

**PERFORMANCE OF STEM BORER RESISTANT MAIZE INBRED LINES UNDER MAIZE WEEVIL INFESTATION (*Sitophilus zeamais*): TOWARDS DEVELOPING MULTIPLE PEST RESISTANT MAIZE**

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**SUMMARY**

Maize inbred lines that showed tolerance to stem borer infestation were screened under maize weevils, *Sitophilus zeamais* infestation to test their performance. Eight maize inbred lines with two checks (one susceptible and one resistant) were screened under open and dark storage conditions at the Entomology Laboratory of the Institute of Agricultural Research and Training, Obafemi Awolowo University in 2019. Seeds of each maize inbred lines recently harvested, were put in a jar and ten unsexed adult weevils were introduced. The adult weevils were removed after ten days after they must have laid eggs. The experiment was laid out in a completely randomized design in three replications. Data were collected on number of emerged weevils, seed damage rating, seed coat thickness and weight, grain weight loss, number of damaged seeds and total number of insects at termination. The experiment was terminated after three months. Mean square of inbred lines (genotypes) was significant for all the traits studied. Some of the inbred lines performed better than the resistant check. Generally, the maize inbred lines performed well under weevil infestation with very little or no seed damage. The results indicate that the maize inbred lines have high level of tolerance to maize weevil infestation. The inbred lines shall further be tested under fall armyworm infestation to develop multiple insect resistant maize varieties.

**Keywords:** Crop improvement, Grain weight loss, Maize weevils, Multiple resistance, Susceptibility

The low yield of maize in Nigeria compared to some other parts of Africa has been attributed to various biotic and abiotic stresses. Among the biotic stresses, insect pests are the most important as numerous insect pests attack maize. They attack virtually all the growth stages of maize crop, from the vegetative stage to cob formation, while some are carried from the field to the store. Stem borers have been reported to cause a yield loss of between 20 and 40% (18) and total yield loss in some cases. Larvae of the fall armyworm were observed to cause as high as 100% damage on

maize fields (Odeyemi *et al.*, 2016). Maize weevil, *Sitophilus zeamais*, has been reported to cause yield loss between 15 and 90% (Derera *et al.*, 2001; Tefera *et al.*, 2016). Weevils also play a role in fungal spread. It was reported that in the presence of weevils, fungal infection increased, but in their absence, fungal infection was low (Bhusal and Khanal, 2019).

Chemical control methods in the control of insect pests and the use of transgenic genes conferred by *Bacillus thuringiensis* (Bt), have limitations in terms of applicability and acceptability (Munyiri *et al.*, 2015; Sylvian *et al.*, 2015). Some of the critical pests can also develop resistance to both control methods (Campagne *et al.*, 2013). Besides, environmental factors particularly climate change, negatively influence plant-insect interaction, hence, affecting the plant defensive mechanisms (Stam *et al.*, 2014) leading to severe yield loss (Kissoudis *et al.*, 2014). Host plant resistance (HPR) has been described as a holistic approach to insect pest control (Garcia-Lara *et al.*, 2010; Murenga *et al.*, 2016).

While developing resistance to a particular insect pest in a maize cultivar, an upsurge of another insect biotype may limit the use of such cultivar. Hence there is need to develop maize cultivars with resistance to two or more insect pests to stabilise their use and enhance maize yield. The foundation of Integrated Pest Management (IPM) programmes should be laid with a crop cultivar that is not only well adapted to the various abiotic constraints but also has some level of resistance to one or more insect and disease pests (Maxwell, 1991).

According to Tefera *et al.* (2016) no report of combined resistance to both stem borers and store pests is available. Badji *et al.* (2018) working on Meta-analysis of Quantitative Trait Loci (MQTL) of maize discovered that most of the MQTL involved resistance to several insect species, therefore generating hope for success of breeding for multiple insect pest resistance. Badji *et al.* (2018) discovered some QTL involved in resistance to European corn borer, sugarcane borer and southwest corn borers in maize. They also discovered some maize varieties that combined

resistance to seed damage by weevil and the Mediterranean corn borer. This study aims at evaluating tropically adapted maize inbred lines with resistance to stem borers, for resistance to maize weevil infestation, toward developing multiple insect pest resistant maize cultivars.

## MATERIALS AND METHODS

Forty maize inbred lines comprising exotic and adapted lines were screened under natural and artificial infestation of stem borers in three locations in two seasons. The data were pooled to select the top lines using 20% selection intensity. The eight lines (20%) selected along with two checks were screened under maize weevil infestation at the Entomology Laboratory of the Institute of Agricultural Research and Training (IAR&T) Ibadan, Nigeria in 2019. The 10 maize lines are listed in Table 1.

**Table 1:** The ten maize inbred lines selected for maize weevil screening

S/N	Inbred lines	Source	Kernel colour
1	4001	IITA	Yellow
2	5057	IITA	White
3	1368	IITA	White
4	ART/98/ILE 1-OB (susceptible check)	IAR&T	White
5	TZEI 2	IITA	White
6	5012	IITA	White
7	KU1414SR/SR (resistant check)	IITA	Yellow
8	TZEI 9	IITA	White
9	TZEI 1	IITA	White
10	9450	IITA	Yellow

ART/98/ILE 1-OB was used as the susceptible check, while KU1414SR/SR was the resistant check as reported by Oloyede-Kamiyo and Adetumbi (2017). The maize lines used were harvested in the same season without insecticide application for preservation. One hundred seeds of each maize line were weighed and put in a jar. Ten unsexed adult maize weevils that were reared at the entomology laboratory were introduced into each jar and covered with a lid containing 40 µm

mesh screen to provide ventilation and prevent the insects from escaping. The adult weevils were introduced to mate and lay eggs on the seeds. After 10 days, all the adult insects were removed from all the jars to allow their eggs to hatch and start fresh infestation on the maize seeds. The experiment was laid out in a completely randomised design in three replications. The screening was conducted concurrently under open and dark storage conditions to compare level of infestation under the two conditions. In the open condition, the jars were displayed on the table in the laboratory where there was fresh air, while the dark condition was in a closed room without light.

Data were collected on weevil damage rating at 4, 8 and 12 weeks after removal of adult weevils (WAR) on a scale of 1-5, 1 for no sign of weevil attack; 2 for few seeds with sign of attack; 3 for 50% of the seeds with holes; 4 for 75% of the seeds with holes and presence of powder remnant; and 5 for severe attack showing holes on all the seeds with powder remnant. Number of weevils at first rating (4 weeks after removal of the introduced weevils) was done by counting. Maize seed coat thickness (mm) and seed coat weight were also determined using vernier caliper and sensitive scale respectively, according to Oloyede-Kamiyo and Adetumbi (2017). Grain weight loss was estimated by removing the final weight of the seeds with the powder after termination from the initial seed weight and expressed as percentage of initial weight according to Oloyede-Kamiyo and Adetumbi (2017). Number of damaged seeds and total number of insects at termination were also counted. Number of damaged seeds was expressed as percentage of number of seeds in the jar. The experiment was terminated after 3 months.

Percentage data were transformed before analysis using arc-sine transformation. Analysis of variance was conducted. Means were separated using Duncan multiple range test. Phenotypic correlation was also conducted to determine relationship among traits.

## RESULTS

Combined analysis of variance under open and dark conditions is presented in Table 2. Mean square of conditions was not significant for any of the traits studied. However, mean square of variety was significant for all the traits at  $P \leq 0.01$ .

Mean performance of the traits under infestation (Table 3) revealed that grain weight loss was significantly higher ( $P=0.05$ ) in the susceptible check, ART/98/ILE 1-OB, (28.2%) compared with any other maize line, followed by 9450 (10.0%). Other lines did not differ significantly from the resistant check (KU1414SR/SR) in grain weight loss. A similar trend was observed in all other resistant traits. However, 9450 did not differ significantly from the resistant check (KU1414SR/SR) for all the traits except in seed coat parameters and total number of insects at termination. Some of the lines were better than the resistant check for number of emerged insects 4 weeks after removal of the introduced adults, damage rating at 8 and 12 WAR, and number of damaged seed at termination. Seed coat thickness was highest in the resistant check (0.07 mm), although this was comparable to the seed coat thickness of inbred 4001, TZEI 1 and TZEI 9. Seed coat thickness of other lines was not significantly different from that of the susceptible check. The proportion of the seed coat weight relative to the seed itself in the susceptible check was comparable to that of the resistant check and some of the inbred lines, such as TZEI 2, TZEI 9, 5057, 1368 and 9450.

The performance of the lines under open and dark conditions for weight loss is presented in Fig. 1. The maize inbred lines behaved differently under both storage conditions. Weight loss was higher under open condition than in the dark for half of the maize inbreds. The value was significantly highest in ART/98/ILE 1-OB followed by 9450 for both conditions. No significant difference was observed in damage rating between the open and dark conditions for most of the maize inbreds especially at rating at 4WAR and 8WAR (Fig. 2). Damage rating was slightly higher in the open condition for inbreds 1, 3 and 5, while it was higher in the dark condition for inbreds 6, 7, 8, 9 and 10 especially at 8 and 12 WAR.

**PERFORMANCE OF STEM BORER RESISTANT MAIZE INBRED LINES UNDER MAIZE WEEVIL INFESTATION  
(*Sitophilus zeamais*): TOWARDS DEVELOPING MULTIPLE PEST RESISTANT MAIZE**

**Table 2:** Mean squares from analysis of variance for the traits studied under both conditions

Source	df	Grain weight loss (%)	Seed coat thickness (mm)	Seed coat weight (%)	Number of emerged weevils 4WAR	Damage rating 4WAR (1-5)	Damage rating 8WAR (1-5)	Damage rating 12WAR (1-5)	Number of damaged seeds at termination	Total number of insects at termination
Storage condition	1	0.02	0.00	0.00	0.03	0.004	0.004	0.15	0.001	1.00
Variety	9	0.12**	0.001*	0.003**	3.74**	3.07**	4.51**	5.81**	1.00**	10.90**
Error	35	0.12	0.00	0.001	0.37	0.15	0.29	0.52	0.04	1.15

df: degree of freedom; \*, \*\*: significant at  $P = 0.05$  and  $0.01$  respectively.

WAR- Weeks after removal of the insects; Scale 1-5: 1 for no sign of infestation; 2 for few seeds with sign of attack; 3 for 50% of the seeds with holes; 4 for 75% of the seeds with holes and presence of powder remnant; 5 for severe infestation

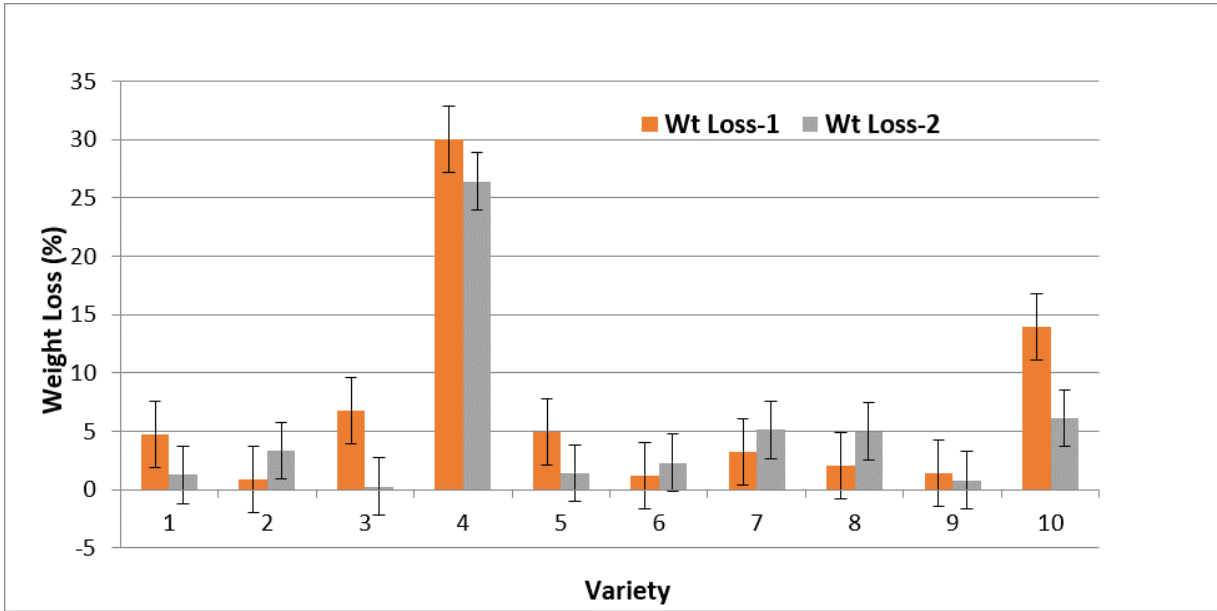
Highly significant positive correlations ( $P \leq 0.01$ ) existed among weevil damage traits (Table 4). Grain weight loss had positive and highly significant correlations with all the damage traits ranging from 0.78\*\* to 0.92\*\* at  $P \leq 0.01$ . Seed coat thickness and seed coat weight had negative but non-significant correlation with weevil damage traits (Table 4).

**Table 3:** Mean performance of the maize inbred lines under weevil infestation

Variety	Grain weight loss (%)	Seed coat thickness (mm)	Seed coat weight (%)	Number of emerged weevils 4WAR	Damage rating at 4WAR (1-5)	Damage rating at 8WAR (1-5)	Damage rating at 12WAR (1-5)	Number of damaged seeds at termination (%)	Total number of insects at termination
4001	3.03b	0.06ab	8.59a	1.67bc	1.17b	1.33bc	2.25bc	4.33c	5.67c
5057	2.13b	0.05b	6.88bc	0.00c	1.00b	1.00c	1.33c	1.00c	1.17c
1368	3.55b	0.04b	6.40bc	1.50bc	1.17b	1.42bc	1.67bc	4.50c	5.50c
ART/98/ILE 1-OB	28.24a	0.05b	5.96cd	14.00a	3.33a	3.83a	4.50a	92.67a	146.70a
TZEI 2	3.18b	0.06ab	5.65cde	0.50bc	1.08b	1.17c	1.42c	2.67c	5.00c
5012	2.04b	0.052b	4.62e	0.40c	1.00b	1.00c	1.40c	1.40c	1.40c
KU1414SR/SR	4.20b	0.07a	5.81cde	1.67bc	1.17b	1.58bc	2.33bc	11.00bc	13.83c
TZEI 9	3.55b	0.05ab	5.25de	0.5bc	1.08b	1.17c	1.42c	1.00c	2.83c
TZEI 1	1.11b	0.04b	7.25b	0.00c	1.00b	1.00c	1.33c	1.00c	1.33c
9450	10.04b	0.05b	6.71bc	3.83b	1.58b	2.08b	2.58b	28.33b	47.00b

Values with the same alphabet within column are not significantly different from each other at  $P = 0.05$

WAR- Weeks after removal of the insects; Scale 1-5: 1 for no sign of infestation; 2 for few seeds with sign of attack; 3 for 50% of the seeds with holes; 4 for 75% of the seeds with holes and presence of powder remnant; 5 for severe infestation.



**Figure 1:** Percentage weight loss of the maize inbred lines under open (wt loss-1) and dark condition (wt loss-2)

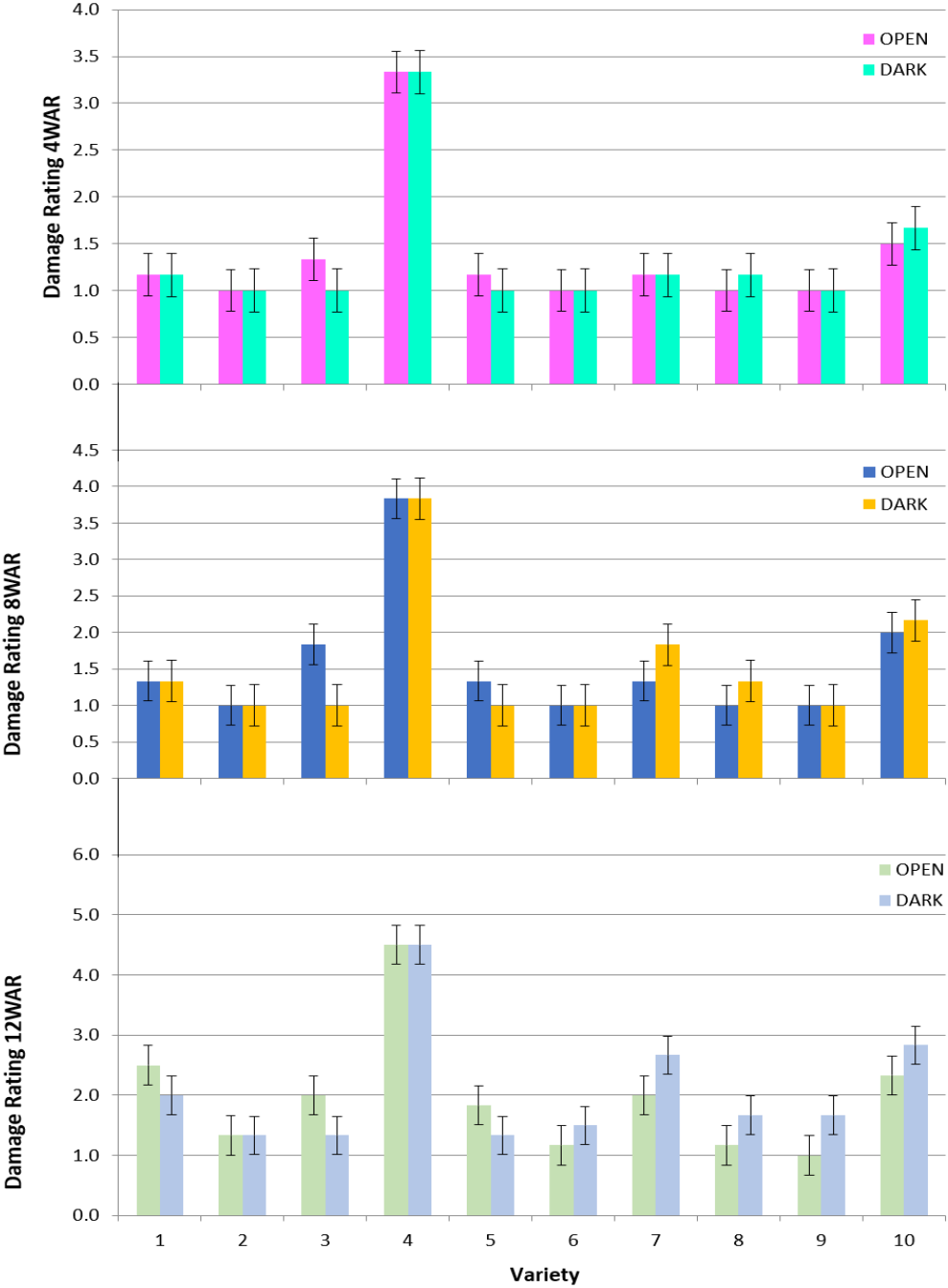


Figure 2: Damage rating of the maize inbred lines under open and dark conditions

**PERFORMANCE OF STEM BORER RESISTANT MAIZE INBRED LINES UNDER MAIZE WEEVIL INFESTATION  
(*Sitophilus zeamais*): TOWARDS DEVELOPING MULTIPLE PEST RESISTANT MAIZE**

**Table 4:** Phenotypic correlation among the traits studied under weevil infestation

Traits	Grain weight loss (%)	Seed coat thickness (mm)	Seed coat weight (%)	Number of emerged weevils 4WAR	Damage rating at 4WAR (1-5)	Damage rating at 8WAR (1-5)	Damage rating at 12WAR (1-5)	Number of damaged seeds at termination (%)	Total number of insects at termination
Grain weight loss (%)	1	-0.07	-0.03	0.90**	0.91**	0.92**	0.87**	0.94**	0.92**
Seed coat thickness (mm)		1	0.16	-0.03	-0.06	-0.01	0.02	-0.07	-0.06
Seed coat weight (%)			1	-0.02	-0.04	-0.04	0.03	-0.001	0.001
Number of emerged adults 4WAR				1	0.99**	0.97**	0.91**	0.96**	0.97**
Damage rating at 4WAR (1-5)					1	0.96**	0.89**	0.96**	0.97**
Damage rating at 8WAR (1-5)						1	0.94**	0.95**	0.95**
Damage rating at 12WAR (1-5)							1	0.89**	0.89**
Number of damaged seeds (%) at termination								1	0.99**
Total number of insects at termination									1

## DISCUSSION

Multiple insect resistance in maize inbred lines enhances creation of a genepool from which multiple pest resistance varieties or hybrids could be developed. This is very essential with the effects of the prevailing climate change especially in the humid zones. The significant mean square for the maize inbred lines selected under stem borer for all the weevil damage traits suggested high variation among the inbred lines for weevil resistance and possible resistance to multiple pests in

the maize inbreds. Badji *et al.* (2018) also observed combined resistance to kernel damage by weevil and the Mediterranean corn borer in some maize varieties. Other inbred lines not being significantly different from the resistant check for all the damage traits suggested that the lines have considerable level of resistant to maize weevil. Therefore, all the lines could further be tested under other insect pests. The non-significant mean square for condition showed that condition does not influence the performance of the lines under weevil infestation.

Significantly high percentage weight loss for the susceptible check, ART/98/ILE 1-OB, was also reported by Oloyede-Kamiyo and Adetumbi (2017), indicating that the maize variety is highly susceptible to maize weevil. ART/98/ILE 1-OB is a quality protein maize variety, while others are maize inbred lines that have proven to be resistance to stem borers. However, Arnason *et al.* (1993) working on quality protein maize (QPM) genotypes from CIMMYT found no indication that QPM genotypes were any more susceptible on average than backcrossed material expressing normal protein in the endosperm. Some of the lines being better than the resistance check in performance suggested that most the maize inbreds are highly resistance to maize weevil and could be used to create a genepool for multiple insect resistance.

Serratos *et al.* (1987) reported that a remarkable feature of the resistant maize was the intense fluorescence of the kernel pericarp as observed by fluorescence microscopy. In the present study, seed coat was thickest in the resistance check. However, seed coat thickness of other lines was not significantly different from that of the susceptible check suggesting that seed coat thickness may not always be a factor in level of resistance of a maize variety to weevil, likewise the seed coat weight. Oloyede-Kamiyo and Adetumbi (2017) also reported that seed coat thickness is an important trait in weevil resistance, but the present study indicated that some other biochemical factors may be responsible for resistance. Nhamucho *et al.* (2017) reported that genotypes with high protein content showed resistance to maize weevils, while those with high starch showed susceptibility. Concentrations of phenolic acids in grain have been reported to have a negative and

highly significant correlation with indices of susceptibility of maize to maize weevil (Serratos *et al.*, 1994).

The susceptible genotypes showing high maize weevil progeny emergence at 4 WAR compared with other lines suggested that they supported more adult oviposition and rapid progeny development which translate to high total number of insect at termination, while resistance genotypes could have had some antibiosis factors that affect progeny emergence (15; 1; 24). Number of emerged weevils during evaluation has been used as key parameter to discriminate between resistance and susceptible lines Nhamucho *et al.* (2017).

The maize inbred lines behaved differently under open and dark condition even though the difference was not significant in the analysis of variance. While some inbred lines performed the same way under both conditions, some performed better under open condition and some performed better under dark condition indicating that condition may not be a factor in performance of maize inbreds under weevil infestation.

Positive and highly significant correlation among weevil damage traits showed that the higher the number of emerged weevil progeny, the higher the damage caused and the higher the total number of weevils at termination of experiment. As the weevils emerge, they multiply rapidly in presence of the food (maize), and this will amount to high number of weevils at termination. Therefore, weevil population at early stage could be used to predict the level of kernel damage in infested maize. Similar result has been reported in earlier studies (Derera *et al.*, 2001; Kim and Kossou, 2003; Abebe *et al.*, 2009; Mwololo *et al.*, 2012; Nhamucho *et al.*, 2017; Khakata, 2018).

## **CONCLUSION**

It can be concluded in this study that the maize inbred lines, earlier selected for resistance to stem borers, have high level of resistance to maize weevils. They could therefore undergo further

screening under the fall armyworm towards developing multiple insect resistant maize hybrids/germplasm.

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