	3.00	4.00	70.67	4.13	12.60	7.87	0.85	45.28	38.47
67N	29.04	28.00	31.00	29.00	7.13	12.93	8.70	5.14	38.17	42.87
19N	71.43	4.00	5.00	51.00	5.13	11.77	9.00	1.37	39.13	36.66
56N	53.65	4.33	4.67	58.00	4.13	11.80	9.23	1.15	37.67	45.54
13N	29.00	12.33	21.33	62.00	4.67	11.60	7.17	2.14	40.82	35.40
45U	41.35	9.67	8.00	73.67	4.33	13.47	8.47	1.23	37.80	43.10
80U	46.35	18.33	30.00	47.00	7.00	12.67	8.20	3.14	43.22	38.50
66U	45.18	13.33	10.67	66.33	6.47	13.30	9.00	1.55	43.07	39.65
46U	45.90	8.67	6.00	67.33	4.87	12.47	8.80	1.16	41.67	43.60
63U	57.14	8.00	14.67	46.00	4.73	12.77	9.23	2.54	43.17	38.67
91U	48.55	7.00	7.00	45.33	4.93	13.87	8.37	1.86	38.50	38.47
12N	26.89	22.00	20.00	32.00	7.40	12.77	7.83	4.07	38.60	34.87
38N	37.34	20.67	5.33	28.00	5.60	12.87	7.73	4.20	39.13	35.25
78N	26.00	32.67	98.00	24.00	19.07	12.10	7.40	8.30	33.78	41.10

95U	33.28	19.67	23.67	33.00	4.60	13.60	7.80	4.16	40.37	40.87
80N	40.11	10.33	9.00	53.00	4.47	12.50	8.40	1.80	38.50	32.00
74U	56.03	5.33	5.00	50.00	5.20	12.00	9.10	1.40	41.13	38.63
Mean	**50.26**	**12.58**	**15.18**	**55.76**	**5.65**	**12.61**	**8.82**	**2.26**	**41.14**	**37.4**
LSD	**1.482**	**1.513**	**1.848**	**7.85**	**0.316**	**0.133**	**0.433**	**0.276**	**1.748**	**0.014**

Key: 100sw = one hundred seed weight, GH =grain hardness, PS = parental survival, F1= f_1 progeny emergence, MC =moisture content, GWL=grain weight loss, PR = Protein, PH = Phenolic, SI =susceptibility index, MDP = Median Development Period

4.2.6 Grain weight loss

Grain weight loss was significantly different among the genotypes ($p < 0.05$). It arose from the feeding of the larvae and / or adults in and on kernels computed from the initial and the final weight. The grand mean weight loss was 5.7%. The greatest weight loss of 19.1% was observed in genotype 78N. The lowest grain weight loss of 2.5% was observed in genotype 74N. Grain weight loss percentage was high among genotypes with more number of F_1 progeny emergencies.

4.2.7 Grain Hardness

Grain hardness showed discrimination among entries. They were significant differences among the genotypes ($p < 0.05$). Grand mean hardness value of 41.1% was observed among genotypes. Genotype 60N was statistically higher than the other genotypes with 51.7% and genotype 1N had similarly a higher grain hardness value of 47.8%. Genotypes 26N (34.5%) and 78N (33.8%) had statistically lower grain hardness values.

4.2.8 Kernel weight

Kernel weight was also significantly different among genotypes ($p < 0.05$). The genotypes had kernel weight values ranging from 0.3g to 0.5g with the grand mean of kernel weight being 0.37g. Highest kernel weight value was observed in genotype 26N (0.51g). Genotypes 56N (0.42g) and 95U (0.42g) were also significantly higher. Lowest kernel weight values were observed in genotype 80N (0.3%).

4.2.9 Dobie index of susceptibility (SI)

Significant differences ($p < 0.05$) were observed on index of susceptibility among the genotypes evaluated (Table 1). The SI in this study ranged from 0.4 to 8.3 for genotypes 60N and 78N respectively. According to the

CIMMYT classification, out of the twenty seven maize genotypes evaluated against *S. zeamais* for resistance, twenty three genotypes were found to be relatively resistant; three genotypes 26N, 12N and 67N were moderately resistant. Only one genotype (78N) was moderately susceptible (Table 3).

Table 3: Classification of Maize weevil resistance among genotypes using Dobie Index

Genotype	Dobie Index	Classification
1N	0.75	Resistant
4N	0.59	Resistant
77N	1.28	Resistant
60N	0.48	Resistant
8N	2.14	Resistant
6N	1.61	Resistant
74N	0.80	Resistant
24N	0.80	Resistant
73U	1.53	Resistant
31N	0.84	Resistant
67N	5.14	Moderately resistant
19N	1.37	Resistant
56N	1.14	Resistant
13N	2.14	Resistant
45U	1.23	Resistant
80U	3.14	Resistant
66U	1.55	Resistant
46U	1.16	Resistant

63U	1.71	Resistant
91U	1.86	Resistant
12N	4.07	Moderately resistant
38N	1.40	Resistant
78N	8.34	Moderately susceptible
95U	2.88	Resistant
80N	1.80	Resistant
74U	1.29	Resistant
26N	4.62	Moderately resistant

Table 4: Means of traits measured on parental lines

LINE	GH	PS	MDP	GWL	PR	PH	SI
151	40.3	35.2	20.0	17.3	7.2	40.3	6.26
152	38.2	41.0	19.0	16.7	7.4	9.3	6.63
917	37.3	46.3	220	16.3	7.3	8.7	5.95
1212	38.0	40.7	20.7	15.2	7.6	7.1	5.92
10075	45.5	11.7	91.0	4.20	8.0	64	0.80
10096	45.3	21.3	74.0	3.7	7.1	66.5	1.11
10111	45.1	13.7	90.0	4.5	8.8	55.3	1.18
10112	44.6	12.8	59.0	6.6	8.2	77.6	2.14
Mean	**41.82**	**27.83**	**49.46**	**10.58**	**7.70**	**36.81**	**3.76**
LSD (5%)	**1.436**	**0.8559**	**1.201**	**0.4786**	**0.292**	**0.6841**	**0.2598**

Key:

GH= grain hardness, PS = Parental survival, MDP = median development period, PR = protein, GWL = grain weight loss, PH = Phenolic, SI = index of susceptibility.

4.3 Association among variables measured or derived

The results (Table 6) shows an inverse relationship between the susceptibility index (SI) and median development period, grain weight loss (%), grain hardness, 100 seed weight, Phenolic and protein. The parental survival (r = 0.612***) and F_1 progeny emergencies (r = 0.197) were positively correlated to susceptibility index. The inter component correlations among traits showed that median development period was positively correlated with grain weight loss (r = 0.189), 100 seed weight (r = 0.171) though they were not significant (p > 0.05). Seed weight was also positively correlated to grain weight loss (r =0.167) but it was not significant(p>0.0

Table 5: Correlation coefficients of *S. zeamais* infestation of the maize genotypes

PAR	SI	F_1E	MDP	GWL	GH	100SW	PS	PH	PR
SI	1								
F_1E	0.197	1							
MDP	-0.312	-0.082	1						
GWL	-0.308	0.083	0.189	1					
GH	-0.361***	0.085	-0.355***	-0.131	1				
100SW	-0.473***	0.093	0.171	0.167	-0.138	1			
PS	0.612***	-0.316	-0.504***	0.248	-0.122	0.052	1		
PH	-0.213	-0.048	-0.27	0.09	0.423***	0.14	-0.225	1	
PR	-0.155	0.172	-0.289	-0.151	-0.042	0.06	-0.095	-0.144	1

Key: PAR = parameter, SI=susceptibility index, F_1E = f_1 progeny emergence, MDP = median development period, GWL = grain weight loss, GH = grain hardness, 100SW = 100 seed weight, M = mortality, PS= parental survival, PH= Phenolic, PR= protein, *** significant at 5% probability.

Parental survival of the maize weevil was strongly positively correlated (r = 0.612***) to susceptibility index. Parental survival was also negatively and significantly associated with the median development period of the progenies (r = -0.504***). From the biochemical tests, it was evident that Phenolic content in maize showed a positive relationship with grain hardness (r = 0.423***) and seed weight (r =0.147).

4.4 Stepwise multiple regression

Significant contributions to the total variation were observed from the four traits namely parental survival of adult weevils, Phenolic content, F_1 progeny emergency and grain hardness. Parental survival had a most significant influence on susceptibility index of 78.5% of the total variation (Table 6). Additional of other variables such as Phenolic, F_1 progeny emergency, and grain hardness also showed significant influence of 10.9%, 8% and 0.5% respectively.

Table 6: Stepwise correlation of susceptibility index and other traits

Variable	Partial square	R-model square	R-F value	Pr >F
Parental survival	0.785	0.785	91.452	0.000
Phenolics	0.109	0.894	24.589	0.000
F_1 emergency	0.08	0.974	71.874	0.000
Grain hardness	0.005	0.979	5.584	0.000

Further addition of other variables did not amount to significant differences according to the total variation in susceptibility index, thus they were left out.

4.5 Genetic parameters

It was observed that the non additive variation controls all the traits that were measured in this study (Table 7). The calculated narrow sense heritability was low in most traits ranging from 5.9 to 22.2% (Table 8). Heritability analysis according to Derera *et al*., (2001) on genetic analyses can be performed only for the index of susceptibility because it incorporates all resistance parameters. Therefore in this study it was estimated that heritability of the traits was 20.9% since this was the heritability based on susceptibility index in this study (Table 8). The heritability estimate was considered low since it was below 30 %.

Table 7: Summary for the average degree of dominance for the traits

Trait	Degree of dominance
F_1 emergence	1.74
Median development period	1.73
Grain weight loss	1.67
Grain hardness	1.88
Mortality (%)	1.69
Parental survival	2.80
Phenolic content	6.33
Protein content	8.67
Susceptibility index	1.52

Table 8: Estimated genetic variances for some traits in the maize genotypes

Variance Component	F_1E	MDP	Grain weight loss	Hardness	Kernel weight	Phenols	Proteins	Susceptibility Index
σ_{Am}^2	157.36	542.2	2.69	38.76	0.000025	107.2	0.156	3.756
σ_{Af}^2	-23.52	-174.8	0.296	13.72	0.00114	-40	-0.4753	-0.648
σ_T^2	66.9	183.68	1.496	26.24	0.000582	33.6	0.3318	1.554
σ_D^2	239	812.2	3.752	68.68	0.006	39.76	5.53	4.3
h^2 (%)	**17.9**	**15.6**	**22.2**	**21.65**	**8.12**	**8.3**	**5.9**	**20.9**

(Source: Derera, 2013)

Key:

σ_{Am}^2 =Additive variance in males, σ_{Af}^2 = Additive variance in females, σ_T^2 = Variance totals, σ_D^2 =Dominance variance, h^2 (%) =Narrow sense heritability

4.6 Combining Ability

The mean squares due to general combining ability were significant for all variables measured. However, the mean squares due to the specific combining ability were not significant for the same traits (Table 9).

Estimates of the Specific Combining Ability (SCA) and the General Combining Ability (GCA) effects for the various traits are presented in tables 10 and 11 respectively. The GCA was significant for all traits measured. Positive and significant GCA effects were exhibited in the Phenolic content among the male lines: Line 10075 (27.19**), Line 10096 (29.70 **), Line 10111 (18.53**) and Line 10112(40.79**). Line 10112 had a positive and higher significant Phenolic content among the male lines. The male lines also showed negatively and significantly GCA effects in terms susceptibility index: Line 10075 (-2.96**), Line 10096 (-2.63**), Line 10111 (-2.57**) and Line 10112 (-1.62**). Line 10112 in terms of susceptibility had a lower susceptibility (-1.616**) indicating that it was more resistant.

Most of the female lines showed a lot of negative and significance in Phenolic content: Line 151 (-29.74**), Line 152 (-27.51**), Line 917 (-28.11**) and line 1212 (-30.84**). All the female lines had a positive and significant GCA effects: Line 151 (2.17**), Line 152 (2.89**), Line 917 (2.20**) and Line 1212 (2.51**).In terms of parental survival, the GCA effects among the lines were negative and significant probability level of 1%. For example in line 10112 (-14.16**) but all female lines showed positive significance at 1% level of probability. Line 151 (12.9**), Line 917 (18.44**).In terms of SCA effects, genotype 1N (24.24**) and genotype 60N (24.91**) had higher positive and significant Phenolic

contents. But these same genotypes had a lower susceptibility values: 1N (-1.51*) and 60N (-1.89**). Genotype 67N (-26.76**) with the lowest Phenolic content had a higher SCA effect on the susceptibility on the same genotype of 2.88**.

Table 9: mean squares for susceptibility index and the other agronomic traits among the parental lines

Source of Variation	df	MDP	PS	F1	PH	GWL	PR	GH	SI
Rep	2	0.79	0.3	0.28	4.12	10.76	9.16	3.26	0.16
Crosses	15	0.21*	0.28*	1.87	22.65**	8.7*	4.61	2.87**	0.41**
GCA males	3	0.68	0.23	0.89	25.46**	6.8*	3.55	1.51*	0.58 *
GCA females	3	0.26	0.17	0.55	11.71**	3.9**	3.33	2.86**	0.56*
SCA	9	0.09	3.55	0.08	12.41*	4.1**	1.81	2.14	0.35
Error	30	0.55	0.99	3.61	3.29	0.34	4.61	4.12	1.4
CV %		1.5	2.2	3.4	4.6	3.2	2.6	3.8	3.6

*, ** significant at 5% and 1% probability level respectively

Table 10 : SCA effects

Entry	CROSS	MDP	PS	F1	PH	GWL	PR	SI	GH	100SW
1N	151 x10075	29.58**	-8.58	-11.18**	24.24**	-1.12	0.7	-1.51	6.69**	-2.16*
67N	151 x 10096	-34.45	15.42**	15.82**	-26.76**	1.48*	0.17	2.88**	-2.97**	5.47**
74U	151 x 10111	5.77*	-7.25	-10.18**	-5.76	-0.45	0.57	-0.86	-0.01	1.23
60N	151 x 10112	9.19**	-9.58	-12.85**	24.91**	-1.38	2.84**	-1.89**	10.53**	-1.96
74N	152 x 10075	27.88**	-9.58	-12.18**	2.24	-3.18	0.67	-1.44	1.93	-0.74
56N	152 x 10096	3.39	-8.25	-10.51**	2.24	-1.52	0.7	-1.11	-3.47	8.14*
63U	152 x 10111	6.88*	-4.58	-0.51	-9.76	-0.92	0.7	0.28	2.03	1.27
38N	152 x 10112	-12.92	8.09**	-9.85**	-27.76**	-0.05	-2.8**	1.94	-2.01	-2.15
24N	1212 x 10075	12.46**	-8.25**	-11.18**	19.57**	-0.72	2.07**	-1.46	6.35	-3.57**
12N	1212 x 10096	-23.37**	9.42**	4.82	-23.76**	1.75	-0.7	1.81	-2.54	-2.53*
77U	1212 x 10111	12.84**	-2.58	-6.18**	2.24	-0.98	0.77	-0.61	-1.43	-7.15*
91U	1212 x 10112	-1.71	-5.58*	-8.18**	-10.43	-0.72	- 0.16	-0.4	-2.64	1.07
6N	917 x 10075	6.15**	-1.58	-0.18	17.24	-0.85*	1.7	-0.65	2.15	-1.13
80U	917 x 10096	-3.91	5.75**	14.82**	-8.76	1.35	-0.33	0.88	2.08	1.1
4N	917 x 10111	27.84**	-3.91	-12.18**	22.91**	-1.05	1.04	-1.65	4.7	-1.96*
80N	917 x 10112	-10.15**	-2.25	-6.18**	-2.76*	-1.18	- 0.13	-0.46	- 2.64	-5.4**
s.e.d		**0.56**	**0.399**	**0.861**	**0.319**	**0.2232**	**0.1361**	**0.1211**	**0.67**	**0**

*, **, significant at 5% and 1% probability level respectively.

Table 11 : GCA effects for the parental lines

Males	**F1**	**GWL**	**SI**	**MDP**	**PH**	**PR**	**PS**
10075	-9.09**	-6.38**	-2.57**	41.54**	27.19**	1.93**	-16.16**
10096	-7.75**	-6.88**	-2.63**	24.54**	29.69**	1.06**	-6.50**
10111	-2.75**	-6.05**	-2.96**	40.54**	18.53**	-0.30*	-14.16**
10112	3.91**	-3.95**	-1.62**	9.54**	40.79**	-0.30*	-14.16**
s.e.d	**0.84**	**0.21**	**0.08**	**0.5**	**0.28**	**0.126**	**0.38**
Females							
151	2.25**	4.62**	2.17**	-28.79**	-29.74**	-0.47*	12.90**
152	3.91**	6.12**	2.51**	-30.46**	-27.51**	-0.87**	7.37**
917	5.91**	5.75**	2.20**	-27.46**	-28.11**	-0.77*	18.44**
1212	3.58**	6.75**	2.89**	-29.46**	-30.84**	-0.87*	7.37**
s.e.d	**0.861**	**0.2232**	**0.1211**	**0.56**	**0.319**	**0.1361**	**0.3991**

*, ** indicates significance at the 0.05 and 0.01 levels of probability respectively.

CHAPTER 5

5.0 DISCUSSION

The study proposed to establish basis for development of weevil resistance in maize *(Zea mays* L). The overall objective of this study was to establish mechanisms of weevil resistance in maize, specifically through characterizing genotypes for traits related to weevil resistance to weevils in maize and also to estimate the genetic basis of the mechanisms of weevil resistance in maize. The genotypes showed significant differences among each other. Results of the study showed genetic variation for all the traits measured. The results were discussed in two parts.

5.1 Factors related to weevil resistance in maize

Protein content was negatively correlated with the susceptibility index of maize genotypes. This was consistent with findings reported by Dobie, 1977, Arnason *et al*., 1993 and Keba and Sori, 2013. Furthermore, genotypes in this study with higher protein content were classified to be resistant based on CIMMYT, (2001) classification. This was evident in genotype 60N which had the highest protein content (11.3%) and the number of adult weevils surviving at the end of the experiment on this genotype was only 3.0. The lowest genotypes in terms of protein content were genotypes 95U (7.8%) and 78N (7.4%) which had parental survival numbers of 19.7 and 32.7 respectively. This was also consistent with what other researchers found out (Arnason *et al*., 1997; Derera *et al*., 2001; Dhliwayo and Pixley, 2003; Garcia- Lara *et al*., 2004).

The above findings about protein content so far showed that higher protein content in genotypes results in more adult weevil mortality and vice versa.

Adult weevil mortality also gave an indication of the number of progenies expected in the genotypes. Keba and Sori (2013) have also reported that less adult weevil's mortality in storage is an indication of more number of eggs laid from the survived adult weevils leading to more number of progenies emerging. Further analysis with the stepwise regression analysis which is a stronger tool than correlation for use in indirect selection showed that protein content was not significant in the observed susceptibility index. This suggests that none of the maize varieties tested was completely resistant for proteins. These findings were consistent with Siwale *et al*., 2007 and Tongjura *et al*., 2010. Although protein may seem to have some antibiosis effect, lack of a definite relationship with physical resistance parameters in this study may indicate other resistance factors in maize studied.

Arnason *et al*., (1994; 1997) also reported on the presence of biochemical compounds, Phenolics especially the ferulic acid in the maize grain in conferring resistance. The level of the Phenolic compounds was negatively correlated with susceptibility index. This was in agreement with the findings reported by Dobie (1974). It was also noticed that genotypes with the highest amount of the phenols like genotype 60N (80.676mg/100g) had less grain weight loss (4.3%) since weevil attack may have been prevented by the amounts of Phenolic compounds particularly ferulic acid component. Genotypes with lower amounts of Phenolic, 78N, had 24mg/100g of grain had a higher grain weight loss of 19%. This means that higher Phenolic content in the grain could have prevented the grain from being attacked by the insects while the lower Phenolic content in the grain resulted into more grain damage. This showed antibiosis that was being conferred by the Phenolics in the maize grain as consistently stated

by Classen *et al*.,(1990), Sen *et al*.,(1994), Arnason *et al*.,(1997) and Singh (2009). Furthermore, Phenolics showed a positive significant association with the kernel hardness (r = 0.423***). This was also consistent with other authors Classen *et al*., 1990, Sen *et al*., 1994, Arnason *et al*., 1997 and Derera *et al*., 2001. These authors reported that phenolic compounds particularly ferulic acid had an influence on the hardness of the grain such that it was able to make the cell walls hard and limit the biodegradability of the cell wall polysaccharides by insects. The Phenolic acids were able to cause adverse effects to weevil feeding behavior and survival. Therefore, biochemical screening of the maize grain may be used as a first step towards selection of genotypes for resistance.

In terms of grain weight loss, resistant maize varieties had a minimum grain damage and small quantity of powder formed. Grain weight loss was highest in genotype 78N in which there was a 19 % grain weight loss. The median development period among the genotypes had an average of 50.3 days. The range of the median development period was wider (26 to 79.8 days). The period was longer in the resistant genotype (60N) in which the median development period was 79.8 days but median development period was shorter in the susceptible genotype 78N with median development period of 26 days. For susceptible genotypes the development period of weevils was shorter and vice versa.

Higher grain weight loss values may have been expected in this study if the young weevils of same age particularly 0 to 3 weeks old were used. This was demonstrated by Dobie (1974; 1977) in which fecundity and the feeding of maize was highest when weevils were in the range of 0 to 3

weeks old after which there was a steady decline. In this study, weevils which were used were of unknown age such that it was possible that some of the weevils used may have been past the 0 to 3 weeks old. Parental survival was negatively and significantly ($p < 0.05$) correlated to the median developmental period ($r = -0.504***$). Through stepwise multiple regression analysis, it was observed that parental survival in terms of explaining total variation had a highest contribution of 78.5%. This means that the number of parent weevils that were alive or dead in given genotypes gave an indication of susceptibility or resistance. It was also clear that the longer the median development period, the less the parental survival of the adult weevils and vice versa. The highest number of F_1 progeny emergence was 98 in genotype 78N with an experimental mean of 15.2. In this study F_1 progeny emergence of the weevil had a small contribution of 8% to the total variation (Table 6).

There was a negative correlation ($r = -0.355$) between grain hardness and the median development period of the weevils which was significant ($p < 0.05$). This means that genotypes with a harder testa took more time for the weevils to develop on the grain as was evident in the low susceptibility index value indicating resistant genotypes (Table 3). Susceptible genotype like genotype 78N with low grain hardness (33.8%) had 98 progenies (Table 2) emerging indicating a high possibility of higher damage by weevils. These results were consistent with the findings reported by Leuschner *et al*.,(2000) who reported a distribution of larger numbers of *Sitophilus oryzea* progenies among genotypes of pearl millet (*Pennisetum glaucum* L) that had a higher proportion of the soft endosperm. The findings were also consistent with Derera *et al*., (1998) who reported that

insect pests develop faster on a more favorable host. The softer the endosperm the easier it is for insect pests to feed on grain.

Grain hardness was further found to be significantly ($p < 0.05$) and positively associated with Phenolic compounds ($r = 0.423^{***}$). Grain hardness contributed 0.5% to the total variation of susceptibility index. Increased Phenolic compounds increased hardness as well which may have contributed to the resistance of genotypes. This was consistent with the study reported by Arnason *et al.,* (1997) in which increased Phenolic content was observed to be concentrated on the cell walls of the grain and then makes the grain harder depending on the concentration of Phenolic content.

The range of susceptibility index values obtained in this study ranged from 0.4 for genotype 60N to 8.4 for genotype 78N. Most genotypes in this study were resistant. Arnason *et al*., (1994) reported susceptibility index values ranging from 0 for resistant check to 15.2 for susceptible check for genotypes which had the moisture ranges of 10.4% to 14.9%. The genotypes used in this study had moisture content ranging from 11.6 % to 13.9%. The grand mean value of the moisture content of the genotypes was 12.6%. This mean value was just about the moisture content the Entomology research team at CIMMYT, Mexico found which is known to provide effective pest control (Bergvinson, 2001).

5.2 Inheritance of weevil resistance in maize

Though previous studies (Widstrom *et al*., 1975; Tipping *et al*., 1989; Widstrom *et al*., 1983; Kang *et al*., 1996; Derera *et al*., 1998) have shown that additive gene action was more important, genetic parameters for all traits in this study reveal that they were controlled by non-additive

variance. This means that in order to develop the host plant resistance, the process would take a very slow progress due to the low heritability estimate (20.9%) as indicated in table 8. From the narrow sense heritability calculated, it indicates a very low gain in selection. To breed for higher Phenolic content among genotypes in a breeding programme it would be necessary so that to do the population improvement through recurrent selection since the trait has the heterotic response. This means that inbred lines will have to be developed in order to come up with hybrids. These hybrids will express heterosis in terms of high weevil resistance. The best combiners then can be selected through determining the one with the good general combining ability and also good specific combining ability (Kang *et al*., 1996). Inbred lines with significant positive GCA in terms of Phenolic content were seen to reduce their susceptibility to weevils as is indicated on male line 10112. This line is likely to reduce weevil attack in its crosses. The general trend that was noticed among the inbred lines in terms of their general combining ability was that they confirmed existence of genetic variability among different maize genotypes with respect to various resistance parameters which breeders may take advantage of. The negative GCA effects of the female inbred lines indicate reduced Phenolic content in the maize results into the increased positive significant susceptibility values. This indicates that female lines are likely to contribute an increased weevil attack in their crosses. Through indirect selection of some of these traits like Phenolic, it is possible to improve maize varieties to weevil attack. It is possible that the contribution of the Phenolic compounds could have been more than 10.9% (Table 8) if parental lines used would have had a higher Phenolic content than the mean Phenolic content of 36.8 mg/100g of grain (Table

4). The evaluated genotypes had a higher mean value of 55.8 mg/100g of grain (Table 2). Female line 151 and male line 10112 could be used as parents in making synthetic populations for recurrent selection. While doing the SCA effects among genotypes for yield, testing for weevil resistance among hybrids can be done because this trait is showing some heterotic response in this study. This is in agreement with the study that was conducted by Serratos *et al*., (1993).

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATION

The study was set up to establish mechanisms of weevil resistance in maize. The specific objectives of the study were to characterize genotypes for traits related to maize weevil attack and also to estimate the genetic basis of the mechanisms of weevil resistance in maize.

With response to characterization of traits to weevil resistance, the study established that genotypes responded differently to maize weevil attack. This shows that through breeding you can identify some hybrids which are more resistant to weevil attack than others. Phenolic content and parental survival were the important mechanisms of resistance which could be used as the indirect selection criteria as one is doing the characterization in a breeding programme.

With response to the second objective, the study was able to establish that it is possible to breed for weevil resistance among the different genotypes that one can use. The low heritability of 20.9% found for the weevil resistance traits indicates that the expected gain from selection would be very low. Non-additive gene action played significant roles in determining resistance among the traits measured. This means that population improvement will have to be done through cyclic selection since the traits were showing the heterotic response through inbred line development.

It can therefore be concluded that Phenolic content and parental survival can be used as indirect selection criteria for weevil resistance during characterization and also during the routinely SCA effect studies for yield

in maize. It is possible to test for weevil resistance on experimental hybrids because this trait shows heterotic response.

It is recommended that it could be interesting to carry out a study in future in which the local maize genotypes with the multi-coloured testa would be characterized to see how they may differ in the Phenolic content and also establish the genetic basis as well.

REFERENCES

Abebe, F., T.Tefera, S.Mugo, Y.Beyene and S.Vidal. 2009. Resistance of Maize varieties to the maize weevil *Sitophilus zeamais* (Motsch) (Coleoptera: Curculionidae). African journal of Biotechnology volume 8: 5937 – 5943.

Abraham, T. and E. Firdissa. 1991. Insect pests of farm stored maize and their management practices in Ethiopia. International Organization for Biological and Integrated Control of Noxious Animals and plants Regional Section Bulletin, 23: 45 – 57.

Adams, J.M., 1976. Weight loss caused by development of *Sitophilus zeamais* Motsch in maize. Journal of stored products research.12 (4): 269 – 272.

Ali, K. and R.H. Smith. 2001. The effect of seed coat on the susceptibility of faba bean to *Callosobruchus chinensis* L. African crop Science journal, 9: 421 – 430.

Alter,T.R., 2013. Weevils on stored grains. Pennstate college of Agricultural sciences. Cooperative extension Entomology notes. Department of Entomology.

Arnason, J.T., J.Gale, B.Conilh de Beyssac, A.Sen, S.Miller, B.Philogene, J.D. Lambert, R.G.Fulcher, A. Serratos and J.A Mihm. 1992. Role of phenolics in resistance of maize grain to stored grain insects, *Prostephanus trancatus* (Horn) and *Sitophilus zeamais* (Motsch). Journal of stored products research, 28: 119 – 126.

Arnason,J.T.,B.Baum, J. Gale,J.D.H. Lambert,D. Bergvison, B.J.Philogene, J.A. Serratos, J.Mihm and D.C. Jewell. 1994**.** Variation in resistance of Mexican landraces of maize to maize weevil *Sitophilus zeamais*, in relation to taxonomic and biochemical parameters. Euphytica 74; 227 – 236.

Arnason, J.T.,Conilh B.B, Philogene B.J.R. and D.J. Bergvinson. 1997. Mechanism of Resistance in Maize grain to the Maize weevil and the Larger Grain Borer. Insect resistance maize: recent advances and utilization, Mihm, J A (Ed). CIMMYT Mexico, pp: 91 – 95.

Arnason, J.T., B. Baum, J. Gale, J.D.H. Lambert, D. Bergivinson, B.J.R. Philogene and D.C. Jewell. 1993. Variation in resistance Mexican landraces of maize weevil *Sitophilus zeamais*, in relation to taxonomic and biochemical parameters. Euphytica, 74, 227 – 236.

ASARECA–TUUSI. 2009. Drought and low-N torelant maize germplasm and varieties – inbred lines, populations, OPVs, Hybrids.

Aylor, D.E., B.M. Baltazar and J.B. Schoper. 2005. Some physical properties of teosinte (*Zea mays Parviglumis*) pollen. Journal of Experimental botany, 56: 2401 – 2407.

Barbano D.M, J.M. Lynch, and J.R. Fleming.1991. Direct and Indirect Determination of True Protein Content of Milk by Kjeldahl Analysis: Collaborative Study. Journal of Association of Official Analytical Chemists 74:281-288.

Bekele, J. and A.Hassanali. 2001. Blend effects in the toxicity of the essential oil constituents of *Ocimum Kilimandscharicum* and *Ocimum kenyense* (Labiateae) on two post harvest insect pest. Phytochemistry 57:385 -391.

Bekele, J.A., D. Obengofori, A.Hassanali and G.H.N Nyamasyo. 1995. Products derived from the leaves of Ocimum Kilimandscharicum as post-harvest grain protectants against the infestation of three major stored product insect pests. Bull. Entomol. Research, 85: 361 -367.

Bergvinson, D.J. 2001. Storage pest resistance in maize.p.32 – 39. In maize programme. Maize research highlights 1999 – 2000. CIMMYT, Mexico D.F, Mexico.

Billy, A.C., L.M.Red, J.H.Taylor, D.Johnston, C.Maouin, B.Bakan,C Regnault-Roger,K.Paul,J.Arnasonand.J.Philogene.2003. Dehydrodimers of ferulic acid in maize pericarp and aleurone: Resistance factors to Fusarium graminearum.Phytopathology, 93: 712 – 719.

Bosque-Perez, N.A. and I.W. Buddenhagen. 1992. The development of host- plant resistance to insect pests: outlook for the tropics. InMenken, S.B.J., et al. (Ed.), Proceedings of the 8th International Symposium. Insect-pest Relationships. Kluwer Publishers, Dordrecht, pp. 235- 239.

Caswell, G.H. 1962. Agricultural Entomology in the Tropics. Edward Arnold, London, pp 40 -76.

Chapman, R.F. 2000. Entomology in the Twentieth Century *Ann. Review Entomology,* vol *45:261 – 285.*

Chapman R.F. 1998. The Insects: Structure and Function. 4th edition. Cambridge University Press.

CIMMYT, 2001. Maize Research Highlights 1999-2000. International Maize and Wheat improvement Center, CIMMYT, Mexico.

Classen, D., J.T.Arnason, J.A.Serratos, J.D.H.Lambert, C.Nozzolillo and B.J. Philogene. 1990. Correlation of phenolic acid content of maize to resistance to *Sitophilus zeamais*, the maize weevil, in CIMMYT's collections. Journal of Chemical Ecology 16, 301-315.

Derera, J., V.Pixley and D.P.Giga. 2001. Resistance of Maize weevil. Antibiosis African Crop Science journal, 9, 431 – 440.

Derera, J., K.V. Pixley and D.P. Giga. 1998. Inheritance of maize weevil resistance in maize hybrids among lines from Southern Africa, Mexico and CIMMYT pp24– 27.

Derera, J., K.V.Pixley and D.P.Giga. 1999. Inheritance of maize weevil resistance in maize hybrids among lines in Southern Africa, Mexico and CIMMYT –production technology for the future: Challenges and opportunities. Proceedings of the South Eastern African Regional Maize Conference, 21 – 25.

Derera, J. 2013. Genetic data handling. University of kwazulu Natal,South Africa.

Dhliwayo, T. and K.V. Pixley. 2003. Divergent selection for resistance to maize weevil in six maize populations. Crop Sci. 43: 2043 – 2049.

Dhuyo, A.R and S. Ahmed. 2007. Evaluation of fungus *Beauveria bassiana* (Bals.) Infectivity to the larger grain borer *Prostephanus truncates* (Horn). Park. Entomolgy 29: 77-82.

Dick, K. 1988. A review of insect infestation of maize in farm storage in Africa with special reference to the ecology and control of *Prostephanus truncatus*. Overseas Development Authority Natural Resources Institute, Bulletin.No.18, pp42.

Dobie, P. 1977. The contribution of the tropical stored products center to the study of insect resistance in stored maize. Tropical stored products information 34: 7 – 22.

Dobie, P. 1974. The laboratory assessment of the inherent susceptibility of maize to post-harvest infestation by *Sitophilus zeamais* Motsch. (Coleoptera, Curculionidae). Journal on Stored products. 10: 183 – 197.

Dobie P., P. Haines, C.P. Hodges and P.F. Prevett. 1984. Insects and Arachnids of Tropical Stored Products, their Biology and Identification (A Training Manual). TDRI. UK.

Eden, W.G. 1952. Effect of kernel characteristics and components of husk cover on rice weevil damage to corn. Journal of Economic Entomology 45, 1084 – 1085.

Falconer, D.S. 1981. Introduction to Quantitative Genetics. Second edition. Longman,New York.

Falconer, D.S. 1989. Introduction to Quantitative Genetics. Third edition. Longman , New York.

FAO. 1993. Maize in Human Nutrition. Agriculture and Consumer Protection. FAO, Rome.

Garcia–Lara, S., D.J .Bergvinson, A.J.Burt, A.I. Ramputh, D.M. Diaz–Pontones and J.T Arnason. 2004. The role of pericarp cell wall components in maize weevil Crop 44:1546 – 1552.

Giga, D.P and U.W.Mazarura. 1991. Levels of resistance to the Maize weevil *Sitophilus zeamais* (Mostch) in exotic, local open pollinated and hybrid Maize Applications 12, 159-169.

Gudrups, I., S.Floyd, J.G.Kling, N.A. Bosque-Perez and J.E.Orchard. 2001. A comparison of two methods of assessment of maize varietal resistance to the maize weevil, *Sitophilus zeamais*, Motschulsky, and the influence of kernel hardness and size on susceptibility. Journal of stored Products Research. 37:287-302.

Gwinner, J., R. Harnisch and O. Muck. 1996. Manual on the prevention of post - harvest seed losses, post-harvest project, GTZ, D-2000, Hamburg, FRG. pp294-295.

Hallauer, A.R. and J.B. Miranda. 1988. Quantitative genetics in maize Breeding Iowa state University Press/ Ames. pp49-52.

Hampl, J.S. and W.S. Hampl. 1997. Pellagra and the origin of a myth: evidence from European literature and folklore. Journal for Royal society, 90: 636 – 639.

Hefyny, M. 2010. Genetic control of flowering traits, yield and it's components in corn inbred lines (*Zea mays* L) at different sowing dates. Asian Journal of crop science. 2: 236 – 249.

IITA. 2013. Weevil damage: a comparison of different maize varieties. International Institute of Tropical Agriculture Annual report and research Highlights, pp 77 –78.

Jansen, D.H. 1977. How Southern cowpea weevil larvae *Callosobruchus maculatus* (Coleoptera: Bruchidae) die on non- host seeds. Ecology 58: 921 – 927.

Kamanula.J., W.Gudeta, S.Mvumi, B.M.Nyirenda, K.C.Greenwell, S.P.Nyirenda and C.P.Stevenson. 2011. Farmers' insect pest management practices and pesticidal plant in the protection of stored maize and beans in Southern Africa. International Journal of Pest Management volume 59.pp 41-49.

Kang M.S, Y.Zhang and R.Magari. 1996. Combining ability for maize weevil preference of grain maize. Crop Science volume 35: 1556 – 1559.

Kiani, G., G.A. Nematzadeh, S.K. Kazemitabar and O. Alishah. 2007. Combining ability in cotton cultivars for agronomic traits. Journal of ecology volume 9: 521–523.

Keba, T. and W.Sori. 2013. Differential resistance of maize varieties to maize weevil (*Sitophilus zeamais* Motschulsky) (Coleoptera: Curculionidae) under laboratory conditions.

Kossou, D.K., J.E. Mareck and N.A. Bosque-Perez. 1993. Comparison of improved and local maize varieties in the Republic of Benin with emphasis on susceptibility to *Sitophilus zeamais* Motschulsky. Journal of Stored Products Research. 29, 333 -343.

Kossou, D.K. and S.K.Kim. 2003. Responses and genetics of maize germplasm resistant to the maize weevil *Sitophilus zeamais* Motschulsky in West Africa. Journal of Stored Products Research 39: 489-505.

Kossou, D.K, N.A. Bosque-Perez and J.H. Mareck. 1992. Effects of shelling maize cobs on the ovipositor and development of *Sitophilus zeamais*, Motschulsky. Journal of Stored Products Research, 28, 187 -192.

Leuschner.K, Monyo E.S , Chinhema. E, Tembo . E and D. Martin. 2000. Pearl Millet Grain Size and Hardness in relation to resistance to Sitophilus Oryzea (L) (Coleoptera : Curculionidae). African Crop Science Journal Volume 8.

Li-Ruming ,M.S Kang , O.J Moreno, L.M. Pollak and R.M.Li. 1998. Genetic variability in exotic X adapted maize (*Zea mays*, L.) germplasm for resistance to maize weevil. Plant Genetic Resources Newsletter volume 114: 22 – 25.

Makate, N. 2010. The susceptibility of different maize varieties to post harvest infestation by *Sitophilus zeamais* (Motsch) (Coleoptera: Cuculionidae). Scientific Essay, 5030-5034.

Makkar, H.P.S. 2003. Quantification of Tannis in tree and shrub foliage, laboratory manual. Kluwer Academic publishers. Vienna, Austria.

Mather, K. and J.L. Jinks. 1971.Biometrical genetics. The study of continous variation . Cornell university press, New York.

Michael, D., McMullen, F. and J.Degenhardt. 2009. Genetics and biochemistry of insect resistance in maize. Springer Science. 10:387-394.

Mugo, S., P. Likhayo, H.Karaya, J.S.Gethi, J.Shuma and T.Tefera. 2005. Screening maize germplasm for resistance to maize weevil *Sitophilus zeamais* (Motsch) and larger grain borer, *Prostephanus truncatus* (Horn). African journal of Biotechnology volume 13:1490 – 1504.

Munjoma, S. 2004. Comparison of maize inbred line per se and test cross performance for maize weevil (*Sitophilus zeamais* Motsch) resistance. MSc Thesis. University of Zimbabwe, Harare, Zimbabwe.

Murata. M and T. Imanura . 2008. Infestation and development of *Sitophilus species*. In pouch – packaged spaghetti in Japan. Journal for Economic Entomology vol 3: 1006 – 1010.

Nigussie, M. and G. Saleh. 2007. Genetic variability and responses to two methods of recurrent selection in two sweet corn (*Zea mays,* L. Saccharata) populations. Asian Journal of Plant Science. 6: 859 - 863.

Organization for Economic Co-operation and Development (OECD). 2003. Consensus document on the biology of *Zea mays* (maize). OECD Environmental and Safety publications. Series on Harmonization of Regulatory Oversight in Biotechnology. No. 27 Environment Directorates. Paris.

Pingali, P.L. and S.Pindey. 2000. Meeting world maize needs: technology opportunities and priorities for the public sector. In P.L. Pingali (ed) CIMMYT 1999 – 2000 world maize facts and trends.

Pixley, K.V. 1997. CIMMYT Mid-altitude maize breeding programme. In: CIMMYT –Zimbabwe Annual Research Report, 1996/97. 7 – 13.

Poehlman, J.M. and D.A.Sleper. 1995. Breeding field crops (4th Ed.) Iowa state University press.

Rashnaw, A.M. 2010. Estimation of some genetic parameters using six populations of two cowpea hybrids. Asian Journal of Crop Science, 2: 261 – 267.

Rugumamu, C.P. 2011. Development and Applications of insect pest management technologies in stored crops: A contribution to integrated pest management. Huria Journal, IX: 17– 32.

Savidan, A. 2002. Tritrophic interactions in maize storage systems. PhD Thesis. University of Neufchatel, Neuchatel, Switzerland.

Sen, A., D.Bergvinson, S.S. Miller, J. Atkinson, G.R. Fulcher and J.T. Arnason. 1994. Distribution and micro-chemical detection of phenolic acids, flavonoids and phenolic acid amides in maize kernels. Journal of Agricultural and Food Chemistry; 42: 1879-1883.

Serratos, A.,J.T. Arnason, C.Nozzolillo, J. Lambert, B.J.Philogene, G.Fulcher, K. Davidson,L. Peacock, J. Atkinson and P. Morand. 1987**.** Factors contributing to resistance of exotic maize populations to maize weevil, *Sitophilus zeamais*. Journal of Ecology, 13, 751 –760.

Serratos, J.A., A.Blanco–Labra, J.A.Mihm, L.Pietrzak and J.T.Arnason. 1993. Generation means analysis of phenolic compounds in maize grain and susceptibility to maize weevil *Sitophilus zeamais* infestation. Canadian journal of botany 71: 1176 – 118.

Serratos, J.A., A. Blanco-Labra, J.T.Arnason and J.A. Mihm. 1997. Genetics of Maize Grain resistance to Maize weevil. pp132 -138. In Mihm J.A. Insect Resistant Maize: Recent advances and utilization. Proceedings of an international symposium. 27 Nov – 3 Dec, 1994.

Singh, D. and F. McCain. 1963. Relationship of some nutritional properties of the corn kernel to weevil infestation. Crop science Journal, 259 – 261.

Singh, D. 2009. Plant breeding: principles and methods. Kalyani publishers. New Delhi.

Siwale, J., K.Mbata, J.Microbert and D.Lungu. 2007. Comparative resistance of improved maize genotype and landraces to maize weevil. African Crop Science Journal. 17: 1 -16.

Tefera, T., S.Mugo and P.Likhayo. 2011. Effects of insect population density and storage time on grain damage and weight loss in maize due to the maize weevil *Sitophilus zeamais* and the larger grain borer *Prostephanus truncatus*. African of Agricultural Research, 6: 2249 – 2254.

Thanda, D. and V.P. Kevin. 2003. Divergent selection for Resistance to maize weevil and six maize populations. Crop science, 43: 2043 – 2049.

Tipping, P.W., D.E.Legg, J.G. Rodriguez and C.G.Poneleit. 1988. Influence of maize pericarp surface relief on resistance to the maize weevil (Coleoptera: Curculionidae). Journal of the Kansas Entomological Society, 61, 237 – 241.

Tongjura,J.D.C.,G.A. Amuga and H.B. Mafuyai. 2010. Laboratory assessment of the susceptibility of some varieties of zeamais infested with *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae).Sci.World J., 5: 55 -57.

Urrelo, R., V.F. Wright, R.B. Mills and E.K. Horber. 1990. An abbreviated procedure to determine the inherent resistance of maize to *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). Journal of Stored Products Research 26: 100.

Van Vleck, L.D., E.J. Pollak and E.A.B.Oltenacu. 1987**.** Genetics for the animal sciences.W.H. Freeman and company. United States of America. pp230-231.

Wannows, A.A., H.K.Azzam and S.A. Al-Ahmed. 2010**.** Genetic variances, heritability, correlation and path coefficient analysis in yellow maize crosses (*Zea mays* L) Agricultural Journal,volume 1: 630-637.

Warnock, D.F., W.D.Hutchison, C.B.Tong and D.W. Davis. 2001**.** Evaluating Maize for Allelochemicals that Affect European corn borer (Lepidoptera: Crambidae) Larval Development. Crop science 41: 1761 – 1771.

Widstrom, N.W., W.D. Hanson and L.M. Redlinger. 1975**.** Inheritance of Maize weevil resistance in maize Crop Science, 15, 467-470.

Widstrom N.W, W.W. Macmillan, L.M. Redlinger and W.J Wiser. 1983. Dent corn inbred sources of resistance to the maize weevil (Coleoptera : Curculionidae). Journal economical Entomology. Volume 76 : 31-33.

Widstrom, N.M. 1989**.** Breeding methodology to increase resistance in maize to corn earworm, Fall Army worm and maize weevil. In: CIMMYT.1989. Towards insect resistant maize for the third world: proceedings of the international symposium on methodologies for developing host plant resistance to maize insects. Mexico, D.F; CIMMYT.

APPENDICES

Appendix 1: Males (Insect Resistant Maize inbred lines)

Entry	Line	Pedigree	Origin
1	CKSPL10075	(CUBA/GUAD C1 F27-4-3-3-B-1-BX [KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-230 -1 –B-3-B-B-B-B-B	CIMMYT
2	CKSPL10096	(CUBA/GUAD C1 F27 -4-3-3-B-1-BX [KILIMA ST94 A] -30/MSV 03-2-10-B-2-B-B) -294-1-B-2-B-B-B	CIMMYT
3	CKSPL10111	(CUBA/GUAD C1 F27 -4-3-3-B-1-BX [KILIMA ST 94A] -30/MSV-03-2-10-B-2-B-B) -306-1-B-1-B-B-B-B-B	CIMMYT
4	CKSPL10112	(CUBA/GUAD C1 F27 -4-3-3-B-1-BX [KILIMA ST 94 A] -30/MSV -03-2-10-B-2-B-B)-306-1-B-2-B-B-B	CIMMYT

5	CKSPL10113	(CUBA/GUAD C1 F27-4-3-3-B-1-BX [KILIMA ST94 A] -30 CIMMYT /MSV -03- 2-10-B-2-B-B)-306-1-B-3-B-B-B-B-B
6	CKSPL10136	(CUBA/GUAD C1 F27-4-3-3-B-1-BX [KILIMA ST 94A] - CIMMYT 30/MSV-03-2 10-B-2-B-B)-386-1-B-B-B-B-B
7	CKSPL10146	(CUBA/GUAD C1 F27-4-3-3-B-1-BX [KILIMA ST 94 A] - CIMMYT 30/MSV-03-210-B-2-B-B) -393-1-B-2-B-B-B-B-B
8	CKSPL10164	(CUBA/GUAD C1 F27-4-3-3-B-1-BX [KILIMA ST94A] - CIMMYT 30/MSV-03-2-10-B-2-B-B)-420-1-B-1-B-B-B-B-B
9	CKSPL10264	(CUBA/GUAD C1 F27-4-3-3-B-1-BX [KILIMA ST94A]- CIMMYT 30/MSV-03-2-10-B-2-B-B)-69-1-B-2-B-B-B

Appendix II: Female inbred lines

Entry	Line	Origin
1	151	ZARI
2	152	ZARI
3	1212	ZARI
4	710	ZARI
5	913	ZARI
6	917	ZARI
7	3234	ZARI
8	5522	ZARI

ZARI = Zambia Agriculture Research Institute

Appendix III: Crosses evaluated for weevil resistance in the study

No	**Entry**	**Cross**
1	1N	151 x 10075
2	67N	151 x 10096
3	74U	151 x 10111
4	60N	151 x 10112
5	74N	152 x 10075
6	56N	152 x 10096
7	63N	152 x 10111
8	38N	152 x 10112
9	24N	1212 x 10075
10	12N	1212 x 10096
11	77U	1212 x 10111
12	91U	1212 x 10112

13	6N	917 x 10075
14	80U	917 x 10096
15	4N	917 x 10111
16	80N	917 x 10112
17	31N	152 X 10146
18	78N	152 X 10164
19	95U	1212 X 10146
20	19N	1212 X 10164
21	73U	913 X 10146
22	8N	913 X 10164
23	87N	5522 X 10164
24	46U	5522 X 10164
25	13N	MRI534*
26	45U	MRI624*
27	26N	DKC 8033*

Key

*= selfed

Appendix IV: ANOVA for F_1 progeny emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	7.6296	3.8148	6.68	
REP.*Units* stratum					
Entry	26	28102.8889	1080.8803	1892.21	<.001
Residual	52	29.7037	0.5712		
Total	80	28140.2222			

Appendix V: ANOVA for moisture content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.5022	0.7511	3.52	
REP.*Units* stratum					
Entry	26	30.3422	1.1670	5.46	<.001
Residual	52	11.1111	0.2137		
Total	80	42.9556			

Appendix VI: ANOVA for Median Development Period

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	3.4321	1.7160	2.10	
REP.*Units* stratum					
Entry	26	20960.0247	806.1548	984.78	<.001
Residual	52	42.5679	0.8186		
Total	80	21006.0247			

Appendix VII: ANOVA for grain weight loss percent

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.12543	0.06272	1.69	
REP.*Units* stratum					
Entry	26	661.16840	25.42955	685.89	<.001
Residual	52	1.92790	0.03708		
Total	80	663.22173			

Appendix VIII: ANOVA for Grain Hardness percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.259	0.129	0.11	
REP.*Units* stratum					
Entry	26	1141.573	43.907	38.60	<.001
Residual	52	59.155	1.138		
Total	80	1200.986			

Appendix IX: ANOVA for Kernel weight

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.00009630	0.00004815	0.59	
REP.*Units* stratum					
Entry	26	0.15746667	0.00605641	74.33	<.001
Residual	52	0.00423704	0.00008148		
Total	80	0.16180000			

Appendix X: ANOVA for Mortality percentage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	6.067	3.033	0.43	
REP.*Units* stratum					
Entry	26	35942.522	1382.405	194.51	<.001
Residual	52	369.573	7.107		
Total	80	36318.162			

Appendix XI: ANOVA for parental survival

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.9630	0.4815	0.56	
REP.*Units* stratum					
Entry	26	5512.6667	212.0256	248.48	<.001
Residual	52	44.3704	0.8533		
Total	80	5558.0000			

Appendix XII: ANOVA for Phenolic content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	34.50	17.25	0.75	
REP.*Units* stratum					
Entry	26	171521.42	6596.98	287.40	<.001
Residual	52	1193.61	22.95		
Total	80	172749.52			

Appendix XIII: ANOVA for protein content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.17821	0.08910	1.28	
REP.*Units* stratum					
Entry	26	164.41969	6.32383	90.67	<.001
Residual	52	3.62679	0.06975		
Total	80	168.22469			

Appendix XIV: ANOVA for susceptibility index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	0.14363	0.07182	2.53	
REP.*Units* stratum					
Entry	26	233.60083	8.98465	316.44	<.001
Residual	52	1.47643	0.02839		
Total	80	235.22089			