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COMPARATIVE RESISTANCE OF IMPROVED MAIZE GENOTYPES AND LANDRACES TO MAIZE WEEVIL

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ABSTRACT

A comparative study of maize (*Zea mays* L.) weevil *(Sitophilus zeamais* Motsch.*)* resistance in selected landraces and improved genotypes of maize, was conducted through a field experiment and laboratory assays. The maize grain characteristics that confer resistance to the maize weevil, *Sitophilus zeamais* Motschulsky, namely; grain hardness, weight and protein content, were compared in 50 improved maize genotypes developed by CIMMYT and two landraces (Cimambwe and Pandawe). The relative susceptibility of all maize experimental material to the weevil, were also compared using Dobie's susceptibility indices, grain weight loss and median development period. Genotypes were significantly different ($p<0.05$) for grain hardness but not for protein content. Genotypic differences in grain weight loss due to feeding by larval and adult *S. zeamais* were highly significant ($P<0.001$). The genotypes did not differ significantly ($P>0.05$) in the Dobie's indices of susceptibility but exhibited a wide index range (0.77 to 9.11). The landrace, Chimambwe, had the second highest Median Development Period, while Pandawe, had the heaviest kernels, though without noticeable advantage in resistance to the weevil. The Open Pollinated Varieties (OPVs) were not superior to hybrids according to the Dobie's index of susceptibility. The possibility of developing maize hybrids or OPVs that are as resistant to the maize weevil, *S. zeamais*, as or even better than landraces is discussed and recommendations made.

Key Words: Open pollinated, *Sitophilus zeamais,* Zambia, *Zea mays*

RÉSUMÉ

Une étude comparative de résistance aux charançons de maïs (*Zea mays* L.) Curculionoidea (*Sitophilus zeamais* Motsch.) des cultivars traditionnels sélectionnés et les génotypes améliorés de maïs avait été réalisée à travers un champ expérimental ainsi que l'expérience et de laboratoire. Les caractéristiques de grains de maïs qui confèrent la résistance aux charançons de maïs à savoir, *Sitophilus zeamais* Motschulsky, dureté de grain; les poids et les contenu en protéines, avait été comparés pour 50 génotypes de maïs améliorés, développés par CIMMYT et deux cultivars traditionnels (Cimambwe et Pandawe). La relative sensibilité aux charançons de tout le matériel expérimental de maïs avaient également été comparé à l'aide de l'indices susceptibilité de Dobie, perte de poids de grain et la période médiane de développement. Les génotypes étaient significativement différents ($p<0,05$) pour la dureté de grains mais pas pour la teneur en protéine. Les différences génotypique en perte de poids grain en raison de la nutrition de larves et des adultes de *S. zeamais*. étaient hautement significative ($P<0,001$). Les génotypes n'ont pas différé significativement ($P>0.05$) dans les indices susceptibilité de Dobie de mais présentaient une gamme large d'index (0,77 à 9,11). Le cultivar traditionnel, Chimambwe, avait la deuxième plus longue période de développement médian, tandis que Pandawe avait les plus lourds amandes, pourtant sans avantage notable dans la résistance au charançon. Les variétés à pollinisation ouvert (OPVs) n'étaient pas supérieures aux hybrides conformément à l'index de susceptibilité de Dobie. La possibilité de développer des hybrides de maïs ou

OPVs qui sont aussi résistants aux charançons de maïs, *S. zeamais*, comme ou même mieux que les cultivars traditionnels est discutée et les recommandations formulées.

Mots Clés: Pollinisation ouverte, *Sitophilus zeamais*, Zambie, *Zea mays*

INTRODUCTION

Maize is the third most produced cereal after wheat and rice in the world. Its principle uses are human consumption and stock feed (Poehlman and Sleper, 1995). In Zambia, maize is the number one cereal in production followed by irrigated wheat (Zulu *et al.*, 2000). The national yield of maize is low and is estimated at 1.5 tonnes per hectare (Pingali, 2001). Commercial yields of maize in the country are well below their potential which are estimated to be in the range of 8.5 t ha^{-1} for early maturing open pollinated varieties to 9.7 t ha^{-1} for intermediate to late maturing hybrids (Vivek *et al.*, 2005).

The low maize yields in Zambia and elsewhere in Africa are due to biotic and abiotic stresses on the crop. The International Maize and Wheat Improvement Centre (CIMMYT), in Harare, Zimbabwe, has classified maize production areas in Africa, into ecological zones and has documented the biotic and abiotic stresses that cause low maize yields in the zones. Zambia belongs to the mid-altitude, sub-tropical ecological zone within the sub-Saharan African region. Abiotic stresses depressing maize yield in this zone are low and declining soil fertility and drought. The biotic stresses include Gray Leaf Spot disease, streak virus disease, and damage by insect stem borers (*Chilo* and *Sesamia* spp.) and other insects including maize weevils. The latter infest maize while the crop is still in the field (Cardwell *et al.*, 2000; Kim and Kossou, 2003; Pendleton *et al.*, 2005; Asawalam and Hassanali, 2006) and destroy the crop during storage (Pingali, 2001).

Despite the increased understanding of the inheritance of weevil resistance and of the resistance mechanisms in the maize grains, there has been very little application of this knowledge in maize breeding programmes (Dhliwayo and Pixley, 2002). Very little work has been done on breeding for maize weevil resistance in storage. In their study of maize weevil resistance involving two synthetic populations and four bi-parental populations, Dhliwayo and Pixley (2002) found that it was possible to improve weevil resistance in maize during storage using conventional breeding methods.

The objectives of this study were to (i) compare the resistance of selected landrace and improved maize genotypes, to the maize weevil *Sitophilus zeamais*, and (ii) characterise grain traits that confer this resistance in the maize genotypes.

MATERIAL AND METHODS

Study area. A field experiment was set up at Golden Valley Agricultural Research Trust (GART) Station, 80 km north of Lusaka, Zambia, during the 2005 - 2006 growing seasons, to multiply the selected landrace and improved maize genotypes for laboratory weevil bioassays and biochemical tests. The experimental set-up was also to advance the F_1 hybrids materials in the study to F_2, the generation normally stored by farmers and which is what should therefore be resistant to the maize weevil damage (Munjoma, 2004).

The Station is located at Latitude14^o 40' South and Longitude 25^o 01' East, and at 1140 m above sea level. The soil type 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 categorized as a Chromic luvisol (FAO, 1998).

Field experiment. Thirty-five F_1 hybrids and 15 Open Pollinated Varieties (OPVs) of maize obtained from CIMMYT and that had in previous studies been classified into various resistance group categories, were used in this study. Furthermore, two local varieties (landraces) from Mbala district of northern Zambia were also included in the experiment (Table 1). The two landraces are locally called Pandawe and Chimambwe. The description of these landraces is given in Table 2.

TABLE 1. Maize materials used in the study

Stock ID	Name	Pedigree	Origin	Comment
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)
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)
A1235-32		[WEEVIL/CML444]-B-5-1-3-BB/[WEEVIL/CML312]-B-18-3-1-B	CIMMYT	Hybrid (Resistant)
A1228-28		[KILIMA ST94A]-19/MSV-03-4-05-B-1-BB-2-2-1-2-B-1//CML395/CML444	CIMMYT	Hybrid Resistant
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
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
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
A1228-29		[KILIMA ST94A]-19/MSV-03-4-05-B-1-BB-2-2-1-2-B-2//MCL395/CML444	CIMMYT	Hybrid Resistant
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
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
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
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
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
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
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
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
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
A1229-7		02SADVE2B-#-45-2//CML312/CML442	CIMMYT	Hybrid Susceptible
V381-4	VH052526	CML395/CML444//[P30/P45//M162W/MSR]97-323-3-1-5-B-1-#-1-B	CIMMYT	Hybrid Susceptible
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
A1011-17		CML443/CML445//CML488	CIMMYT	Hybrid Susceptible
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
V298-25	VH052534	CML312/CML442//NIP25-230-2-1-B-1-B	CIMMYT	Hybrid Susceptible
V317-11	VH053014	CML312/CML442//ZM301c1F2-72-#-1-3-B	CIMMYT	Hybrid Susceptible
V319-38	VH05615	CML181dent/CML182//GQL5	CIMMYT	Hybrid Susceptible
V381-2	VH051960	CML312/CML442//P100C6-61-1-4-##1-3-1-BBB	CIMMYT	Hybrid Susceptible
A1123-4		CML489/CML444//ZM621A-10-1-1-1-2-BBBBB	CIMMYT	Hybrid Susceptible
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
A660-20		CML440/CML444//CML445	CIMMYT	Hybrid Susceptible
A1118-1		CML488/CML444//CML312/CML442	CIMMYT	Hybrid Susceptible
J15-9		CML312/CML444//CML489	CIMMYT	Hybrid Susceptible
A1238-16		CML444/CML197//CML488	CIMMYT	Hybrid Susceptible
A923-7		CML443/CML445//[CML444/ZSR923S4BULK-2-2-X-X-X-X-1-BB]-1-1-1-1-1-B	CIMMYT	Hybrid Susceptible
A923-8		CML443/CML445//[EARLY/ZM621A]-14-1-1-2-2-B	CIMMYT	Hybrid Susceptible

TABLE 1. Contd.

Stock ID	Name	Pedigree	Origin	Comment
A1229-6		02SADVE2B-#-45-1//CML312/CML442	CIMMYT	Hybrid Susceptible
J11-12		SYN[SZ/Elite01]	CIMMYT	OPV Resistant
A1142		SYN[WEEVIL-A(FSINDEX)]	CIMMYT	OPV Resistant
A1143		SYN[WEEVIL-A(FSWEEVRESIST)]	CIMMYT	OPV Resistant
A1145		SYN[WEEVIL-B(FSINDEX)]	CIMMYT	OPV Resistant
A1146		SYN[WEEVIL-B(FSWEEVRESIST)]	CIMMYT	OPV Resistant
J11-42		04WEEVILA-#	CIMMYT	OPV Resistant
J11-44		04WEEVILB-#	CIMMYT	OPV Resistant
A1037	VP0535	ZM305	CIMMYT	OPV Susceptible
A1147		SYN[WEEVIL-B(FSWEEVSUSCEPT)]	CIMMYT	OPV Susceptible
A771-3		02SADVL	CIMMYT	OPV Susceptible
A1174		02SADVL	CIMMYT	OPV Susceptible
A1043		ZM621	CIMMYT	OPV Susceptible
A545-8		ZM623	CIMMYT	OPV Susceptible
A1176		02SADVL2	CIMMYT	OPV Susceptible
A1144		SYN[WEEVIL-A(FSWEEVSUSCEPT)]	CIMMYT	OPV Susceptible
	PANDAWE	Not applicable	Mbala	Landrace
	CIMAMBWE	Not applicable	Mbala	Landrace

CML = CIMMYT maize line; 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 Colour	Grain size	Maturity	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 Magorokosho, 2006

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

A field experiment was laid out using a randomised complete block design with 3 replications. Each plot consisted of 4 rows measuring 5 m long. The inter- and intra-row spacing was 90 cm and 25 cm, respectively. Two maize 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 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 and elsewhere in central Zambia. Atrazine was applied for weed control 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 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.

Laboratory assays. The maize weevils used in this study were collected from bags of weevil-infested maize, harvested from field trials at Mount Makulu Central Research Station, Chilanga, Zambia, during the previous growing season. The genotypes that were multiplied at GART were evaluated for maize weevil resistance by maize weevil bioassays using a modified Dobie's method (Dobie, 1977; Serratos *et al*., 1993). This was conducted at Mount Makulu Central Research Station.

Three cobs of each genotype were hand-shelled and the grain packed into 5 x 8 polythene bags. The bags were closed with rubber bands and then stored in a deep freezer for one week to kill any previous infestation by insects, including adults, larvae or eggs (Kossou *et al*., 1993). The temperature in the freezer was minus -16 ^{o}C.

Samples of 50 g of grains of each genotype were taken into new 350-ml plastic jars. 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. The tops of the lids of these jars were cut out, leaving only the screw-top rings. Forty 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) were poured into each jar.

To close the jars after the introduction of weevils, a piece of 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.

Grain of SC 513 maize variety from Seedco, an international seed company operating in Zambia, was included in the experiment as a susceptible variety check. This brought the total number of treatments to 53. Genotype entry numbers written on stickers were used to label the jars.

The jars were placed in controlled temperature and relative humidity room and laid out in an RCBD with each shelf constituting a block. The temperature in the room was maintained at 29 ± 1 ^{o}C by a thermostat-controlled heater mounted on a wall. The relative humidity ranged from 43 to 60%; the humidity being provided by water placed in four troughs (Bekele and Hassanali, 2001).

After an oviposition period of ten days, the adult (parents) maize weevils were removed from the samples by sieving with a Standard U.S.A. Testing Sieve set (VWR Scientific, West Chester, PA 19380,U.S.A.). The powder, where present, 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 were collected on the No. 18 sieve; while the grain remained on the No. 8 sieve.

Live and dead weevils were counted using tweezers and a tally counter. Tweezers were also used to probe immobile weevils to establish whether they were dead. Weevils, like some other beetles, tend to feign death when disturbed (Baker,2007).

Sieving and checking for emergence of the F_1 progeny started 3 weeks following the removal of the parents (Serratos *et al*., 1993). Sieving and counting the F_1 progeny was done every 2 days (Derera *et al*., 2001) and the sieved insects were discarded. This interval of counts did not pose a risk of the F_1 progeny laying eggs in the maize samples to produce the F_2 generation, considering the fact that individuals of S*itophilus zeamais* do not mate before they are three days old (Danho *et al*., 2002).

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 kernel weight.

Grain hardness. 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 Retsh Laboratory Mill, 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 number 11 sieve 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 cm 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.

Kernel weight. The number of kernels contained in a 50 ± 0.1 g grain sample of each genotype was determined and this number was divided into 50 g to obtain the weight per kernel.

Protein content. Twenty-grammes 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 used as the criterion to separate genotypes into different resistance groups (Dobie, 1977; 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 (i.e. 50 percent emergence) of the F1 progeny.

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

The Dobie 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 index of $\leq$ 4 was classified as resistant;
Dobie index of 4.1 to 6.0 was moderately resistant;
Dobie index of 6.1 to 8.0 was moderately susceptible;
Dobie index of 8.1 to 10 was susceptible; and
Dobie index of >10 was classified as highly susceptible.

Statistical analyses. The Analysis of Variance (ANOVA) for all the measured parameters was done using the Mstat-C Programme (Freed *et al*., 1988). Total progeny emergence data were transformed to log base 10 before subjecting them to ANOVA (Dhliwayo and Pixley, 2003). Before conducting the log transformation, a value of 1 was added to all data points because of the presence of zeros in some data points in the data set. The mean separation, in cases where there were significant differences among treatments, was done using LSD (0.05) to facilitate the comparison of all pairs of treatment means (Montgomery, 2001).

RESULTS

Protein content. Genotypes were not significantly different (P>0.05) for protein content (Table 3). However, 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 4).

Grain hardness. Grain hardness showed discrimination among the 52 genotypes (P < 0.05). However, genotypes exhibited a higher or lower degree of hardness regardless of whether they were hybrids or OPVs. The two landraces were ranked low in hardness in relation with 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 5).

Parent survival. Table 6 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.

TABLE 4. 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
Mean	9.8	
LSD (5%)	2.6	

TABLE 3. 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			

Grand Mean = 9.801~ Grand Sum = 1529.000~ Total Count = 156. Coefficient of Variation = 16.21%

TABLE 5. Comparative hardness between the top 5 and the least 5 genotypes

Entry	Grit (%)	Classification
Hybrids		
Top 5		
9	72.3	Resistant
6	71.8	Resistant
1	71.4	Resistant
13	71.2	Resistant
8	71.1	Resistant
Least 5		
27	62.9	Susceptible
26	62.6	Susceptible
24	61.0	Susceptible
33	60.0	Susceptible
25	59.7	Susceptible
OPVs		
Top 5		
39	74.0	Resistant
48	71.3	Susceptible
41	71.2	Resistant
46	71.0	Susceptible
43	70.0	Susceptible
Least 5		
36	67.5	Resistant
50	66.6	Susceptible
37	66.3	Resistant
49	65.8	Susceptible
40	65.4	Resistant
Landraces		
Pandawe	62.6	Unknown
Chimambwe	63.9	Unknown
Mean	67.76	
LSD (5%)	6.65	

Progeny emergence. Emergence of the F_1 progeny was different among genotypes. Figure 1 shows an F_1 adult progeny emerging from a kernel and an exit hole left by another F_1 adult weevil. The total of all the F_1 progeny adult weevils for each genotype is presented in Table 6. The grand emergence mean was 13.43 and the range was 1.67 for entry number 8 and 50 to 86.67 weevils for the susceptible check. For the total weevil emergence data, the differences were highly significant ($P<0.001$) but for the transformed data, the differences were not significant ($P>0.05$).

Grain weight loss. The greatest weight loss of 8.563 g occurred in entry 53, a susceptible check (Table 6), while the lowest weight loss of 1.7 g was recorded in entry number 2.

Dobie index of susceptibility. The Dobie Index of susceptibility ranged from 0.77 for entry numbers 8 and to 9.11 for the susceptible check (Table 6). The trial mean was 3.77. However, the Dobie index re-classified the susceptible genotypes to be resistant or moderately resistant, except for the check which was susceptible (Table 7).

DISCUSSION

Protein content. Although genotypes were not statistically different for protein content (Table 1), a closer look at the best 5 and worst 5 genotypes revealed 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 (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 resistance parameters in this study may indicate that there are other resistance factors in maize studied. Arnason *et al*. (1994; 1997) reported the presence of biochemical compounds, particularly ferulic acid in the kernels.

Grain hardness. The differences among genotypes for grain hardness in this study were expected when there is a large number of genotypes in an experiment being evaluated. 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 5). 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.

TABLE 6. Maize weevil bioassay for selected maize weevil resistance parameters for 53 maize genotypes

Kernel weight (mg)	Parent survival (%)	F_1 progeny emergence (no.)	F_1 Progeny Emergence (Log 10)	Median development period (days)	Grain weight loss (g)	Weight loss (%)	Dobie index
373.1	40.83	6.667	0.81	64.667	2.093	4.2	3.12
333.3	19.17	2.333	0.233	99.333	1.763	3.6	0.867
409.8	35.83	9.667	0.743	80	2.182	4.3	2.893
362.3	30	3.667	0.553	60	2.130	4.3	2.18
335.6	17.5	8.667	0.817	58.333	1.850	3.7	3.37
359.7	41.67	6.667	0.8	57.333	2.277	4.5	3.29
373.1	25.83	11.00	0.593	74.667	2.390	4.8	2.607
312.5	13.33	1.667	0.2	79.333	2.027	4.1	0.777
387.6	27.5	5.667	0.62	49.333	2.170	4.4	2.763
400.0	50.83	4.667	0.533	51.333	1.823	3.6	2.62
365.0	48.33	19.00b	1.19	49	2.407	4.8	5.547
403.2	40	4.333	0.627	47.333	3.237	6.5	3.093
420.2	53.33	4.667	0.577	59.667	1.950	3.9	2.7
354.6	13.33	4.000	0.56	50	2.120	4.2	2.713
438.6	39.17	7.000	0.833	66.667	2.122	4.2	2.92
403.2	38.33	7.667	0.693	74.333	2.130	4.2	3.04
384.6	21.67	8.667	0.793	46	2.407	4.8	3.77
409.8	37.5	12.33	1.003	42.333	2.413	4.8	5.353
375.9	10	5.667	0.6	74.667	2.317	4.6	2.607
393.7	17.5	11.33	0.793	75.333	2.460	4.9	3.387
406.5	16.67	11.67	0.603	68.333	2.672	5.3	2.86
427.4	38.33	15.67	1.15	48	2.440	4.9	5.453
406.5	30	23.67	1.03	72.667	2.392	4.8	4.737
335.6	51.67	13.33	1.057	55	2.393	4.8	4.68
314.5	20.83	16.33	1.03	54.667	2.377	4.7	4.73
416.7	39.17	27.00	1.333	53	2.887	5.8	5.89
357.1	42.5	13.00	0.763	75	2.227	4.5	3.333
431.0	50	25.67	1.053	73.333	3.382	6.8	4.757
393.7	28.33	6.000	0.583	44	2.017	4.1	3.02

TABLE 6. Contd.

	Kernel weight (mg)	Parent survival (%)	F_1 progeny emergence (no.)	F_1 Progeny Emergence (Log 10)	Median development	Grain weight loss (g)	Weight loss (%)	Dobie index
	406.5	24.17	11.33	1.01	43.333	2.117	4.2	5.507
	416.7	36.67	9.333	0.917	41	2.060	4.1	5.393
	362.3	15.83	8.667	0.813	58.333	1.997	4.0	3.45
	375.9	40.83	14.67	0.977	39.667	2.277	4.5	5.683
	277.8	21.67	7.000	0.793	58.667	2.313	4.6	3.147
	362.3	8.	29.00	1.057	73	2.487	5.0	4.81
	390.6	45	35.33	1.373	51.333	3.673	7.4	6.197
	243.9	48.33	17.00	1.197	51	2.790	5.6	5.417
	393.7	33.33	12.33	0.813	73.667	3.202	6.4	3.623
	373.1	23.33	3.667	0.443	76.667	2.647	5.3	1.797
	340.1	33.33	20.33	0.98	74.667	2.950	5.9	4.257
	354.6	19.17	10.33	0.757	74.333	2.070	4.1	3.347
	318.5	25.83	21.00	0.973	43.667	2.260	4.5	5.137
	480.8	38.33	14.00	0.96	52	2.393	4.8	4.32
	365.0	50	6.667	0.733	57.667	2.320	4.6	3.017
	400.0	40.83	4.667	0.653	64.667	2.150	4.3	2.343
	396.8	29.17	20.00	0.897	75.667	2.380	4.8	3.927
	416.7	36.67	6.667	0.78	66.667	2.023	4.0	2.717
	384.6	49.17	24.67	1.27	50	2.497	5.0	6.02
	438.6	32.5	8.000	0.703	71.667	2.160	4.3	3.457
	347.2	17.5	1.667	0.2	66	2.102	4.2	1.377
	495.0	34.17	19.00	0.963	74.667	2.980	5.9	4.177
	312.5	31.67	22.00	0.603	96.333	2.963	5.9	2.617
	Not assessed	84.17	86.67	1.92	48.667	8.563	17.1	9.11
MMean	363.4	33.76	13.43	0.829	62.019	2.49	5	3.772
LSD (5%)	61.2	30.82	23.06	0.834	40.92	0.9	6.773	3.73

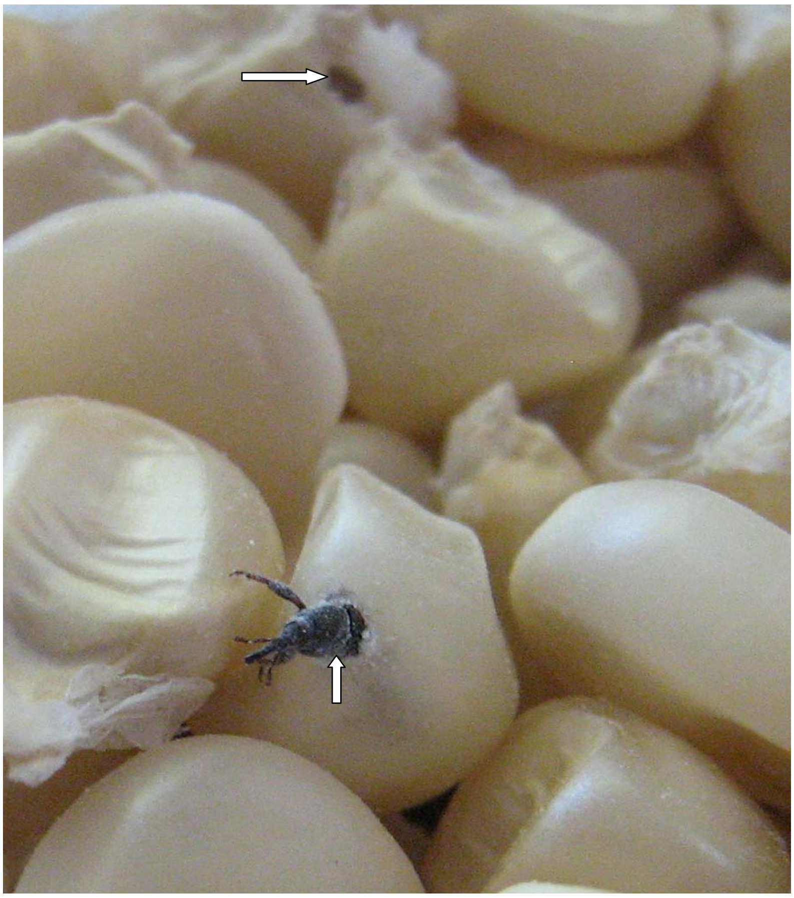

Figure 1. An F_1 adult weevil progeny emerging from a kernel (lower arrow) and an emergence hole left by another weevil (upper arrow).

Parent survival. Parent weevil survival tended to be higher in susceptible than resistant genotypes. 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 F_1 progeny. The susceptible check yielded 86 F_1 progeny compared to a grand mean of 13 and means of less than 1 in treatment 2 and 8 (Table 6).

Progeny emergence. Progeny emergence tended to be higher in susceptible genotypes than in resistant ones (Garcia-Lara *et al*., 2004). In this study, the susceptible check had the highest number of the total F_1 emergence, numbering up to 87 weevils, against the experimental mean of 13 weevil emergencies. The total F_1 progeny emergence may have been reduced in the whole experiment by mechanical disturbance of the samples through the action of sieving every 2 days (Ungunantwiwat and Mills, 1979).

Median development period (MDP). The median development period in this study was very high, ranging from 39.667 in entry number 33, to 99.3 in entry number 2 (Table 6). Other workers have reported lower ranges MDPs, for example, MDP ranges of about 4 to 40 days. The longer MDPs in this study may be attributed to the less than optimal relative humidity in the constant climate room.

Grain weight loss. The highest loss again occurred in the susceptible check, in which 8.5 g

TABLE 7. Comparative classification of maize to maize weevil resistance using Dobie index

Class	CIMMYT classification	Dobie index	New classification§
Hybrid	Resistant	3.12	Resistant
Hybrid	Resistant	0.867	Resistant
Hybrid	Resistant	2.893	Resistant
Hybrid	Resistant	2.18	Resistant
Hybrid	Resistant	3.37	Resistant
Hybrid	Resistant	3.29	Resistant
Hybrid	Resistant	2.607	Resistant
Hybrid	Resistant	0.777	Resistant
Hybrid	Resistant	2.763	Resistant
Hybrid	Resistant	2.62	Resistant
Hybrid	Resistant	5.547	Moderately resistant
Hybrid	Resistant	3.093	Resistant
Hybrid	Resistant	2.7	Resistant
Hybrid	Resistant	2.713	Resistant
Hybrid	Resistant	2.92	Resistant
Hybrid	Resistant	3.04	Resistant
Hybrid	Resistant	3.77	Resistant
Hybrid	Susceptible	5.353	Moderately resistant
Hybrid	Susceptible	2.607	Resistant
Hybrid	Susceptible	3.387	Resistant
Hybrid	Susceptible	2.86	Resistant
Hybrid	Susceptible	5.453	Moderately resistant
Hybrid	Susceptible	4.737	Moderately resistant
Hybrid	Susceptible	4.68	Moderately resistant
Hybrid	Susceptible	4.73	Moderately resistant
Hybrid	Susceptible	5.89	Moderately resistant
Hybrid	Susceptible	3.333	Resistant
Hybrid	Susceptible	4.757	Moderately resistant
Hybrid	Susceptible	3.02	Resistant
Hybrid	Susceptible	5.507	Moderately resistant
Hybrid	Susceptible	5.393	Moderately resistant
Hybrid	Susceptible	3.45	Resistant
Hybrid	Susceptible	5.683	Moderately resistant
Hybrid	Susceptible	3.147	Resistant
Hybrid	Susceptible	4.81	Moderately resistant
OPV	Resistant	6.197	Moderately susceptible
OPV	Resistant	5.417	Moderately resistant
OPV	Resistant	3.623	Resistant resistant
OPV	Resistant	1.797	Resistant
OPV	Resistant	4.257	Moderately resistant
OPV	Resistant	3.347	Resistant
OPV	Resistant	5.137	Moderately resistant
OPV	Susceptible	4.32	Moderately Resistant
OPV	Susceptible	3.017	Resistant
OPV	Susceptible	2.343	Resistant
OPV	Susceptible	3.927	Resistant
OPV	Susceptible	2.717	Resistant
OPV	Susceptible	6.02	Moderately susceptible
OPV	Susceptible	3.457	Resistant
OPV	Susceptible	1.377	Resistant
Landrace	Unclassified	4.177	Moderately Resistant
Landrace	Unclassified	2.617	Resistant
Hybrid	Susceptible check	9.11	Susceptible
Mean		3.772	
LSD (5%)		3.73	

The classification was based on the Dobie index 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 0 to 3 weeks after which there is a steady decline. Since the weevils that were used in this experiment were of unknown age, it is possible that some of them were older than the optimum age for feeding and reproduction.

Dobie index of susceptibility. The range of values of indices obtained in this experiment (0.77 to 9.11) was lower than those obtained by other investigators. Arnason *et al*. (1994) obtained indices as high as 14 in susceptible varieties. One possible explanation is that the previous studies dealt with much more susceptible genotypes than in this experiment. Another cause could be the differences in moisture content. Most researchers infest their samples at about 14% moisture content. For instance, the maize samples that Arnason *et al*. (1994) used in Canada had moisture content ranging from 10.4 to 14.90%. The Dobie Index of Susceptibility from such maize samples then ranged from 0 for a resistant check to 15.2 for a susceptible check. The maize samples in the present study had moisture content of 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 for grain moisture content below 12%, the resistant genotype, population 84, provided effective control for both insect species. However, once the moisture content reached 16%, 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. Previous studies (Dobie, 1974) have shown that the fecundity of weevils is highest when they are 0 - 21 days old.

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 contained anthocyanin, and/or flint, as well as dent grain. The departure from the CIMMYT classification observed in some genotypes could be due to the effect of environment. Kim and Kossou (2003) studied the response and genetics of maize germplasm resistant to the maize weevil in Nigeria and found highly significant ($P<0.01$) data of crosses x location interactions for number of egg plugs, F_1 weevils, damaged kernels and percent weevil survival. They concluded that the interactions indicated environmental effects on maize weevil resistance to weevils. Similarly, Duarte *et al*. (2005), in a study of nitrogen 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.

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