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COMPARATIVE DEVELOPMENT OF COWPEA BRUCHID, (*Callosobruchus maculatus* (F.) (COLEOPTERA:BRUCHIDAE) ON PODS AND SEEDS OF SOME COWPEA CULTIVARS (*Vigna unguiculata*

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ABSTRACT

The seeds of high-yielding and earlier-maturing cowpea cultivars, IT83E-761, TVNu-72, TVx3236 and Ife Brown ... compared for their pods resistance to *Callosobruchus maculatus*, a cosmopolitan field-to-store pest and principal post-harvest pest of cowpeas. Parameters used for comparison were mean number of eggs laid on each cultivar, mean percent egg hatched, mean percent larval mortalities, mean percent pupal mortalities, mean percent emerging adult mortalities, mean percent adult emergence, developmental periods and percent weight loss of seed. TVNu-72, Tvu 3236 and TVu 3000 demonstrated significant resistance to *C. maculatus* compared to IT83E-716 and Ife brown with pods showing more resistance than seeds. These cultivars caused both larval mortalities and significant deterrence to oviposition. In comparison, *C. maculatus* showed significant preference for Ife brown and IT83E-716 compared to others as significantly more eggs were laid on the former than the latter (cultivars). It is suggested that pod wall hairiness and thickness, seed coat oviposition deterrent factor(s) and cotyledonal antibiotic factor(s) are involved in conferring polygenic resistance on TVNu-72 and Tvx 3236 cultivars.

KEYWORD: ***Callosobruchus maculatus, Vigna unguiculata*, cultivar**

INTRODUCTION

Cowpea, *Vigna unguiculata* (L) Walp of the family Fabaceae is one of the most important grain legumes in the tropics where it serves as a major source of protein in human as well as animal diets. It also constitutes the cheapest source of dietary protein for most people in many developing countries where per capital income and the daily intake of animal protein are both very low (Lale and Efevbokhan, 1991; Ogunwolu and Idowu, 1994). Its high protein and lysine contents make it a natural supplement to staple diets of cereals, root tubers and fruits. The mature seeds are an important pulse, chiefly in Africa and are often ground into a meal, whereas the immature pods are eaten fresh, frozen or canned. The dried seeds are sometimes used as substitutes for coffee beans while the young shoots and leaves are eaten like spinach. The seeds may be used as concentrates for farm animals. The food is highly palatable to all type of livestock and the crop is used as hay, silage, pasture, soil cover and green manure.

The dried pulse contains approximately 12.0% moisture, 24.6% protein, 0.7% fat, 55.7% carbohydrate, 3.8% fibre and 3.2% ash (Kochhar, 1981). According to Blade *et al* (1997), Nigeria accounts for 70% of the world's production. Total production in Nigeria is 700,000 – 800,000 tonnes with 80% from Northern Nigeria (Kochhar, 1981).

The availability and utilization of cowpeas in tropical countries are reduced due to seed destruction by larvae of the cowpea seed bruchid, *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae), a cosmopolitan field-to-store pest ranked as the principal post harvest pest of cowpea. (Gatehouse *et al*, 1985; Ofuya, 1987, Lale and Efeovbohan, 1991).

Its initial infestation begins in the field and continues in storage where its population grows rapidly causing substantial qualitative and quantitative damage or losses (Messina and Renwick, 1985; Singh and Ntare, 1985). An individual bruchid is capable of causing substantial qualitative and quantitative weight loss after only four months of storage (Tanzubil, 1987). In Nigeria alone, the estimated value is in excess of 30 million US dollar (Singh *et al* 1983). Attack by this bruchid not only causes reduced seed weight, but also affects the aesthetic and nutritional qualities of the grain and reduce seed viability (IITA, 1983). For instance, Pingale *et al* (1956) reported a reduction in the amount of thiamne in chickpeas and green grain infested with *C. maculatus* roughly proportional to the amount of pest damage to the seed. In addition, insect damage caused slight denaturation of protein, cowpea seeds harbouring any stage of the bruchid are unpalatable and seeds riddled with emergence holes are unmarketable (Southgate, 1979). It has also been reported that loss in seed germination due to bruchid attack may reach 100% for grains with four emergence holes (Santos, 1971).

Over the years, individual methods of controlling this bruchid with differing levels of effectiveness have been devised. Chemical control methods such as fumigation of the store or commodity with carbon disulphide or phosphine, or dusting with malathion, carbaryl, pirimphos-methyl or permetherine, have been reported to be effective against *C. maculatus* (Ogunwolu and Idowu, 1994; Onolemhemhen and Oigiangbe, 1991). However, such chemicals are seldom used by African peasant farmers due to financial, technical and safety considerations. Plant oils and powders obtained especially from the seed of various plants have also been used to protect cowpea from damage in storage (Lale and Mustapha, 2000). Different storage devices which provide hemetic conditions have also been reported to be effective. Traditionally, products such as ashes, sand and clay minerals are also used. However, from the point of view of the farmer, the cheapest and most effective method of protecting cowpea against *C. maculatus* in storage is the use of resistant varieties of cowpea. Thus, the use of cowpea varieties whose seeds could deter and/or reduce oviposition or significantly delay insect development would offer farmers an economical and ecological acceptable means of controlling bruchids in tropical storage (Ofuya and Credland, 1995). The thickness of the pod wall has also been reported to confer varying degrees of protection against the cowpea bruchids. Akingbohungbe (1976) and Fatula and Badaru (1983 a,b) reported that

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cultivars with abnormal thick pods provide useful resistance to attack before the pod is threshed thus lessening subsequent infestation in the store. Pod wall resistance on selected lines and its impact on population suppression and damage (IITA, 1983) suggested that pod wall resistance of one variety if combined with seed resistance of another variety could supress more than 97% of the insect population. These mechanisms would be an advantage because it would diminish the risk of *C. maculatus* overcoming resistance that depends on a single factor. Consequently, the International Institute of Tropical Agriculture (IITA) has pioneered the development of several cowpea lines with varying degrees of resistance for the management of *C. maculatus* in tropical countries.

This research therefore examines the oviposition and development of *C. maculatus* on the pods and seeds of some cowpea cultivars with a view to testing the susceptibility or otherwise of (some) already developed cowpea lines to attach by *C. maculatus*.

MATERIALS AND METHODS

A stock culture of males and females emerging adults of the cowpea bruchid, *C. maculatus* was obtained from the International Institute of Tropical Agriculture (IITA), Ibadan and maintained in the laboratory for three months.

Healthy seeds and pods of cowpea were obtained from IITA, Ibadan. The seeds were planted and watered at 7.00 a.m. and 6.00p.m. daily till they poded. The harvested pods and seeds were then used for the experiment.

Into each rearing cage on which a wire guage was placed at the top before the lid was fitted by turning and the bottom covered with filter paper, three fresh pods of each variety IT83E-71-6, TVNU-72, TVX 3236, Ife brown and TVU 3000 were introduced and labelled appropriately. Each cage also contained a pair of freshly emerged adult male and female *C. maculatus*. Also 5% glucose solution was provided. Five rearing cages with their content were provided for the trials and replicated four times.

The experiment was repeated with 5g of fresh seeds of same variety in separate rearing cages and replicated four times. Daily observations were made in order to monitor mating periods and fecundity rate of females. The adult male and female were removed after 5 days of egg laying but replaced when a male died before a female and recently emerged adult male was introduced. The number of eggs deposited in each raring cage was counted and monitored for hatching.

First filial generation adult *C. maculatus* emerged from pods and seeds in each replicate batch, and each variety was counted and removed to avoid second generation emergence, starting from the day emerged adult beetle appeared. These counts continued until 14^{th} day when it was terminated. All seeds in each replicate for all varieties were opened and dead larvae and pupae were counted. Adults emergence, larval and pupal mortalities were expressed as percentage of the total number of eggs that hatched.

RESULTS

FECUNDITY AND OVIPOSITION

Fecundity of *C. maculatus* was obtained by counting the number of eggs laid per variety of cowpea. This was however influenced by feeding the adult female beetle with glucose solution.

Generally, oviposition was highest in seeds than pods with Ife brown having 48.0 and 79.8 mean eggs on pods and seeds respectively while the least mean eggs was laid on TVNU – 72 that had 13.8 on pods and 19.3 on seeds (Table 1). Analysis of variance showed that there was no significant difference in oviposition on the seed and pod (P<0.05). The number of eggs laid decreased with age, freshly laid eggs being translucent but turns milky white in a few hours. Oviposition by the insect was best on Ife brown followed by IT83E-716 , TVX 3236 , TVU 3000 and TVNU-72.

EGG HATCHABILITY

Hatching occurred between day six and seven. Mean egg hatched on seeds ranged from 13.8 to 74.5 and 11.3 to 45.3 on pods of TVNU-72 and Ife Brown respectively (Table 1). Analysis of variance (P=0.05) revealed that there was no significant difference in the hatchability on seeds and pods.

Percentage hatchability was generally high in all varieties with the pods having a range of 81.9 - 94.4 and seeds 71.5 - 93.4. Correlation test showed that there was no significant difference in percentage hatchability on the different varieties of seeds and pods.

TABLE 1: COMPARATIVE PERFORMANCE OF *C. maculatus* ON DIFFERENT COWPEA CULTIVARS

Cultivars	Mean Oviposition		Mean Hatching		Mean Adult Emergence	
	Seed	Pod	Seed	Pod	Seed	Pod
IT83E-716	70.5	47.5	63.3 (89.4)	42.5 (89.5)	47.3 (75.1)	22.5 (52.9)
TVNU-72	19.3	13.8	13.8 (71.5)	11.3 (81.9)	3.3 (23.9)	2.0 (17.7)
TVU 3000	63.3	39.8	55.0 (87.7)	34.8 (87.4)	32.5 (59.1)	15.8 (45.4)
TVX 3236	64.8	41.5	54.0 (83.3)	35.0 (84.3)	30.3 (56.1)	34.3 (40.9)
Ife brown	79.8	48.0	74.5 (93.4)	45.3 (94.4)	58.5 (78.5)	28.3 (62.5)

Figures in parentheses are percent values of means.

TABLE 2. COMPARATIVE MORTALITY OF *C. maculatus* ON DIFFERENT COWPEA CULTIVARS

Cultivars	Mean No. of Dead Larvae		Mean No. of Dead Pupae		Mean No. of Dead Emerging Adult	
	Seed	Pod	Seed	Pod	Seed	Pod
IT83E-716	10.3 (16.4)	15.7 (36.9)	3.3 (5.2)	2.3 (5.4)	2.0 (3.2)	1.2 (2.8)
TVNU-72	8.3 (60.1)	6.2 (60.)	1.0 (8.6)	2.0 (17.7)	1.0 (7.3)	1.0 (8.9)
TVU 3000	17.5 (31.8)	13.8 (39.7)	3.0 (5.5)	2.0 (5.7)	2.0 (3.6)	1.0 (2.9)
TVX 3236	18.0 (35.2)	17.0 (48.6)	3.4 (6.3)	2.1 (7.4)	2.3 (4.2)	1.1 (3.1)
Ife brown	11.0 (14.8)	13.8 (30.4)	3.0 (4.0)	2.0 (4.4)	2.0 (2.7)	1.2 (2.6)

Figures in parentheses are percent values of means.

LARVAL MORTALITY

Percentage larval death was highest in pods of TVNU-72 with 60.2 followed by TVX 3236 with 48.6 and least in Ife Brown with 30.4 (Table 2). Similar trend was observed in seeds with highest percentage mortality in TVNU-72 that had 60.1 followed by TVU 3236 with 35.2 and least in Ife brown with 14.8 (Table 2).

Significant difference (P=0.05) exists in death in the different varieties of cowpea seeds and pods.

PUPAL MORTALITY

Pupal mortality was higher in pods than seeds varieties. This ranged from 4.4% to 17.7% in pods and 4.0% to 8.6% in seeds (Table 2). This result also showed that pupal mortality varied significantly from one variety to another with TVNU-72 ranking highest and Ife brown the least in percentage death. At P=0.05, there was a significant difference between varieties.

EMERGING ADULT MORTALITY

Death of emerging adults on all varieties varied significantly with highest percent death in TVNU-72, followed by TVX 3236 and least in Ife brown (Table 2). Statistical analysis showed that there was a significant difference between the varieties and between pods and seeds.

ADULT EMERGENCE

The number of adults emerging with days show a negative or non-linear correlation. Adult emergence generally was higher in seeds than pods with mean emergence ranging from 2.0 (2.7%) – 1.05 (7.3%) and 1.2 (2.6%) – 1.0 (8.9%). There was a significant difference between varieties of pods and seeds ($P<0.05$).

DEVELOPMENTAL PERIODS

The mean incubation period from infestation, egg laying to adult emergence ranged from 26-32 days in seeds

TABLE 3: COMPARATIVE WEIGHT LOSS OF SEED AND DEVELOPMENTAL PERIOD OF *C. maculatus* ON DIFFERENT COWPEA CULTIVARS

Cultivars	Weight Loss Of Seed		Development Period (Days)	
	Mean (g)	%	Pod	Seed
IT83E-716	0.94	(18.8)	27-30	26-29
TVNU-72	0.32	(6.4)	30-34	29-32
TVU 3000	0.84	(16.8)	28-30	27-29
TVX 3236	0.69	(13.8)	29-32	28-31
Ife brown	0.98	(19.6)	27-30	27-29

and 27-34 days in pods. IT83E-716 and Ife brown had same developmental period of between 27-30 days, IT83E-716 had the least developmental period of 26-29 days while beetles took much longer time to develop in TVNU-72, between 29-32 days (Table 3).

SEED WEIGHT LOST

Only TVNU-72 showed less than 10% seed weight lost having a mean of 0.32 (6.4%), while the highest was recorded in Ife Brown with a mean of 0.98 (19.6%) (Table 3).

Analysis of variance indicated significant difference in seed weight loss between varieties at P=0.05. Weight loss in pods was not obtainable as this could be influenced by drying out of the pods.

DISCUSSION

The result demonstrated significant varietal effect on the oviposition, percent adult emergence, percent larval mortality, percent pupal mortality and mean hatchability.

The general preference for the IT83E-716 and Ife Brown cultivars for oviposition by *C. maculatus* compared with TVNU-72, TUX 3236 and TVU 3000 may be a reflection of the chemical rather than the physical characteristics of the seed coat and pod wall. From earlier reports on the preference for oviposition by *C. maculatus*, different workers have demonstrated that this beetle prefers to lay its eggs on smooth-coated seeds rather than on seeds with wrinkled coats (Howe and Curier, 1964; Singh *et al*, 1977; Dobie, 1981). In contrast, our findings is at variance with these earlier workers as one of the two most preferred cultivars IT83E-716 has a rough surface and this agrees with the work of Lale And Efeovbokhan (1991) and Lale and Kolo (1998). Chemical factors associated with host plants resistance have been shown to be related to the presence or absence of secondary plant substances (Epino and Rejesus, 1983). These substances are involved in feeding and oviposition stimulation and deterrency. It is possible that cultivars TVNU-72, TUX3236 and TVU 3000 have certain chemical deterrents or certain oviposition stimulants in their seed coat and pod wall, hence *C. maculatus* preferred to oviposit on the IT83E-716, and Ife Brown.

Similarly, pod hairiness has been demonstrated as a major cause of reduced oviposition (Talekar and Lin, 1981). The TVNU-72 pod surface is occupied by numerous hairs. It is likely that low oviposition on TVNU-72 was probably due to pod surface hairiness as it may have acted as a barrier to egg attachment (Naliby *et al*, 1998).

Several bruchid species utilize oviposition markers that influence distribution of eggs on seeds, to prevent intra-specific competition that will occur if eggs are crowded on a few seeds (Messina and Renwick, 1985; Mbata, 1992). Antibiosis and antixenosis have also been reported to play a role (Fenemore, 1984; Kumar, 1984). This work recorded 13.8-79.8 range of number of eggs laid. This agrees with Hill (1975) but disagrees with the findings of Mbata (1994) and Brooker (1967) that reported 64 eggs and 70 eggs respectively. Glucose solution has been found to enhance fecundity in insects (Nwanze, 1973). This relates positively to this work.

Mean egg hatchability showed significant difference between cultivars but correlation analysis of the percent hatchability reveals that there is no such difference at P=0.05. Egg hatchability of *C. maculatus* is determined by the failure of fertilization and mortality during egg stage, which seems to be caused mainly by the trampling by adults. According to Utida (1972) hatchability is a function of collision among adults and that between adults and deposited eggs. This assertion may not hold in this work since only a female and a male pair per replicate was used. Therefore, hatchability may also be influenced by a resistant factor.

The high larval mortalities observed on TVNU-72, TVX3236 and TVU 3000 may suggest the presence, in this varieties, of chemical substance(s) in the cotyledons, which may be poisonous to feeding larvae, leading eventually to their death. Janzen (1977) had earlier reported the presence of some poisonous substances in the cotyledons of certain varieties of other grains legumes. The poisonous chemical substances probably confer antibiosis on these varieties. This explains at least, in part, the significantly higher larval moralities in these varieties. Varieties IT83E-716 and Ife Brown probably either lack this chemical(s) completely or have low level of it so that *C.maculatus* larval found them to be more suitable source of food. Consequently, significantly higher proportion of hatched eggs emerged as adults from these varieties. Such high larval mortalities were earlier observed in TVU 2027, which has an exceptionally high level of seed resistance (Dobie, 1981; IITA, 1983). An abnormally high content of trypsin inhibitor is associated with the mechanism of resistance in this variety (Gatehouse *et al*, 1979; Gatehouse and Boulter, 1983). Whether varieties TVNU-72, TUX 3236 and TVU 3000 employ the same mechanism of anitbiosis in achieving this level of resistance is uncertain. What is significant, however, is that compared to TVU 2027, which has been shown not to deter oviposition by *C. maculatus* (Gatehouse *et al;* 1985) varieties TVNU-72, TVX 2326 and TVU 3000 combine prolongation of larval development, high larval mortality with significant oviposition deterrence. This is of particular significance as it has already been shown that the number of *C. maculatus* which can emerge from the seed, depends on the number of eggs initially present (Dick and Credland, 1986a,b). The resistance of TVNU-72, TVX3236 and TVU3000 is likely to be of greater value since it appears to be more polygenic, and may therefore be more difficult for resistance breaking biotypes of *C.maculatus* to break down compared with TVU2027 which has already been demonstrated to be ineffective against certain strains of *C. maculatus* (Dick and Credland, 1986a).

The finding that *C.maculatus* showed a significantly higher preference for cultivars IT83E – 716 and Ife Brown both for oviposition and as a source of food, than TVNU – 72 is of considerable significance. The use of such susceptible varieties albeit higher – yielding and early – maturing by farmers, would necessitate a change from traditional methods of cowpea storage to more effective and sophisticated methods. It may also lead to intensification of control of *C. maculatus* in store. Both changes may require more effort and money than most tropical farmers can afford at present.

The discovery that varieties TVNU – 72 and TVX 3236, both of which are high yielding and earlier maturing than the traditional varieties, also possess higher oviposition deterrent and antibiotic factors, offers great hope to farmers in the struggle to mitigate the menace of *C. maculatus* on stored cowpeas in the near future.

Oviposition, hatchability and adult emergence were generally lower in pods than seeds. Also mortality was higher in pods than seeds in all varieties examined. This followed the pattern observed by Caswell (1980) and Nalidy *et al* (1998). According to them adult emergence is four times greater when grains are stored as seeds than when stored as pods. Initial drop in *C.maculatus* population in threshed as compared to unthreshed cowpea is attributed to egg loss during threshing. Very soon thereafter during storage, however, infestation in threshed seeds built up much more rapidly that in pod-stored cowpea (Caswell, 1980). He attributed the slower rate of infestation of pod-stored grains to reduced penetration by first instar larvae and reduced adult emergence through the pod walls. In contrast, when cowpea is stored as seeds, infestation and reinfestation continues unhindered resulting in multiple fold increase. This work suggests that the low adult emergence rate from pods compared to seeds could be as a result of the barrier posed by pod wall thickness and hardness combined with a possible reduction in reinfestation due to a low penetration rate by first instar larvae and pod surface hairs. Clearly, one way of increasing the shelf life of cowpea is to store intact dry pods and not seeds. This would only be effective if pods are not damaged and the variety does not shatter once dry.

On the other hand, the pod-wall and seed coat resistance of TVNU-72 if combined with the seed resistance TVX 3236 and some susceptible varieties of cowpea, more than 97% of the insect population could be suppressed. This would be an advantage because it would diminish the risk of *C. maculatus* overcoming resistance that depended on a single factor.

The incorporation of the genes controlling the seed and pod resistance observed particularly in TVNU-72 into susceptible varieties with desirable agronomic traits is likely to lead to the development of more cowpea varieties with greater seed resistance to *C. maculatus* infestation in storage.

The mode of action of any chemical factor(s) present in the seed coats and pod walls of resistant cowpea cultivars on bruchid eggs and its/their influence on the oviposition and developmental behaviour of gravid females and immature stages certainly merit further investigation.

The influence of stock cultures of *C. maculatus* maintained on specific cowpea varieties in laboratory on other or same cowpea varieties is also worth investigating.

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