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EFFECTS OF FIVE IMPROVED COWPEA SEED GENOTYPES ON OVIPOSITION AND PROGENY DEVELOPMENT OF *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

This study was aimed at determining the effects of genotype differences on egg laying, larval, pupal development and adult emergence of the cowpea seed bruchid, *Callosobruchus maculatus* (F.). Five cowpea genotypes (SAMPEA 6, SAMPEA 7, SAMPEA 9, SAMPEA 10 and SAMPEA 11) developed by the Institute for Agricultural Research (IAR) in Samaru, Zaria, Nigeria were used. Oviposition, larval development and pupal development bioassays were carried out under ambient laboratory conditions by sampling 20 seeds at the 7th, 14th and 19th day after infestation (DAI) respectively. The number of eggs, larvae and pupae in sampled seeds were counted and recorded. Adult emergence bioassay was done by counting daily the number of emerged adults until 35 DAI when adult emergence stopped. Results from the study showed that the highest mean number of eggs (41.3), larvae (28.3), and pupae (25.7) per 20 seeds at 7DAI, 14 DAI and 19 DAI respectively occurred in SAMPEA 6. Also, more adults (80.6) were observed to have emerged from SAMPEA 6 than from any other genotype. With respect to all bioassays in this study, SAMPEA 6 was significantly different ($P<0.05$) from all other genotypes. On the other hand, SAMPEA 10 had the least mean number of eggs (14.0) and larvae(8.0) per 20 seeds at 7DAI and 14DAI respectively and was significantly different ($P<0.05$) from the other genotypes. However, at 19DAI, SAMPEA 9 was observed to have the lowest mean number of pupae (4.7) per 20 seeds and was significantly different ($P<0.05$) from the other genotypes. There was no significant difference ($P>0.05$) in the number of adults that emerged from SAMPEA 7, SAMPEA 9, SAMPEA 10 and SAMPEA 11 at $P = 0.05$.

Keywords*:* *Cowpea* genotypes, SAMPEA, *Callosobruchus maculatus*, oviposition and development.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp., is native to central Africa. It is widespread throughout the tropics and most tropical areas between 40°N to 30°S and below 2000 m altitude (Ecocrop, 2009). Cowpea grows in savannah vegetation at temperatures ranging from 25°C to 35°C and in areas where annual rainfall ranges from 750 mm to 1100 mm (Madamba *et al*., 2006). Cowpea is primarily grown as a pulse, but also as a vegetable both for the green leaves and green seeds. Cowpea is the main edible leguminous plant which is cultivated all over the West of Africa (Mondedji *et al*., 2002). Trade in dry cowpea grains 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 level (Singh, 2002; Langyintuo *et al*., 2003). Nigeria is the largest producer and consumer of cowpeas and accounts for about 45 percent of the world's cowpea production (USAID, 2008). However, the crop suffers heavily from insects, both on the field and when grains are stored after harvest. Yield reduction caused by the insects can reach as high as 95%, depending on location, year, and cultivar (Carlos, 2000). Insect infestation during storage markedly reduces the quality, viability and market value of the grains (Emeasor and Emosairue, 2002). The highest losses of grain legumes during storage are due to bruchids. In storage, *Callosobruchus maculatus* (F.), is regarded as the most important and common pest of cowpea both in Africa and Asia (Deshpande *et al*., 2011). Infestation starts in the field, but heavy damage is done in storage (Swella and Mushobozy, 2007). Infestation on stored grains may reach 50% within 3-4 months of storage (Pascual-Villalobus and Ballesta-Acosta, 2003). The use of conventional insecticides, inert materials, extracts, oils and powders of plants parts with insecticidal properties as well as development of cowpea varieties resistant to *C. maculatus* are some of the several tools being used in the fight against bruchids in stored cowpea. However, the development and use of resistant legume varieties offers a simple, cheap and attractive approach to the reduction of bruchid damage (Ofuya and Lale, 2001). The expenses to the farmer are limited because he only has to buy the seeds and no environmental hazards are involved (Ahmed and Yusuf, 2007). Consequently, several local and improved cultivars of cowpea are continuously being screened for resistance to the cowpea beetle *C. maculatus*. One of these, TVu 2027, led to the development of several lines such as IT81D-1032, IT81D-1064 and IT81D-1045, that show different levels of resistance to the bruchid (Singh and Singh, 1990). IT89KD-391, IT90K-82-2, IT97K-499-35, IT98K-1092-2,IT99K-491-7,IT98K-1111-1, Ife Brown, Kanannado brown, Banjara, Borno brown, Kanannado brown and Saddam etc., are some cultivars in Nigeria that have been screened and reported (Oludare, 2011; Maina *et al*., 2012). This study was therefore aimed at evaluating some improved cowpea (*Vigna unguiculata*) seed genotypes developed in Nigeria for resistance to *C. maculatus.*

MATERIALS AND METHODS

Study area, sources and types of cowpea seeds

The study, which was carried out at the height of the dry season, was conducted in a laboratory within the professorial suite of the Faculty of Agriculture, University of Ilorin, Ilorin (8.5'N 4.53'E). The improved genotypes of cowpea (*V. unguiculata* (L.) Walp) seeds used for the experiments were obtained from the Institute for Agricultural Research (IAR), Samaru, Zaria, Nigeria. The genotypes include SAMPEA 6, SAMPEA 7, SAMPEA 9, SAMPEA 10 and SAMPEA 11. The morphological characteristics of the different genotypes are shown in Table 1. SAMPEA 6 is one of the parents of the American black eye beans. SAMPEA 6 and SAMPEA 7 are the most popular of the lot with yield potential of 2.5t/ha and resistance to many stress factors (http://globalbiofuelsnig.org, 2008). SAMPEA 9 is dual purpose (high grain and fodder yields). SAMPEA 10 is a genotype resistant to Striga and bacteria. SAMPEA 11 is a dual-purpose cowpea genotype with a rough seed coat. It has combined resistance to major diseases including septoria leaf spot, scab, and bacterial blight, as well as to nematodes and tolerance for Nigeria's strain of *Striga gesnerioides* (http://r4dreview.org, 2010).

Sterilization of seeds

The seeds were sterilized by keeping inside the freezer compartment of a Thermocool-250 refrigerator for 4 days at 0^oC. This was carried out to disinfest the seeds.

Rearing of *Callosobruchus maculatus*

Callosobruchus maculatus beetles used for the experiment were got from an insect culture sourced from the Nigerian Stored Products Research Institute (NSPRI), Ilorin. From the parent insect culture, a new generation of *C. maculatus* was reared inside a Kilner jar in the laboratory at 29±1 ^oC and 75±5 R.H. About 50 adult beetles (male and female) were introduced into a 1 litre plastic jar containing about 500 'clean' cowpea seeds i.e. unbroken seeds that were devoid of holes or eggs. The seeds occupied about one-third of the jar leaving two-third of free space above them. The mouth of the jar was covered with a piece of muslin cloth held with rubber bands. This set-up allowed for the free movement of air in and out of the jar while preventing the beetles from escaping. The culture was left on the laboratory table and the beetles were allowed to mate and oviposit on the seeds for 7 days. After 7 days of oviposition, many of the seeds were observed to have eggs on them. Both the dead and living beetles were then removed from the jar and the culture left and observed for adult emergence. A first filial generation (F_1) of adult *C. maculatus* began emerging at 20-21 days after oviposition.

Experimental Procedure

Oviposition bioassay

First filial generation (F_1) adult *C. maculatus* beetles that emerged within 48 hours from the insect culture were used for the experiment. Ten (10) of these (5 males and 5 females) were introduced into five small plastic jars each containing 80 unwrinkled, uninfested seeds of a genotype. Each genotype was taken as a treatment and each treatment was replicated three times. The beetles were allowed to mate and oviposit for 7 days on the seeds. At the 7th day after infestation (DAI), the beetles were removed. Thereafter, 20 seeds were randomly sampled from each container. The number of eggs laid on each of the 20 seeds sampled were counted and recorded. Each set of 20 seeds sampled at 7DAI, were not returned into the jar from which they were taken but were placed in a Petri-dish and covered.

Progeny development and emergence bioassay

At 14DAI, another set of 20 seeds were sampled randomly from each replicate and then each seed was dissected with a razor blade in order to count and record the number of larvae within it. Likewise, at 19 DAI, another set of 20 seeds were sampled randomly from each replicate and then each seed was again dissected and the number of pupae in it counted and recorded. The remaining 20 seeds in each container as well as the 20 seeds of each genotype placed in Petri-dishes at day 7 were left and observed for adult emergence. Adult emergence started to occur from 21 DAI. Total number of emerged adults from each treatment was counted and recorded after all emergences had ceased (at about 35 DAI).

Data Analysis

Data collected were subjected to a one-way ANOVA in a Completely Randomized Design (CRD) model using SPSS computer package. The separation of treatment means was done using New Duncan's Multiple Range Test (DMRT) at 5% level of probability.

RESULTS

Effect of genotype differences on oviposition at 7 Days After Infestation (DAI)

Table 2 shows results from the oviposition bioassay at 7DAI. The highest mean number of eggs (41.3/20seeds) observed in SAMPEA 6 was significantly different ($P<0.05$) from the lowest mean number of eggs (14.0/20seeds) observed in SAMPEA10. Other genotypes had intermediate values and were not significantly ($P>0.05$) different from each other. With the exception of SAMPEA 10 whose seeds were averagely oviposited on (48.3%), all other genotypes had more than half of their seeds oviposited. Notwithstanding, there was no significant difference ($P>0.05$) in the percentage of seeds oviposited in all the genotypes at 7DAI.

Effect of genotype differences on larval development at 14 Days After Infestation (DAI)

Table 2 also shows results from larval development bioassay at 14DAI. SAMPEA 6 had the highest mean number of larvae (28.3/20seeds) while SAMPEA 10 had the lowest (8.0/20seeds). There was a significant difference ($P<0.05$) between the mean number of larvae per 20 seeds of SAMPEA 6 and SAMPEA 10. Other genotypes had intermediate values and were not significantly different ($P>0.05$) from one another. Though SAMPEA 6 had the highest percentage of seeds with larvae (73.3%) at 14DAI, it was not significantly different ($P>0.05$) from SAMPEA 7 (66.6%), SAMPEA 9(55.0%) and SAMPEA 11(58.3%). SAMPEA 10 had the lowest percentage (35.0%) of seeds with larvae and was significantly different ($P<0.05$) from SAMPEA 6 and SAMPEA 7 only.

Table 1: Morphological characteristics of the cowpea seed genotypes

Genotype	Source of seed	Seed* Size	Seed coat colour
SAMPEA 6	IAR	Large	White
SAMPEA7	IAR	Medium	Brown
SAMPEA 9	IAR	Medium	White
SAMPEA 10	IAR	Large	White
SAMPEA 11	IAR	Large	White

*Small= <12g/100 seeds, Medium=12-18g/100seeds, Large= >18g/100seeds (Edde and Amatobi, 2003).

Table 2: Oviposition and larval development in different genotypes of cowpea

Genotype	Number of eggs laid/20seeds at7DAI	Percentage of seeds oviposited at 7DAI	Number of larvae/20seeds at 14DAI	Percentage of seeds with larvae at 14DAI
SAMPEA 6	41.3b	85.0a	28.3b	73.3b
SAMPEA7	30.6ab	71.6a	23.0ab	66.6b
SAMPEA 9	34.0ab	78.3a	16.6ab	55.0ab
SAMPEA 10	14.0a	48.3a	8.0a	35.0a
SAMPEA 11	33.6ab	81.6a	16.3ab	58.3ab

Values in the same column with the same letters following them are not significantly different at P=0.05

Table 3: Pupal development and adult emergence in different genotypes of cowpea

Genotype	Number of pupae/20seeds at 19DAI	Percentage of seeds with pupae at 19DAI	Mean number of emerged adults
SAMPEA 6	25.7c	81.6c	80.6b
SAMPEA7	16.6bc	50.0b	48.0a
SAMPEA 9	4.7a	18.3a	22.6a
SAMPEA 10	14.0ab	55.0b	36.3a
SAMPEA 11	14.0ab	43.3ab	40.0a

Values in the same column with the same letters following them are not significantly different at P=0.05

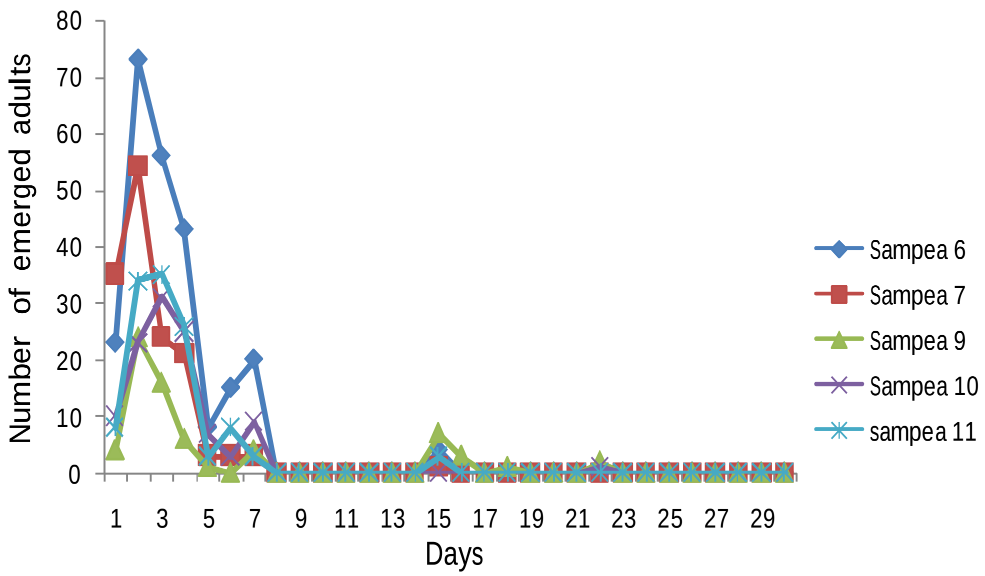

Figure 1: Daily adult emergence of *C. maculatus* in the different genotypes of cowpea over a period of 30 days

Effect of genotype differences on pupal development at 19 DAI

Table 3 shows results from pupal development bioassay at 19DAI. The highest mean number of pupae (25.7/20seeds) and lowest mean number of pupae (4.7/20 seeds) occurred in SAMPEA 6 and SAMPEA 9 respectively. Other genotypes had intermediate values. There was a significant difference (P<0.05) between the mean number of pupae per 20 seeds of SAMPEA 6 and that of SAMPEA 9. Furthermore, 81.6% of seeds of SAMPEA6 were found with pupae while only 18.3% of seeds of SAMPEA 9 had pupae at 19DAI. Other genotypes had intermediate values. There was a significant difference (P<0.05) between the percentage of seeds with pupae of SAMPEA 6 and SAMPEA 9.

Effect of genotypes differences on total adult emergence of *C. maculatus*

Results presented for adult emergence bioassay (table 3) show that SAMPEA 6 had the highest mean number of emerged adults (80.6) from 40 seeds while SAMPEA 9 had the lowest (22.6). There was a significant difference (P<0.05) between the mean number of emerged adults from SAMPEA 6 and all the other genotypes. However, no significant difference (P>0.05) existed between SAMPEA 7, SAMPEA 9, SAMPEA 10 and SAMPEA 11.

Figure 1 shows the daily emergence of adults from the genotypes over a period of 30 days. SAMPEA 6 had the highest number of emerged adults within the first 6 days followed by SAMPEA 7. SAMPEA 6 still had more adult emergence above the other varieties within the 6^{th} and 9^{th} day after emergence was first observed.

DISCUSSION

All the genotypes of cowpea seeds (SAMPEA 6, SAMPEA 7, SAMPEA 9, SAMPEA 10 and SAMPEA11) used for the experiment were oviposited on. This agrees with Cope and Fox (2003) that the female *Callosobruchus maculatus* will deposit eggs on nonviable surfaces especially if there are few or no hosts available. However, some genotypes were observed to have more eggs than others. This observation also agrees with the statement made by Ofuya and Bamigbola (1991) that the realized fecundity of the female *C. maculatus* is variable and is influenced by the kind of host infested. SAMPEA 6 and SAMPEA 10 had the highest and the lowest mean number of eggs respectively. Both are large seed genotypes showing clearly that the variation in oviposition amongst the genotypes used was not due to seed size. This observation corroborates Edde and Amatobi (2003) who stated that oviposition by the bruchid varies from one cowpea variety to another but that seed size was not a likely explanation for the observation. In addition, SAMPEA 7 (with brown coloured seeds) did not show a significant difference in mean number of eggs laid when compared to SAMPEA 9 and SAMPEA 11(both with white coloured seeds). Work by Edde and Amatobi (2003) on different cultivars of cowpea seeds with different colours did not also show any correlation between seed colour and the number of eggs laid on cowpea varieties.

Varieties that express resistance have physical or biochemical attributes that modify behavioural responses (xenobiosis) or that adversely affect development or survival of the pest insect species through metabolic aberrations (antibiosis). It was determined by Tarver ***et al***. (2006) that cowpea bruchids feed differently when living in resistant cowpea seeds compared to susceptible ones. Using the cowpea bruchid resistant variety – TVu 2027 as a case study, these authors showed that wild-type larvae penetrated much more deeply into susceptible seeds and created larger cells than they did in TVu 2027. They attributed the presence of a physical barrier in the interior of the TVu 2027 seeds that the insects cannot penetrate; a zone in the interior of the seed that is poor in nutritional value, and thus does not support normal larval growth and development; a toxin that is more concentrated toward the interior of the cotyledon; a repellant factor in the interior of the seeds; or a combination of the foregoing as possible factors responsible for the resistance exhibited by TVu 2027. Either of these factors may hold the explanation for the variation in the number of larvae in the seeds of the different genotypes screened in this study. Since it is only living larvae that are capable of pupating, then whatever factor(s) inhibiting larvae development within the different seed genotypes must have indirectly affected pupation within each seed variety hence the variation observed in the number of pupae within the different seeds. Though all five varieties had their highest mean number of emerged adults within the first 7 days, SAMPEA 6 had the highest mean number of emerged adults during the period. The results obtained show that genotype differences has some effect on adult emergence. This agrees with Adam and Baidoo (2008) who reported that adult emergence was indeed influenced by cowpea varieties and that more adults emerge from less resistant varieties. Consequently, SAMPEA 6 which had the highest mean adult emergence may be said to be the most susceptible of the five varieties.

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