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Comparing the Effectiveness of the "weevil warehouse" and "laboratory bioassay" as Techniques for Screening Maize Genotypes for Weevil Resistance

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Abstract The maize weevil (*Sitophilus zeamais* Motschulsky), causes devastating post-harvest grain losses, especially in tropical countries. Development of weevil resistant maize hybrids requires a rapid, inexpensive but effective screening method which can easily be incorporated in a maize breeding program without any advanced training in entomology. The current study compared the efficacy of weevil warehouse which is a kind of free-choice test with laboratory bioassay following a no-choice test, for discriminating maize genotypes into different weevil resistance/susceptibility classes. Fourteen maize genotypes were simultaneously screened using the weevil warehouse and the laboratory bioassay techniques. Results from both shelled grain and suspended ears under weevil warehouse assessments were compared with those from laboratory bioassay technique. Grain damage and grain weight loss were measured. High levels of consistency were detected during grouping of maize genotypes. The shelled grain option of the weevil warehouse and the laboratory bioassay screening methods were equally effective towards discriminating maize genotypes for their response to weevil attack (CVs of 7.1% vs 6.5% for grain damage and 12% vs 13% for grain weight loss, respectively). Therefore, the "weevil warehouse" technique, which is simple, inexpensive, time saving and precise would be recommended for rapid screening of maize germplasm for maize weevil resistance.

Keywords: *weevil screening method, weevil warehouse, grain resistance*

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1. Introduction

Maize is one of the principal cereal food crops in the tropics and subtropics [1] and forms an essential component of the global food security as a major part of the diet of millions of people in Africa [2]. It grows under a wider range of ecological conditions depending on the varieties [3]. The crop is versatile in its use, environmental adaptation and it is also consumed all over the world by both human beings and animals [4]. Increasing maize production and productivity has been achieved through development of high yielding stress tolerant varieties. Despite this intervention at production level, there is evidence of food insecurity arising from storage losses. Postharvest losses particularly due to pests threaten the livelihoods of farmers across Africa [2]. The maize weevil (*Sitophilus zeamais* Motschulsky) is the most prevalent and hence destructive storage pest of maize in the tropics [5,6,7,8]. The maize weevil attacks the crop before harvest and multiplies further during storage [9].

Cugala *et al*. [10] reported grain loss of 20 - 90% worldwide due to the maize weevil. Stored crop insect management technologies among rural communities include the application of chemical pesticides that are expensive to buy, unreliable in terms of time availability and inappropriate handling practices [11]. Germplasm screening is a vital step when breeding for weevil resistance in maize [12]. However, for effective identification of weevil resistant genotypes, fast, cheap and precise maize weevil screening techniques would be required [7,8,13,14,15]. Various weevil screening parameters and procedures have been used to discriminate maize

germplasm into different susceptibility classes [16,17]. Makate [1] reported that F_1 weevil progeny emergence was a more consistent parameter for discriminating maize genotypes into different weevil susceptibility classes, as opposed to parental weevil mortality. Derera *et al.* [18] developed one of the most popular weevil screening methods that utilizes F_1 weevil progeny emergence and the median development period (MDP) to calculate the Dobie index of susceptibility (DIS). The DIS discriminates maize genotypes into resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible, based on the magnitude of the index in comparison with the resistant and susceptible checks [15]. The DIS deploys laboratory-based bioassay procedure that exploits the "no-choice" screening procedure. Generally, the Dobie [19] method being laboratory based, it is expensive as it is labour intensive and requires laboratory equipment, in addition to the long screening period of at least 100 days. Breeders require a rapid method for screening genotypes so that they can select and plant in the same year [15]. Many attempts have been made to modify Dobie's methodology so as to reduce the screening period while maintaining the accuracy of the index [16,20]. Urrelo *et al.* [20] modified Dobie's [19] technique by substituting F_1 progeny emergence with the number of egg plugs, and the MDP with days to first emergence. However, their method requires more labour for counting the egg plugs especially at the initial stages [21]. Urrelo *et al.* [20] method also requires more skills when identifying the egg plugs through staining and thus it is expensive. In similar regard, Derera *et al.* [16] modified the Dobie's methodology by using a shorter period for oviposition and also determining the minimum number of adult maize weevils required for screening maize without sexing. Generally, most of the weevil screening methods reported are laboratory-based protocols requiring much capital investment in terms of equipment and skilled personnel, in addition to a long period requirement. Furthermore, laboratory bioassay system entails artificial screening conditions, which may not fully simulate the natural conditions under which maize would respond to weevil attack [15]. Consequently, genotype response to weevil infestations ought to be affected by the screening conditions [22]. Therefore, validation of a fast, simple, cheap and effective weevil screening technique simulating farmers' storage conditions would enhance maize screening and ultimately breeding for weevil resistance. Reports indicate that the free-choice weevil warehouse screening method is effective as a preliminary screening procedure for identifying donor parents for weevil resistance breeding [23]. However, its effectiveness has not been fully explored in advanced stages of maize evaluations. The weevil warehouse ought to be cheaper and time saving, since it does not require expensive laboratory equipment and artificial conditions, as opposed to the laboratory bioassay technique. The objectives of the study were to i)- Compare the effectiveness of shelled grain and suspended maize ears under weevil warehouse method with laboratory bioassay technique in discriminating maize genotypes into different weevil susceptibility classes; ii)- Determine the minimum time required by the weevil warehouse technique to discriminate maize genotypes into different susceptibility classes.

2. Materials and Methods

2.1. Germplasm

The study involved 11 inbred lines, one single cross hybrid and two open pollinated varieties (OPV) obtained from CIMMYT and National Crops Resources Research Institute (NaCRRI). Characteristics of entries used are given in Table 1. Entries M9 and M10 were used as resistant checks, while M11 and M12 were used as susceptible checks.

In order to obtain sufficient quantities of experimental materials, the entries were first planted at NaCRRI, Namulonge (0°32'N, 32°34'E, 1200 mm) to produce enough ears/kernels required for screening purposes. To maintain the genetic purity of the genotypes, open pollinated varieties were grown in isolated fields, while inbred lines were sib-mated and covered with paper bags to exclude outcrossing.

Table 1. Characteristics of maize entries used in the study

Genotype code	Genotype name	Genotype category	Origin	Response to weevil infestation
M1	CL106507	Inbred line	CIMMYT	Not known
M2	CL106508	Inbred line	CIMMYT	Not known
M3	CL106509	Inbred line	CIMMYT	Not known
M4	CL106511	Inbred line	CIMMYT	Not known
M5	CL106512	Inbred line	CIMMYT	Not known
M6	CL106513	Inbred line	CIMMYT	Not known
M7	CL106514	Inbred line	CIMMYT	Not known
M8	CL106515	Inbred line	CIMMYT	Not known
M9	[weevil/CML312]-B-13-2-1-BBB/[weevil/CML387]-B-9-1-1	Single cross Hybrid	CIMMYT	Resistant
M10	07WEEVIL	Inbred line	CIMMYT	Resistant
M11	Longe5	OPV	NaCRRI	Susceptible
M12	Popcorn	OPV	NaCRRI	Susceptible
M13	WL-118-9	Inbred line	NaCRRI	Resistant
M14	WL-118-3	Inbred line	NaCRRI	Resistant

2.2. Maize Weevil Rearing

Maize weevils were first reared to get adequate numbers of the same age (0 to 7 days). To provide for weevil acclimatization, rearing was done under the same conditions as the screening environment. Thus, weevils used for screening under laboratory conditions were reared in the laboratory and those for the warehouse technique were reared in the maize crib. Weevils for laboratory bioassay technique were reared by incubating a weevil-maize grain culture for 14 days in the laboratory at a temperature of 28±2°C and a relative humidity of 70±5%, to enhance oviposition. The weevil-maize grain culture was established by introducing about 300 to 400 unsexed adult weevils into 3000 cm^3 plastic jars containing about 1.5 Kg of susceptible maize grain. Ventilation in the plastic jars was achieved by perforating the lids of the plastic jars and fitting them with gauze-wire mesh of pore size <1 mm to prevent the weevils from escaping. A fan heater and a humidifier were used for regulating the temperature and relative humidity, respectively. After two weeks the maize-weevil cultures were sieved to separate weevils from the grain. The maize grain was returned to the plastic jars and incubated under the same conditions to allow the oviposited eggs to hatch to F_1 weevil progenies. The emerging F_1 weevil progenies were collected for one week to produce the 0 to 7 days' old weevils which were used for grain screening.

On the other hand, weevils used for screening maize genotypes under free choice screening techniques were reared in the maize crib in the same conditions under which screening was done. Usually, the prevailing temperature and relative humidity ranges from 16.2 to 32.3°C and 55 to 89%, respectively. Weevils used for screening in season 2011B were reared between July and August of 2011, while those used in 2012A season were reared between January and February 2012.

2.3. Preparation of Maize Genotypes for Screening against Weevil Infestation

Adequate cobs for each genotype were harvested and sun dried to a moisture content range of 13 – 15%. Cobs for shelled grain and laboratory bioassay techniques were shelled while those for suspended ears were left intact. All experimental materials were initially subjected to cold treatment at -20°C for 14 days to eliminate field infestations and later were acclimatized for 7 days at room temperature under weevil free environment.

2.4. Screening under Laboratory Bioassay Technique

The quantity of maize grain used per genotype was 50 grams, these were weighed into 250 cm^3 glass jars with perforated lids fitted with <1 mm gauze wire mesh for ventilation and blockage of weevil escape. The grain was infested by 32 unsexed weevils of age 0 to 7 days. The experiment was arranged in a randomized complete block design with each genotype (weevil-grain culture) replicated six times. The weevil-grain cultures were first incubated for 14 days to allow oviposition as described by Derera *et al.*, [18]. The weevils were then sieved out of the cultures and the grain maintained at temperatures 28±2°C and relative humidity 70±5% until the end of the screening exercise. During the incubation period, the grain was monitored every two days to record and remove any F_1 weevil progenies which emerged [24]. Recording continued until no more weevils were emerging from any of the genotypes. The data recorded included: the total number of parental weevils which were alive and/or dead, the total number of F_1 weevil progenies that emerged from each entry, and the median development period (MDP), which was calculated as the period in days between the middle of oviposition to 50% emergence of the F_1 weevil progenies. Other data collected were grain damage and grain weight loss [12].

2.5. Weevil Warehouse Technique

This technique embraces free-choice weevil screening methods, which for this study entailed evaluation of both unshelled ears suspended in the maize crib in nylon mesh bags and shelled maize grain put in paper bags following procedures of Bergvinson [25], modified by adding adult maize weevils to the test genotypes to enhance grain infestation.

2.6. Screening Suspended Ears

Four uniformly sized ears for each genotype were put in gauze wire mesh bags, weighed to determine their original weights and suspended on rafters (suspended ears) of the maize crib. For purposes of experimental precision, the distance from one sample to another was maintained at 30 cm. Similarly, the height from the crib floor to the suspended maize ears, was maintained at 1 m. Overall, six replications were used per genotype and were arranged in a randomized complete block design, thus totaling 84 sampling units used for the entire experiment. The experiment was divided into two sets, each comprising of three replications. The first set was left intact for the 4month and then weighed and scored for the maize weevil damage. The second set was examined every month to record the monthly weight loss and grain damage up to 4 months when the experiment was discontinued. The second set was designed to determine the minimum period required to characterize maize genotypes based on their response to weevil attack. Infestation was initiated by opening six plastic jars each containing 1500 adult weevils. The plastic jars were arranged in the crib in such a way that all suspended ears had equal chances of being infested at the same time and by the same number of weevils, when searching for food (suspended ears). Unwanted infestations from other insects were minimized by fitting a gauze-wire mesh of pore sizes ≤1 mm at the sides of the maize crib. Scoring for ear damage by the weevils was done using the scale of 1 to 10 while following procedures of Tadele *et al.* [26]: where 1 = 0 to ≤ 10% damage, 2 = 11 to 20% damage, 3 = 21 to 30% damage, 4 = 31 to 40% damage, 5 = 41 to 50% damage, 6 = 51 to 60% damage, 7 = 61 to 70% damage, 8 = 71 to 80%, 9 = 81 to 90% damage, and 10 = 91 to 100% damage.

2.7. Screening Shelled Grain in Paper Bags

Grain amounting to 100 grams was weighed for each entry and separately put into paper bags of size 10 x 10 x 15 cm for the length, width and height, respectively. Small holes that could not allow the maize grains to fall out were punched at the sides of the paper bags to enhance aeration. Fourteen paper bags, each containing one genotype were randomly assigned into plastic buckets. These were put at the sides (walls) of the bucket to maintain a uniform distance between them and the source of weevils that was placed in the center of the bucket to ensure that an equal chance of being infested at the same time by all genotypes during the experiment. Then 250 cm^3 glass jars containing 700 adult weevils were opened to allow the weevils to attack the genotypes of their choice in an experiment of six replications with each replication contained in one big bucket. The six replications were equally divided into two sets of three replications. The first set was left intact until the end of the experiment while the other one was used for monthly data collection, to determine the minimum period in months required to characterize genotype susceptibility to weevil infestation. For both sets, data was recorded on grain weight, grain damage, weight of damaged and undamaged grains. Grain weight loss was determined as the difference between the original (100 g before infestation) and the new weight after infestation. Percent weight loss was then calculated following procedures of Gwinner *et al.* [27]; Percent gain weight loss = (Wu x Nd) – (Wd x Nu) x 100 / Wu x (Nd + Nu); where Wu: weight of undamaged grain, Nd: number of damaged grains, Wd: weight of damaged grains, Nu: number of undamaged grains.

2.8. Data Analysis

The data obtained on the various parameters (grain weight loss, median development period, Dobie's index of susceptibility, percent grain damage) were subjected analysis of variance using GenStat Statistical Software (14^{th} Edition). The differences between means were detected using least significant differences at a 5% probability level (LSD = 0.05). Overall genotypic responses to the maize weevil infestation were derived from the following models: $Y_{ij} = \mu + R_i + E + e_{ij}$. Where Y_{ij}: Observed value of the trait; μ: Overall mean of the trait; R_i: Effect of the i^{th} replication; E_j: Effect of the j^{th} entry; and e_{ij}: Residual effect. The monthly period response to maize weevil infestation was estimated from the following model: $Y_{ijk} = \mu + R_j + P_j + E_k + EP_{ik} + e_{ijk}$. Where Y_{ijk}: Observed value of the trait; μ: Overall mean of the trait; R_i: Effect of the i^{th} replication; P_j: Effect of the j^{th} period; E_k: Effect of the k^{th} entry; EP_{ik}: Effect of the interaction of the k^{th} entry in the j^{th} period; and e_{ijk}: Residual effect.

3. Results and Discussion

3.1. Response of the Maize Genotypes to Weevil Infestations

The mean squares for grain damage and grain weight loss exhibited in the 14 genotypes under laboratory bioassay, shelled grain and suspended ears techniques are presented in Table 2. The results indicated that the genotypes were highly significant ($P<0.001$) for the two weevil susceptibility parameters assessed. The significant entry mean square for grain damage and grain weight loss indicated that the genotypes responded differently towards weevil infestations under the three screening techniques [18].

The response to weevil infestation and the ranks of the 14 maize genotypes as exhibited by grain damage and weight loss encountered under laboratory bioassay and weevil warehouse conditions (shelled grain and suspended ear) are shown in Table 3. The results revealed the variations in response to weevil attack that existed among the 14 genotypes, and this provided the basis for genotype discrimination. Wide variations were observed among the 14 genotypes for the three weevil screening techniques. As regards to grain damage, more damage was encountered under the "shelled grain" option of the weevil warehouse screening technique than that encountered under the laboratory bioassay and suspended ear techniques. Generally, higher grain damage levels encountered under shelled grain were probably due to the larger surface area exposed to weevils for attachment and subsequent boring, as compared to the relatively smaller surface area exposed to weevils for attachment under unshelled (suspended) ears. These results agree with Kossou *et al.* [28] who reported reduced oviposition, increased median development period and subsequently less damage in unshelled maize ears than in shelled ears in Benin. The results are also consistent with the traditional practices by farmers who store unshelled maize.

Table 2. Mean squares for grain damage and grain weight losses exhibited by the study genotypes under laboratory bioassay and weevil warehouse techniques

SOV	DF	Laboratory bioassay		Weevil warehouse method			
		Shelled grain		Shelled grain		Suspended ears	
		GD (%)	GWL (%)	GD (%)	GWL (%)	GD (1-10)	GWL (%)
Rep	5	7.48	19.73	32.24	5.45	8.55	5.79
Entry	13	819.25***	261.23***	704.02***	550.52***	1883.94***	206.42***
Error	65	13.27	10.06	19.71	15.29	12.32	10.76
R^2		0.93	0.84	0.88	0.88	0.97	0.80
CV (%)		6.55	13.27	7.11	12.24	6.87	17.55

***: Significant at 0.001, SOV: Source of variation, DF: Degree of Freedom, GD: Grain damage, GWL: Grain weight loss.

For parameter grain damage under laboratory bioassay technique, genotype M13 encountered the least damage of 35.83%, while the susceptible control genotype M12 encountered the highest damage of 70.67%. The damage encountered by genotype M8 (38.83%) was not significantly (P>0.05) different from the least damaged genotype M13 therefore, the two genotypes were categorized as resistant. On the other hand, genotypes M1 and M7 encountered high damages that were not significantly (P>0.05) different from the most damaged entry i.e. susceptible control M12 and therefore genotypes M1 and M7 were categorized as susceptible genotypes. The rest of the genotypes encountered grain damage that was significantly higher than the least damaged genotype (M13) and at the same time significantly lower than the most damaged genotype (M12) and hence they were categorized as moderately resistant. Accordingly, the moderately resistant genotypes were M2, M3, M4, M5, M6, M9, M10, M11 and M14. According to this classification, the resistance control genotypes M9 and M10 and the susceptible control genotype M11 were grouped as moderately resistant thus suggesting that they are not the most suitable control genotypes for weevil screening studies. Therefore, appropriate control genotypes would be required for future screening tasks.

For grain damage under shelled grain technique, genotypes M8, M9, M13 and M14 were categorized as resistant genotypes; genotypes M1, M3, M4, M5, M6, M10 and M11 were categorized as moderately resistant; while genotypes M2, M7 and M12 were categorized as susceptible. For grain damage under suspended ears, genotypes M3 and M13 were grouped in the resistant class, genotypes M1, M4, M5, M6, M8, M9, M10, M12 and M14 were grouped in the moderately resistant class, while genotypes M2, M7 and M11 were grouped in the susceptible class. Based on this grouping criterion, several genotypes were observed to be consistently grouped in the same response category by the three screening techniques. For example, genotypes M3, M8 and M13 were consistently grouped in the resistant category by at least two of the three screening techniques. Similarly, genotypes M4, M5 and M10 were consistently grouped in the moderately resistant category, whereas genotype M7 was consistently grouped in the susceptible category by the three screening techniques. These results are in agreement with Giga *et al.* [22] who reported comparable results between laboratory and on-farm maize evaluation techniques against weevil attack in Zimbabwe.

Regarding grain weight loss (%) the response trend exhibited was almost similar to that manifested under grain damage. Under laboratory bioassay technique, genotypes M4, M6, M8 and M9 were categorized as resistant, genotypes M3, M5, M10, M13 and M14 were categorized as moderately resistant, whereas genotypes M1, M2, M7, M11 and M12 were categorized as susceptible. For shelled grain, genotypes M4, M5 and M14 were categorized as resistant; genotypes M2, M3, M6, M8, M9, M10, M12 and M13 were categorized as moderately resistant, whereas genotypes M1, M7 and M11 were categorized as susceptible.

For suspended ears, genotypes M3, M4 and M8 were categorized as resistant, genotypes M5, M6, M9, M10, M13 and M14 were categorized as moderately resistant, while genotypes M1, M2, M7, M11 and M12 were categorized as susceptible. From the response grouping based on grain weight loss parameter, it was also observed that some of the genotypes were consistently grouped in the same category under the three screening techniques. For instance, genotype M4 was consistently grouped in the resistant category, genotypes M10 and M13 were consistently grouped in the moderately resistant category, whilst genotypes M1, M7 and M11 were consistently grouped in the susceptible category.

The reasonably high levels of consistency observed on grouping the 14 genotypes according to their response to weevil attack based on grain damage and grain weight loss, as assessed by the laboratory bioassay technique, the shelled grain and suspended ears techniques of the weevil warehouse manifested the high discrimination power of the two weevil warehouse techniques as compared with the laboratory bioassay techniques. (). For instance, in all the three techniques, genotypes M8, M13 and M14 were ranked among the best six genotypes. Genotype M13 was ranked first by both laboratory bioassay technique and suspended ear option, but ranked third by shelled grain option under grain damage. In a similar trend, genotype M8 was ranked fourth by both weevil warehouse techniques, and ranked second by the laboratory bioassay technique. Genotype M14 was ranked fifth by all the three techniques, thereby further portraying the high level of consistency manifested by the three weevil screening techniques. On the other hand, genotypes M7 and M2, together with the susceptible checks M11 and M12 were consistently ranked among the worst six performing genotypes for grain damage encountered under the three screening techniques. In this regard, genotype M7 was ranked as the fourth last entry for the laboratory bioassay technique and ranked the last by shelled grain, whereas it was ranked third last under suspended ears. All these ranks portray a reasonable level of consistency in genotype discrimination. These results agree with Kang *et al.* [23] who observed reasonable levels of consistency exhibited in experimental hybrids evaluated for response to weevil attack using the free-choice (shelled grain) weevil screening technique. Furthermore, the similar data range of 34.4% and 34.9% exhibited by the laboratory bioassay and the shelled grain option of the weevil warehouse, respectively; and the similar CVs observed for grain damage under the two techniques, portrayed the same potential by the two techniques to discriminate maize genotypes according to their responses to weevil attack.

Similarly, the higher data range of 56.7% exhibited by the suspended ear option of the weevil warehouse also indicated its higher potential to discriminate maize genotypes, despite the few inconsistencies manifested in the foregoing study.

3.2. Correlation among Genotype Ranking by the Three Screening Methods

The data of the correlations for the ranks of the 14 genotypes by the laboratory bioassay, and the weevil warehouse methods (shelled grain and suspended ear techniques) are shown in Table 4. Significant positive rank correlations (P<0.05 – P<0.01) were exhibited

between the laboratory bioassay and the weevil warehouse methods based on the grain damage data. The rank correlation between shelled grain technique and the laboratory bioassay was not significant (P>0.05) for both grain damage and grain weight loss data.

The significant positive correlations among genotype ranking by laboratory bioassay, shelled grain (only under grain damage) and suspended ear techniques also emphasized the consistency exhibited during genotype categorization into different weevil resistance or susceptibility classes. The insignificant correlation between shelled grain under grain weight loss parameter and the rest of the techniques suggested that this technique was not an appropriate for discriminating the 14 genotypes under the current study conditions.

3.3. Monthly Genotype Response to Weevil Infestation

The mean squares for the monthly response of genotypes to weevil infestation, under weevil warehouse screening technique (shelled grain and suspended ears) are shown in Table 5.

Table 3. Mean performance of maize genotypes under laboratory bioassay and weevil warehouse methods based on grain damage and grain weight loss

Entry	Grain damage (%)						Grain weight loss (%)					
	Laboratory bioassay method		Weevil warehouse method				Laboratory bioassay method		Weevil warehouse method			
			Shelled grain		Suspended ears				Shelled grain		Suspended ears	
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
M1	70.17	13	60.83	7	54.67	8	30.08	12	45.03	14	22.17	10
M2	63.00	10	73.00	12	74.83	13	32.33	14	22.00	4	26.17	13
M3	45.17	4	59.33	6	25.00	2	27.17	9	39.17	11	8.00	1
M4	64.17	11	65.83	9	48.33	6	24.00	6	20.17	3	10.67	2
M5	57.67	9	67.83	10	63.00	11	24.50	7	17.33	1	18.50	7
M6	55.33	6	50.17	2	59.17	10	18.67	4	25.00	5	18.50	7
M7	69.83	12	83.67	14	72.50	12	29.17	10	40.67	12	23.33	11
M8	38.83	2	52.50	4	37.00	4	12.33	1	32.33	6	11.67	3
M9	56.83	7	49.33	1	49.17	7	13.67	2	35.83	9	18.60	9
M10	41.50	3	62.17	8	35.00	3	21.67	5	33.33	7	18.33	6
M11	57.67	8	70.67	11	76.67	14	30.83	13	45.17	13	24.00	12
M12	70.67	14	75.83	13	55.50	9	29.33	11	38.00	10	28.17	14
M13	35.83	1	50.33	3	20.00	1	15.67	3	33.50	8	18.20	5
M14	51.67	5	52.67	5	44.00	5	25.17	8	19.50	2	15.33	4
Mean	**55.59**		**62.44**		**51.06**		**23.90**		**31.95**		**18.69**	
LSD (0.05)	**4.20**		**5.12**		**5.05**		**3.66**		**4.51**		**4.78**	

Table 4. Correlation among genotype ranking

TEST METHOD	LABORATORY BIOASSAY		WEEVIL WAREHOUSE		
	GD	GWL	SGD	SED	SGWL
Grain damage					
Shelled grain under Weevil warehouse	0.67**				
Suspended ears under weevil warehouse	0.67**		0.59*		
Grain weight loss					
Laboratory bioassay	0.66*		0.75**	0.61*	
Shelled grain under Weevil warehouse	0.23	0.35	0.16	0.09	
Suspended ears under weevil warehouse	0.65*	0.61*	0.55*	0.76**	0.37

GD: Grain damage, GWL: Grain weight loss, SGD: Shelled grain damage, SED: Suspended ears damage, SGWL: Shelled grain weight loss.

Table 5. Mean squares of hybrids for grain damage and weight loss under the weevil warehouse

SOV	DF	Shelled grain		Suspended ears	
		GD (%)	GWL (%)	GD (Score 1-10)	GWL (%)
Replication	2	8.13	74.13	1.60	11.29
Period (P)	3	180.53***	6349.83***	177.48***	3974.85***
Entry (E)	13	6.62*	338.29***	21.96***	140.12***
PXE	39	0.21	85.29***	1.86***	14.11***
Error	110	3.25	29.73	0.55	6.56
R^2		0.65	0.89	0.94	0.95
CV (%)		46.76	35.79	17.31	12.19

****: Significant at 0.05 and 0.001, GD: Grain damage and GWL: Grain weight loss, SOV: Source of variation.

The mean squares of the monthly response of genotypes to weevil infestation, under weevil warehouse screening techniques indicated significant (P<0.05 – P< 0.001) periods and entries for grain damage and grain weight loss, under both shelled grain and suspended ears. This implied that the 14 genotypes exhibited significant differences in their response to grain damage resulting from weevil attack. The significant (P<0.05) interaction between the infestation (incubation) period and entries implied that the 14 genotypes exhibited entry-dependent variations in the rate of grain damage and associated weight loss during the storage period. Genotype-dependent variations in response to weevil attack provided the basis for discriminating the 14 genotypes. These results are consistent with results reported by Giga *et al.* [22].

3.4. Grain Damage and Weight Loss under the Weevil Warehouse

Results of the monthly grain damage and weight loss under the weevil warehouse screening technique (shelled grain and suspended ears) are shown in Table 6. The results indicated significant differences among infestation periods for both grain damage and weight losses. Results of the monthly grain damage and/or weight loss indicated that the longer the grain was subjected to weevil infestations, the more grain damage was encountered and consequently the more weight of the grain was lost, however, the monthly responses (rate of grain damage and weight loss) were genotype dependent. The increased grain damage and subsequent weight losses could probably be explained by the exponential increase in weevil population density resulting from multi-generation reproduction and continuous feeding on the same quantity of grain. Due to time constraint, the experiment was prematurely discontinued, therefore, the trend was not observed beyond four months; however, the rate of grain damage and weight loss was expected to decline as the evaluation period progressed, until a point it would level-off, when the food reserves in the grains are depleted. These results are consistent with those reported by Giga *et al.* [22], who observed the narrowing of the gap between (decline in the variations) weevil susceptible and resistant genotypes as the incubation (storage) period increased.

Table 6. Means of grain damage and weight loss under the weevil warehouse

Period (Month)	Shelled grain	
	Grain damage (%)	Grain weight loss (%)
1	12.52	10.26
2	32.69	16.51
3	48.45	24.67
4	60.45	32.62
LSD (0.05)	**7.79**	**1.11**
Period (Month)	**Suspended ears**	
	Grain damage (Score 1-10)	**Grain weight loss (%)**
1	1.80	2.61
2	3.59	9.38
3	5.21	17.78
4	6.56	31.16
LSD (0.05)	**0.32**	**2.36**

Results also indicated that a minimum of one month's storage was required to begin differentiating genotypes according to resistance or susceptibility. Genotype discrimination into different response classes would continue up to a point when susceptible genotypes cannot easily be distinguished from resistant ones; Giga *et al.* [22] reported this period to be seven months of storage. To save on the screening time and associated screening costs including labour, discrimination of genotypes at early storage/incubation periods would be desirable. However, based on the screening protocols developed by Dobie [19] and Derera *et al.* [16], assessments of genotype response to weevil infestations were done using mainly the F_1 weevil progenies, which are expected to emerge from the grain between 28 and 60 days after oviposition under favourable conditions [26]. Therefore, a minimum period of two months of evaluation would be required for effective discrimination of genotypes, whereby at least the first F_1 weevil progenies would be involved in pest activities. The evaluation period could be extended up to six months (although more costs would be incurred) beyond which the effectiveness in genotype discrimination tends to decline [22]. Furthermore, a possibility of cross-infestation of weevils from the more susceptible genotypes (in which the food reserves are expected to be depleted faster) to the less susceptible genotypes may not be ruled out as the evaluation period progresses.

4. Conclusion

High levels of consistency displayed by the grain damage and grain weight loss parameters under laboratory bioassay technique, and the shelled grain and suspended ear options of weevil warehouse technique suggested that the two weevil susceptibility parameters can effectively be used to discriminate genotypes under the three weevil screening methods. Thus, weevil warehouse techniques can effectively be used to group maize genotypes into different susceptibility classes based on their response to weevil infestation.

The minimum period required for effective discrimination of genotypes was two months. During this period, F_1 weevil progenies emerge to begin feeding on the maize grain and subsequently causing cultivar-dependent damages. Based on these results, it is evident that the weevil warehouse technique is a simple and time saving weevil screening technique that would effectively be used to discriminate maize genotypes. Therefore, the weevil warehouse technique would be an appropriate screening technique for evaluating large numbers of maize genotypes within a period of two months as compared to three months, the minimum period required by the laboratory bioassay technique.

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References

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

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

[3] ASARECA. 2009. Drought and low-N tolerant maize germplasm and varieties – inbred lines, populations, OPVs, Hybrids.

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

[5] Adedire, C. O. 2001. Biology, ecology and control of insect pests of stored cereal grains. In: Ofuya, T. I.and Lale, N. E. S. (eds.) Pests of Stored Cereal and Pulses in Nigeria: Biology, Ecology and Control. Dave Collins Publications, Akure, Nigeria. pp. 59-94.

[6] FAO 2002. FAO Global Early Warning System on Food and Agriculture: FAO/WFP Crop and Food Assessment Mission to Zimbabwe. FAO Publications, Harare, Zimbabwe. pp. 1-3.

[7] Nwosu, L.C., Adedire, C.O., Ogunwolu, E.O. 2015a. Screening for new sources of resistance to *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) infestation in stored maize genotypes. Journal of Crop Protection, 4: 277-290.

[8] Nwosu, L.C., Adedire, C.O., Ogunwolu, E.O. (2015b). Feeding site preference of *Sitophilus zeamais* (Coleoptera: Curculionidae) on maize grain. International Journal of Tropical Insect Science, 35: 62-68.

[9] Lale, N.E.S., and Ofuya, T.I. 2001. Overview of pest problems and control in the tropical storage environment. In: Ofuya, T.I. and Lale, N.E. S. (eds.) Pests of Stored Cereals and Pulses in Nigeria: Biology, Ecology and Control. Dave Collins Publications, Akure, Nigeria. pp. 1-23.

[10] Cugala, A., Sidumo, L., Santos, N. 2007. GiváUso do método de controlo biológico contra a broca maior do grão do milho armazenado, Prostephanus truncatus (horn) (*coleoptera: bostrichidae*) nos celeiros das famílias rurais em Moçambique

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

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

[13] Adedire, C. O., Akinkurolere, R. O., Ajayi, O. 2011. Susceptibility of some maize cultivars in Nigeria to infestation and damage by maize weevil, *Sitophilus zeamais* (Motsch.) (*Coleoptera: Curculionidae*). Nigerian Journal of Entomology, 28: 55-63.

[14] Mwololo, J.K., Mugo, S., Okori, P., Tefera, T., Otim, M., Munyiri, S. W. 2012. Sources of resistance to the maize weevil *Sitophilus zeamais* in tropical maize. Journal of Agricultural Science, 4: 206-215.

[15] Nwosu, L.C., Adedire, C.O., Ogunwolu, E.O., Ashamo, M.O. 2015c. Relative susceptibility of 20 elite maize varieties to infestation and damage by the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). International Journal of Tropical Insect Science, 35: 185-192.

[16] Derera, J., Pixley, K., Giga, D.P. 2010. Appraisal of protocol for the rapid screening of maize genotypes for maize weevil resistance. African Entomology 18, 8-16.

[17] Kasozi, L.C., Derera, J., and Tongoona, P. 2015. Response of maize population "Longe5" to two cycles of modified S1 recurrent selection for resistance to maize weevil. Euphytica.

[18] Derera, J., Pixley, K. and Giga, D. 2001. Resistance of maize to the maize weevil. I. Antibiosis African Crop Science Journal, 9 (2) (2001), pp. 431-440.

[19] Dobie, P. 1974. The laboratory assessment of the inherent susceptibility of maize varieties to postharvest infestation by Sitophilus zeamais. Journal of Stored Products Research 10, 183-197.

[20] Urrelo, R., Wright, V.F., Millis, R.B., Horber, E.K., 1990. An abbreviated procedure to determine the inherent resistance of maize to Sitophilus zeamais Motsch. (Coleoptera: Curculionidae). Journal of stored Products Research 26, 97-100.

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

[22] Giga, D.P., Mazarura, U.M., Canhao, J. 1999. Review of resistance mechanisms and assessment of susceptibility of various maize genotypes to Sitophilus zeamais stored in small farm stores. Sixth Eastern and Southern Africa Regional Maize Conference 21st - 25th September, 1998. pp. 19-23.

[23] Kang, M., Zhang, Y., Magari, R. 1995. Combining ability for maize weevil preference of maize grain. Crop Science 35, 1556-1559.

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

[25] Bergvison, D., 2006. The "Weevil Warehouse": A cost-effective means of screening large quantities of germplasm against primary storage pests. CIMMYT. Unpublished.

[26] Tadele, T., Mugo, S., Tende, R., Likhayo, P. 2010. Mass rearing of stem borers, maize weevil, and larger grain borer insect pests of maize. CIMMYT: Nairobi, Kenya.

[27] Gwinner, J., Harnisch, R., Mueck, O. 1996. Manual on the prevention of post harvest seed losses, post harvest project, GTZ, D-2000, Hamburg, FRG, p. 294.

[28] Kossou, D.K., Bosque-Pérez, N.A., Mareck, J.H. 1992. Effects of shelling maize cobs on the oviposition and development of (*Sitophilus zeamais* Motschulsky). Journal of Stored products Research 28, 187-192.