

Effects of Varietal Differences and Severity of Seed Damage by *Callosobruchus maculatus* (F.) on the Weight and Germination of Some Improved Cowpea Seeds

OJUMoola, A.O.* AND ADESIYUN, A.A.
Department of Crop Protection, University of Ilorin,
Ilorin, Nigeria.

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ABSTRACT. The effects of varietal differences and severity of seed damage by the cowpea bruchid, *Callosobruchus maculatus*, on the weight and germination of some improved cowpea seeds were investigated in a 5 x 4 factorial experiment laid out in a completely randomized design replicated three times. Five improved cowpea seed varieties, namely, - SAMPEA 6, SAMPEA 7, SAMPEA 9, SAMPEA 10 and SAMPEA 11, were exposed to infestation and damage by *C. maculatus*. The damaged seeds were sorted, based on the number of exit hole(s) in them, into – no-hole, one hole, two-hole, and three-hole groups and thereafter placed on moist tissue paper in Petri dishes. The emergence of radicles and primary leaves 96 hours after placement (HAP) were used as germination indicators. SAMPEA 6 and SAMPEA 7 had the highest and lowest mean weight per seed (207.5mg and 159.2mg) respectively and both differed significantly ($P<0.05$) from other varieties. SAMPEA 6 and SAMPEA 7 both had a significantly higher ($P<0.05$) mean percentage germination (100.0%) than other varieties based on the emergence of radicles. Similarly, SAMPEA 7 and SAMPEA 11 had the highest mean percentage germination (25.0%) based on the emergence of primary leaves but were significantly different ($P<0.05$) from SAMPEA 6 and SAMPEA 10 only. The mean weight value (193.7mg) of seeds with no holes differed significantly ($P<0.05$) from those of seeds with one or more holes. Based on the emergence of radicles and primary leaves, seeds with no hole had highest percentage germination (93.33% and 61.33%) respectively and were significantly ($P<0.05$) different from those with hole(s). Significant interaction effects were also observed between variety and number of holes at $P=0.05$.

Keywords: *Callosobruchus maculatus*, SAMPEA, seed damage, Cowpea seeds.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) belongs to the subfamily Papilionoideae, and the family Fabaceae (formerly Leguminosae). Cowpea grains are a cheap source of dietary protein (Alabi *et al.*, 2003), that is consumed in several forms. Like other pulses, the protein in

cowpea grain is rich in the amino acids- lysine and tryptophan, when compared with cereal grains (Hall *et al.*, 2003). The protein in cowpea grain has also been shown to reduce low-density lipoproteins that are implicated in heart disease (Phillips *et al.*, 2003). Cowpea grain is also a rich source of minerals and vitamins (Hall *et al.*, 2003). It also provides a high quality feed, both fresh and as hay, for livestock, (Elzebroek and Wind, 2008). Cowpea is a valuable component of farming

*Corresponding author:

E-mail: ojumoolaolusegun@gmail.com

systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Carsky *et al.*, 2002). Trade in dry cowpea grain and cowpea hay is important to the economy of West Africa in particular, with substantial quantities of cowpea grain being traded at the local and regional levels (Singh, 2002; Langyintuo *et al.*, 2003).

The crop is attacked by insect pests at different stages of its growth as well as during storage. The cowpea bruchid, *Callosobruchus maculatus* (F.), is a key pest to several grain legumes (Lale, 2002). It starts infestation in the field, but heavy damage is done in storage (Swell and Mushobozy, 2007). The main method of transmission is through infested seed, either from the field into storage, or in contaminated seeds within and between storage facilities (Brier, 2008). In the early stages of attack, the only symptom is the presence of eggs glued to the surface of the pulses (CABI, 2011). The eggs hatch into scarabeiform larvae after three days and bore into the seed beneath the oviposition site. There are four larval instars. These larval stages are most notorious and destructive causing great damage to seeds (Messina, 1998; Ojianwuna and Umoru, 2012). As larval development occurs entirely within the seed, these immature stages can normally not be seen. However, they can be detected after pupation takes place; although the seed coat of the bean is still intact, a round 1 to 2 mm window is apparent at the location where the beetle is pupating. The adults emerge through these windows leaving round holes or exit holes in the grain that are the main evidence of damage (CABI, 2011). This study was thus carried out to determine the effect of variety and seed damage caused by *C. maculatus* on the weight and germination of some improved cowpea seeds.

MATERIALS AND METHODS

Source and Disinfestation of Seeds

The study was conducted in a laboratory within the professorial suite of the Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria. Improved varieties of cowpea seeds—SAMPEA 6, SAMPEA 7, SAMPEA 9, SAMPEA 10 and SAMPEA 11, obtained from the Institute for Agricultural

Research (IAR), Samaru, Zaria, Nigeria were used. The seeds were disinfested in the freezer compartment of a Thermocool-250 refrigerator for seven days to terminate the development of any immature stage of the insect.

Insect Culture

Callosobruchus maculatus adults were obtained from an original stock culture sourced from the Nigerian Stored Products Research Institute (NSPRI), Ilorin, Nigeria. From this parent stock, a new generation of *C. maculatus* was raised in the laboratory at 29 ± 1 °C and 75 ± 5 relative humidity. About 50 unsexed adult beetles were introduced into a 1L jar containing about 100 g of a susceptible cowpea seed variety (Ife brown). The open top of the jar was covered with a piece of muslin held in place with rubber bands to allow for ventilation and prevent insect escape. The beetles were allowed to mate and oviposit for seven days after which dead and live beetles were removed and discarded. *C. maculatus* adults that emerged from the seeds in the jar were used for the study.

EXPERIMENTAL PROCEDURE

Seed damage bioassay

The experiment was set up as a 5 x 4 factorial in completely randomized design replicated three times. Five pairs of newly emerged (1-2 days old) *C. maculatus* adults were introduced into 250 ml clear round plastic containing 40 wholesome and pristine seeds of one of SAMPEA 6, SAMPEA 7, SAMPEA 9, SAMPEA 10 and SAMPEA 11. After the seven days allowed for oviposition, the bruchids were removed from the containers; infested seeds were left undisturbed and observed until F¹ adult emergence ceased.

Weight loss assessment

Each set of 40 seeds was disinfested by freezing for seven days and sorted, based on the number of holes on them, into four groups, namely, seeds with no hole, seeds with one, two, and three holes. A random sample of four seeds was taken for seed weight determination on a sensitive OHAUS balance (Model Pro Av114).

Germination bioassay

Five seeds randomly sampled from the no-hole, one-hole, two-hole, and three-hole groups were placed on moist tissue paper in covered Petri dishes. The tissue paper was moistened daily and germination was assessed based on the emergence of radicles and of primary leaves at 96 hours after placement (HAP).

Data Analysis

Data collected were subjected to a two-way ANOVA while separation of treatment means was done using the Duncan Multiple Range Test (DMRT) at 5% level of probability. All statistical calculations were done using IBM SPSS Statistics 21.

RESULTS

The main effects of variety and number of exit holes are presented in Table 1. The highest mean seed weight (207.5) was observed in SAMPEA 6 while SAMPEA 7 had the least (159.2) mean seed weight. SAMPEA 7 and SAMPEA 9 were not significantly different ($P>0.05$) from each other but both had significantly lower ($P<0.05$) seed weights than other varieties.

With respect to the mean percentage germination based on radicle emergence,

SAMPEA 6 and SAMPEA 7 had the highest percentage germination (100.0%). Both differed significantly ($P<0.05$) from SAMPEA 9, SAMPEA 10 and SAMPEA 11. Among these three varieties, differences were due to random variation ($P>0.05$). Table 1 further shows that SAMPEA 7 and SAMPEA 11 had the highest mean percentage germination (25.0%) based on the emergence of primary leaves at 96 HAP. The least mean percentage germination (5.0%) was observed in SAMPEA 6 and it differed significantly ($P<0.05$) from values for other varieties except SAMPEA 10.

Seeds with no holes had significantly higher ($P<0.05$) mean seed weight than seeds with hole(s). However, differences in seed weight among groups of seeds with holes were not significant ($P>0.05$). The same trend was observed in the mean percentage germination based on the emergence of radicles and primary leaves at 96 HAP.

Table 2 shows the interaction effect of both variety and the number of exit holes on the mean weight of seed, the mean percentage germination based on the emergence of radicle and primary leaves at 96 HAP. The mean weight of seed decreased as the number of holes increased for all varieties. Trends for percentage germination based

Table 1: Main Effects of Varietal Differences and Severity of Damage on Mean Seed Weight and Mean Percentage Germination 96 HAP^a

FACTOR	Mean weight ^b per seed (mg)	Mean percentage germination ^b based on the emergence of radicles at 96 HAP	Mean percentage germination ^b based on the emergence of primary leaves at 96 HAP
VARIETY			
SAMPEA 6	207.5a	100.0a	5.0c
SAMPEA 7	159.2d	100.0a	25.0a
SAMPEA 9	160.4d	75.83b	16.7ab
SAMPEA 10	182.5c	70.0b	13.3bc
SAMPEA 11	197.4b	75.0b	25.0a
SEM (±)	2.52	3.63	2.98
NUMBER OF EXIT HOLES			
No hole	193.7a	93.3a	61.3a
One hole	178.2b	81.3b	6.7b
Two holes	177.7b	84.0b	0.0b
Three holes	176.1b	78.0b	0.0b
SEM(±)	2.25	3.25	2.67

^aHAP: Hours After Placement

^bMean values in a column followed by the same letter(s) are not significantly different from each other at $P=0.05$

Table 2: Interaction Effects of Varietal differences and Severity of Damage on Mean Seed Weight and Mean Percentage Germination 96 HAP^a

FACTORS			Mean percentage germination ^b based on the emergence of radicles at 96	Mean percentage germination ^b based on the emergence of primary leaves at
Variety	Number of exit holes	Mean weight ^b per seed (mg)		
			HAP	96 HAP
SAMPEA 6	No hole	230.9a	100.0a	20.0c
	One hole	203.4bc	100.0a	0.0c
	Two holes	202.9bc	100.0a	0.0c
	Three holes	192.8cd	100.0a	0.0c
	No hole	158.46ghi	100.0a	93.3a
SAMPEA 7	One hole	156.4hi	100.0a	6.7c
	Two holes	156.1hi	100.0a	0.0c
	Three holes	165.9fgh	100.0a	0.0c
	No hole	173.7efg	80.0abc	53.3b
SAMPEA 9	One hole	164.71fghi	80.0abc	13.3c
	Two holes	154.0hi	73.3bc	0.0c
	Three holes	149.1i	70.0bc	0.0c
	No hole	188.6cde	93.0ab	53.3b
SAMPEA 10	One hole	176.8def	80.0abc	0.0c
	Two holes	187.4cde	60.0cd	0.0c
	Three holes	177.4def	46.7d	0.0c
	No hole	217.0ab	93.3ab	86.7a
SAMPEA 11	One hole	189.6cde	86.7ab	13.3c
	Two holes	187.9cde	60.0cd	0.0c
	Three holes	195.2c	60.0cd	0.0c
SEM(±)		5.03	7.27	5.96

^aHAP: Hours After Placement^bMean values in a column followed by the same letter(s) are not significantly different from each other at P=0.05

on the emergence of radicles and primary leaves deviated from this. Unlike in the main effects, there was no significant difference ($P>0.05$) in the mean percentage germination based on radicle emergence of seeds with no hole and those with one hole. On the other hand, just like in the main effects, there was a significant ($P<0.05$) difference in the mean percentage germination based on primary leaves emergence of seeds with no holes and those with at least one hole for all varieties.

DISCUSSION

During germination, water diffuses through the seed coat into the embryo, which has been almost completely dry during the period of

dormancy, causing a swelling of the seed. The swelling is often so great that the seed coat is ruptured. With the absorption of oxygen by the seed, energy is made available for growth. The foodstuffs stored in the cotyledons are broken down by enzymes into simpler substances that are transported through the embryo to the various centres of growth. The radicle (embryonic root) is the first portion of the embryo to break through the seed coat. It develops into root with root hairs that absorb water and attach the embryo to the particles of the soil. Thereafter, the embryonic shoot (plumule) emerges. This embryonic shoot comprises of three main parts - the cotyledons (seed leaves), the hypocotyl, and the epicotyls (Anon., 2013). In the case of the bean seed where

the cotyledons are brought into light, they develop chlorophyll and carry on photosynthesis until the true foliage leaves develop from the plumule.

Baidoo *et al.*, (2010) reported that weight loss in cowpea seeds was as a result of the larvae eating up the inside of the seeds. Results from this study show that the more the number of larvae feeding within the seeds, the more the holes made in the seed and consequently, the more the weight lost by the seed. The relatively lower mean weight per seed observed in SAMPEA 7 and SAMPEA 9 may be ascribed to the size of their seeds which has been described to be medium sized i.e. between 12 and 18g/100seeds (Ojumoola and Adesiyun, 2014). Based on radicle emergence at 96 HAP, SAMPEA 6 and SAMPEA 7 had the best germination. Both SAMPEA 6 and SAMPEA 7 have been reported to possess resistance to many stress factors (Anon., 2008). This quality may have enabled the two varieties to overcome the limitations posed by the exit holes to radicle emergence.

Furthermore, results obtained from this study also showed that germination (assessment based on the emergence of radicle and primary leaves at 96 HAP) was poor in seeds with one or more holes. This corroborates the report by Ofuya (2001) that infestation of cowpea seeds by *C. maculatus* also caused poor seed germinability. The failure of seeds with holes to germinate may be due to the inability of the seeds to imbibe sufficient water because their cotyledons had been decimated by the larval instars. The extent to which imbibition occurred has been reported to be dependent on the composition of the seed amongst other factors. It has been reported that cowpea seed contains 20-24% protein, 63.3% carbohydrates and 1.9% fat (Davis *et al.*, 1991). The principal component of seeds responsible for the imbibition of water is protein. Proteins are zwitter-ions that exhibited both negative and positive charges that attracted the highly charged polar water molecules. Starches like amylase have little affinity for water while lipids have no affinity for water (Mc Donald). The feeding of the larval stages on the cotyledon may have reduced these imbibition-aiding components thus the failure to germinate even in the presence of suitable

environmental conditions (adequate moisture, temperature and oxygen).

After the imbibition of water by seeds, erstwhile inactive enzymes are activated, which break down the sugar containing compounds within the cotyledon to produce energy molecules such as ATP needed for the emergence of the radicle (Mc Donald, n.d.). It might thus be inferred that by feeding on the seed's cotyledon, the larval stages of the beetle brought about a reduction in the quantity of energy molecules contained in it. Consequently, the seed was able to produce radicle after imbibition but unable to produce epicotyls and primary leaves because the energy produced was insufficient to drive the growth of these latter parts. This might explain the good to excellent germination (based on radicle emergence) and the poor to nil germination (based on primary leaves emergence) observed in seeds with holes.

The feeding activities of the larvae might be the main factor responsible for the failure of the seeds with holes to germinate well but it might not be the only factor. Several authors (Bewley, 1997; Duran and Retamal, 1989; Powell, 1989) have reported that the seed coat helped to restrict the rate at which imbibitions occurred. According to them, this restrictive function of the seed coat was important because rapid uptake of water could cause imbibition damages to the embryos of germinating seeds, particularly those that imbibe water quickly. Mc Donald *et al.*, (1988) also reported that in soybean, an intact seed coat is important to slow the uptake of water into the seed. In the cowpea seed, damage is caused to the seed coat during adult emergence. This may have resulted in imbibition damages to the embryo especially in those seeds with more than one hole hence their failure to germinate like those without holes.

From the foregoing, it can be seen that apart from suitable environmental conditions needed for germination, the good condition of the seed is an important factor without which germination might not occur or come to completion. This statement agrees with that made by Sehirali (1997) that although all factors affecting plant growth and development are at an optimum level, obtaining higher yield

depends on seed quality. However, in this study, the interaction of variety and number of exit holes had some effect on germination. In other words, the intactness of the seed's structure played a vital role in determining how well the seed manifested its innate agronomic potentials. Thus, a cowpea seed variety with high yield potentials will very likely not perform well if its structure has been damaged.

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