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# EVALUATION OF SOME ACCESSIONS OF BAMBARA GROUNDNUT (*VIGNA SUBTERRANEAN* L. VERDC) FOR RESISTANCE TO BRUCHID INFESTATION, BASED ON GRAIN SOURCE AND SEED COAT COLOUR

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**Abstract:** The relative susceptibility of different accessions of Bambara groundnut (*Vigna subterranean* L. Verdc) to *Callosobruchus maculatus* (F.) was assessed in a laboratory trial in Nigeria. Treatments were comprised factorial combinations of four grain sources from Nigeria (Enugu, Anambra, Benue and Kogi state) and three predominantly contrasting seed coat colours (black, brown, and milky-colour) laid out in a completely randomized design (CRD). There were four replications of each treatment. Egg depositions by adult *C. maculatus* were affected by grain sources such that ovipositions on those sourced from the state of Anambra were significantly ($p < 0.05$) higher than those from other sources. Similarly, black coloured grains harboured more insects and eggs compared to other seed coat colours. Accesssions collected from Benue and/or those with a milky-coloured seed coat showed some levels of oviposition deterrence. However, the interaction of grain source and seed coat colour was not significant based on oviposition, adult emergence, and mortality counts. Grain sources and seed coat colour were, therefore, important traits to be considered while selecting ideotypes for resistance to *C. maculatus.*

**Key words:** Bambara groundnut, *Callosobruchus maculatus,* grain source, resistance, seed coat colour

## INTRODUCTION

Bambara groundnut (*Vigna subterranean* L. Verdc) is believed to have originated from several areas of the African continent, notably between Jos and Yola, in Northern Nigeria (Mkandawire 2006). The crop is presently grown throughout the country, except in the riverine and swampy areas of the Niger delta. The Bambara groundnut is one of the most widely eaten grain legumes in Nigeria, and provides 14–24% of the protein intake for most Nigerians (James 2003). The high protein and lysine contents of the grains make them a natural supplement to staple diets of cereals, roots, tubers, and fruits (Ocran *et al.* 1998; Ijarotimi and Olapade 2009).

The Bambara groundnut grains are often ground into flour which is used to prepare the following dishes: alele, alelen, ganye, danwake, gauda, kosai, tuno, and waine (Linnemann 1988). In Igboland and Idomaland, Nigeria, the flour is also used to prepare a local delicacy called Igba or Okpa. The fresh immature seeds can be eaten raw but become too hard when mature. The seeds can be roasted or boiled, though, which make them sweet and pleasant-tasting.

Despite the importance of this crop in Nigeria, the availability of its seeds for planting, and its grains for consumption, is always limited by bruchid [*Callosobruchus maculates* (F.)] attack. Such attacks occur not only at the ripening stage in the field but also in storage. Presently, conventional synthetic insecticides are used to abate losses due to *C. maculatus*. There are problems, however, associated with the use of insecticides ranging from prohibitive costs and non-availability when needed, to the farmers' lack of technical skill or competence in the safe handling of the pesticides. The result is that the consumer is exposed to a lot of risks. The misuse of pesticides can result in poisoning.

Bambara groundnut is known to come in a variety of colours. The grain colours used in this research are the predominant grain colours in Nigeria which are mainly: milky-coloured, brown, black, and variegated colours. Grain location became necessary in this study, to evaluate the impact of the environment on the pre-and post-harvest physiology of the grains, which can predispose grains to storage pests. The specific objective of this research was to evaluate the effects of the grain source and seed-coat colour on the susceptibility of Bambara groundnut to *C. maculatus* attack.

## MATERIALS AND METHODS

The experiment was conducted at the Laboratory of the Department of Crop Science, the Faculty of Agriculture, the University of Nigeria, Nsukka, Nigeria. The latitude of Nsukka is 06°52′N and the longitude is 07°24′E. The altitude is 447 m above sea level. The mean tempera-

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ture range of the area is from 29°C to 31°C, while the relative humidity is between 69 and 79% (Uguru *et al*. 2011).

**Insect culture**

A culture of *C. maculatus* adults used for the study was obtained from naturally infested stored Bambara groundnut. The culture was maintained and reared in the laboratory (28±2°C and 78±20% RH – relative humidity) on dry susceptible Bambara groundnut. The groundnut was in plastic buckets covered with pieces of muslin cloth. The muslin cloths were held in place with a rubber band to ensure good aeration and to prevent the escape of the bruchids. The culture was kept in the laboratory cupboard, at room temperature and pressure, for 3 weeks. The sexes of *C. maculatus* were determined by examining the elytral pattern as described by Southgate *et al.* (1957). The females are usually dark coloured and possess the elytral spots, while the males are pale brown and less distinctly spotted. Furthermore, males have a comparatively shorter abdomen, and the dorsal side of the terminal segment is sharply curved downwards and inwards. In contrast, the females have comparatively longer abdomens, and the dorsal side of the terminal segment is only slightly bent downwards (Bandara and Sexana 1995).

**Sources of the tested seed varieties**

Different Bambara groundnut grain lots with three distinct colours namely: black, brown, and milky-coloured were obtained from four different locations in Nigeria. The locations (sources) were the Nigerian states of Enugu, Anambra, Benue and Kogi. These states were purposively selected because they share common boundaries with each other, and are major places where Bambara groundnut is being grown (Benue and Kogi) and consumed (Enugu and Anambra). All the varieties were fumigated in airtight polythene bags with aluminium phosphide for four days, and aired for four days, to disinfect them of any incipient infestation.

**Set-up of treatments**

Treatments comprised all possible combinations of the Bambara groundnut sourced from four locations and three different seed coat colours, as indicated. Hence, there were 12 treatments and these were replicated four times and arranged in a completely randomized design (CRD). The grains were placed in plastic cylindrical containers. The containers had a 30 cm diameter and 10 cm depth. Each container had three side perforations (5 cm radius) equidistant to one another. A muslin cloth was used to seal each container for proper ventilation. The only open end on a container was covered with a replaceable plastic lid, to aid data collection. Inside each plastic container 60 g of the appropriate grain sample was placed and was then infested with ten adult bruchids (5 males: 5 females). Grains were properly selected to ensure that they were smooth with no wrinkles or blemish on their seed coats. The containers were placed on a laboratory (28±2°C and 70±20% RH) bench and arranged in a CRD manner. Oviposition and mortality counts were taken at 2, 6, 10, and 12 days after infestation (DAI). Oviposition counts were taken by counting the number of eggs on 10 randomly selected grains per treatment. A magnifying lens (× 20) was used to make the counts. The mean count was later obtained by dividing counts obtained by the number of grains sampled. The number of adults emerging (F1 generation) was also recorded as well as the percentage of damaged grains, by calculating the ratio of infested grains to the total number of grains introduced.

**Statistical analysis**

All the data were transformed using appropriate procedures, before analysis of variance was carried out on them. The statistical analyses were done in a factorial manner to determine the main effects of the grain source and seed coat colour as well as their interaction. The means were separated using Fisher's least significant difference at a 5% level of significance. All statistical analyses were carried out using the GenStat (2003).

## RESULTS

Oviposition increased with the time after infestation of grains of Bambara groundnut with bruchids (Table 1). Throughout the sampling period, fewer egg counts were sampled from grains sourced from Benue compared to other sources. Also, the number of eggs deposited on the various grains differed significantly ($p < 0.05$) amongst the various seed coat colours. Ovipositions on black grains were significantly ($p < 0.05$) higher than those on the milky-coloured coat, which in turn was significantly ($p < 0.05$) less than those with a brown coat (Table 2).

On the effect of grain source on the adult emergence count of *C. maculatus*, Benue accessions maintained the lowest value (Table 3). The trend black > brown > milk also repeated itself in the adult emergence count after infestation, although this was not significantly different ($p > 0.05$) (Table 4).

There were no significant differences ($p > 0.05$) in the mortality counts of *C. maculatus* among the various grain sources in all the sampling periods. Similarly, the main effect of different Bambara groundnut colours on the mortality of *C. maculatus* was not significantly different ($p > 0.05$) (Table 5). However, the milky-coloured accessions showed some promise, with respect to *C. maculatus* attack resistance.

The interaction of the grain source and seed-coat colour was not significant. However, oviposition count, adult emergence, and mortality were consistently higher for grains sourced from Anambra and with black or brown grain coat colours (Tables 6, 7). On the contrary, grains sourced from Benue with milky-coloured coats were recorded as having the least egg count, while Enugu samples with the milky-colour harboured the least adult emergence and mortality counts.

The percentage of grain damage by *C. maculatus* on Bambara groundnut followed the same trend as adult emergence and oviposition as shown clearly in figures 1 and 2. Thus, the least damage was recorded for Benue sourced lines with the milky-colour, although the differences were not significantly different ($p > 0.05$).

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Table 1. Main effect of grain source on oviposition count of *C. maculatus*

| Source | Oviposition count | | | |
|---|---|---|---|---|
| | 2 DAI | 6 DAI | 10 DAI | 12 DAI |
| Enugu | 1.22 | 1.71 | 2.60 | 3.84 |
| Anambra | 1.22 | 1.71 | 2.58 | 4.02 |
| Benue | 0.91 | 1.16 | 1.33 | 2.53 |
| Kogi | 1.18 | 1.44 | 2.27 | 3.40 |
| Mean | 1.13 | 1.52 | 2.30 | 3.45 |
| F-LSD (0.05) | 0.29 | 0.39 | 1.01 | 0.38 |

DAI – days after infestation;
F-LSD (0.05) – Fisher's least significant difference at a 5 percent probability level

Table 2. Main effect of Bambara groundnut grain colour on oviposition count of *C. maculatus*

| Grain colour | Oviposition count | | | |
|---|---|---|---|---|
| | 2 DAI | 6 DAI | 10 DAI | 12 DAI |
| Black | 3.77 | 3.98 | 4.12 | 4.58 |
| Brown | 2.76 | 2.92 | 3.02 | 3.57 |
| Milky-coloured | 2.12 | 2.32 | 2.40 | 2.83 |
| Mean | 2.88 | 3.07 | 3.18 | 3.66 |
| F-LSD (0.05) | 0.87 | 1.01 | 0.95 | 0.96 |

Table 3. Main effect of grain source on adult emergence of *C. maculatus* on Bambara groundnut

| Source | Emergence count | | | |
|---|---|---|---|---|
| | 24 DAI | 26 DAI | 30 DAI | 32 DAI |
| Enugu | 0.80 | 0.89 | 2.69 | 3.36 |
| Anambra | 1.40 | 1.84 | 4.35 | 5.38 |
| Benue | 0.71 | 0.86 | 2.23 | 2.76 |
| Kogi | 1.02 | 1.48 | 3.74 | 4.29 |
| Mean | 0.99 | 1.27 | 3.25 | 3.95 |
| F-LSD (0.05) | 0.33 | 0.70 | 1.60 | 1.76 |

Table 4. Main effect of grain colour on adult emergence of *C. maculatus* on Bambara groundnut

| Grain colour | Emergence count at days after infestation | | | |
|---|---|---|---|---|
| | 24 DAI | 26 DAI | 30 DAI | 32 DAI |
| Black | 0.79 | 1.41 | 3.57 | 4.84 |
| Brown | 0.79 | 1.22 | 2.75 | 3.97 |
| Milky-coloured | 0.71 | 1.18 | 1.83 | 3.03 |
| Mean | 0.76 | 1.67 | 2.72 | 3.95 |
| F-LSD (0.05) | ns | ns | ns | ns |

ns – not significant

Table 5. Main effect of different Bambara groundnut grain colours on mortality count of *C. maculatus*

| Grain colours | 2 DAI | 6 DAI | 10 DAI | 12 DAI |
|---|---|---|---|---|
| Black | 4.08 | 8.83 | 9.92 | 10.00 |
| Brown | 3.92 | 8.75 | 9.92 | 10.00 |
| Milky-coloured | 3.00 | 8.75 | 9.83 | 10.00 |
| Mean | 3.67 | 8.78 | 9.89 | 10.00 |
| F-LSD (0.05) | ns | ns | ns | ns |

Table 6. Interaction effect of grain source x colour on oviposition counts of *C. maculatus* on Bambara groundnut

| Source | Colour | 2 DAI | 6 DAI | 10 DAI | 12 DAI |
|---|---|---|---|---|---|
| Enugu | black | 1.20 | 1.73 | 4.07 | 4.60 |
| | brown | 1.40 | 1.73 | 3.40 | 3.80 |
| | milky-coloured | 1.07 | 1.67 | 3.27 | 3.67 |
| Anambra | black | 1.33 | 2.20 | 5.70 | 6.00 |
| | brown | 1.20 | 1.60 | 3.20 | 3.80 |
| | milky-coloured | 1.13 | 1.47 | 2.13 | 2.60 |
| Benue | black | 1.07 | 1.13 | 2.53 | 3.00 |
| | brown | 1.00 | 1.20 | 2.73 | 3.27 |
| | milky-coloured | 0.67 | 1.13 | 1.80 | 2.13 |
| Kogi | black | 1.27 | 1.53 | 4.20 | 4.73 |
| | brown | 1.20 | 1.60 | 2.87 | 3.53 |
| | milky-coloured | 1.07 | 1.20 | 2.27 | 2.80 |
| | mean | 1.13 | 1.52 | 3.18 | 3.66 |
| | F-LSD (0.05) | ns | ns | ns | ns |

Table 7. Interaction effect of grain source x colour on the adult emergence of *C. maculatus* on the Bambara groundnut

| Source | Colour | 24 DAI | 26 DAI | 30 DAI | 32 DAI |
|---|---|---|---|---|---|
| Enugu | black | 0.71 | 0.71 | 3.43 | 5.19 |
| | brown | 0.88 | 1.25 | 2.12 | 3.34 |
| | milky-coloured | 0.71 | 0.71 | 1.09 | 1.55 |
| Anambra | black | 0.71 | 2.27 | 4.59 | 5.81 |
| | brown | 0.88 | 1.25 | 3.51 | 5.60 |
| | milky-coloured | 0.88 | 2.00 | 2.87 | 4.70 |
| Benue | black | 0.88 | 1.17 | 2.16 | 2.77 |
| | brown | 0.71 | 0.71 | 2.06 | 2.59 |
| | milky-coloured | 0.71 | 0.71 | 1.70 | 2.93 |
| Kogi | black | 0.71 | 1.47 | 4.11 | 5.59 |
| | brown | 0.71 | 1.49 | 2.68 | 4.34 |
| | milky-coloured | 0.71 | 1.47 | 2.26 | 2.94 |
| | mean | 0.77 | 1.27 | 2.72 | 3.95 |
| | F-LSD (0.05) | ns | ns | ns | ns |

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Table 8. Interaction effect of grain source x colour on the bruchid mortality.

| Source | Colour | 2 DAI | 6 DAI | 10 DAI | 12 DAI |
|---|---|---|---|---|---|
| Enugu | black | 5.67 | 8.33 | 10.00 | 10.00 |
| | brown | 3.00 | 8.00 | 9.67 | 10.00 |
| | milky-coloured | 3.00 | 8.00 | 9.67 | 10.00 |
| Anambra | black | 4.00 | 9.33 | 9.67 | 10.00 |
| | brown | 5.33 | 9.33 | 10.00 | 10.00 |
| | milky-coloured | 3.67 | 9.33 | 10.00 | 10.00 |
| Benue | black | 2.33 | 9.00 | 9.67 | 10.00 |
| | brown | 4.33 | 8.00 | 10.00 | 10.00 |
| | milky-coloured | 2.67 | 8.67 | 10.00 | 10.00 |
| Kogi | black | 3.67 | 8.67 | 10.00 | 10.00 |
| | brown | 3.67 | 9.67 | 10.00 | 10.00 |
| | milky-coloured | 2.67 | 9.00 | 10.00 | 10.00 |
| | mean | 3.67 | 8.78 | 9.89 | 10.00 |
| | F-LSD (0.05) | ns | ns | ns | ns |

DAI – days after infestation; ns – not significant;
F-LSD (0.05) Fisher's least significant difference at a 5 percent probability level

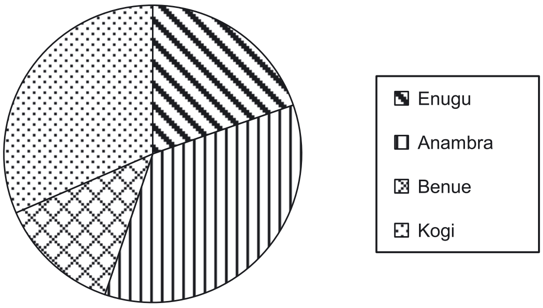

Fig. 1. Main effect of grain source on percentage damage by *C. maculatus* on Bambara groundnut

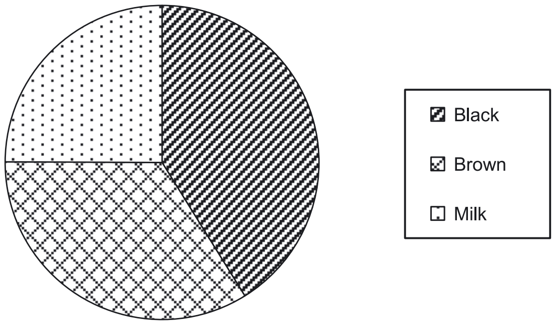

Fig. 2. Main effect of grain colour on percentage damage by *C. maculatus* on Bambara groundnut

## DISCUSSION AND CONCLUSION

The results from this study clearly showed that grain sources glaringly affected oviposition and adult emergence counts of *C. maculatus*. The highest counts were obtained from Enugu and Anambra accessions on most of the sampling days, while the least was from the Benue accession. This suggests that Benue accessions probably had a better genetic constitution to either reduce oviposition or hatchability of the deposited eggs.

Also, grain colours could affect oviposition of bruchids. Grains having a milky-coloured coat recorded lower oviposition and emergence counts. This suggests that milky-coloured grains may have attracted fewer *C. maculatus* than the other evaluated colours, thus harbouring fewer *C. maculatus* to deposit their eggs on such-coloured grains. It could also be inferred, that *C. maculatus* prefers darker backgrounds to lay their eggs than against the brighter milky-coloured backgrounds. This reference might be an adaptation for the survival of the young; as darker surfaces, besides being a better radiator of heat than brighter surfaces, are also a better absorber of heat (Anyakoha 2010). Therefore, eggs dropped on such dark backgrounds would have better incubation conditions in terms of warmth required for better hatchability of the eggs. The better egg hatchability conditions of the darker surfaces could also explain the higher adult emergence recorded amongst these black-coated grains.

The effect of seed-coat colour in the present study is in line with the reports by many researchers like Orzolek and Murphy (1993), Vacha *et al*. (2008), and Echezona and Offordile (2011), who state that insects have a preference for colours. However, reports specifically on the effect of seed coat on oviposition and survival of *C. maculatus* are contradictory. For instance, Eddie and Amatobi (2003) in their experiments on twenty-two cowpea varieties (five resistant, four moderately resistant, and thirteen susceptible) with and without seed coat, reported that seed coat had no value in protecting cowpea seeds against attack by *C. maculatus*. They suggested that the growth and development of *C. maculatus* in cowpea depends on the nutritional value of the seeds. The preference for Enugu and Anambra to other grain sources by the bruchid, therefore, suggests that grains sourced from the different locations in this study may have differed in their nutritional contents. This, however, was not within the confines of our study.

Our study concludes that although other attributes of seeds such as size, nutritional value, and chemical composition (Dasbak *et al*. 2008; Lattanzia *et al*. 2005) are known to affect their resistance to insect attacks, attributes like grain source and coat colour can still contribute to the susceptibility of grains to storage pests. Grains sourced from the state of Benue showed better resistance or immunity to *C. maculatus* attack. For this reason, the efforts of farmers and researchers should be concentrated more on these grains, especially the milky-colour varieties, which hold a better promise for bruchid resistance than the black or brown varieties.

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