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SUSCEPTIBILITY OF SOME COWPEA VARIETIES TO THE SEED BEETLE *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: CHRYSOMELIDAE)

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Abstract: The study evaluated seven cowpea *Vigna unguiculata* L. Walp. varieties: IT89K-568-18, IFE-98-12, IT81P-994, IT89K-288, IT96-660, 'Milk' and 'Drum' for susceptibility to the seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). Seed samples (100 g) of each variety were infested with 5 pairs of the seed beetle (1–2 days old) with three replications at 30±2^{0}C and relative humidity of 69–72%. The parameters evaluated included: number of damaged and undamaged seeds, percentage seed weight loss, mean number of emerged adults, median developmental period and susceptibility index. The percentage seed weight loss in IT96-660 was significantly ($p<0.05$) higher than in IT89K-568-18, IT89K-288 and 'Milk'. The mean number of seeds damaged in IFE-98-12 was significantly ($p<0.05$) higher than in IT89K-568-18, IT81P-994, IT89K-288, IT96-660 and 'Milk'. The median developmental period in 'Milk' was significantly ($p<0.05$) higher than in IT89K-568-18, IFE-98-12, IT96-660 and 'Drum'. IFE-98-12 and IT89K-568-18 with susceptibility index of 10.4 and 7.8 were rated highly susceptible and moderately susceptible, respectively. The number of emerged adults was highly significant and positively correlated with the percentage seed weight loss. Results obtained showed that these cowpea varieties were relatively susceptible to *C. maculatus* attack in storage suggesting the need for their proper preservation against the insect.

Key words: *Callosobruchus maculatus,* cowpea, seeds, seed weight, susceptibility, variety.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is a leguminous crop grown in the tropical and sub-tropical regions of Africa (Kolawole et al., 2014). Cowpea is grown on an estimated 14.5 million hectares of land across the world. Global production of dried cowpeas in 2010 was 5.5 million metric tons, with Nigeria producing 2.2 million metric tons of dried grain in 2010 (CGIAR, 2011). Harvested

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cowpea seeds are mainly stored for subsequent use as human food, a cash crop product (Maina et al., 2012) and for the following season planting.

Seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae), is the most important and common pest of stored cowpea seeds in Africa. The attack of this pest of stored seeds resulted in it having many common names which include: bean beetle, pulse beetle, bean weevil, storage beetle, cowpea bruchid among others in different parts of Sub-Saharan Africa. The insect pest is capable of contaminating infested seeds with its faeces and causing physical damage through its post-harvest feeding and reproductive activities. It is therefore a major constraint to food security and income generation in the region.

There is evidence in the literature that most cowpea varieties are susceptible to *C. maculatus* in storage. For example, Badii et al. (2013) have reported that in order to reduce both seed loss due to bruchid attack and over-dependence on chemicals for control, the search for host plant resistance in cowpea varieties has increasingly become important. In Nigeria, control strategies to reduce post-harvest seed losses caused by insects still rely mainly on the use of synthetic insecticides which are not only expensive, but also cause environmental contamination and insect resistance. Appleby and Credland (2003) have reported that control methods have included development of 'resistant' varieties as an environmentally benign alternative to insecticides. This study examines the relative response of cowpea varieties to seed beetle, *C. maculatus*, in storage in order to provide a basis for development of reliable and sustainable resistance to the insect pest.

Materials and Methods

Sources and disinfestation of cowpea varieties: Improved cowpea varieties (IT89K-568-18, IT81P-994, IT89K-288 and IT96-610) were sourced from the International Institute of Tropical Agriculture, Ibadan, Nigeria. One improved cowpea variety, IFE-98-12, was obtained from the Institute of Agricultural Research and Training (IAR&T), Ibadan. Seeds of two local varieties ('Milk' and 'Drum') were purchased from Agro-allied Shop at Tipper Garage, Tanke, Ilorin, Nigeria. Seeds of all the cowpea varieties were separately wrapped in polyethylene bags and disinfested in a deep freezer at -18^{0}C for 3 days to kill developing insects and air-dried prior to the study.

Rearing colony of the seed beetle: Adult seed beetles were sourced from a well-conditioned culture kept in the Crop Protection laboratory, University of Ilorin, Nigeria. Twenty-five pairs of *C. maculatus* were randomly picked with a pooter and introduced into each of three 500-ml Kilner jars containing 200 g of cowpea seeds. The jars were covered with perforated lids to allow aeration and prevent escape of the insects. The adult seed beetles were allowed to lay eggs and multiply at ambient temperature of 30±2^{0}C and relative humidity of 69–72%. The

seeds in different jars were changed as required to prevent collapse of the culture. Freshly emerged adults (1–2 days old) were used for the study.

Experimental procedure: One-hundred-gram sample of each cowpea variety was measured into a transparent plastic container (14.3×11.5×9.6 cm) and then infested with 5 pairs of freshly emerged adult beetles (1–2 days old). A completely randomised design with three replications was used. The containers were covered with muslin cloth to allow aeration and prevent insect escape. The number of dead beetles was monitored, counted and recorded 5 days after infestation (DAI). The dead beetles were removed and discarded. Samples of 20 seeds were randomly selected from each replicate and examined daily for the number of eggs laid.

Data on damage indices were collected from the infested cowpea varieties 90 DAI and analysed for the following parameters:

Numbers of damaged and undamaged seeds: Cowpea seeds in each sample were separated into damaged (those with holes) and undamaged (those without holes) and then counted and recorded. The procedure of Amusa et al. (2014) was adopted. They considered a seed with at least a perforation from an adult bruchid emergence as a damaged seed.

Percentage seed weight loss: The seed sample of each container was reweighed and the percentage seed weight loss was determined as the difference between the initial seed weight and final seed weight in each replicate divided by the initial seed weight multiplied by 100.

Mean number of emerged adults: The daily number of emerged adults in each seed sample was counted using a tally counter and recorded from 23 DAI. The observation was terminated 46 DAI to avoid an overlap between emerged adults in the second generation.

Susceptibility index: The susceptibility index was calculated using the equation of Dobie (1977) as follows:

$$SI = {}^{\log_e F}/_{MDP} \times 100 \quad (1)$$

where: F = Total number of emerged adults;

$\log_e$ = Natural logarithm;

MDP = Median developmental period (days estimated as the time from the middle of the oviposition period to the emergence of 50% of the adults).

The Dobie's index of susceptibility was used to classify the cowpea varieties into different groups (Dobie, 1974; 1977) using the following scales:

Scale index of < 4.1 as highly resistant;

Scale index of 4.1 – 6.0 as moderately resistant;

Scale index of 6.1 – 8.0 as moderately susceptible;

Scale index of 8.1 – 10 as susceptible;

Scale index of >10 as highly susceptible.

Data analysis: Data were subjected to analysis of variance using the GENSTAT software version 5 and significantly different means were separated using Duncan's multiple range test at p = 0.05. Correlation coefficients of the damage parameters were also compared for the cowpea varieties.

Results and Discussion

Table 1 shows the various parameters considered in the determination of responses of seven cowpea varieties to *C. maculatus* infestation. Adult mortality of *C. maculatus* was not significantly different ($p>0.05$) among the varieties. Table 1 shows significant differences in the number of seeds damaged among the varieties, with a higher number of seeds damaged in IFE-98-12 than in 'Milk'. This indicates that the local variety ('Milk') could be visually rated as less susceptible when compared to IFE-98-12 which was highly susceptible to the seed beetle attack. It was observed that there was no significant difference in the percentage seed weight loss in IT96-660 and IFE-98-12. There was a significant difference ($p<0.05$) in the percentage seed weight loss among the varieties, namely IT96-660 had the highest percentage of seed weight loss (13.6) while 'Milk' had the lowest percentage seed weight loss (3.7). The percentage seed weight loss in IT96-660 was significantly different ($p<0.05$) from the percentage weight loss in IT89K-568-18, IT89K-288 and 'Milk'. However, IFE-98-12 also had the highest number of damaged seeds and consequently the highest susceptibility index.

There was a significant difference ($p<0.05$) in the mean number of eggs laid on the seeds of 'Drum' (34.7) when compared to other varieties (6.7 to 18.3).

The median development periods in IT81P-994 and IT89K-288 were not significantly different ($p>0.05$) from each other. The median development periods in IT89K-568-18, IFE-98-12, IT96-660 and 'Drum' were significantly lower than in 'Milk'. The least median development period and highest mean number of emerged adults were observed in IFE-98-12. In this study, the median development period ranged from 23 to 31 days while Beck and Blumer (2007) reported that the mean life cycle of *C. maculatus* ranged between 21 and 25 days on a susceptible variety.

The results also showed that there was a significant difference ($p<0.05$) in the mean number of emerged adults among the varieties, namely IFE-98-12 and 'Milk' had the highest (250.3) and lowest numbers (34.7) of emerged adults, respectively. The numbers of emerged adults in the varieties IT89K-288 and 'Milk' were significantly lower ($p<0.05$) than in IFE-98-12. A higher number of adult beetles emerged in cowpea variety with the shortest median development period, suggesting that the median development period played an important role in cowpea seed infestation (Table 1).

The results also showed significant differences between the varieties with respect to weight loss and the number of emerged adults. The results of the mean number of emerged adults and the susceptibility index showed varietal differences in the same weight of seed samples infested with the same number of insects. The susceptibility index and the mean number of emerged adults were higher on IFE-98-12 than on other varieties. The relatively shorter developmental period and higher number of emerged adults were recorded on the highly susceptible variety (IFE-98-12) while the longer developmental period and lower number of emerged adults were observed in moderately resistant varieties. This report shows that the high number of emerged adults and high seed weight loss can be used as traits of the seeds susceptible to *C. maculatus* attack. Abebe et al. (2009) have used the median developmental period and the percentage of seed damage as indicators of seeds susceptible to the insect attack. The size of the seeds may have been responsible for provision of a favourable site and nutriments for egg-laying and subsequent development. Other authors have reported that IT89K-288 which was noticed to be moderately resistant in this report was found to be resistant to bruchid (Singh et al., 1997) while Obadofin (2014) found that IT89K-288 was highly resistant to *C. maculatus*. In another investigation, Akintola and Oyegoke (2004) reported that IT89K-288 was moderately susceptible. Ileke et al. (2013) reported that IT96-610 was the least susceptible to *C. maculatus*.

Rating of cowpea seed varieties based on Dobie's scale: IT81P-994, IT96-660, and 'Drum', with susceptibility indices of 8.3, 8.5 and 9.0, respectively, were rated as susceptible and IT89K-568-18 with the susceptibility index of 7.8 was rated as moderately susceptible while IFE-98-12 with the susceptibility index of 10.4 was rated as highly susceptible. 'Milk' and IT89K-288 with susceptibility indices of 4.8 and 5.7, respectively, were rated as moderately resistant.

The number of damaged seeds in the cowpea varieties was significantly different from each other (Table 1). The mean numbers of damaged seeds in IT89K-568-18, IT89K-288 and 'Milk' were significantly lower than in other varieties. The numbers of damaged seeds in IT81P-994 and IT96-660 were significantly ($p<0.05$) higher than in IT89K-568-18, IT89K-288 and 'Milk'. The results of this study have corroborated the findings of other authors (Adam and Baidoo, 2008; Amusa et al., 2013; Mogbo et al., 2014) that *C. maculatus* has the ability to cause severe damage to cowpea seeds. This is probably the reason why the insect is a serious pest of stored cowpea seeds in Nigeria. Oke and Olajide (2012) reported that cowpea varieties exhibited some levels of resistance and susceptibility to *C. maculatus*. With recent advances in biotechnology and plant breeding, it is possible to remove desirable characters from resistant varieties and transfer them to other cowpea varieties to improve their resistance to cowpea bruchid (Ileke et al., 2013). Visual observation of the characteristics of the cowpea varieties and behaviour of the beetle showed that IFE-98-12 (an improved variety)

was the most susceptible and 'Milk' (a local variety) was the least susceptible to bruchid infestation. The results showed a highly significant difference among the varieties with respect to all the parameters studied except adult mortality.

Table 1. Responses of improved and local cowpea varieties to *Callosobruchus maculatus* attack.

Cowpea varieties	Adult mortality ± SD	Mean No. of eggs/20 seeds ± SD	Mean No. of emerged adults ± SD	MDP±SD (days)	% Seed wt. Loss (30DAI) ± SD	No. of damaged seeds ± SD	No. of undamaged seeds ± SD	Susceptibility index ± SD
IT89K-568-18	5.33±0.94^{a}	16.3±7.59^{b}	79.0±1.24^{ab}	24.3±0.82^{b}	5.8±0.91^{b}	55.0±1.63^{c}	548.7±12.65^{a}	7.8±1.38
IFE-98-12	5.67±1.70^{a}	18.3±3.86^{b}	250.3±101.65^{a}	23.0±0.00^{b}	9.9±3.69^{ab}	170.3±53.54^{a}	416.3±19.04^{b}	10.4±0.93
IT81P-994	7.00±0.82^{a}	10.0±3.32^{b}	177.7±50.17^{ab}	27.0±1.41^{ab}	7.5±2.85^{ab}	100.7±7.41^{b}	441.7±48.80^{b}	8.3±1.94
IT89K-288	7.00±2.16^{a}	6.7±3.86^{b}	39.3±38.06^{b}	27.7±3.09^{ab}	6.1±0.76^{b}	28.7±1.25^{c}	447.3±55.51^{b}	5.7±1.96
IT96-660	6.33±2.62^{a}	8.7±8.29^{b}	145.7±62.29^{ab}	25.3±0.47^{b}	13.6±5.99^{a}	111.7±5.73^{b}	553.3±66.02^{a}	8.5±0.75
Milk	6.00±1.73^{a}	8.0±3.56^{b}	34.7±9.18^{b}	31.0±4.24^{a}	3.7±1.83^{b}	21.0±1.41^{c}	447.0±44.73^{b}	4.8±1.66
Drum	6.33±1.25^{a}	34.7±8.18^{a}	111.0±42.13^{ab}	24.0±0.00^{b}	8.2±0.97^{ab}	135.7±4.50^{ab}	255.7±36.17^{c}	9.0±0.80
SEM	1.18	3.82	57.0	2.07	2.13	14.59	31.2	

Values with the same superscript(s) within the same column are not significantly different at p=0.05 using Duncan's multiple range test.

This study demonstrates considerable variation among the cowpea varieties in their response to *C. maculatus* infestation. It was observed that none of the cowpea varieties was highly resistant to the insect attack. The variation in response of the cowpea varieties to *C. maculatus* infestation suggests inherent variation in genetic factors among the seed varieties screened. This offers opportunity for researchers to differentiate between highly susceptible varieties such as IFE-98-12 reported in this study and other less susceptible varieties. The moderately resistant varieties, 'Milk' and IT89K-288, were not significantly different from each other in most of these parameters and had low numbers of emerged adults, seeds damaged, and percentage seed weight loss. It has been reported that variables such as adult emergence, growth index, developmental period and weight loss are the most reliable indicators for resistance of cowpea to damage by *C. maculatus* (Reden and McGuire, 1983; Jackai and Asante, 2003; Ewedairo et al., 2015).

Table 2 indicates the correlation coefficient of damaged parameters considered in this study. An inverse relationship existed between the susceptibility index and the median development period. However, the number of emerged adults was highly significant and positively correlated with percentage seed weight loss. It

was also observed that the mean number of emerged adults and percentage seed weight loss were highly significant and positively correlated with SI. The correlation coefficient showed that the number of damaged seeds and the susceptibility index were significant but negatively correlated with the median development period. The number of undamaged seeds was highly significant but negatively correlated with the mean number of eggs laid.

Table 2. Correlation coefficient of damage parameters used to determine susceptibility of cowpea seed varieties to *Callosobruchus maculatus.*

	Mean developmental period	Mean No. of eggs	% Seed wt. loss	No. of emerged adults	Adult mortality	No. of damaged seeds	No. of undamaged seeds	Susceptibility index
Mean developmental period	-							
Mean No. of eggs	-0.3688	-						
% Seed wt. loss	-0.4365	0.0457	-					
No. of emerged adults	-0.4866*	0.1756	0.6069**	-				
Adult mortality	0.2939	-0.0313	-0.4101	-0.0902	-			
No. of damaged seeds	-0.5618**	0.5220*	0.5178*	0.4964*	-0.1517	-		
No. of undamaged seeds	0.0314	-0.5972**	0.0291	-0.1613	-0.1059	-0.3205	-	
Susceptibility index	-0.7483**	0.3647	0.6216**	0.8578**	-0.2635	0.6891**	-0.1324	-

*Significant at p=0.05; **Highly significant at p=0.01.

Conclusion

In this study, differences were observed among cowpea varieties regarding their susceptibility to *C. maculatus* attack. This report shows that the most susceptible was an improved variety (IFE-98-12) and that none of the varieties was highly resistant to *C. maculatus* attack. To some extent, the moderately resistant varieties ('Milk' and IT89K-288) can be suggested for inclusion in the pest management technique aimed at reducing *C. maculatus* attack. A further empirical investigation is in progress to assess the role of physical characteristics and phenol content of these cowpea varieties in relation to the seed damage indices. In the end, plant breeders should be encouraged to offer farmers cowpea varieties resistant to insect infestation.

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OSETLJIVOST NEKIH VARIJETETA VIGNE NA CRNI ŽIŽAK *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: CHRYSOMELIDAE)

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R e z i m e

Studijom se procenjuje sedam varijeteta vigne *Vigna unguiculata* L. Walp.: IT89K-568-18, IFE-98-12, IT81P-994, IT89K-288, IT96-660, „Milk" i „Drum" imajući u vidu osetljivost na crni žižak vigne, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). Uzorci semena (100 g) svakog varijeteta bili su inficirani pomoću 5 parova crnog žižka (starosti 1–2 dana) sa tri ponavljanja na temperaturi 30±2^{0}C i relativnoj vlažnosti 69–72%. Vrednovani parametri su uključivali: broj oštećenih i neoštećenih semena, procentualni gubitak mase semena, srednji broj izleglih odraslih, srednji razvojni period i indeks osetljivosti. Procentualni gubitak mase semena kod varijeteta IT96-660 bio je značajno ($p<0,05$) viši nego kod varijeteta IT89K-568-18, IT89K-288 i „Milk". Srednji broj semena oštećenih kod varijeteta IFE-98-12 bio je značajno ($p<0,05$) više nego kod varijeteta IT89K-568-18, IT81P-994, IT89K-288, IT96-660 i „Milk". Srednji razvojni period žižka kod varijeteta „Milk" bio je značajno ($p<0,05$) viši nego kod varijeteta IT89K-568-18, IFE-98-12, IT96-660 i „Drum". IFE-98-12 i IT89K-568-18 sa indeksom osetljivosti 10,4 i 7,8 ocenjeni su kao veoma osetljivi odnosno umereno osetljivi. Broj izleglih odraslih žižaka bio je veoma značajan i pozitivno je korelirao sa procentualnim gubitkom mase semena. Dobijeni rezultati su pokazali da su ovi varijeteti vigne relativno osetljivi na napad insekta *C. maculatus* prilikom skladištenja sugerišući potrebu za njihovim odgovarajućim čuvanjem radi zaštite od ovog insekta.

Ključne reči: *Callosobruchus maculatus,* vigna, semena, masa semena, osetljivost, varijetet.

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