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Research Paper

Screening of Some Cowpea Varieties for Resistance to *Callosobruchusmaculatus*

A.A. Obadofin

Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa

Corresponding author, e-mail: (fathom434@yahoo.com)

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Abstract: *Seventeen (17) cowpea varieties suitable for intercropping with cereals were assessed for their resistance to Callosobruchusmaculatus attack under laboratory conditions. Parameters used for comparisons were number of eggs laid on each variety, larva mortality, live pupa, adult emergence, number of holes and weight of cowpea at the end of the experiment. There were significant differences ($p<0.05$) among the varieties tested with respect to the parameters used. IT89KD- 288, IT99K-429-2 and IT97K-356-1 were resistant to Callosobruchusmaculatus while IT97K-461-4, IT97K-570-18, IT99K-1122, IT98K- 205-8, IT99-1060, IT98-1399 and Ife Brown were susceptible. This indicates that the level of resistance in these varieties is sufficient to provide reasonable protection during storage.*

Keywords: Cowpea, *Callosobruchusmaculatus*, screening, storage and resistance.

Introduction

Cowpea (*Vignaunguiculata*(L) Walp), is one of the most important grain legume crops throughout the tropics and serves as a major source of protein in human as well as animal cell (Okigbo, 1978; FAO, 2002 and Islam *et al.*, 2006). Cowpea is widely cultivated in the tropics. Nigeria remains the largest producing country in the world, followed by Niger and Brazil (Quin, 1977 and Adaji*et al.*, 2007). Cowpea is also an ancient crop grown for its green pods, ripened seeds and green leaves.

It is an annual crop cultivated from seed (Dugje*et al.*, 2009), germination is epigeal and the germination is very high. Dry and wet season cultivation is possible because it is tolerant to a reasonable range of rain and most cultivars withstand high temperature. Cowpea seeds vary in their seed colour (black, white, light brown, brown), growth habits (erect or spreading), duration of maturity (early, intermediate, or late), as well as preference from region to regions. In West Africa for

example, white and brown seeds with rough seed coat are preferred. In East Africa, the varieties preferred are those with red or brown seeds with smooth coat while the black types are most preferred in Cuba and the Caribbean (IITA, 1981; Agbogidi, 2010).

Cowpea constitutes the cheapest source of dietary protein for most people in the tropical world where per-capital income and the consumption of animal protein are both very low (Raine, 1970, Okigbo, 1978). The fresh seeds and the immature seeds are eaten and they may be frozen or canned. All the plant parts that are used for food are nutritious providing protein, vitamins and minerals.*Callosobruchusmaculatus* (ColeopteraBruchidae) is the most important insect pest attacking stored cowpea, and is known to be of economic importance (Jackai and Singh, 1980; Van Alebeek, 1996). Another bruchid pest of cowpea, ranked by Van Alebeek (1996) as next in importance to *Callosobruchusmaculatus,* is*C.chinensis* L. The principal storage pest of cowpea in sub-Sahara Africa is the "cowpea weevil" (Van Alebeek, 1996). In low resource farms, Callosobruchus infestation starts in the field and continues in storage. Adult beetles lay eggs on pods in the field or on seed causing extensive damage in storage (Singh and Singh, 1990).

The female prefer natural green pods but will oviposit on dry, mature pods as well (Messina, 1984). Female oviposit more readily on exposed grain as in cowpea lines which pods dehisce easily. Larvae hatching from eggs on either seeds or pods use their mouthparts to bore through the button of the chorion. Within the seed, the larva undergoes four (4) instars, the longest of which is the fourth (Shade et al 1990). The development from eggs to adult at 26^{o}Cand 55% RH takes place about 36 days in susceptible seeds. Cowpea on sale in market in sub-Sahara Africa often has bruchid emergence holes. Emerging females mate and lay viable eggs on the same day they emerge. Presently, *C. maculatus*is controlled in storage mainly by fumigation. Pods of cowpea stored for 8 months could have as much as 50% of the grains damaged by *C. maculatus*(Caswell, 1984).

Resistance to insect pest is potentially a valuable means of pest control, either as a sole control measure or as an adjunct to other control measures. The term bruchid-resistant is often used for cowpea lines with resistance to *C. maculatus*only, but not bruchids. When for example, such varieties are planted in areas with *Bruchidusatrolineatus*or another bruchid; cowpea grain may develop as many holes as bruchid-susceptible varieties. An important component of crop improvement in the world over is the development of pest resistant crop varieties. The ability of a host to resist attack by another is known as Host Plant Resistance (HPR). Seed resistance is a valuable tool against *C. maculatus* but must be carefully deployed to avoid the rapid development of a virulent bruchid biotype. Kitch*et al*(1991), SilimNalidy*et al* (1999) and Kitch*et al* (2011)reported that pod-wall thickness might be responsible for reduced adult emergence from resistant pods. The objective of the study is to evaluate the extent of resistance and /or susceptibility of seventeen new cowpea varieties to *Callosobruchusmaculatus*in storage.

Materials and Methods

Sources of Materials

Seventeen cowpea varieties were obtained from germplasm of the International Institute of Tropical Agriculture (IITA), Ibadan. They comprise sixteen (16) new varieties, which have not been screened for cowpea resistant to *C. maculatus* and are yet to be released to the market and one screened variety which has been identified to be resistant to *C. maculatus* (IT89KD-288) (Table 1).

Insect Culture

All screening exercises were carried out at the Plant Biology Laboratory of the College of Agricultural Sciences, OlabisiOnabanjo University, Ago-Iwoye. Infested cowpea seeds were obtained from the market and used to raise a culture of *C. maculatus.* A stock culture of *C. maculatus*was reared in a container filled with susceptible cowpea varieties, which was obtained from the local

market. Cowpea samples used in raising stock culture were heated of any mites or insect by storing in oven at 50^{o} C for 1 hour.

Insect Sexing

The bruchids that emerged from the culture were sexed by examining the pygidium, which has two large patches separated from each other by a vertical line on the dorsal surface of the female. The dark spots absent in the pygidium are covered dorsally by a whitish pubescence.

Screening Method

Two newly emerged male and two female adult beetles *C. maculatus* were randomly selected and transferred using a camel hairbrush (with moistened tip) into the container containing the culture medium. The adult were allowed to mate and lay eggs for 24 hours, after which the weevils were sieved out and discarded.

To ensure that there is no hidden infestation prior to carrying out this procedure; the test material was disinfected of mites or insect by storing in an oven at 50 ^{o}C for one hour. Twenty seeds (an equal number for each test line) were weighed out into a specimen tube (vials). The vials were arranged out in a Completely Randomized Design. Each vial was provided with a fine nylon mesh covering the mouth and held in place with a rubber band. There were seventeen (17) of such vials per replicate. One of each of these seventeen (17) treatments served as a control. Each was labeled with the appropriate symbol representing the variety and replicate number. Five (5) days after infestation the number of eggs laid per seeds lot was counted. All insects were sieved out of the infested vial and then returned and left undisturbed, in the laboratory under the prevailing temperature and relative humidity. Starting from 25 days after infestation, the adults that emerged each day from seeds were counted and removed. This was done up to four (4) weeks, or until no further emergence occurred on the susceptible control. The seeds were weighed at the end of the experiment. In screening for resistance to storage pest, Ife Brown, (a susceptible line) is used as a check.

Data Collection and Analysis

Data were collected using the following;

1. Number of eggs laid.
2. Larva number.
3. Number of live pupa.
4. Adult emergence.
5. Number of holes.
6. Final weight of cowpea at the end of experiment.

Data collected were subjected to analysis of variance (ANOVA) using Completely Randomized Design (CRD). Means were tested by Duncan Multiple Range Test (DMRT) at 5% level of significance.

Results

Statistical analysis carried out on insect development indices showed that significant differences ($p<0.01$) exist among the 17 cowpea varieties tested for all the measured indices (i.e. number of eggs laid, larva number, number of pupa, adult emergence, number of cowpea weight) (Table 2).

Effect of Cowpea Varieties on Number of Eggs Laid by *Callosobruchus Maculatus*

The mean number of eggs on the screened cowpea varieties differed significantly ($p<0.05$) ranging from 27 to 110. The least mean number of eggs laid (27) was recorded on IT99K-429-2 and II97-356-1 and highest value (110) record for IT98K-205-8

Effect of Cowpea Varieties on Larva

Significant differences ($p<0.05$) exist among the 17 varieties tested such that the range of mean values was between 15 and 101 (Table 2). The variety that recorded the lowest number of larva was Ife Brown while IT97K-570-18 and IT98k-205-8 varieties had the highest larva number of 101.

Effect of Cowpea Varieties on Live Pupa

The least significant live pupa was recorded on IT98K-428-3 with a mean number of 2 while Ife brown had the highest mean number of 18 (Table 2).

Effect of Cowpea Varieties on Adult Emergence of *C. Maculatus*

Adult emergence varied significantly among the cowpea varieties with IT97K-356-1 and TK99K-429-2 varieties having the values of 1.1 and 1.2, respectively (Table 2). There were significant differences ($p<0.05$) to the value of 4.2 (IT99K-1122), 4.3 (IT98DK-1399), 4.9 (IT97K-570-18) and 4.8 (Ife Brown) recorded on the varieties so indicated.

Effect of Cowpea Varieties on Number of Holes

The most significantly affected cowpea varieties were IT97K-356 and IT97K-461-4 with mean the highest mean number of holes of 5.2 recorded (Table 2). The least affected of the cowpea varieties were IT97K-461-4 and IT97K-356-1 with a mean value of 1.3 and 1.4 respectively which were not significantly different from each other.

Final Weight of Cowpea (gm)

The final weight of cowpea ranged from 2.6 to 4.21gm with significant difference ($p<0.05$) among the varieties (Table 3). The least significant ($p<0.05$) weight was recorded on Ife Brown (2.6gm) while higher significant weight were recorded on IT97K-356-1 (3.8gm) and IT89KD-288 (4.21 gm) respectively.

Coefficient of Correlation among Insect Development Indices Measured on 17 Cowpea Varieties

The correlation matrix as shown in Table 4 shows that a high positive relationship existed among number of holes laid larva number, live pupa and adult emergence. They were all significant at 1% probability level.

Resistant Rating on Seventeen Varieties of Cowpea

Table 5 shows the rating of the 17-cowpea varieties as adjudged by the indices used in this experiment. The varieties that were rated as highly resistant were three (3) IT97K-356-1, IT99K-429-2 and IT89KD-288. Those in the moderately resistant group are seven (7) (IT98K-428-3 to

IT89K131-12). While those in the last group of susceptible varieties are also seven (7) (IT99K-1060 to Ife Brown).

Discussion

The results from the study carried out on cowpea resistance to *Callosobruchusmaculatus*in storage demonstrated significant varietal differences in all parameters used. These include the number of eggs laid, larva number, pupa and adult emergence, number of holes bored and final weight of the cowpea varieties.

As regards the coat texture of the different cowpea varieties, the number of eggs laid on the cowpea varieties showed that there was no preference for oviposition by *C. maculatus*. The number of eggs laid on Ife Brown, which has a rough coat texture and one of the most preferred, was not significantly different from IT98K-570-18, which has a smooth coat texture. Early report indicated that this insect prefers to lay eggs on smooth-coated seeds rather than on seed with wrinkled coat (Nwanze and Horber, 1976; Dorbie and Prevetts, 1984). The finding of this experiment is at variance with the finding of the earlier workers. The general preference for oviposition by C. maculatus on Ife Brown, IT99K-205-8, IT97K570-18, compared with IT99K-429-2 and a few other varieties may be a reflection of the chemical composition in these varieties rather than the physical characteristics of the seed coat.

The lowest larva number for IT99K-429-2, IT97K-356-1 and IT89KD-288 might suggest the presence in these varieties of some active ingredient in the cotyledons, which may be toxic to the feeding larvae leading eventually to their death. Janzen (1977) had earlier reported the presence of some poisonous substances in the cotyledon in certain varieties of other grain legumes. This poisonous chemical probably confers antibiosis on these varieties, and may explain in part the significantly lower larva number in the affected varieties. *C. maculatus* reared on susceptible varieties such as Ife Brown, IT98K-1399, IT98K-205-8, IT99K-1122 and IT97-570-18 had early developmental rate compared to the highly resistant varieties such as IT89K-288 and IT99K-429-2 which were characterized by slow but staggered development and low adult emergence rate. This is in support of Singh and Singh (1990) who reported similar observation. The highly resistant varieties such as IT98KD-288 and IT99K-429-2 probably possess attributes that may lead to the non-use or reduced use of the cowpea seeds for food, shelter and oviposition by *C. maculatus*.

This modality has been rechristened by Kogan and Ortman (1978) as "antixenosis" meaning "bad host" to reflect a characteristic of the plant. The data on the final weight of the different varieties due to *C.maculatus* infestation showed that susceptible varieties significantly lost more weight than the others. It has been shown that cowpea stored without control of *C.maculatus* can record up to 30% reduction in weight (Jackai and Daoust, 1986; Tanzubil, 1991). The need therefore to look for varieties that are resistant to *C.maculatus* in storage becomes more important. It is suggestive that varieties such as IT(&KD-288, IT99K-2 and few other that were found to be resistant to *C. maculatus*may provide reasonable protection during storage.

Conclusion

The response of *C. maculatus*is different to various varieties of cowpea in storage. The seed coat of the varieties tested did not play any significant role in resistant varieties observed in this study. Varieties such as IT89KD-288, IT99K-2 and IT97K-356-1 in combination with other forms of measures could be used by low resource farmers to prevent infestation of *C. maculatus* to cowpea in storage.

Tables:

Table 1: Characteristics of Cowpea Varieties Screened for Resistance to *Callosobruchusmaculatus*

S/N	Variety	Coat / Color	Size	Coat / Texture	Eye Color
1	IT89KD-288	White	Medium	Rough	Black
2	IT96D-610	Brown	Medium	Rough	White
3	IT99K-1060	Brown	Medium	Rough	White
4	IT97K-461-4	Brown	Large	Smooth	White
5	IT98K-205-8	White	Medium	Rough	Black
6	IT99K-492-2	White	Medium	Rough	Black
7	IT98K-506-1	White	Small	Rough	Black
8	IT97-568-18	Brown	Medium	Rough	White
9	IT97-499-38	White	Small	Rough	Black
10	IT97K-494-3	White	Medium	Rough	Black
11	IT97K-356-1	White/Brown	Medium	Rough	Black
12	IT98K-428-3	White	Medium	Rough	Black
13	IT97K-570-18	Black	Large	Smooth	Brown
14	IT89K-131-2	Brown	Medium	Rough	White
15	IT99K-1122	Brown	Small	Smooth	White
16	IT98D-1399	White	Small	Rough	Black
17	Ife Brown	Brown	Medium	Rough	White

Table 2: Mean number of egg-s laid, larva number and number of live pupa of C. maculatus

S/N	Variety	Number of Eggs Laid	Number of Larvae	Number of Pupa	Adult Emergence	Number of Holes
1	IT89KD-288	33ab	27a	6e	3.7de	4.1e
2	IT96D-610	37abc	30a	7e	4.7f	5.2f
3	IT99K-1060	67d	63d	4abc	2.9cd	3.2d
4	IT97K-461-4	87e	79e	8ef	1.3a	1.4a
5	IT98K-205-8	110f	101f	9f	1.5a	1.7abc
6	IT99K-492-2	27a	22a	5cd	2.1abc	2.4bc
7	IT98K-506-1	50bcd	46c	4abc	2.1abc	2.4bc
8	IT97-568-18	50bcd	45c	5cd	2.6b	2.3bc
9	IT97-499-38	40abc	37abc	3acd	2.1abc	2.0abc
10	IT97K-494-3	37abc	32abc	5cd	1.5a	1.7abc
11	IT97K-356-1	27a	23a	4abc	1.1a	1.3a
12	IT98K-428-3	37abc	35abc	2ab	1.6ab	1.8abc
13	IT97K-570-18	107fg	101f	6e	4.9f	5.2f
14	IT89K-131-2	53cd	45bc	8ef	1.9abc	2.5cd
15	IT99K-1122	93ef	87e	6e	4.2ef	4.5cf
16	IT98D-1399	93ef	89e	4abc	4.3ef	4.5ef
17	Ife Brown	99f	18f	18f	4.8f	5.1f

Means with similar alphabets are not significantly different at $p<0.05$

Table 3: Weight loss of 17 cowpea varieties to *C. maculates* in storage (gm)

S/N	Variety	Initial Weight	Final Weight of Cowpea	Weight Loss	Percentage Weight Loss
1	IT89KD-288	10.0	4.21j	5.7	57.0
2	IT96D-610	9.8	3.3cdef	.6.6	. 67.3
3	IT99K-1060	8.7	3.6gh	5.1	58.6
4	IT97K-461-4	10.0	4.01hi	6.0	60.0
5	IT98K-205-8	7.5	3.3ef	4.2	56.0
6	IT99K-429-2	9,4	3.1bcd	6.3	67.0
7	IT98K-506-1	8.3	3.6gh	4.7	56.6
8	IT97K-568-18	8.5	3.5efg	5.0	58.8
9	IT97K-499-38	9.8	3.2bcde	6.6	67.3
10	IT97K-494-3	8.3	3.5fgh	4.8	57.8
11	IT97-356-1	9.5	3.8hi	4.8	60.0
12	IT98K-428-3	8.9	3.1bcde	5.8	65.2
13	IT97K-670-18	7.5	2.9ab	4.6	61.3
14	IT98K-131-12	8.5	3.0bcd	5.5	64.7
15	IT99K-1122	7.5	3.1bcd	4.4	58.7
16	IT98K-1399	7.1	2.9bc	4.2	59.2
17	Ife Brown	6.7	2.6a	4.1	61.2

Means with similar alphabets are not significantly different at $p<0.05$

Table 4: Aggregate correlation coefficient among insect development indices measured on 17 varieties of cowpea

No of Eggs Laid	No of Holes	Larva Number	Live Pupa	Adult Emergence
No of eggs laid	0.99**	0.739**	0.987**	0.985**
No of holes	-	0.737**	0.994**	0.990**
Larva	-	-	0.727**	0.747**
Pupa	-	-	-	0.996**

** Correlation is significant at $P<0.01$

Table 5: Resistance rating on seventeen varieties of cowpea

S/N	Varieties	Resistance Level
I	IT97K-356-1	Highly Resistant
2	IT99K-429-2	Highly Resistant
3	IT89KD-288	Highly Resistant
4	IT98K-428-3	Moderately Resistant
5	IT96D-610	Moderately Resistant
6	IT97K-494-3	Moderately Resistant
7	IT97K-499-38	Moderately Resistant
8	IT98K-506-1	Moderately Resistant
9	IT97K-568-18	Moderately Resistant
10	IT98K-131-12	Moderately Resistant
11	IT99K-1060	Susceptible
12	IT97K-461-4	Susceptible

13	IT98K-1399	Susceptible
14	IT99K-1122	Susceptible
15	IT97K-570-18	Susceptible
16	IT98K-205-8	Susceptible
17	Ife Brown	Susceptible

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