Comparative Resistance of Maize Populations to the Maize Weevil, *Sitophilus zeamais* Motschulsky.

by

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A dissertation submitted in Partial Fulfilment of the requirements for the degree of Master of Science in Agronomy (Plant Breeding) of the University of Zambia.

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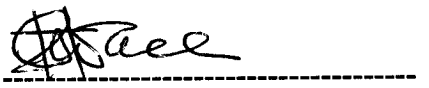
2007

DECLARATION

I, **Julius Siwale**, hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or any other University.



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APPROVAL

This dissertation of **Siwale Julius** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy (Plant Breeding) by the University of Zambia.

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DEDICATION

I dedicate this dissertation to my parents who have always encouraged me in my academic endeavours and to my wife, Roster and children Wankumbu, Lenganji and Salifyanji, who have endured my long absence from home during my study.

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ABSTRACT

Storage losses present a major threat to food security among the small-holder farmers in Africa. The Maize weevil, *Sitophilus zeamais* Motschulsky, is one of the most important storage pest for which breeding for resistance is the best option for reducing storage losses. A study was conducted to identify maize cob and grain characteristics conferring resistance to the maize weevil in selected maize genotypes. Field and laboratory experiments were conducted using 52 maize genotypes of varying resistance to the pest. Field experiments looked at husk cover length and husk cover rating, while laboratory experiments focused on grain hardness, protein content, cob and grain weight loss and Dobie's susceptibility indices. Results from the field showed that there was no initial maize weevil infestation from the field on the cobs harvested. Genotypes were significantly different for husk length and husk cover rating scores ($p<0.001$), with landraces having longer husks (mean, 88 mm) and better husk cover score ratings (mean, 1.8) than hybrids (mean, 35 mm and 3.0, for husk length and cover rating scores, respectively) and open pollinated varieties (OPVs) (mean 48 mm cover length and score 2.1). Genotypes were significantly different ($p<0.05$) for grain hardness. Grain protein content was not significantly different among the genotypes ($p>0.05$). There was no correlation between protein content and grain hardness across genotypes ($r=0.14$ among all genotypes; $r=0.20$ among hybrids; and $r=0.26$ among OPVs). However, there was significant correlation between protein content and grain hardness for resistant OPVs ($r=0.82$) only. Husk parameters measured did not discriminate the tested genotypes for weevil resistance because there was no initial infestation from the field, while the grain

protein content tended to be related to hardness, which could be used as a proxy for resistance to the maize weevil. Genotypic differences in grain weight loss due to feeding by the larvae and adults of the maize weevil were highly significant. The genotypes also differed significantly in the Dobie's index of susceptibility. The two landraces used in the study did not show any superiority in resistance over the hybrids or OPVs. Similarly, OPVs were not necessarily superior to hybrids according to the Dobie's index of susceptibility. Out of the best nineteen genotypes according to Dobie's index of susceptibility fifteen were hybrids. It was therefore concluded that it is possible to develop hybrids or OPVs that are as resistant as or even better than some landraces. Since only two landraces were included in this study, and these were from the same district, it is recommended that a larger number of land races and from different parts of Zambia be screened for resistance to the maize weevil. The landraces with superior resistance could then be used in crosses to develop hybrids and OPVs with increased resistance to the maize weevil. An attempt at analysing for ferulic acid content in the maize grain failed due to mechanical faults on the only available Gas chromatography/mass spectropy machine in the chemistry department at the University of Zambia. The analysis of ferulic acid could have provided further information on the mechanisms of resistance in the maize materials studied. It is necessary that in future Zambian parental maize lines and landraces get characterized for ferulic acid content.

CHAPTER 1: INTRODUCTION

1.1 Background

Maize, *Zea mays* L., is the principal staple food crop in Zambia and other countries in Southern Africa. Annual consumption of maize per capita for Zambia is estimated at 113 kilogrammes (OECD, 2003). Maize production is, however, characterised by low yields caused by biotic and abiotic stresses as well as poor crop management (Jewell, 1997). In addition, to low yields, there is further loss of the harvested maize crop during storage. There are many storage insect pests, one of which is the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Weight loss on maize grain arising from feeding by pests varies, depending on crop variety, storage conditions and duration, and on pest combination in storage. Studies conducted in Zambia gave weight losses from insect pests at a small-holder storage level, a range of 1 to 9 percent depending on the variety. The main focus of improvement in maize breeding programmes has traditionally been concerned with increased yield and resistance to field pests and diseases. Since varieties are rarely assessed for resistance to stored-product pests, the introduction of improved varieties has in the past often been accompanied by reports of increased susceptibility to stored-product pests (Gudrups et al., 2001).

Losses in local maize landrace varieties are reported to be lower (1 to 3 percent) than in most improved varieties (5 to 6 percent) and in hybrids (8 to 9 percent) and this is attributed to the local varieties' resistance to insect attack, because of their hard kernels and their complete and better husk coverage of the cobs, which protect the grain in the field, during drying and in storage considerably (Mejía, 2005).

Maize weevil control in maize has been through use of various measures, the most common one being chemical applications. The use of chemicals though effective, comes with some concerns. In

the first instance chemicals are expensive for the generally resource poor farmers in Zambia. In addition, the safety concerns in handling chemicals and residue on the grain along with the associated environmental pollution make this control option less attractive.

Resistance to the maize weevil damage as would be conferred by the genetics of maize on the other hand provides a better option under the small scale farmer setting. However, developing maize varieties with weevil resistance requires that grain and/or cob characteristics that confer resistance are identified. Development of weevil resistant varieties is important in ensuring food security for small scale farmers, while contributing to environmental friendly agricultural production.

Some work has been done in recent years on maize weevil resistance and this has focused on the grain. However, a lot of local farmers store their maize on intact cob in the husk. The weevil infestation starts in the field, especially when harvesting is delayed, which is quite common (Tigar et al., 1993; Pendleton et al., 2005). "Stooking" of maize, a common practice in Africa that involves putting stalks of mature maize in standing conical heaps prior to harvesting, encourages field infestation of the crop by the maize weevil (Tigar et al., 1993). It is therefore also important to determine resistance of the maize grain on the cob.

Painter (1968) described three-fold mechanisms of grain resistance to weevils, namely; antibiosis, non-preference and tolerance. Antibiosis resistance is the ability of the host to injure the pest, reduce its reproduction potential , retard its development rate and /or kill the pest (Dent, 1991) as cited by Derera et al., (2001). Non-preference, according to Painter (1968) "denotes a group of plant characters and insect responses that lead insects away from the use of a particular plant or variety, for oviposition, for food, or for shelter, or for combinations of the three". Tolerance is defined by Painter (1968) as "a basis of resistance in which a plant shows an ability to grow and reproduce itself or to repair injury to a marked degree in spite of supporting an insect pest

population approximately equal to that damaging a susceptible host". Tolerance as a resistance mechanism, does not apply to stored products because for a stored product any damage by a pest is terminal (Kang et al., 1995).

A larger number of modern high-yielding maize varieties coming from the breeding programmes have proved to be more susceptible to infestation by storage pests, of which the maize weevil, *Sitophilus zeamais,* is one of them. This makes farmers lose a lot of their maize or, makes them sell their maize in a hurry when prices are still very low (Tigar et al., 1993).

In selecting for weevil resistance in maize, it is necessary to identify the particular traits that confer this resistance on the crop.

1.2 Objectives

The overall objective of this study was to determine the comparative resistance of 52 selected maize genotypes, to the maize weevil *Sitophilus zeamais.*

The specific objectives were to:

(i) characterize grain traits that confer weevil resistance in selected maize populations, and

(ii) compare storage forms of maize for protection against maize weevil damage.

CHAPTER 2.0: LITERATURE REVIEW

2.1 Overview of the Maize Crop

2.1.1 Importance and origin of the Maize Crop.

Maize is the principal staple food crop for the people of Zambia, and it is also widely consumed in other countries of the sub-Saharan Africa region (Diop et al., 1996; OECD, 2003). Diop et al., 1996, reported that in Zambia, maize is also used for brewing beer and making stock feeds. The picture is the same in many other developing countries: in Latin America, Mexico, for instance, one of the main uses of maize is for food (OECD, 2003). Also, maize production in Africa is similar to the production of the crop in some Latin American countries because the peasants of less developed rural areas in the countries of both regions grow maize in small plots, using negligible amounts of inputs or technology and use no improved varieties (OECD, 2003).

Maize originated in the Americas (Hallauer and Miranda, 1988; Poehlman and Sleper, 1995; OECD, 2003). There are many speculations as to how maize evolved, but it is now generally accepted that maize originated from teosinte (*Zea mexicana* L.), the closest known wild relative of maize (Hallauer and Miranda, 1988; Poehlman and Sleper, 1995; Aylor et al., 2005). There are two types of teosinte. The basal branching teosinte sub-species is called *Zea mays parviglumis* (L.) Iltis and Doebley (OECD, 2003). The lateral branching sub-species is named *Zea mays mexicana* (Schrader) Iltis (Poehlman and Sleper, 1995; OECD, 2003). Mexico and Guatemala are the native countries of teosinte. This plant grows wild in cultivated maize fields in its natural habitat. It is similar to maize by having a monoecious flowering habit; it has the same number of chromosomes and crosses readily with maize. The difference from maize lies in the number of kernels. It has 6 to

12 kernels contained in hard triangular, shell-like structure borne in pistillate spikes (Poehlman and Sleper, 1995).

2.1.2 Structure and types of the Maize Kernel in relation to Maize Weevil Resistance.

Seeds of teosinte are enclosed in a fruit case. This protects the kernels from infestation by weevils (Savidan, 2002). The kernel of modern maize comprises three main components; the pericarp, the endosperm and the germ. It lacks the fruit case, which its closest relative, the teosinte possesses (Mangelsdorf, 1974; Diop et al., 1996; Savidan, 2002).

There are broadly two types of maize kernels in terms of kernel texture, namely; The flint type which has higher proportions of hard endosperm, and the dent type which, on the other hand, has higher proportions of the soft endosperm. Generally the dent type tends to be more susceptible to insect attack on account of its softness than the flint type (Diop et al, 1996; Kim and Kossou, 2003)

As a result of lack of the fruit case, maize is susceptible to attack by the maize weevil and other stored maize insect pests. Savidan (2002) tested the susceptibility of teosinte to the maize weevil. She did this by leaving the teosinte fruit case intact in one treatment and removing it in the other. The result was that, the treatment without the casing had seeds attacked by weevils, while the seeds in that treatment with the fruit casing, were not attacked by the weevils.

2.2 Biology and Ecology of the Maize Weevil, *Sitophilus zeamais* Motschulsky

The maize weevil belongs to the insect beetle family, Curculionidae (the true weevils). A total of 60,000 species has been described world wide of which approximately 30 species are pests of stored products, three of which are *Sitophilus* spp. and are of extreme importance. The fourth

stored product pest species in this family, but of less importance than the *Sitophilus* spp group, is *Caulophilus latinasus* (Say), the broad-nosed weevil (Semple *et al.,* 1992).

Adult weevils may be distinguished from all other stored products insect pest species by having the head protruding in front of the eyes to form a well defined snout, the antennae are geniculate (elbowed) and clubbed, and all tarsi are 4-segmented. The larvae are apodous scarabaeiform, stout and slightly curved, and are creamy white with a pale brown or yellowish head.

Three species of Sitophilus are important pests of whole cereals; however, they are of little significance as pests of milled cereals because the larvae require a hard substrate in which to develop. As primary pests, these weevils are of additional importance in that their entry into whole grains provides access for secondary stored products insect pests such as the flour beetle, *Tribolium spp.*

The S*itophilus spp* include:

(i) *Sitophilus granarius* (L.), the granary weevil.

(ii) *Sitophilus oryzae* (L.), the rice weevil.

(iii) *Sitophilus zeamais* Motschulsky, the maize weevil.

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2.2.1 Description of the maize weevil

The maize weevil, *Sitophilus zeamais* is small, 3 mm long (range 2,5 to 4.5 mm) insect that is somewhat larger than the rice weevil, *S. oryzae* (L.). It is often confused with the rice weevil, *S. oryzae* (L.) and is sometimes referred to as *S. oryzae* (L.) 'large strain' by some people. Although generally darker than the rice weevil, the variation that exists within each species makes separation on the basis of external characters alone rather difficult and inaccurate. According to Coombs and Porter (1986), these difficulties in the nomenclature make it often impossible for one to be sure

whether it is *Sitophilus oryzae* (L.) or *Sitophilus zeamais* that is being referred to in some records of infestation before 1959. According to these authors records of the species should be more accurate after a publication by Floyd and Newsom in 1959 and more so by Kuschel in 1961 who provided characters to enable the separation of the species with greater certainty and in 1964 Halstead compared the variability of the various characters (Coombs and Porter, 1986).

The *Sitophilus zeamais* may be distinguished from *S. oryzae* (L.) by examining the curve of the aedeagus (the copulatory organ of a male insect [Kim and Sota, 2006]). In *S. oryzae* (L.) the curve of the aedeagus is more or less uniform at the tip whereas in *Sitophilus zeamais* the tip of the aedeagus is distinctly hooked (Proctor, 1971).

The *Sitophilus zeamais* is an active flier resulting in many field infestations in areas adjacent to infested stores. Sexing is done using their rostral characteristics (Halstead, 1963) or by examination of genitalia (Throne and Eubanks, 2002). In the male the rostrum is distinctly shorter and wider than that of the female, and it is rough; in the female the rostrum is distinctly longer and narrower than that in the male, and it is smooth and shining (Halstead, 1963).

2.2.2 The Life Cycle of *Sitophilus zeamais*

The female bores a hole in a grain with her mandibles, lays her egg at the bottom of the hole, which is then sealed with a gelatinous plug (Diop *et al.,* 1996; Semple, 1992. After the larva has hatched it undergoes four moults, before pupating within a single grain. The life cycle is completed in 37 days at 25°C and 110 days at 18°C (at 70% R.H.) (Diop et al., 1996). A female lays an average 200 eggs during its life cycle of 5 to 9 months (Diop et al., 1996).

2.2.3 Feeding habits and host range of *Sitophilus zeamais*

When the larva hatches it feeds within the grain until it pupates. Upon development into an adult it still feeds inside the grain until it emerges, leaving a round emergence hole in the grain. The adult continues to feed voraciously (Semple, 1992; Diop et al., 1996).

Sitophilus zeamais attacks a wide range of cereals although it is particularly a pest of maize. Thus it also breeds in rice and wheat (Semple, 1992).

2.2.4 Ecology and Pest Status of *Sitophilus zeamais*

Sitophilus zeamais occurs throughout the warm humid areas of the world (Okelana and Osuji, 1985). The ideal temperature for its development is about 28°C and relative humidity of 70 %. These conditions are ideal for oviposition, fecundity and kernel damage for all maize cultivars (Okelana and Osuji, 1985). Ovipostition is inhibited in grain with less than 12.5% moisture content.

At the global level, the maize weevil is probably the most serious stored maize pest. In Zambia this was the most important and most common insect pest of stored maize before the coming of the Larger Grain Borer, *Prostephanus truncatus* (Horn) in the country in 1993 (Diop et al., 1996).

2.2.5 Factors Affecting Maize Infestation by Weevils

Some of the factors affecting maize infestation by the weevil are storage method (form), that is, whether stored as loose grain, on cob without husks or on cob with husks (Savidan, 2002); moisture content of the grain, and environmental factors such as temperature and relative humidity. Choosing the right storage method is therefore one strategy of integrated pest management (IPM). Husk cover protection is however limited to initial infestation (Savidan, 2002).

2.3 Effects of Damage by the Weevil on the Maize Grain

There are two types of damage that weevils inflict on the maize grain namely, direct and indirect damage

2.3.1. Direct damage

Maize weevils cause direct damage in stored maize when their feeding results in a reduction in weight or seed viability of the grain.

2.3.1.1. Reduction or loss in weight

The direct feeding of insect pests on stored grains results in food and weight losses. Weevils, which feed mainly on the carbohydrate portion of maize, remove a considerable amount of the calorie potential but little of the protein and vitamins, which are mainly in the germ and bran.

2.3.1. 2 Reduction or loss of seed viability

A seed grain whose germ has been attacked will not germinate. This may reduce future maize production for farmers who use saved grain as seed, a common practice in eastern and southern Africa (Dhiliwayo and Pixley, 2003; Semple, 1992).

2.3.2. Indirect damage

Maize weevils cause different kinds of indirect damage due to their presence and feeding action in stored maize.

2.3.2.1. Quality loss

The loss in the quality of maize caused by maize weevils and other stored-product insect pests can take many forms, and the important ones are described below.

(i) Nutrient loss. When grains are attacked by insect species which feed selectively on the germ leaving the endosperm almost untouched, food loss is not apparent; weight loss is also small compared to loss of vitamins, proteins, etc.

(ii) Heating and spoilage. Heating from insect infestation (caused mainly by the immature stages) accelerates further infection by microflora and bacteria. This results in spoilage of grain, which often causes more serious economic damage than loss in weight by insect consumption.

(iii) Contamination, tainting or discoloration. Contamination and tainting of foodstuffs with frass materials such as insect fragments, excreta, secretions, webbings and dusts also contributes

to deterioration in quality. Insect secretions or entomoxins, such as quinone, may also prove to be serious contaminants.

(iv) Production of off-flavours and odours. Cereal, after prolonged storage, but particularly when ground into a meal or flour or when infested by insects, show an increase in free-fatty acid content. If, prior to milling, the maize has been attacked by adults of the flour beetle, *Tribolium castaneum*, the initial free-fatty acid content of the resultant meal is much higher than that of uninfested meal, and the free-fatty acid content rises to a much higher level.

(v) Predisposition to disease. Since maize weevil infestation of maize starts in the field (Kang, et al., 1995; Asawalam and Hassanali, 2006) the damaged cobs are predisposed to kernel infection by disease pathogens notably the fungus, *Aspergillus favus* link:Fr. the causal organism of aflatoxin contamination in maize (Li *et al.*, 2004).

2.3.2.2. Monetary loss

Financial losses are not simply in terms of that lost in reduction in weight, but also the downgrading or absolute rejection of the maize grain due to the presence of live insects or signs of their activity. The cost of any applied control measures, as well as all the agricultural inputs invested in growing the grain, become important "hidden" costs, if grain is consumed or destroyed by insects before reaching the consumer.

2.4 Mechanisms of Resistance to the Maize Weevil

Resistance mechanisms of maize genotypes to the maize weevil have been investigated by many researchers and over a long time period. They have been found to include grain hardness, grain size

and biochemical compounds. (Arnason et al., 1994; Arnason et al., 1997; Derera et al., 2001; Dhliwayo and Pixley, 2003; Giga et al., 1999).

2.5 Methods of 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 hardness

This criterion is based on the premise that when the pericarp or endosperm is hard the female weevil will find it difficult to puncture a hole to oviposit. The hatched larva will also find it difficult to feed on the hard endosperm resulting in a retarded rate of development and hence reduced rate of weevil multiplication in the grain lot.

2.5.2 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, Derera et al, 2001). However, Adams, (1976) found frass weight to be of no use in estimating weight loss.

2.5.3 Biochemical Compounds

Biochemical compounds function through mechanical resistance and antibiosis (Arnason et al., 1994; Kang et al., 1995; Arnason et al., 1997; Derera et al., 2001; Dhliwayo and Pixley, 2003; Garcia-Lara et al., 2004;). Maize genotypes with higher levels of protein content tend to be more resistant to the maize weevil than those with lower levels of protein. Similarly, maize varieties that

are rich in phenolic compounds, particularly hydroxycinnamic acids are reported to be more resistant to this maize storage beetle than those varieties that are poor in these biochemicals. This tendency has been observed not only in the maize weevil but also in other stored products beetles such as the larger grain borer, and neither is it restricted to maize only but to other crops as well, for instance sorghum, rice, bulrush millet, and others. Among the hydroxycinnamic acids, *E*-ferulic acid (Figure 1) is the most abundant in the maize grain, constituting 90 percent, seconded by *p*-coumaric acid which is about 10 % of all the phenolic acids in the maize kernel (Classen et al., 1990 as cited by Serratos, et al., 1993),

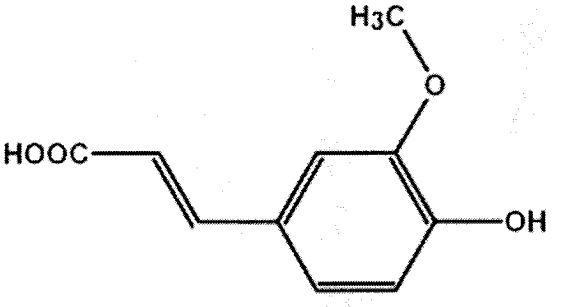

Figure 1: The structure of Ferulic acid
(Source: Phytochemicals. 2007).

Ferulic acid makes the kernel harder through the formation of cross linkages with carbohydrates in the pericarp tissue. It is also an antifeedant. It is found more in the cell walls of the pericarp and the aleurone layer. The endosperm is very poor in ferulic acid content. 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 acid also protects maize against Fusarium ear rot, a fungal disease caused by *Fusarium graminearum* (Schwabe) (Bily et al., 2003). Ferulic acid has health properties as well. It is reported to help in the reduction of risks to various disease conditions such as heart disease and many cancers in man (Phytochemicals, 2007). The chemical name of

ferulic acid is 3-(4-Hydroxy-3-methoxyphenyl)-2-propenoic acid and the chemical formula is $C_{10}H_{10}O_4$ (Martens, 2002; Phytochemicals, 2007). The structure of ferulic acid is given in Figure 1.

2.5.4 Grain size

Many researchers have found that larger grained maize genotypes tend to be more resistant to weevil attack than smaller-sized ones. This is not only true for the maize weevil in maize but for the rice weevil S*itophilus oryzea* (L.) in rice and in other cereals as well, for instance in millet (Leuschner et al., 2000; Gudrups et al., 2001). It is only Widstrom (1989) who reported that grain size was not an important factor.

2.5.5 Susceptibility indices

Susceptibility indices are used as additional measures of the susceptibility of maize varieties to infestation by *S. zeamais*. The higher the index, the greater the susceptibility of the maize to the weevil. Two Susceptibility indices have been developed. These authors, in their screening of 52 maize varieties, (Gudrups et al., (2001) compared Dobie's and Urrelo's Susceptibility indices and found that the two indices gave similar results.

(i) Dobie's Susceptibility Index

Dobie developed this method in 1974 and it has been extensively used since then (Dobie, 1977; Kossou et al., 1993; Derera et al., 2001; Gudrups et al., 2001; Dhliwayo and Pixley, 2003).

The index is given by the formula:

I = 100 log e (no of adult weevil progeny emerged)/MDP.

Where:

I = Dobie's Susceptibility Index

MDP = Median Development period, and this is the period (days) from the middle of the oviposition period to the middle of the emergence of the F1 progeny.

Log e (sometimes written as log n) = the natural logarithm.

(ii) Urrelo's Susceptibility Index

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

I = Ln E x 100/DFE

Where:

E = the total number of egg plugs on the grain, and

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

Dobie's index is the most widely used of the two indices. It is preferred because of the lower total time required for assessment of relative susceptibility of maize varieties. The biggest disadvantage of the Urrelo method lies in 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 F1 adult (Gudrups *et al.*, 2001).

2.6 Methods (Forms) of Presentation

In maize weevil resistance studies of maize, investigators have used two methods or forms of presentation of the maize samples to the weevils, namely the free-choice and the no-choice feeding methods. In the free-choice method, different genotypes of maize are put in containers such that the

weevils, placed in some central place, are able to enter and leave as they wish. A susceptible genotype will attract and retain more weevils than a resistant one. Under the no-choice feeding method, maize samples of different genotypes are placed in containers such as jars, and a predetermined number of weevils is introduced in each container. More eggs will be laid and generally more F1 weevil progeny will emerge from a susceptible genotype than from a resistant one. Kang et al., (1995) reported that the free-choice feeding method was useful in the screening of nursery materials for non-preference and is the first step to obtain no-choice resistant hybrids.

2.7 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 et al., (1978) cited by Horber (1989) and 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. Based on their test results they found and recommended that 20 unsexed adult weevils were needed to infest test samples containing at least 1 g of seed / weevil.

2.8 Maize materials

Maize samples used in testing for maize weevil resistance should normally come from the same location and grown in the same season. The samples should not be treated with chemicals. Field infestation (weevils or any other pest) is normally killed by freezing the samples at $0^{o}C$ or below, or by fumigating with phostoxin. Tablets of phostoxin are placed in paper envelopes to avoid contamination with residues. Phostoxin has been reported to have no effect on the weevils infested (or introduced to) on the previously fumigated maize (Dobie, 1974). Prior to infestation, the samples are conditioned for 3 weeks or more in order to have uniform temperature and moisture content.

2.9 Weevils

The weevils used for infestation of maize samples should be of known age. They should be young adults of 0 to about 21 days. Dobie (1974) found that fecundity of weevils rose sharply from week 1 reaching a peak at week 3, and then declining steadily up to almost zero at week 12. Prior to infestation the weevils are conditioned on the same genotype for at least 5 days (Savidan, 2002).

2.10 Common Methods of Storing Maize in Zambia

Farmers store their maize in three forms, namely; shelled grain, maize grain on cobs with husks removed and maize grain on cobs with intact husks (Diop et al., 1996). Maize grain on the cob is commonly stored in cribs. Therefore in studying maize weevil resistance all the three forms of maize storage have been used by various scientists (Derera et al., 2001; Dhliwayo and Pixley, 2003; Gudrups et al., 2001; Kossou et al., 1993; Savidan, 2002).

2.11 Methods of Maize Weevil Control

Weevils are commonly controlled using chemicals. Other methods involve use of various plant materials and good sanitation. Use of resistant cultivars was proposed many years ago when scientists found that different cultivars responded differentially to maize weevil infestation. Dhliwayo and Pixley (2003) suggested that previously breeders would not attempt to breed for resistance because they were not sure that it would work. However, from their study involving two synthetic populations and four bi-parental populations, these authors concluded that it is possible to improve maize populations for resistance to the maize weevil.

2.12 The Need for Resistant Varieties of Maize to the Maize Weevil

Resistant maize varieties are an important component in the integrated pest management strategy of maize against the maize weevil (Dhliwayo and Pixley, 2002; Savidan, 2002). In his study on "The Fate and Efficacy of Spinosad for Insect Management in Farm Stored Corn", Szabela (2005) found that "Progeny production for all treatments was 12 to 24 times greater for maize weevil than for the other three insect species" (the lesser grain borer (*Rhyzopertha dominica* (Fabricius)), red flour beetle (*Tribolium castaneum* (Herbst)) and the Indian meal moth (*Plodia interpunctella* (Hubner)) . This was consistent with its life cycle of larvae emerging as adults from the inside of corn kernels before coming in contact with externally applied insecticides. Resistant maize varieties are able to reduce the rate of development of the larvae into adults due to hardness of the endosperm or to biochemical antifeedants or a combination of these, and hence reduce the multiplication rate of the weevils (Szabela, 2005).

Issues of insecticide resistance and environmental concerns also make the use of resistant varieties a necessity (Dhliwayo and Pixley, 2003; Throne et al., 2003).

2.13 Inheritance of Maize Weevil Resistance

In order for the resistance mechanisms to be useful to a breeder they must be heritable. Studies have been conducted on this subject and the mechanisms involved have been found to be heritable (Derera et al., 1999). Studies utilising the Diallel Design or the North Carolina design II, using either free- or no- choice tests have found significant additive, non-additive, and maternal effects, with additive being more important than non-additive. Cytoplasmic effects were not important (Dhliwayo and Pixley, 2003).

2.14 Breeding schemes

Kim et al., (1988) cited by Kim and Kossou (2003) reported that the conventional recurrent selection breeding scheme is the most widely used for developing maize genotypes resistant to the maize weevil.

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

Maize

Thirty five (35) F_1 hybrids and 15 Open Pollinated Varieties (OPVs) of maize from the International Centre for Maize and Wheat Improvement (CIMMYT) in Harare, Zimbabwe, and two local varieties (landraces) of maize from Mbala in Zambia were used in this study (Table 1). The two landraces used are locally called Chimambwe and Pandawe. The description of these landraces is given in Table 2.

Table 1. Maize materials used in the study

Entry	Stock ID	Name	Pedigree	Origin	Comment
1	A1228-20		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-415-1-B-1//CML395/CML444	CIMMYT	Hybrid (Resistant)
2	A1228-16		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-354-1-B-1//CML395/CML444	CIMMYT	Hybrid (Resistant)
3	A1235-32		[WEEVIL/CML444]-B-5-1-3-BB/[WEEVIL/CML312]-B-18-3-1-B	CIMMYT	Hybrid (Resistant)
4	A1228-28		[KILIMA ST94A]-19/MSV-03-4-05-B-1-BB-2-2-1-2-B-1//CML395/CML444	CIMMYT	Hybrid Resistant
5	A1228-9		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-160-1-B-1//CML395/CML444	CIMMYT	Hybrid Resistant
6	A1228-19		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-411-1-B-1//MCL395/CML444	CIMMYT	Hybrid Resistant
7	A1227-14		[CML312/CML444//[DTP2WC4H255-1-2-2-BB/LATA-F2-138-1-3-1-B]-1-3-2-3-B]-2-1-3-B//MCL395/CML444	CIMMYT	Hybrid Resistant
8	A1228-29		[KILIMA ST94A]-19/MSV-03-4-05-B-1-BB-2-2-1-2-B-2//MCL395/CML444	CIMMYT	Hybrid Resistant
9	A1228-7		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-127-1-B-1//MCL395/CML444	CIMMYT	Hybrid Resistant
10	A1228-17		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-394-1-B-2//MCL395/CML444	CIMMYT	Hybrid Resistant
11	A1228-2		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-47-1-B-1//MCL395/CML444	CIMMYT	Hybrid Resistant
12	A1228-15		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-342-1-B-1//MCL395/CML444	CIMMYT	Hybrid Resistant

13	A1228-8		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-127-1-B-2//MCL395/CML444	CIMMYT	Hybrid Resistant
14	A1228-12		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-206-1-B-1//MCL395/CML444	CIMMYT	Hybrid Resistant
15	A1228-18		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-393-1-B-1//MCL395/CML444	CIMMYT	Hybrid Resistant
16	A1228-24		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-445-1-B-1//MCL395/CML444	CIMMYT	Hybrid Resistant
17	A1228-23		(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-442-1-B-1//MCL395/CML444	CIMMYT	Hybrid Resistant
18	A1229-7		02SADVE2B-#-45-2//CML312/CML442	CIMMYT	Hybrid Susceptible
19	V381-4	VH052526	CML395/CML444//[P30/P45//M162W/MSR]97-323-3-1-5-B-1-#-1-B	CIMMYT	Hybrid Susceptible
20	V381-4	VH051584	[89[G27/TEWTSRPool]#-278-2-X-B/[COMPE2/P43SR//COMPE2]F#-20-1-1]-B-32-2-B-4-#-B// CML395/CML444	CIMMYT	Hybrid Susceptible
21	A1011-17		CML443/CML445//CML488	CIMMYT	Hybrid Susceptible
22	V381-6/1	VH052530	CML312/CML442//[Ent2:92SEW1-EarlySel-2/[DMRESR-W]EarlySel-#I-3-2-B/CML390]-B-26-1-B-1-#-1-B	CIMMYT	Hybrid Susceptible
23	V298-25	VH052534	CML312/CML442//NIP25-230-2-1-B-1-B	CIMMYT	Hybrid Susceptible
24	V317-11	VH053014	CML312/CML442//ZM301c1F2-72-#-1-3-B	CIMMYT	Hybrid Susceptible
25	V319-38	VH05615	CML181dent/CML182//GQL5	CIMMYT	Hybrid Susceptible
26	V381-2	VH051960	CML312/CML442//P100C6-61-1-4-##1-3-1-BBB	CIMMYT	Hybrid Susceptible
27	A1123-4		CML489/CML444//ZM621A-10-1-1-1-2-BBBBB	CIMMYT	Hybrid Susceptible
28	V381-7	VH052531	CML312/CML442//[Ent320:92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML386]-B-22-1-B-2-#-1-B	CIMMYT	Hybrid Susceptible
29	A660-20		CML440/CML444//CML445	CIMMYT	Hybrid Susceptible
30	A1118-1		CML488/CML444//CML312/CML442	CIMMYT	Hybrid Susceptible
31	J15-9		CML312/CML444//CML489	CIMMYT	Hybrid Susceptible
32	A1238-16		CML444/CML197//CML488	CIMMYT	Hybrid Susceptible
33	A923-7		CML443/CML445//[CML444/ZSR923S4BULK-2-2-X-X-X-X-1-BB]-1-1-1-1-1-B	CIMMYT	Hybrid Susceptible
34	A923-8		CML443/CML445//[EARLY/ZM621A]-14-1-1-2-2-B	CIMMYT	Hybrid Susceptible
35	A1229-6		02SADVE2B-#-45-1//CML312/CML442	CIMMYT	Hybrid Susceptible
36	J11-12		SYN[SZ/Elite01]	CIMMYT	OPV Resistant
37	A1142		SYN[WEEVIL-A(FSINDEX)]	CIMMYT	OPV Resistant
38	A1143		SYN[WEEVIL-A(FSWEEVRESIST)]	CIMMYT	OPV Resistant
39	A1145		SYN[WEEVIL-B(FSINDEX)]	CIMMYT	OPV Resistant
40	A1146		SYN[WEEVIL-B(FSWEEVRESIST)]	CIMMYT	OPV Resistant
41	J11-42		04WEEVILA-#	CIMMYT	OPV Resistant
42	J11-44		04WEEVILB-#	CIMMYT	OPV Resistant
43	A1037	VP0535	ZM305	CIMMYT	OPV Susceptible
44	A1147		SYN[WEEVIL-B(FSWEEVSUSCEPT)]	CIMMYT	OPV Susceptible
45	A771-3		02SADVL	CIMMYT	OPV Susceptible
46	A1174		02SADVL	CIMMYT	OPV Susceptible
47	A1043		ZM621	CIMMYT	OPV Susceptible
48	A545-8		ZM623	CIMMYT	OPV Susceptible
49	A1176		02SADVL2	CIMMYT	OPV Susceptible
50	A1144		SYN[WEEVIL-A(FSWEEVSUSCEPT)]	CIMMYT	OPV Susceptible
51		PANDAWE	Not applicable	Mbala	Landrace
52		CIMAMBWE	Not applicable	Mbala	Landrace

Legend

CML = CIMMYT maize line
CIMMYT = International Maize and Wheat Improvement Centre, Zimbabwe.
Mbala = a district in the Northern Province of Zambia.

Table 2: Description of the landraces

Acce-ssion	Var. Code	Variety Name	District	Ear Length	Grain Color	Grain Size	Mat-urity	Grain Type	Rows	Sample Size	Farmer Name	**Years grown**	**Special Traits**
212	Z 080	Chimambwe	Mbala	L	W,Y	L	L	SD	8-10	-	Simfukwe	4	high milling %, very sweet when fresh
213	Z 081	Chimambwe	Mbala	M, L	W	M	M	D	12	10	Sichimba	25	heavy maize, tolerates low soil fertility
214	Z 082	Chimambwe/ Kalimwa	Mbala	M	W Y, P	L	EE	F,SD	10	10	Namkoko	10	tolerates low soil fertility, heavy mealie meal, good for "samp"
218	Z 086	Pandawe	Isoka	-	W, P	M	E	SD	-	-	Simwanza	10	heavy mealile-meal, tolerates low soil fertility, high yield
219	Z 087	Pandawe	Isoka	-	W,R,P	M	L	SD	-	-	Simukoko	30	does not rot easily, heavy grain, sweet when fresh

Adapted from Mugorokosho, 2006

Legend

L = Long or large or late with reference to ear length, grain size and maturity period, respectively.
M = Medium in the above characteristics
E = early maturing
EE = Very early maturing
W = White
P = Purple
Y = Yellow
D = Dent grain texture
SD = Semi-dent grain texture
F = Flint grain texture
- = no information

Note: Where more than one letter, separated by a comma, is found under one character this implies a mixture, e.g., W,Y in grain colour of accession

212 implies that a mixture of white and yellow grain is found on the same cob.

Maize Weevils

The maize weevils were collected from bags of weevilled maize harvested from trials at Mount Makulu Central Research Station, Chilanga, Zambia. To obtain young weevils for use in the weevil bioassay, 200 hundred grams of SC 513, a white dent maize variety from Seedco (Seedco, 2006) were weighed into each of 70 one-litre jars and then 200 weevils were introduced into each jar. These jars had lids with their to parts removed to allow adequate ventilation when the jars are closed, but to prevent the weevils from escaping circular brass screens were put inside the lids. The temperature in the room was maintained at 29 ± 1°C. The relative humidity ranged from 43 to 50 %. The room was humidified by water placed in an open dish (Bekele and Hassanali, 2001).

3.2 Methods

The research was done in two phases. The first phase involved the multiplication of hybrids, the OPVs and the landraces. In the second phase weevil bioassay and other laboratory analyses were conducted.

3.2.1 Phase I: Multiplication of hybrids, the OPVs and the landraces.

The maize materials were planted at Golden Valley Agricultural Research Trust (GART) 80 km north of Lusaka, Zambia, during the 2005/2006 growing season. The station is located at latitude14° 40' South, longitude 25° 01'East and at an altitude of 1140 m above sea level. The soil is described as Makeni Series which is a fine, mixed, isohyperthemic ultic Paleustalf (Ti jmons, 1988). According to the World Reference Base (WRB) this soil is described as a Chromic luvisol (FAO, 1998). The trial was laid out in a Randomized Complete Block Design with 3 replications (Appendix 1). This design was used in order to remove the effects of soil heterogeneity, considering that the total experimental area was large (about 3,492 m^2). Each plot consisted of 4

rows measuring 5 m long. The inter- and intra- row spacing were 90 cm and 25 cm, respectively. Two seeds were planted per station. In addition to the standard crop management practices for maize, furadan was applied pre-plant in planting holes for the control of cutworms and stem borers; azodrin was applied against stalk borers; and confidor (imidacroprid) was applied at grain filling stage for the control of termites which had already been observed to have caused wide spread damage in the neighbouring maize trials. Methomyl, a carbamate, was applied against the armoured cricket *Acanthoplus speiseri* Brancsik, a pest of grain crops, common in the study area (Appendix 4). For weed control, atrazine was applied and supplemented with hand-weeding.

For weevil evaluation, 12 plants were isolated from the two middle rows in each plot and upper ears covered with plastic bags to prevent free pollination. Pollen was collected from the isolated plants, bulked and then used to pollinate the same plants (12 plants). Where plant emergence was poor the number of isolated plants was less than 12.

3.2.1.1 Husk Cover Rating Scores and Husk Length measurement

Husk cover rating and husk cover length were measured on the samples of freely pollinated cobs and not on cobs used for weevil evaluation, to reduce the handling of the latter as this would disturb the husks on the cobs. Husk cover rating was estimated on a scale of 1 to 5 by putting a hand around the husk leaves as they extend beyond the ear tip. The rating of one was assigned, if the husk leaves were longer than 4 fingers, two if 3 fingers long and so on until the rating of five, which was given if the husk leaves were not longer than 1 finger and the tip was exposed (Kossou *et al.*, 1993).

Husk length was measured in millimetres with a 30-cm ruler. This involved measuring the length of husk leaves as they extend beyond the tip of the ear.

3.2.2 Phase II. Evaluation of Genotypes for Maize Weevil Resistance.

Maize genotypes were evaluated for maize weevil resistance by assessing maize weevil infestation in the field and by maize weevil bioassay.

3.2.2.1 Assessment of maize weevil infestation in the field

To assess maize weevil infestation in the field, 200 g of hand-shelled grain from randomly (uncontrolled) pollinated plants of all the 52 genotypes were weighed into 350 ml plastic jars, the type used for packing 500 g of jam in Zambia. These were incubated in a Controlled Temperature and Relative Humidity room for three months. The temperature in the Controlled Temperature and Relative Humidity room was maintained at 29±1 ° C by a thermostat-controlled fan-heater mounted on the wall. The relative humidity was in the range of 45 to 50 percent. The ideal 70±5 % RH was not achieved. This was because the humidifier had broken down and only an open water trough was being used to raise the humidity.

3.2.2.2 Maize weevil bioassay

This work was done at Mount Makulu Central Research Station using modified Dobie's method (Dobie, 1974; 1977; Serratos et al., 1993).

Three experiments were set up according to the three common forms of maize storage in Zambia namely: loose grain, husked cobs (cobs with husks on), and dehusked cobs (cobs with husks removed).

3.2.2.2.1 Experiment 1: Loose grain.

Three cobs of each genotype were hand-shelled and the grain packed into 5 x 8 polythene bags purchased from Zambia Polythene Products limited, Lusaka Zambia. The bags were closed with rubber bands and then stored in a deep freezer for one week to kill previous infestation of any insects including adults, larvae or eggs (Kossou et al., 1993). The temperature in the freezer was minus (–) 16°C. The grain was then conditioned in the Controlled Temperature and Relative Humidity room for 14 days to equilibrate grain temperature and, more importantly, moisture content (Munjoma, 2004) while still in the polythene bags.

After conditioning, duplicate samples of 50 g of each genotype were weighed into new 350 ml plastic jars, the type used for packing 500 g of jam in Zambia. These jars, purchased from Polymer Mouldings Limited in Lusaka, measured 11.7 cm in height and about 5.2 cm in diameter at the mouth. One set was for acclimatization of the weevils to the maize genotype and the other for the bioassay (Dobie, 1974). The tops of the lids of these jars were cut out, leaving only the screw-top rings. Forty (40) unsexed weevils of mixed age, initially counted into vials with the help of pairs of tweezers and a Denominator Multiple-Tally tally counter (The Denominator Company, Inc. Woodbury, Connecticut, USA) (Figure 2), were poured into each acclimatization jar (Figure 3).

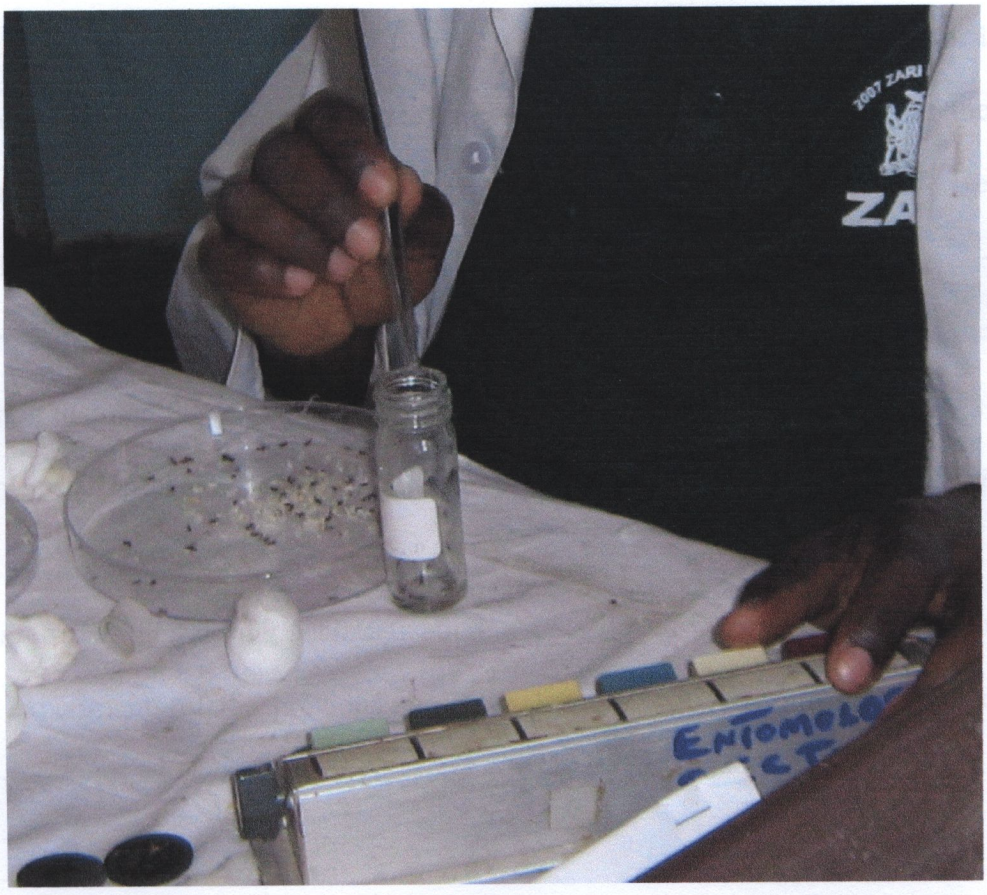

Figure 2: Picking and counting maize weevils into vials using a pair of tweezers and a tally counter

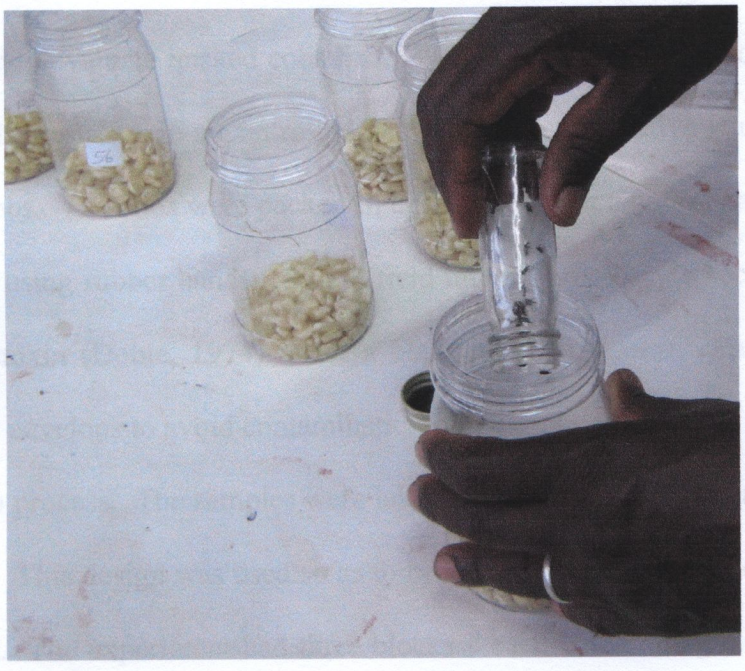

Figure 3: Introducing weevils into a jar containing a maize sample

To close the jars after the introduction of weevils a piece calico cotton cloth, 15 cm x 15 cm, was put on top of the jar and the ring of the lead screwed on to the jar over the cloth or the cloth was fastened to the jar with a rubber band. The cotton cloth was used to prevent the weevils from escaping and to provide ventilation.

Weevils were put on the samples of each genotype to get the insects accustomed to the new food before infesting them on the test samples after 7 days of feeding (Dobie, 1974). However, for some unknown reasons all insects were found dead after the 7 days of supposed acclimatization.

Since the insects had died during acclimatization, another lot of insects were sieved from infested maize from experiments of the previous season. These insects were put directly into the grain samples for weevil bioassay, 40 unsexed weevils per jar.

Experiments 2: Husked cobs.

An attempt was made to evaluate husked cobs for MWR under No-choice feeding regime (NCFR) using artificial weevil infestation in a Controlled Temperature and Relative Humidity (CTH) room. Each sample had 3 cobs. The cobs were packed in tailor-made calico cotton bags measuring 50 cm by 30 cm and closed using rubber bands. To kill field infestation of any insects, the samples were fumigated with Phostoxin (Dobie, 1974) under a black polythene sheet. Phostoxin tablets were placed in small paper envelops to avoid contamination of the sample bags with phostoxin dust at the end of the fumigation process. The samples were laid out in a Randomised Complete Block Design (Derera et al., 2001). This design was used so as to be consistent with the experimental design that was used in the field. The experiment had three blocks – each block being a representation of the field block in terms of the randomization of treatments. The samples were conditioned in the CTH

room as for the grain samples. Thereafter the bags were opened and 40 unsexed weevils of mixed age, initially counted into vials as described above, were poured into the bags (Figure 3).

The bags were again tightly closed with rubber bands to avoid weevils escaping and entry of any insects from outside. The bags were hanged on hooks under shelves (Figure 4) – a modification of the method of Dr.D. Bergivinson (unpublished correspondence, 2006) for a "weevil warehouse".

Figure 4: Bags of cob-samples hanging on hooks in a CTH room, with plot numbers matching with those on the tags

After ten days (Dhliwayo and Pixley, 2001) oviposition period the bags were opened to take count of live and dead weevils and to discard them. There were no live weevils found after ten days for unknown reasons.

Experiment 3: Dehusked cobs.

The samples of dehusked cobs were prepared as in Experiment 1. Again all parent weevils died during the 10-day ovipostion period.

3.2.2.3 Ancillary Experiments Conducted to Determine the Cause (s) of Weevil Mortality in the Initial Maize Weevil Bioassay Set Up Attempts.

Following the initial failure of setting up the weevil bioassays due to deaths of weevils for unknown reasons, two ancillary experiments were sequentially set up to determine the cause (s) of the weevil mortality observed.

Ancillary Experiment set 1

The first experiment compared storage materials and storage rooms. The treatments were:

(i) Washed maize grain in a washed jar,

(ii) Washed maize grain in an unwashed jar

(iii) Unwashed maize grain in a washed jar

(iv) Unwashed maize grain in an unwashed jar

(v) Local maize variety from an individual within Mt. Makulu in a washed jar

(vi) Local maize variety from an individual within Mt. Makulu Research Station in an unwashed jar

These treatments were prepared in duplicate, so that one set was stored in the Controlled Temperature and Relative Humidity room at the Maize Research Section and the other in a Controlled Temperature and Relative Humidity room at the Entomology Research Section. The treatments were not replicated. Tap water was used in all the washings in the treatments.

The results from this test showed that weevil survival was better in washed grain regardless of whether the jar or cotton bag was washed or not. A Controlled Temperature and Relative Humidity room did not have any effect on the weevil survival. This led to suspicion that the samples may

have had chemical contamination either from the fumigation conducted on them earlier, in the case of grain from cobs, or from confidor (imidacroprid) which was applied at grain filling stage in the field. Therefore all the samples were washed in tap water in an attempt to remove the suspected contamination. Grain samples were then air dried at ambient temperature within a large well aerated room. All samples in cobs were washed in woven bags, the type used for packing Irish potatoes in Zambia, and then packed back into calico cotton bags for drying in an oven. The temperature was set at 50°C and the samples were left to dry for about 24 hours.

Following this washing and drying of the samples the experiments were repeated. This time the insects were of mixed age and sex as the stock of weevils that had been raised in preparation for weevil bioassay, and was of known age, was depleted during the first attempt of screening of the maize samples for weevil resistance.

Ancillary Experiment 2

Another unreplicated ancillary experiment was set up to determine whether there was any problem with the aeration because of the use of cotton cloth as a covering for the jars. This had the following 18 treatments:

(i) Entry 51 (landrace) from previously shelled grain sample, in an unwashed 350 ml plastic jar.

(ii) Entry 51 in a washed plastic jar.

(iii) Entry 51 in glass jar.

(iv) Entry 27 (hybrid) from previously shelled grain sample, in an unwashed 350 ml plastic jar.

(v) Entry 27 (hybrid) from previously shelled grain sample, in a washed 350 ml plastic jar.

(vi) Entry 27 (hybrid) from previously shelled grain sample, in a glass jar.

(vii) Local variety from within Mt.Makulu in an unwashed plastic jar.

(viii) Local variety from within Mt.Makulu in a washed plastic jar.

(ix) Local variety from within Mt.Makulu in a glass jar.

(x) Grain from a cob of plot 73 in an unwashed plastic jar.

(xi) Grain from a cob of plot 73 in a washed plastic jar

(xii) Grain from a cob of plot 73 in a glass jar.

(xiii) Grain from a husked cob of plot 18 in an unwashed plastic jar.

(xiv) Grain from a husked cob of plot 18 in a washed plastic jar.

(xv) Grain from a husked cob of plot 18 in a glass jar.

(xvi) Blank (without maize) unwashed plastic jar.

(xvii) Blank (without maize) washed plastic jar.

(xviii) Blank (without maize) glass jar.

Circular brass screens were used on all the jars instead of the cotton cloth.

Ancillary Experiments Results and Final Weevil Bioassay Set Used

This test showed a highest weevil survival rate in the grain from the non-experimental local variety followed by grain from a husked cob of a study genotype, entry 28, harvested from plot 18.

Based on these results it was decided that the maize samples on cobs with husks for all genotypes under study get shelled and the weevils get introduced into the shelled grain, for the bioassay experiments.

Fifty grams (50±0.15 g) of maize kernels of each genotype were weighed into new plastic jars of the type described above. Grain for plots 73, 80 and 86 (entries 7, 46 and 52, respectively) was taken from the remnants of the samples that were originally shelled for grain infestation as these

plots did not have enough cobs to carter for all the three forms of storage and so there were no cobs with husks. Grain of SC 513 maize variety from Seedco, an international seed company operating in Zambia, was included in the experiment as a susceptible check, bringing the total number of treatments to 53. Forty weevils (40) were introduced into each jar as before. The jars were labelled by writing a plot number on a paper label and sticking it on the jar. Additionally, the plot numbers were also written on the covering cloth with a marker pen. The labels on the covers made identification of the jars easier than the paper labels on the jars as these got partly covered by the cloth. Ten days later, the adult (parents) maize weevils were removed from the samples by sieving with a U.S.A. Standard Testing Sieve set (VWR Scientific, West Chester, PA 19380,U.S.A.). The powder, if any, went through the No.8 (2.36 mm mesh opening) and the No. 18 (1.00 mm opening) and collected in the pan. Weevils went through the No 8 sieve and collected on the No. 18 sieve, while the grain remained on the No. 8 sieve. Live and dead weevils were counted and their numbers recorded. Counting was done using tweezers and a tally counter. Tweezers were also used to probe immobile weevils to establish whether they were dead or alive. Weevils, like some other beetles, have a tendency of feigning death when disturbed (personal observation).

Sieving and checking for emergence of the F1 progeny started about 3 weeks following the removal of the parents (Serratos et al., 1993). Sieving and counting the F1 progeny was done every 2 days. Figure 5 shows one of the sieving sessions, along with a U.S.A. Standard Testing Sieve set, the incubation jars and how these were closed with the cotton cloth and rubber bands or rings of cut-out lids. An F1 adult progeny found emerging from a kernel during sieving and an mergence hole left by another weevil are shown in Figure 4.

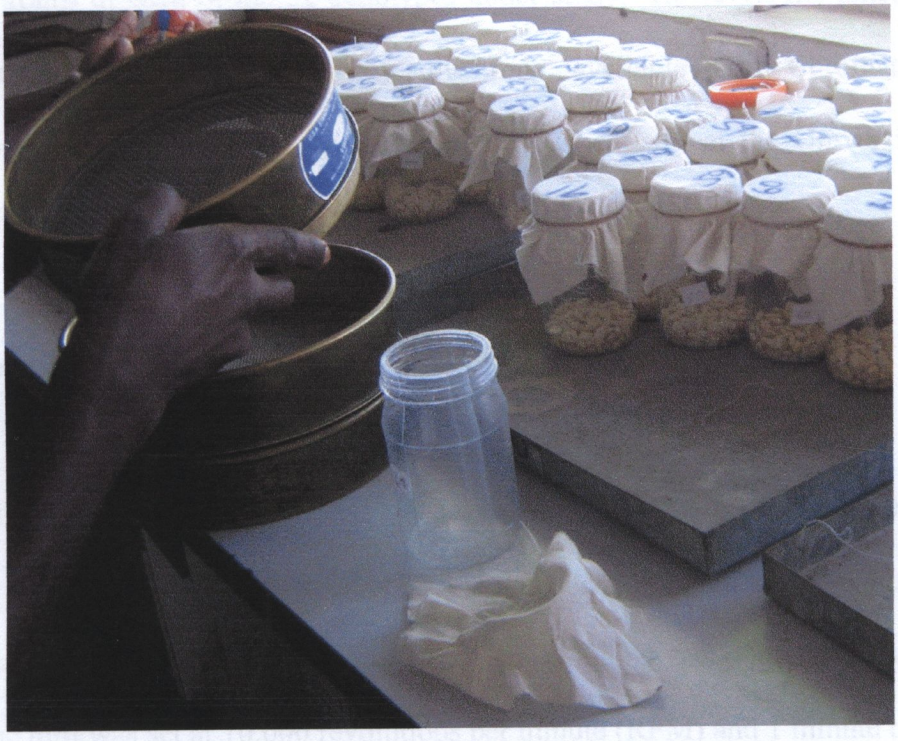

Figure 5: One of the sieving sessions, along with a U.S.A. Standard Testing Sieve set, the incubation jars closed with the cotton cloth and rubber bands or rings of cut-out lids.

3.2.2.5 Physical and biochemical parameters

Important maize kernel physical and biochemical parameters that have been reported to confer resistance to the maize weevil in the literature (Arnason et al., 1997) were analysed using appropriate methods. These parameters were grain hardness, protein content and number of kernels, a proxy of kernel weight. An attempt was also made to characterize the ferulic acid composition of grains of each of the 52 genotypes under study. However, the latter proved futile due to problems encountered when using the only available Gas Chromatography/Mass spectrometer (MS/GC) equipment in the Department of Chemistry at the University of Zambia (UNZA) Great East Road Campus.

Grain hardness

The grain hardness test was done by weighing a sample of 50 ±0.1 g of maize kernels for each genotype. The sample was ground in a laboratory mill under the brand name of Retsh, Type ZM 1000 (GmbH & Co. KG 5657 HAAN 1, Germany). The grinding was done in two stages. During the first stage the mill was set at 10,000 revolutions per minute (RPM) and 1 minute time setting for duration, with the sieve removed. This was done just to break the kernels into smaller fragments to make the next stage easier. The collected fragments were put back into the hopper and the sieve, number 11, replaced. The speed and time setting was the same as above. The collected meal was put back in labelled plastic bags. The meal was then hand-sifted in a 0.5 mm DIN 4188 sieve (ANALYSENSIEB Retsck, W. Germany). The collected flour and retained grit were emptied in separate labelled 5 x 8 cm white plastic bags, and these were subsequently weighed and data recorded. The weight of the grit and flour were added together for each genotype to get the total weight, which was about the same as the original weight of the grain from where the flour and grit samples were derived.

Grain hardness was expressed as percent grit of the total weight of the sample (grit plus flour after sieving a 50±0.1 g ground maize sample). Thus grit percentage was the proxy for grain hardness.

Number of kernels

The number of kernels contained in a 50±0.1 g grain sample of each genotype was determined by counting. This parameter was the proxy for kernel weight. Thus the higher the number of kernels in the 50±0.1 g grain sample the lighter the kernels, and vice versa.

Protein content

Twenty-gram samples of whole maize kernels were ground in a laboratory mill for each genotype. Protein content was determined using the Kjeldahl procedure.

Dobie's Susceptibility Index

The Dobie Index of susceptibility was calculated as described in section 2.5.5. The Relative Dobie Index for each genotype was then computed by taking the susceptibility of that genotype as a proportion of the susceptibility index of the susceptible check and multiplied by 10 (Dobie, 1974). The Dobie Relative Index was then used to classify the genotypes into susceptibility groups following the scales used at CIMMYT in Zimbabwe (Pixley, 1997) which were 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.

3.3 Statistical Analyses

The Analysis of Variance (ANOVA) for all the measured parameters was done using the Mstat-C Programme (Freed et al., 1988). The mean separation, in cases where there were significant differences among treatments, was done using the Duncan's Multiple Range Test (DMRT) to facilitate the comparison of all pairs of treatment means (Montgomery, 2001).

CHAPTER 4: RESULTS

4.1 Husk cover rating scores and husk length

Genotypes were significantly different ($P<0.001$) for husk-cover rating scores and husk length, with landraces having longer husks (mean length, 88 mm) and better husk-cover rating scores (mean, 1.8) than hybrids (mean length, 35 mm and mean score 3.0, respectively) and open pollinated varieties (OPVs) (mean length, 48 mm and mean score 2.1).

Table 3: Husk rating scores and husk length

Entry	Husk Cover Score	Husk Cover Length (mm)
1	2.00 cd	45.67 bcdefghij
2	3.33 abc	31.67 cdefghijk
3	2.33 bcd	50.33 bcdefgh
4	3.33 abc	32.33 cdefghijk
5	3.00 abcd	38.67 cdefghijk
6	3.33 abc	33.67 cdefghijk
7	2.00 cd	56.67 bcde
8	3.00 abcd	44.67 bcdefghij
9	3.00 abcd	35 cdefghijk
10	3.00 abcd	39.33 bcdefghijk
11	3.67 ab	29 efghijk
12	2.67 abcd	38 cdefghijk
13	2.67 abcd	47.33 bcdefghi
14	3.00 abcd	40 bcdefghijk
15	2.67 abcd	46.33 bcdefghij
16	3.33 abc	31.67 cdefghijk
17	3.67 ab	29 efghijk
18	4.00 a	24.33 hijk
19	3.00 abcd	41.33 bcdefghij
20	3.00 abcd	37.67 cdefghijk
21	4.00 a	27 fghijk
22	3.33 abc	37.33 cdefghijk
23	2.67 abcd	45.67 bcdefghij
24	3.33 abc	36.67 cdefghijk
25	3.00 abcd	30.33 defghijk
26	3.67 ab	18.67 jk
27	3.67 ab	12.67 k
28	3.67 ab	33.33 cdefghijk
29	4.00 a	20.33 ijk
30	2.67 abcd	42 bcdefghij
31	2.67 abcd	46.33 bcdefghij
32	2.33 bcd	48 bcdefghi
33	3.00 abcd	30.33 defghijk

34	3.00 abcd	38.67 cdefghijk
35	3.00 abcd	37.67 cdefghijk
36	2.67 abcd	42 bcdefghij
37	2.33 bcd	54.67 bcdef
38	1.67 d	57.67 bcd
39	2.67 abcd	46 bcdefghij
40	2.33 bcd	57.33 bcd
41	3.33 abc	25.67 ghijk
42	2.33 bcd	50.67 bcdefgh
43	2.67 abcd	47 bcdefghi
44	2.00 cd	59 bc
45	2.33 bcd	52.67 bcdefg
46	2.33 bcd	45.67 bcdefghij
47	3.00 abcd	31.67 cdefghijk
48	2.33 bcd	48.67 bcdefgh
49	3.33 abc	39.33 bcdefghijk
50	2.00 cd	58.67 bc
51	1.67 d	109.7 a
52	2.00 cd	66.67 b

4.2 Field infestation

An interesting observation was that immediately after introducing them into a jar, most of the weevils would start climbing the walls of the jar to try to escape. But after a short time, within an hour, the insects would go down to the bottom of the jar. Further observation of the maize samples infested for weevil culture revealed that weevils start feeding from the bottom of the sample and come upwards as they exhaust the food below and probably the microclimatic conditions also become unfavourable.

After incubating the grain samples from uncontrolled pollinated cobs for a period of more than three months there was no emergence of any weevils. This experiment therefore showed that there was no problem of field infestation by the maize weevil at the study site, regardless of the cob husk cover characteristics.

4.3 Protein content

Genotypes were not significantly different from each other for protein content (mean = 9.8 %) at 5% probability level (Table 4).

Table 4. Analysis of variance table of Protein Content of all 52 Genotypes.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Block	2	12.01	6.006	2.38	0.0976
Entry	51	95.51	1.873	0.78	0.8801
Error	102	257.32	2.523		
Non-additivity	1	9.07	9.069	3.69	
Residual	101	248.25	2.458		
Total	155	364.84			

Note: Grand Mean = 9.801; Grand Sum = 1529.000; Total Count = 156
Coefficient of Variation = 16.21%

Grain protein contents of resistant hybrids, susceptible hybrids, resistant OPVs, susceptible OPVs and of the landraces are presented in Table 5.to 9

Table 5. Protein content of resistant hybrids

Entry	Pedigree	Protein (%)
1	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-415-1-B-1//CML395/CML444	10.6
2	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-354-1-B-1//CML395/CML444	9.8
3	[WEEVIL/CML444]-B-5-1-3-BB/[WEEVIL/CML312]-B-18-3-1-B	10.6
4	[KILIMA ST94A]-19/MSV-03-4-05-B-1-BB-2-2-1-2-B-1//CML395/CML444	9.0
5	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-160-1-B-1//CML395/CML444	9.4
6	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV -03-2-10-B-2-BB)-411-1-B-1//MCL395/CML444	9.2
7	[CML312/CML444//[DTP2WC4H255-1-2-2-BB/LATA-F2-138-1-3-1-B]-1-3-2-3-B]-2-1-3-B//MCL395/CML444	8.5
8	[KILIMA ST94A]-19/MSV-03-4-05-B-1-BB-2-2-1-2-B-2//MCL395/CML444	9.2
9	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-127-1-B-1//MCL395/CML444	9.9
10	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-394-1-B-2//MCL395/CML444	8.6
11	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-47-1-B-1//MCL395/CML444	10.0
12	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-342-1-B-1//MCL395/CML444	10.7
13	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV03-2-10-B-2-BB)-127-1-B-2//MCL395/CML444	10.9
14	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-206-1-B-1//MCL395/CML444	9.7
15	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-393-1-B-1//MCL395/CML444	10.4
16	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-445-1-B-1//MCL395/CML444	9.9
17	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-442-1-B-1//MCL395/CML444	10.2
Mean		9.8

Table 6. Protein content of susceptible hybrids

Entry	Pedigree	Protein (%)
18	02SADVE2B-#-45-2//CML312/CML442	9.4
19	CML395/CML444//[P30/P45//M162W/MSR]97-323-3-1-5-B-1-#-1-B	11.2
20	[89[G27/TEWTSRPool]#-278-2-X-B/[COMPE2/P43SR//COMPE2]F#-20-1-1]-B-32-2-B-4-#-B// CML395/CML444	9.5
21	CML443/CML445//CML488	9.5
22	CML312/CML442//[Ent2:92SEW1-EarlySel-2/[DMRESR-W]EarlySel-#I-3-2-B/CML390]-B-26-1-B-1-#-1-B	10.6
23	CML312/CML442//NIP25-230-2-1-B-1-B	9.4
24	CML312/CML442//ZM301c1F2-72-#-1-3-B	8.3
25	CML181dent/CML182//GQL5	9.4
26	CML312/CML442//P100C6-61-1-4-##1-3-1-BBB	10.5
27	CML489/CML444//ZM621A-10-1-1-1-2-BBBBB	11.4
28	CML312/CML442//[Ent320:92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML386]-B-22-1-B-2-#-1-B	9.8
29	CML440/CML444//CML445	10.3
30	CML488/CML444//CML312/CML442	9.6
31	CML312/CML444//CML489	10.0
32	CML444/CML197//CML488	10.0
33	CML443/CML445//[CML444/ZSR923S4BULK-2-2-X-X-X-X-1-BB]-1-1-1-1-1-B	10.3
34	CML443/CML445//[EARLY/ZM621A]-14-1-1-2-2-B	10.2
35	02SADVE2B-#-45-1//CML312/CML442	9.0
Mean		9.9

Table 7. Protein content of Resistant OPVs

Entry	Pedigree	Protein (%)
36	SYN[SZ/Elite01]	10.5
37	SYN[WEEVIL-A(FSINDEX)]	10.8
38	SYN[WEEVIL-A(FSWEEVRESIST)]	10.1
39	SYN[WEEVIL-B(FSINDEX)]	9.1
40	SYN[WEEVIL-B(FSWEEVRESIST)]	8.9
41	04WEEVILA-#	9.9
42	04WEEVILB-#	10.6
Mean		9.9

Table 8. Protein content of Susceptible OPVs

Entry	Pedigree	Protein (%)
43	ZM305	9.9
44	SYN[WEEVIL-B(FSWEEVSUSCEPT)]	8.9
45	02SADVL	9.0
46	02SADVI	9.6
47	ZM621	10.6
48	ZM623	10.0
49	02SADVL2	8.3
50	SYN[WEEVIL-A(FSWEEVSUSCEPT)]	8.3
Mean		9.3

Table 9. Protein content of Landraces

Entry	**Name**	**Protein (%)**
51	Pandawe	9.2
52	Chimambwe	10.0
Mean		9.6

When the top 5 and least 5 genotypes in protein content in each group (hybrids and OPVs) were considered it was found that resistant genotypes had a tendency of containing higher levels of protein than susceptible ones (Table 10.

Table 10. Comparative protein content between the best and the worst genotypes.

Entry	Protein content	Classification
Hybrids		
Top 5		
27	11.4	Susceptible
19	11.2	Resistant
13	10.9	Resistant
12	10.7	Resistant
1	10.6	Resistant
Least 5		
4	9.0	Resistant
35	9.0	Susceptible
10	8.6	Resistant
7	8.5	Resistant
24	8.3	Susceptible
OPVs		
Top 5		
37	10.8	Resistant
42	10.6	Resistant
47	10.6	Susceptible
36	10.5	Resistant
48	10.0	Susceptible
Least 5		
45	9.0	Susceptible
40	8.9	Resistant
44	8.9	Susceptible
49	8.3	Susceptible
50	8.3	Susceptible
Landraces		
Pandawe	9.2	Unknown
Chimambwe	10.0	Unknown

4.4 Grain hardness

Grain hardness showed discrimination among the 52 genotypes ($P < 5$ %; mean = 67.8 %). However, genotypes exhibited a higher or lower degree of hardness regardless of whether they were hybrids or OPVs. The two landraces ranked low in hardness, averaging 63.3 % in relation to the top 5 hybrids and 5 OPVs. Separation of the genotypes into groups of the top 5 and the least 5 in grain hardness again showed that most of the harder genotypes were from the resistant class (Table 11).

Table 11. Comparative hardness between the top 5 and the least 5 genotypes

Entry	Grit %	Classification
Hybrids		
Top 5		
9	72.3 ab	Resistant
6	71.8 ab	Resistant
1	71.4 ab	Resistant
13	71.2 abc	Resistant
8	71.1 abc	Resistant
Least 5		
27	62.9 cdef	Susceptible
26	62.6 def	Susceptible
24	61.0 ef	Susceptible
33	60.0 ef	Susceptible
25	59.7 f	Susceptible
OPVs		
Top 5		
39	74.0 a	Resistant
48	71.3 abc	Susceptible
41	71.2 abc	Resistant
46	71.0 abcd	Susceptible
43	70.0 abcd	Susceptible
Least 5		
36	67.5 abcdef	Resistant
50	66.6 abcdef	Susceptible
37	66.3 abcdef	Resistant
49	65.8 abcdef	Susceptible
40	65.4 bcdef	Resistant
Landraces		
Pandawe	62.6 def	Unknown
Chimambwe	63.9 bcdef	Unknown

4.5 Correlation among kernel resistance parameters

Protein content did not have an overall (for 52 genotypes) definite relationship with the physical resistant parameters, namely: grain hardness and number of kernels (Table 12), and among hybrids (Table 12), but it was related to hardness for the resistant OPVs, only ($r = 0.81$) (Table 12).

Table 12 Simple linear correlations of protein content with hardness and weight per kernel

Trait	r
Across all genotypes	
Hardness	0.14^{ns}
Weight per kernel	0.05^{ns}
Resistant Hybrids	
Hardness	0.20^{ns}
Weight per kernel	0.01^{ns}
Tolerant Hybrids	
Hardness	0.26^{ns}
Weight per kernel	-0.0^{ns}
Resistant Open Pollinated Varieties	
Hardness	0.81*
Weight per kernel	-0.82*
Susceptible Open Pollinated Varieties	
Hardness	-0.32^{ns}
Weight per kernel	0.45^{ns}

^{ns} = Not significant at 5 % level
* = Significant at 5 % level of confidence
** = Significant at 1 % level of confidence

4.6 Weevil Bioassay

4.6.1 Parent survival

The number of parent weevils found alive and those found dead in each maize sample after the ten-day oviposition period was recorded. Table 13 presents the number of live weevils out of the 40 introduced in each sample. The difference is the number of weevils that were found dead in each incubation jar per genotype. The overall mean survival number for the parent weevils at the end of the oviposition period was 13.5, while the range was 4.0 to 33.7 weevils.

4.6.2 Progeny emergence

Emergence of the F_1 progeny was different among genotypes. Figure 6 shows an F_1 adult progeny emerging from a kernel and an exit hole left by another F_1 adult weevil.

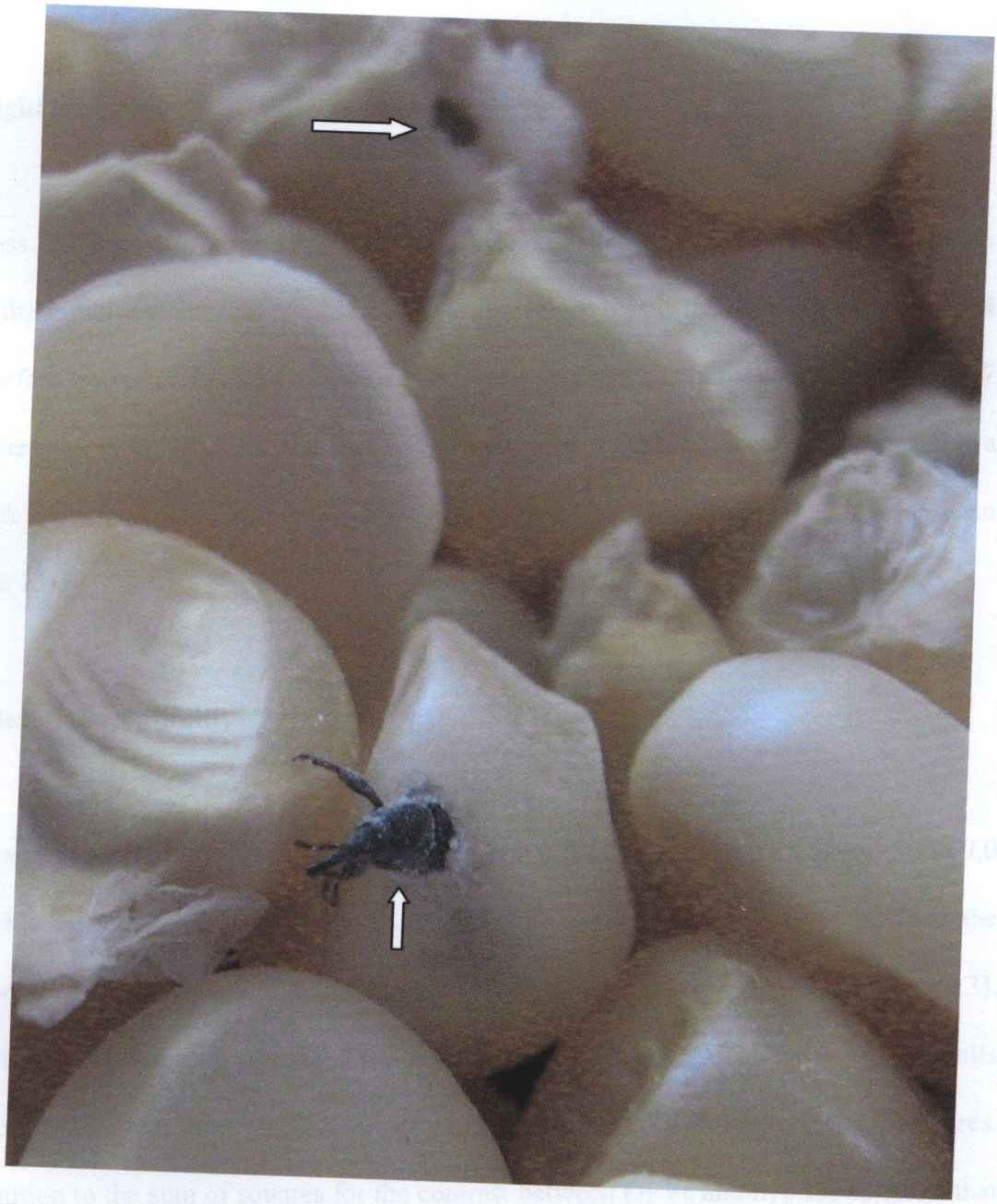

Figure 6: An F1 adult weevil progeny emerging from a kernel (lower arrow) and an emergence hole left by another weevil (upper arrow)

The total of all the F1 progeny adult weevils for each genotype is presented in Table 13. The grand emergence mean was 9.9 and the range was 0.7 to 72.3 weevils, the latter figure being that of the susceptible check.

4.6.3 Grain weight loss

Grain weight loss arising from the feeding of the larvae and/or adults in and on the kernels was computed from the initial and the final weight. Thus grain weight loss is the difference in weight of grain samples before and after the infestation of weevils in no-choice experiments (Serratos, 1987 as reported in Serratos et al., 1997). The greatest weight loss of 8.563 g occurred in entry 53, a susceptible check. The lowest weight loss of 1.7 g was recorded in entry number 2. The trial mean was 2.5 g (Table 13).

4.6.4 Dobie index of susceptibility

The Dobie Index of susceptibility (Table 13) ranged from 0.0 for entry numbers 2, 8 and 50 to 9.0 for the susceptible check. The trial mean was 3.1. However, the Dobie index re-classified the susceptible genotypes to be resistant or moderately resistant, except for the check (Table 13). Therefore, the treatment sums of squares were partitioned, as shown in Table 14. The results indicate that the resistant and moderately resistant genotypescontributed much to the sum squares. The low contribution to the sum of squares for the contrast between OPVs and hybrids suggests that weevil resistance may not be influenced by the type of genotype.

Table 13. Means of weevil bioassay for selected maize weevil resistance parameters for 53 maize genotypes

Entry no.	Parent survival (number, out of 40)	Total F1 Progeny Emergence (number)	Grain weight Loss (g)	Wt loss%	Relative Dobie index
1	16.33 bcd	3.333 c	2.093 defg	4.2defg	1.9
2	7.667 bcd	0.3333 c	1.763 g	3.6g	0.0
3	14.33 bcd	4.000 c	2.182 defg	4.3defg	2.2
4	12.00 bcd	1.333 c	2.130 defg	4.3defg	0.7
5	7.000 bcd	5.000 c	1.850 fg	3.7efg	2.6
6	16.67 bcd	3.333	2.277 cdefg	4.5cdefg	3.7
7	10.33 bcd	9.000 bc	2.390 cdefg	4.8cdefg	2.5
8	5.333 cd	0.3333 c	2.027 fg	4.1efg	0.0
9	11.00 bcd	3.000 c	2.170 defg	4.4defg	1.4
10	20.33 bc	3.333 c	1.823 fg	3.6fg	2.0
11	19.33 bcd	15.00 bc	2.407 cdefg	4.8cdefg	5.6
12	16.00 bcd	2.667 c	3.237 bcd	6.5bcd	2.2
13	21.33 ab	1.000 c	1.950 fg	3.9efg	1.1
14	5.333 cd	1.667 c	2.120 defg	4.2defg	1.8
15	15.67 bcd	2.333 c	2.122 defg	4.2defg	1.6
16	15.33 bcd	5.667 bc	2.130 defg	4.2defg	4.9
17	8.667 bcd	6.333 bc	2.407 cdefg	4.8cdefg	2.9
18	15.00 bcd	10.67 bc	2.413 cdefg	4.8cdefg	5.3
19	4.000 d	4.000 c	2.317 cdefg	4.6cdefg	2.5
20	7.000 bcd	6.667 bc	2.460 cdefg	4.9cdefg	5.2
21	6.667 bcd	9.000 bc	2.672 bcdefg	5.3bcdefg	2.5
22	15.33 bcd	11.67 bc	2.440 cdefg	4.9cdefg	5.5
23	12.00 bcd	21.67 bc	2.392 cdefg	4.8cdefg	5.4
24	20.67 bc	9.333 bc	2.393 cdefg	4.8cdefg	3.8
25	8.333 bcd	14.33 bc	2.377 cdefg	4.7cdefg	4.6
26	15.67 bcd	21.67 bc	2.887 bcdefg	5.8bcdefg	5.9
27	17.00 bcd	9.000 c	2.227 defg	4.5defg	3.2
28	20.00 bc	22.33 bc	3.382 bc	6.8bc	5.3
29	11.33 bcd	3.333 c	2.017 fg	4.1efg	1.9
30	9.667 bcd	8.000 bc	2.117 defg	4.2defg	4.5
31	14.67 bcd	7.333 bc	2.060 efg	4.1efg	5.4
32	6.333 bcd	6.667 bc	1.997 fg	4.0efg	3.0
33	16.33 bcd	12.67 bc	2.277 cdefg	4.5cdefg	5.6
34	8.667 bcd	2.333 c	2.313 cdefg	4.6cdefg	1.9
35	15.33 bcd	24.33 bc	2.487 cdefg	5.0cdefg	5.5
36	18.00 bcd	29.67 b	3.673 b	7.4b	6.2
37	19.33 bcd	13.33 bc	2.790 bcdefg	5.6bcdefg	5.6
38	13.33 bcd	9.667 bc	3.202 bcde	6.4bcd	5.4
39	9.333 bcd	1.333 c	2.647 bcdefg	5.3bcdefg	1.8
40	13.33 bcd	13.67 bc	2.950 bcdef	5.9bcdef	4.2
41	7.667 bcd	7.333 bc	2.070 efg	4.1efg	3.0
42	10.33 bcd	19.67 bc	2.260 cdefg	4.5defg	5.1
43	15.33 bcd	12.33 bc	2.393 cdefg	4.8cdefg	4.0
44	20.00 bc	4.333 c	2.320 cdefg	4.6cdefg	4.2
45	16.33 bcd	2.000 c	2.150 defg	4.3defg	1.2
46	11.67 bcd	15.00 bc	2.380 cdefg	4.8cdefg	3.9
47	14.67 bcd	2.333 c	2.023 fg	4.0efg	1.4
48	19.67 bcd	21.00 bc	2.497 cdefg	5.0cdefg	6.2
49	13.00 bcd	6.000 bc	2.160 defg	4.3defg	5.5
50	7.000 bcd	0.6667 c	2.102 defg	4.2defg	0.0
51	13.67 bcd	12.33 bc	2.980 bcdef	5.9bcde	4.0
52	12.67 bcd	12.00 bc	2.963 bcdef	5.9bcde	2.2
53	33.67 a	72.33 a	8.563 a	17.1a	10.0
Grand Mean	13.5	9.96	2.49	5.0	1.5

Means followed by the same letter in a column are not significantly different from each other (P<0.05) by Duncan's Multiple Range test.

Table 14. Partitioning of the treatment sums of squares for relative Dobie index.

Source of variation	Df	Mean squares	probability
Replication	2	110.521	
Entry	52	11.934	0.016
Check vs resistant	1	131.044	<0.001
Resistant vs moderately resistant	1	322.050	<0.001
Hybrids vs OPVs	1	6.116	0.361
Landraces vs hybrids	1	0.043	0.939

CHAPTER 5: DISCUSSION AND CONCLUSIONS

5.1 Discussion

5.1.1 Husk cover rating scores and husk length

Good husk coverage on the cob protects maize from weevil infestation in the field and in storage if maize is stored with husks on, which is quite a common storage method in Zambia (Appendix 1). This study has shown that the husk cover rating scores and husk lengths for the 52 maize genotypes give the same result about the quality of husk cover on the cob. Cobs with longer husk covers had higher husk cover rating scores than those with shorter husk covers. So the two parameters could be used interchangeably when assessing maize genotypes for husk cover protection against the maize weevil. However, the scoring method is faster, although it would require that the same person takes the scores for a particular experiment since the size of the fist differs among individuals. Although measuring husk length is slower than scoring, it is more objective. It also has the advantage that you can have more than one person taking the measurements in the same experiment.

In studies conducted in Benin on four maize varieties: two international improved, one partially improved local variety and a local variety obtained from farmers, Kossou et al. (1993) made assessments of husk coverage by using husk cover rating scores and husk extension. They measured husk extension as the length (cm) of the husk extending beyond the tip of the ear. The findings of these workers were that the Benin varieties had significantly better husk coverage of the ear, as measured by the husk rating scale and by the length of the extension of the husk beyond the ear tip. Although these authors did not suggest that researchers could use any one of these two parameters to make assessment of husk coverage of different maize genotypes, their findings agree with the results of this study in as far as the two assessment methods are concerned.

It is evident from this research that landraces have superiority in the husk cover parameter, followed by OPVs. Therefore landraces have more protection against infestation by the maize weevil and other insects, as well as diseases, in the field than hybrids and OPVs. This finding is also in agreement with the findings of Kossou et al. (1993) who found that the Benin local varieties had better husk coverage than the improved maize varieties as mentioned above.

5.1.2 Field infestation

In this study maize samples were not infested in the field, regardless of the quality of husk cover. This could simply mean that the study site did not have a high prevalence of weevils. Mr. Kabamba Mwansa (personal communication, 2006) of ZARI and stationed at GART, the study site, did in fact indicate that from his experience infestation of maize in the field by the maize weevil does not occur at that site. However, a substantial amount of literature on this subject indicates that the maize weevil starts its infestation of maize right in the field particularly when harvesting is delayed such as happens when maize is left in the "stooks" for a long time (Appendix 2) (Giles and Ashman, 1971; Kossou et al., 1993; Kang et al., 1995; Perez-Mendoza, 1999; Pendleton et al., 2005; Asawalamu and Hassanali, 2006)

5.1.3 Protein content

Although genotypes were not statistically different from each other (Tables 1-6), a closer look at the best 5 and worst 5 genotypes in terms of protein content revealed that there was a tendency for genotypes with higher protein content to be resistant (based on the classification of the genotypes done at CIMMYT, Zimbabwe). This is consistent with what other investigators have found (Arnason et al., 1994; Kang et al., 1995; Arnason et al., 1997; Derera et al., 2001; Dhliwayo and

Pixley, 2003; Garcia-Lara et al., 2004;). The fact that protein content did not have a definite relationship with physical resistant parameters in this study may indicate that there are other resistant factors in the maize studied. Arnason et al., 1994 and 1997 have reported the presence of biochemical compounds, particularly ferulic acid, in the kernels.

5.1.4 Grain hardness

The statistical differences among genotypes with respect to grain hardness observed in this study is expected when there is a large number of genotypes in an experiment being compared as was the case in this study. This is so because the genotypes had different grain textures. When grinding grain samples, genotypes with softer endosperms yielded more flour than those with harder endosperms. Grain hardness was closely related to maize weevil resistance (Table 8). These results are consisted with those of 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 soft endosperm. The study was conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Zimbabwe.

5.1.5 Correlation among kernel resistance parameters

Protein content did not have an overall definite relationship with the physical resistant parameters (Table 9) and among hybrids (Table 10), but it was related to hardness for the resistant OPVs, only ($r = 0.81$) (Table 10). Other researchers have found high levels of correlation of protein with other resistance parameters. For example, Dobie, (1977), reported a positive correlation of 0.50 between total protein content and grain hardness and 0.60 between endosperm protein and grain hardness. The proxy of grain hardness in Dobie's studies was the average power used by an electric motor to grind a sample of grain in a 'Brabender Farinograph' fitted with a grinding head. In the same report,

the correlation between total protein and litre weight, and between endosperm protein and litre weight were 0.46 and 0.50, respectively. In their study of the role of cell wall components in maize weevil resistance, Garcia-Lara et al., 2004, found a negative correlation between whole kernel nitrogen, which is directly related to protein content, and susceptibility parameters. The absence of a clear correlation in this case could be due to the fact that there were no significant differences in protein content among the genotypes studied.

5.1.6 Weevil Bioassay

5.1.6.1 Parent survival

Parent weevil survival tends to be higher in susceptible than resistant genotypes. This was the case in this study also. Thus the susceptible check had the highest number, 33.7, of surviving parent weevils compared to the trial mean of 13.5. The larger number of parental survival generally leads to a larger number of eggs and ultimately the F1 progeny. The susceptible check in this study yielded 72 F_1 progeny compared to a grand mean of 10 and means of less than 1 in treatment 2 and 8 (Table 13).

5.1.6.2 Progeny emergence

Progeny emergence tends to be higher in susceptible genotypes than in resistant ones (Dobie, 1974; Garcia-Lara et al., 2004). In this study the susceptible check had the highest number of the total F1 emergence, numbering up to 72 weevils, against the experimental mean of about 10 weevil emergencies. The total F1 progeny emergence may have been reduced in the whole experiment by the mechanical disturbance of the samples through the action of sieving every 2 days (Ungunantwiwat and Mills, 1979).

5.1.6.3 Grain weight loss

Grain weight loss is an important resistant parameter (Dobie, 1974, 1977; Serratos et al., 1997). In this study the highest loss again occurred in the susceptible check in which 8.5 g were consumed against the experimental mean of 2.5 g. Grain weight loss values might have been higher than those obtained in this study if the weevils had been only young ones, 0 to 3 weeks. In his extensive experiments on the subject of maize weevil resistance, Dobie (1974, 1977) demonstrated that the fecundity and feeding of the maize weevils is highest when they are in the age range of) to 3 weeks after which there is a steady decline. Since the weevils that were used in this experiment were of unknown and mixed age it is possible that some of them were older than the optimum age for feeding and reproduction.

5.1.6.4 Dobie index of susceptibility

This index (Dobie, 1974, 1977) has been popular in maize weevil resistance screening experiments since its development in 1974. Several researchers have used it, such as, Arnason et al., 1994, Bervignson, 2001, and many others.

The range of values of indices obtained in this experiment was lower than those obtained by other investigators. In this experiment the range was from 0 to 9.0. Some researchers have obtained indices as high as 14 in susceptible varieties (Arnason et al., 1994). One possible explanation is that these researchers may have dealt with much more susceptible genotypes than I did in this experiment. Another difference could lie in the differences in moisture content. Most researchers infest their samples at about 14 percent moisture content. For instance, the maize samples that Arnason et al., (1994) used in Canada had moisture contents ranging from 10.40 % to 14.90 %. The

Dobie index of Susceptibility from such maize samples then ranged from 0.00 for a resistant check to 15.20 for a susceptible check. The maize samples in the present study had moisture content of about 10.5 to 12.5 percent. The Entomology Research Team at CIMMYT, Mexico, conducted a study to quantify the relationship between grain moisture content, kernel hardness, and resistance to *S. zeamais* and the larger grain borer *Prostephanus trancatus* (Coleoptera: Bostrichidae) (Bergvinson, 2001). They found that at grain moisture content below 12 %, the resistant genotype, population 84, provided effective control for both insect species. However, once the grain moisture content reached 16 percent the resistant (population 84) and susceptible (CML 244xCML349) entries showed similar damage levels.

The age of weevils in the bioassay might also have contributed to the lower indices. When the weevils that had been cultured in the laboratory and which were of a known age range, 0 to 4 weeks, died during the first bioassay tests, weevils for the repeated experiment were used directly after collection from weevilled maize samples. Previous studies (Dobie, 1974) have shown that the fecundity of weevils is highest when they are zero to twenty one days of age.

The resistance/susceptibility of the genotypes in this study matched the classification of CIMMYT to a great extent in the case of hybrids whereby 15 out of the 17 hybrids classified as resistant by CIMMYT were still found to be resistant

However, there was no definite pattern for OPVS. This could be attributed to the variability in character of the OPVs. It was observed during the study that some OPVs had a mixture of normal white grain and some grain containing anthocyanin, and/or flint as well as dent grain (Table 15). The departure from the CIMMYT classification observed in some genotypes could be due to the effect of environment. Kim and Kossou (2003) in their study of responses and genetics of maize germplasm resistant to the maize weevil in Nigeria found highly significant mean squares of crosses

x location interactions for all the four variables they had studied, namely: number of egg plugs, F1 weevils, damaged kernels and weevil survival (%). They concluded that the interactions indicated environmental effects on maize weevil resistance to weevils. Similarly, Duarte, et al., (2005), in their study of nitrogen level effects on grain quality of Brazilian maize genotypes, found that nitrogen application increased kernel hardness and decreased breakage susceptibility to a minor extent. However, according to these authors, genotype had a much larger influence on grain quality parameters than environment.

Table 15. Comparative classification of maize to maize weevil resistance using Relative Dobie index.

Entry	Pedigree	Class	CIMMYT classification	Relative Dobie index	New classification[§]
1	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-415-1-B-1//CML395/CML444	Hybrid	Resistant	1.9	Resistant
2	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-354-1-B-1//CML395/CML444	Hybrid	Resistant	0.0	Resistant
3	[WEEVIL/CML444]-B-5-1-3-BB/[WEEVIL/CML312]-B-18-3-1-B	Hybrid	Resistant	2.2	Resistant
4	[KILIMA ST94A]-19/MSV-03-4-05-B-1-BB-2-2-1-2-B-1//CML395/CML444	Hybrid	Resistant	0.7	Resistant
5	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-160-1-B-1//CML395/CML444	Hybrid	Resistant	2.6	Resistant
6	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-411-1-B-1//MCL395/CML444	Hybrid	Resistant	3.7	Resistant
7	[CML312/CML444//[DTP2WC4H255-1-2-2-BB/LATA-F2-138-1-3-1-B]-1-3-2-3-B]-2-1-3-B//MCL395/CML444	Hybrid	Resistant	2.5	Resistant
8	[KILIMA ST94A]-19/MSV-03-4-05-B-1-BB-2-2-1-2-B-2//MCL395/CML444	Hybrid	Resistant	0.0	Resistant
9	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-127-1-B-1//MCL395/CML444	Hybrid	Resistant	1.4	Resistant
10	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-394-1-B-2//MCL395/CML444	Hybrid	Resistant	2.0	Resistant
11	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-47-1-B-1//MCL395/CML444	Hybrid	Resistant	5.6	Moderately resistant
12	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-342-1-B-1//MCL395/CML444	Hybrid	Resistant	2.2	Resistant
13	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-127-1-B-2//MCL395/CML444	Hybrid	Resistant	1.1	Resistant
14	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-206-1-B-1//MCL395/CML444	Hybrid	Resistant	1.8	Resistant
15	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-393-1-B-1//MCL395/CML444	Hybrid	Resistant	1.6	Resistant
16	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-445-1-B-1//MCL395/CML444	Hybrid	Resistant	4.9	Moderately resistant
17	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-BB)-442-1-B-1//MCL395/CML444	Hybrid	Resistant	2.9	Resistant
18	02SADVE2B-#-45-2//CML312/CML442	Hybrid	Susceptible	5.3	Moderately resistant
19	CML395/CML444//[P30/P45//M162W/MSR]97-323-3-1-5-B-1-#-1-B	Hybrid	Susceptible	2.5	Resistant
20	[89[G27/TEWTSRPool]#-278-2-X-B/[COMPE2/P43SR//COMPE2]F#-20-1-1]-B-32-2-B-4-#-B// CML395/CML444	Hybrid	Susceptible	5.2	Resistant
21	CML443/CML445//CML488	Hybrid	Susceptible	2.5	Resistant
22	CML312/CML442//[Ent2:92SEW1-EarlySel-2/[DMRESR-W]EarlySel-#I-3-2-B/CML390]-B-26-1-B-1-#-1-B	Hybrid	Susceptible	5.5	Moderately resistant
23	CML312/CML442//NIP25-230-2-1-B-1-B	Hybrid	Susceptible	5.4	Moderately resistant
24	CML312/CML442//ZM301c1F2-72-#-1-3-B	Hybrid	Susceptible	3.8	Resistant
25	CML181dent/CML182//GQL5	Hybrid	Susceptible	4.6	Moderately resistant
26	CML312/CML442//P100C6-61-1-4-##1-3-1-BBB	Hybrid	Susceptible	5.9	Moderately resistant
27	CML489/CML444//ZM621A-10-1-1-1-2-BBBBB	Hybrid	Susceptible	3.2	Resistant
28	CML312/CML442//[Ent320:92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML386]-B-22-1-B-2-#-1-B	Hybrid	Susceptible	5.3	Moderately resistant
29	CML440/CML444//CML445	Hybrid	Susceptible	1.9	Resistant
30	CML488/CML444//CML312/CML442	Hybrid	Susceptible	4.5	Moderately resistant
31	CML312/CML444//CML489	Hybrid	Susceptible	5.4	Moderately resistant
32	CML444/CML197//CML488	Hybrid	Susceptible	3.0	Resistant
33	CML443/CML445//[CML444/ZSR923S4BULK-2-2-X-X-X-X-1-BB]-1-1-1-1-1-B	Hybrid	Susceptible	5.6	Moderately resistant
34	CML443/CML445//[EARLY/ZM621A]-14-1-1-2-2-B	Hybrid	Susceptible	1.9	Resistant
35	02SADVE2B-#-45-1//CML312/CML442	Hybrid	Susceptible	5.5	Moderately resistant
36	SYN[SZ/Elite01]	OPV	Resistant	6.2	Moderately resistant
37	SYN[WEEVIL-A(FSINDEX)]	OPV	Resistant	5.6	Moderately resistant
38	SYN[WEEVIL-A(FSWEEVRESIST)]	OPV	Resistant	5.4	Moderately resistant
39	SYN[WEEVIL-B(FSINDEX)]	OPV	Resistant	1.8	Resistant
40	SYN[WEEVIL-B(FSWEEVRESIST)]	OPV	Resistant	4.2	Moderately resistant
41	04WEEVILA-#	OPV	Resistant	3.0	Resistant

42	04WEEVILB-#	OPV	Resistant	5.1	Moderately resistant
43	ZM305	OPV	Susceptible	4.0	Resistant
44	SYN[WEEVIL-B(FSWEEVSUSCEPT)]	OPV	Susceptible	4.2	Moderately resistant
45	02SADVL	OPV	Susceptible	1.2	Resistant
46	02SADVL	OPV	Susceptible	3.9	Resistant
47	ZM621	OPV	Susceptible	1.4	Resistant
48	ZM623	OPV	Susceptible	6.2	Moderately resistant
49	02SADVL2	OPV	Susceptible	5.5	Resistant
50	SYN[WEEVIL-A(FSWEEVSUSCEPT)]	OPV	Susceptible	0.0	Resistant
51	Not applicable	Landrace	Unclassified	4.0	Resistant
52	Not applicable	Landrace	Unclassified	2.2	Resistant
53	SC 513	Hybrid	Susceptible	10.0	Susceptible

[§] The classification was based on the Dobie relative index.

An attempt was made to analyze for ferulic acid content in all the 52 genotypes and a susceptible check in this study. This attempt failed due to problems encountered with the equipment.

During the course of this study it was evident that in Zambia in general and the university in particular there is lack of some important basic research infrastructure and equipment. Those pertaining to my study include constant climate rooms with the accompanying equipment that include humidifiers, dehumidifiers and thermostat - controlled fan heaters. The scientific institutions I visited either did not have constant climate rooms or the equipment was not functional. Thus for my study I had to use a malfunctional humidifier and supplemented it with water troughs (Bekele and Hassanali, 2001) in an attempt to raise the relative humidity to the ideal range of 70±5%. The highest R.H. achieved was 60 %.

5.2 Conclusions

This study achieved the overall objective of determining the comparative resistance of the selected maize populations to the maize weevil. It was confirmed that various genotypes included in the study along with the susceptible check responded differently to maize weevil infestation.

The genotypes also differed in grain hardness, and to some extent in protein content. In this respect the first specific objective was met to some extent. However, ferulic acid determination would have given additional information about the biochemical differences of the genotypes studied.

The second specific objective was not met since the experiments on grain on husked and dehusked cobs were not successfully implemented.

From this study it was concluded that there is genetic variability with respect to various resistance parameters. These parameters can be improved upon in the high yielding hybrids in order to meet

on-farm as well as commercial storage requirements. It is also necessary to screen a wide range of landraces in Zambia for weevil resistance so that those with superior resistance characteristics could be used in the development of hybrids. The land races should also be screened for their content of phenolic acids, particularly ferulic acid, which is known to confer not only resistance to the maize weevil but also to stem borers and fungal diseases in the field.

Some resistance variables, such as grain hardness were able to discriminate the genotypes in terms resistance but others like protein content were not, although a trend could be seen in the analysed data. Cob husk characteristics also easily discriminated the maize genotypes for resistance. But since some sites, such as GART, may not have high levels of natural infestation, field infestation by the maize weevil could be determined by planting the experiment at more than one location.

Some biochemical analyses such as ferulic acid are very important in characterizing genotypes for weevil resistance, but the cost of acquiring the standards and analysing the samples may be quite high. However, these costs may be worthwhile if the analysis is limited to parental maize lines and landraces.

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APPENDICES

Appendix 1. Maize stored as intact cobs in a crib (lower arrow) and outside on logs (upper arrow).

Appendix 2. Maize in "stooks" waiting to be harvested. Irrigated spring wheat is already growing in the top right corner back ground.

FIELD PLAN – Golden Valley Agricultural Research Trust (2005/2006 Season)

REP III

G	156	155	154	153	152	151	150	149	148	147	146	145	144
G	131	132	133	134	135	136	137	138	139	140	141	142	143
G	130	129	128	127	126	125	124	123	122	121	120	119	118
G	105	106	107	108	109	110	111	112	113	114	115	116	117

OTHER EXPERIMENT

G	52	51	50	49	48	47	46	45	44	43	42	41	40
G	27	28	29	30	31	32	33	34	35	36	37	38	39
G	26	25	24	23	22	21	20	19	18	17	16	15	14
G	1	2	3	4	5	6	7	8	9	10	11	12	13

REP I

104	103	102	101	100	99	98	97	96	95	94	93	92	G
79	80	81	82	83	84	85	86	87	88	89	90	91	G
78	77	76	75	74	73	72	71	70	69	68	67	66	G
53	54	55	56	57	58	59	60	61	62	63	64	65	G

REP II

LEGEND
G= Guard Plots

Appendix 4. Feeding damage by the Armoured groundcricket