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The Role of Seed Coat and Its Pigmentation on the Acceptance of Bambara Groundnut (*Vigna subterranea* L. Verdc.) Cultivars by the Cowpea Beetle, *Callosobruchus maculatus* (F.)

P. K. Baidoo*, N. A. Kwansa, C. P. Annin

Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
Email: *pkbaidoo2002@yahoo.com

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Abstract

Bambara groundnut, *Vigna subterranea* is an important legume crop that is adaptable to the dry regions of Africa. It is a major source of protein to people in many parts of Africa. One major constraint to increased production of the crop is the incidence of the storage pest *Callosobruchus maculatus* which causes significant weight loss to seeds in storage. The preference of the cowpea beetle *C. maculatus* for a particular seed coat colour was determined using four different colour types of bambara groundnut in an arena. The olfactory attractiveness of testa against cotyledon and cotyledon against whole grain was carried out in an olfactometer. Oviposition preference of *C. maculatus* on the different seed colours was determined. In the arena experiment significantly more of the beetles showed preference for cream and mottled seeds than red and black seeds. Significantly more beetles chose seeds with testa over decorticated seeds as well as whole grain over decorticated seeds. Colour significantly affected the seeds as oviposition sites. Significantly fewer eggs were laid on red and black seeds than cream seeds in both the choice and no choice tests ($P < 0.0001$). Thus for the purpose of reducing losses of seeds in storage it is advisable to cultivate red- and black-coloured seeds which showed lower acceptability to *C. maculatus* as oviposition site.

Keywords

Callosobruchus maculatus*, Cotyledon, Oviposition, *Vigna subterranea

*Corresponding author.

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1. Introduction

Bambara groundnut (*Vigna subterranea*), (L.) (Verdc.), also known as bambara nut, has many agronomic and other attributes which make it an important crop to cultivate. Although less popular in many parts of the world compared to other crops, the popularity of bambara groundnut has never been in doubt throughout the West African sub-region. The seed contains about 20% soluble carbohydrates and 8% fats [1] and is high in protein, but unlike ordinary groundnuts contains very little oil [2]. It is a highly adaptable crop well suited for hot, dry regions where many other crops fail to survive and thrive due to lack of water and nutrients [3]. Areas which are too dry to support sorghum, maize and peanuts are able to support bambara groundnut production. The crop is adapted to a wide range of soils, and performs better on poor soils and low rainfall than groundnuts [2] [4].

Despite the ability of bambara groundnut to do well on poor soils and with little water, several constraints impede the increased production of the crop, the most important of which is the incidence of pests and diseases. Pests attacking bambara groundnuts include leafhopper, *Hilda patruelis* and the larvae of *Diacrisia maculosa* and *Lamprosema indicata*. Developing pods of bambara groundnuts are damaged by the moth beetle, *Piezotrachelus ugandum*. However, the most serious damage is caused to the crop during storage by the bruchid *Callosobruchus maculatus* [5]. *Callosobruchus maculatus* causes overall weight loss, reduces the nutritional quality and the presence of insect frass reduces the market value of the crop. Cowpea beetles in stored grain are a major problem because of their ability to re-infest stored seeds. A single beetle is able to cause a 3.5% weight loss in bambara groundnut seed. Infestation can cause up to 60% loss in seed weight and up to 60% in protein content of pulses [6]. Even slight *C. maculatus* feeding damage to the embryo impairs germination; however, according to Talekar and Lee [7], feeding on the cotyledon will not affect germination though it can reduce the vigour of the young plant.

Seeds of bambara groundnuts exhibit a high phenotypic diversity and selection of varieties for cultivation usually depends on seed coat colour and pattern. Seed coat colour is attributed to the presence and amount of phenolic derivatives [8] [9], which have been reported to have antimicrobial activity [10], and disease resistance [11] [12]. Disease and pest resistance and high yield are some requirements of commercial farmers. Thus varieties of bambara groundnuts which can withstand infestation by *C. maculatus* tend to be favoured by subsistence and commercial farmers. Different varieties of the crop may respond differently to *C. maculatus* infestation and also vary genetically in terms of susceptibility. The present study was carried out to assess the preference of *C. maculatus* to seed coat colour, olfactory attractiveness or otherwise of testa and whole grain as oviposition sites.

2. Materials and Methods

Four different colour types of bambara groundnuts were used for the study. These were cream, mottled, red and black. Five hundred grams (500 g) of each colour type was obtained from Ejura market in the Ashanti Region, air-dried and deep frozen for 2 weeks to get rid of any storage insect pest. A culture of *C. maculatus* was obtained from the Entomology Section of Crop Research Institute, Kwadaso, Kumasi. One hundred adult *C. maculatus* were introduced into each of four 1L Kilner jars containing 500 g of red colour type of bambara groundnut. The beetles were allowed to oviposit for three days after which they were sieved out. The Kilner jars were covered with metal gauze and muslin net to firmly secure them and prevent possible escape and re-infestation. The first filial generation (F_1) that emerged was introduced into other Kilner jars containing bambara groundnuts and the resulting second filial generation (F_2) adults were used for the experiments. Fresh adults which emerged from 0 - 10 days were sieved out and used to infest the experimental groundnuts at 28˚C and 70% relative humidity.

2.1. Colour Effect on Choice of Nuts for Oviposition—The Arena

The arena was set up and used to determine colour preference of *C. maculatus*. It consisted mainly of an open ended Perspex ring which measured 20 cm in diameter. The ring was placed on a flat surface to create the arena. The top of the arena was closed with a glass plate that allowed for visibility within the arena and also prevented the beetles from escaping. For easy observation of beetle activity during the experiment, the bottom of the arena was lined with a clean white paper. Four circles were drawn equidistant on the circular ring and labeled A, B, C and D. The various bean colours were rotated from position A through to D to ensure that position did not affect choice and that the beetles were able to choose the respective bean colour irrespective of their positions. The experiment was replicated four times.

2.2. Olfactory Bioassays

Olfactory bioassays were performed using an olfactometer to determine the role of seed coat and its pigmentation on the acceptance of bambara groundnut by cowpea beetles. Samples of each colour type of bambara groundnut were decorticated (removal of testa). The testa and cotyledon obtained from each sample were tested against each other. Five grammes (5 g) of testa was weighed and placed in a container whilst 5 g of cotyledon was placed in another container. Ten (10) adult *C. maculatus* were introduced into the middle of each container. The various positions of the testa and cotyledons were interchanged to ensure that position did not influence the direction of movement of the beetles but rather the smell of the groundnuts. The set-up was placed in the dark under a hard card box to ensure uniform surroundings and to avoid differences in light intensity which could influence the behaviour of the beetles. Sample materials used in each experiment were discarded to avoid the influence of any hormone left over from *C. maculatus*. The experiment was replicated four times and at the end of each experiment the olfactometer was wiped with 10% alcohol to ensure neutral scent and get rid of any hormonal residue.

2.3. Oviposition-Choice and No Choice Tests

Experiments were set-up to ascertain the effect of seed colour on egg deposition preference of C. *maculatus*. Seventy (70) seeds of each colour type were selected and placed in Kilner jars. Beans with holes and signs of egg deposition were discarded. The seeds were infested with 30 *C. maculatus* and left to stand for 48 hrs after which the beetles were sieved out and the number of eggs deposited on each colour type was counted with the aid of a magnifying glass. For the choice test, 20 seeds of each colour type were placed in Kilner jars and infested with 30 adult *C. maculatus.* The beetles were sieved out after 48 hr and the numbers of eggs deposited were counted with the aid of a magnifying glass.

2.4. Data Analysis

Data obtained were analyzed using the Statistical Analysis Systems [13]. Analysis of variance was performed and where the differences were significant, the Student-Newman-Keuls (SNK) was used to separate the means. Significant difference was set at $P \leq 0.05$.

3. Results

3.1. Beetle Attraction to Different-Coloured Nuts

Beetle choice as affected by position indicated that cream coloured-nuts were most preferred by *C. maculatus*. At all positions, cream-coloured nuts attracted the largest number of beetles. On the other hand red-coloured nuts attracted the least number of beetles at all the positions (**Table 1**). The differences in beetle numbers attracted to different nut colours at different positions were significant at positions A (P = 0.007), B (P = 0.026) and C (P = 0.002). At position D however, beetle preference for the different colours in different positions in the arena did not differ significantly (P = 0.057). Overall, cream seeds recorded significantly more beetles than any of the colours (**Table 1**).

At point A the numbers of beetles attracted to cream and mottled nuts did not differ significantly, but both differed significantly form the number of beetles attracted to both black and red nuts (**Table 1**). On the other hand at position C, the numbers of beetles attracted to cream, mottled and black did not differ significantly but all three differed significantly from beetles attracted to the red-coloured nuts.

3.2. Olfactory Bioassays

3.2.1. Testa against Decorticated Seed

Olfactory preference of beetles for nuts with intact testa and decorticated seeds showed that more of them were attracted to nuts with intact testa than the decorticated nuts (**Table 2**). In the nuts with testa the number of beetles attracted to the different colours ranged from 5.0 in black nuts to 6.0 in cream and red-coloured nuts.

Table 1. *C. maculatus* attraction to different colours of bambara groundnuts at different positions in the arena.

	Number of beetles				
	Position A	Position B	Position C	Position D	Mean
Colour of nut					
Cream	$3.50^a \pm 0.28$	$5.25^a \pm 0.47$	$4.50^a \pm 0.95$	$4.00^a \pm 0.70$	$4.31^a \pm 0.56$
Mottled	$3.00^a \pm 0.40$	$2.25^b \pm 0.25$	$2.75^a \pm 0.25$	$3.50^a \pm 0.28$	$2.87^b \pm 0.30$
Black	$1.75^b \pm 0.25$	$1.50^c \pm 0.50$	$2.75^a \pm 0.25$	$2.00^a \pm 0.47$	$2.19^b \pm 0.35$
Red	$1.00^c \pm 0.70$	$1.50^c \pm 0.28$	$0.75^b \pm 0.25$	$1.75^a \pm 0.81$	$1.25^c \pm 0.48$

Within columns, means with the same letter are not significantly different (P > 0.05).

Table 2. Numbers of *C. maculatus* attracted to testa and decorticated seeds of different colours of Bambara groundnuts.

	Testa	Decorticated seed	Whole grain	Decorticated seed
Colour of seed				
Cream	$6.00^a \pm 0.57$	$2.50^a \pm 0.29$	$6.75^a \pm 0.85$	$2.50^a \pm 0.64$
Mottled	$5.00^a \pm 0.71$	$2.00^a \pm 0.41$	$6.00^a \pm 0.91$	$2.50^a \pm 0.65$
Black	$5.75^a \pm 0.85$	$2.00^a \pm 0.71$	$5.50^a \pm 0.29$	$3.25^a \pm 0.48$
Red	$6.00^a \pm 0.41$	$2.75^a \pm 0.48$	$5.75^a \pm 0.85$	$3.25^a \pm 0.85$

Within columns, means with the same letter are not significantly different (P > 0.05).

Colour of nuts did not significantly affect its acceptability by the beetles (P = 0.45). Similarly, in the decorticated seeds, number of beetles attracted did not differ significantly. There was no significant difference in beetle choice for different colours when the various colour positions were varied (P = 0.35).

3.2.2. Whole Grain against Decorticated Seed

When beetles were presented with the option of choosing between whole grain and decorticated seed, it was observed that many more chose whole grain over decorticated seeds. Cream seeds recorded the largest number of *C. maculatus*, even though the differences were not significant (P > 0.05) (**Table 2**). In the decorticated seeds, red and black attracted larger numbers of beetles than cream and mottled seeds, even though the differences were not significant.

3.3. Effects of Seed Colour on Oviposition by *C. maculatus*

In the no choice test, cream-coloured seeds recorded the largest number of eggs deposited whilst black seeds recorded the least number of eggs (**Table 3**). The differences in the number of eggs deposited were highly significant (P = 0.001). Numbers of eggs deposited on cream and mottled seeds differed significantly (P < 0.05), however, the numbers of eggs deposited on red and black seeds were not significantly different. Results from the choice test showed that the largest number of eggs was deposited on cream seeds whilst the least number of eggs were deposited on black seeds (P < 0.05). Numbers of eggs deposited on mottled, red and black nuts were not significantly different but all three differed from the number of eggs deposited on cream seeds.

4. Discussion

Bambara groundnut is attacked during storage by *C. maculatus*, which occurs before the crop is carried into storage. The infested seeds are then carried into storage. Significant damage is done to the crop during the storage period if no control measures are applied to the seeds. Female lay their eggs on the seed and after hatching the larvae bore into the seed and feed on the stored food. The crop is able to support this storage pest due to its high nutritional value [14]-[17]. In the arena experiment, it was observed that the choice of the beetles for the different colours varied with positions. Majority of the beetles chose lighter-coloured seeds over dark-coloured ones.

Table 3. Numbers of eggs deposited on different colours of bambara groundnut seeds by *C. maculatus*.

	No choice test	Choice test
Colour of seed		
Cream	$10.27^a \pm 0.40$	$4.90^a \pm 0.34$
Mottled	$5.18^b \pm 0.47$	$2.53^b \pm 0.23$
Black	$3.45^c \pm 0.15$	$1.83^b \pm 0.16$
Red	$3.84^c \pm 0.17$	$2.06^b \pm 0.17$

Within columns, means with the same letter are not significantly different ($P > 0.05$).

This was indicated by the significant differences in the number of beetles attracted to different seed colours. Certain compounds in the seed coat of bambara groundnuts such as p-aminophenylealanine have been demonstrated to impart resistance to bruchids. Birch *et al*. [18] focused on the significance of *p*-aminophenylalanine (PAPA) in bambara groundnuts as a defence mechanism against bruchids. However, Bressan [19] demonstrated that bambara accessions that differed in resistance to bruchids did not have significantly different levels of PAPA in the mature seeds.

Tannins impart colour to seeds and their concentrations are correlated with seed colour. Tannins are located mainly in the seed coat. It has been observed that black and red seeds contain the highest level of tannins whilst the lowest level was found in cream-coloured seeds [17]. Tannins may affect the growth and development of insects in the sense that high concentrations in the seed makes it unpalatable thereby reducing feeding by *C. maculatus*. This explains why larger numbers of the pests were attracted to cream seeds than red and black-coloured seeds. Tannins are poisonous to beetles and therefore low concentrations in cream and mottled seeds made them acceptable to *C. maculatus*.

Apart from tannins, other seed protective mechanisms include some storage proteins such as enzyme inhibitors that act on insect gut digestive hydrolases. Alpha-amylase of legume seeds, located in the cotyledons negatively affects the development of *C. maculatus*. This insect is highly dependent on starch as a source of energy; hence decortications of the seeds exposed the beetles to α-amylase which is toxic and therefore negatively affected their development. This explains why the beetles avoided the decorticated seeds and rather chose the seeds with testa. According to Chapman [20], the ways by which an insect receives information concerning potential host are through smell, touch and taste of the host. After the initial attraction, the insect feeds and oviposits on the host. When cotyledons were tested against whole grains, beetles showed preference for whole grains, an indication that cotyledon is harmful to them.

Female bruchids lay egg on the surface of beans and according to Blumer and Beck [21] this is the most important choice a female makes for her offspring because it will influence their growth, survival and reproduction. Therefore any wrong choice of oviposition site will significantly reduce the survival rate of their progenies and subsequently the perpetuation of the species. Seeds of bambara groundnuts are known for their hard texture. The larvae, after hatching from the eggs have to bore through this hard seed coat in order to feed. The seed coat of cream and mottled seeds are thinner than that of red and black [22] making them better accepted for oviposition than red and black seeds. Seed coat colour is due to varying amounts of phenolic compounds [8] [9] and has been shown to have antimicrobial activity [10] and pest and disease resistance [11] [12]. A study by Schaefer and Rolshausen [23] showed that anthocyanins which are responsible for the purple colour of maize are not perceived by most insects. Most insects that have been studied possess only three types of photoreceptors which are most sensitive to green, blue and ultraviolet light. In the absence of a red light receptor, insects would be unable to perceive visual cues of anthocyanins [24]. Dark-coloured nuts are sometimes covered with a network of parallel and transverse ridges compared with the smooth surface of cream nuts. These transverse ridges make the dark-coloured seeds unacceptable for oviposition [25]. This explains the results obtained in the choice and no-choice tests where majority of egg deposition occurred in the cream nuts rather than the black and red nuts.

5. Conclusion

The protective mechanisms employed by seeds against insect pests' attack include the nature, colour and chemical composition of seed coats. Light-coloured seeds, even though higher in protein and carbohydrates have poor resistance to cowpea beetle infestation during storage. This was due to thinner seed coats and lower levels of tannins. Dark-colored seeds attracted fewer beetles for oviposition and therefore showed relatively higher resistance to beetle infestation. Thus for the purpose of cultivation it is advisable for farmers to cultivate red and black seeds of bambara groundnuts because they showed lower acceptability and therefore least preferred as sites for oviposition by *C. maculatus*.

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