

Effect of substrate type on egg laying by females and embryonic development of *Callosobruchus maculatus*

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Abstract

The objective of this article is to determine the effect of seed type, as an egg-laying substrate, on the biodemographic parameters of the cowpea weevil (*Callosobruchus maculatus*). The parameters studied here are female egg laying and embryonic development. To this end, one group of individuals was allowed to lay eggs on Barkedji seeds and another group on Fouta seeds. These two localities belong, respectively, to the Sylvopastoral and Senegal River Valley agro-ecological zones (ZAE).

The effect of seed origin on the embryonic development of *C. maculatus* is negligible. Indeed, with Barkedji seeds, females lay an average of 55.60 ± 19.144 eggs, whereas Fouta seeds result in only 45 ± 18.577 eggs per female. However, egg fertility and larval survival are higher with Fouta seeds than with Barkedji seeds (72.666% and 62.589%, respectively, with a p-value of 0.148 for fertility; 84.403% and 78.448%, with a non-significant p-value of 0.5428). The emergence rate obtained from Barkedji seeds (49%) is lower than that of Fouta seeds (61%), with a p-value of 0.364.

Ultimately, this study has shed light on the processes underlying changes in biodemographic parameters through the egg-laying tests conducted. These parameters are influenced by the nature of the substrate. Although our tests are not statistically significant, the nature of the seed appears to be a determining factor in the embryonic development of *C. maculatus*.

Keywords: Agroecological zone, Senegal, biodemographic parameter, embryonic development, *Vigna unguiculata*, crossbreeding, gene, *Callosobruchus maculatus*

Introduction

By contributing to the biodiversity of cereal agrosystems, promoting bacterial fixation of atmospheric nitrogen, stimulating the development of mycorrhizae, and providing ground cover, legumes support the development of more profitable, sustainable, and resilient agriculture (Franke *et al.*, 2018) [1]. Among these is the cowpea, *Vigna unguiculata* (L.) Walp. The cowpea is a legume native to warm regions, cultivated for human and livestock feed. Global cowpea production amounts to over 5.7 million tons of dry seeds per year, grown on 7.5 million hectares in 2008, 70% of which is produced in Africa (Tengo, 2011) [2]. The annual global cultivated area is estimated at over 12.5 million hectares, of which approximately 9.8 million hectares are in West Africa, making this region the world's leading producer and consumer of cowpeas (CGIAR, 2001) [3]. In Senegal, cowpea cultivation has evolved and is practiced throughout the country, across all agroecological zones. Within these zones, the varieties grown may differ from one area to another.

Cowpea production, which is already vulnerable to climatic fluctuations, faces further challenges in storage due to losses caused by Bruchidae beetles, which make post-harvest storage very difficult. *Callosobruchus maculatus*, a cosmopolitan insect, is the primary pest affecting cowpea stocks. It is found in all agro-ecological zones of Senegal, as in many other countries.

However, its adaptability to agro-climates could influence its biodemographic characteristics.

This led us to ask whether the nature of the egg-laying substrate might affect the biodemographic parameters of this insect.

To answer this question, we sought to determine the egg-laying patterns of females, as well as egg fertility rates, larval survival rates, and adult emergence rates among populations from two distinct agro-ecological zones: the Barkedji population in the sylvopastoral zone and the Fouta population in the Senegal River Valley zone. In these two zones or localities, the seeds used are of different varieties and therefore differ in nature from one zone to another.

To this end, we paired individuals from the same region and placed them in a laying substrate also sourced from that region. Specifically, individuals from Barkedji were placed in Barkedji seeds, and those from Fouta were placed in seeds from Fouta, in order to determine the relevant parameters.

Materials and Methods

1. Differences in the Characteristics of the Seeds Used

Barkedji seeds differ from those of Fouta in several ways. Among these, we have identified three: color, size, and the appearance of the seed coats (Table 1).

Table 1: Some Comparative Characteristics of the Seeds Used

	Color	Zise	Shell	Density
Barkedji seeds	White	Larger	Less rough	Less dense
Fouta seeds	Gray-black	Smaller	Rougher	Denser

2. Collection of cowpea seeds and mass cultivation

To conduct the experiments, cowpea seeds were collected from the localities of Barkedji and Fouta, located in the sylvopastoral zone and the Senegal River Valley, respectively. These locations were chosen because the cowpea varieties cultivated in these zones or localities

differ. The seeds from these locations were obtained from farmers or at the market. Some were stored in the freezer for testing (sterile group), and the rest were placed in jars for mass cultivation (infested group).

These samples (infested parts) were stored in the laboratory in ventilated jars with a volume of approximately 1 liter until emergence. Each strain was reared on cowpeas sourced from the same locality. The rearing was conducted at the Entomology and Acarology Laboratory of the Faculty of Science and Technology at Cheikh Anta Diop University of Dakar (UCAD) under ambient temperature and humidity conditions. The rearing was continuously monitored until a sufficiently large population of insects was obtained.

3. Experimental Protocol

3.1. Selection of Mated Individuals

Mass rearing provided us with a large number of insects. Thus, at the last emergence observed, the contents of the boxes were sieved to remove all adults that had emerged. Six (06) hours later, the newly emerged insects were isolated. This process allowed us to obtain adults less than 12 hours old. These adults, which had emerged from the jars and were less than 12 hours old, were isolated individually, identified by sex and locality, and used in the mating tests. The most distinctive characteristic between males and females is the coloration of the plate covering the pygidium (last abdominal segment). In females, the plate is enlarged and dark-colored on both lateral sides. Males have a reduced pygidium with no coloration (Beck and Blumer, 2014) [5].

3.2. Experimental Conditions

The experiments were conducted under natural climatic conditions at the Entomology and Acarology Laboratory of the Faculty of Science and Technology at UCAD. They took place between August 15 and October 15. During this period, average temperatures ranged between 29 and 36°C, and relative humidity between 70 and 75%. Sterilized cowpea seeds from the localities of Fouta and Barkedji were used as the substrate for the tests. Sterilization involves first sorting out the seeds that appear uninfested to the naked eye. These seeds are then placed in a freezer at 0°C for 15 days to eliminate any potential infestation. Before use, the sterilized seeds are left at room temperature for at least 24 hours to warm up.

3.3. Mating Tests

Mating tests were conducted. To do this, we selected males and females from each locality that were no more than 6 hours old. These insects were isolated individually and then paired up.

The mating tests were conducted as follows: each male from a locality was mated with a female from the same locality in a box containing 25 sterile seeds. For each locality, and thus for each agroecological zone, a batch of ten (10) pairs was formed. The two batches obtained, along with their code names, are as follows:

- **Set 1:** Barkedji seeds; Barkedji males × Barkedji females (GBMBFeB) or the Barkedji population.
- **Set 2:** Fouta seeds; Fouta males × Fouta females (GFMFFeF) or the Fouta population.

The fertilized females in each batch constitute a population. This means we have a total of two populations.

The containers used are made of plastic and have a volume of approximately 85 ml with an average diameter of 5 cm. The lids of these airtight containers have been perforated with small holes for ventilation.

B = Barkedji; Fe = Female; F = Fouta; G = Seed; M = Male.

3.4. Determination of the biodemographic parameters of *C. maculatus*

The determination of the biodemographic parameters studied here begins on the first day of infestation. Every day for 8 to 9 days, the seeds infested by the females are removed and replaced with new, sterile seeds. The removed seeds are placed in another box bearing the same label or code name, and the laid eggs are counted using a magnifying glass. This procedure allowed us to determine the daily egg-laying rate of each female. After counting the eggs, the properly labeled boxes are stored on a lab bench. Between the seventh (D7) and tenth day (D10) after the start of the experiments, sterile or infertile eggs and fertile eggs are counted using a magnifying glass or even with the naked eye. Sterile eggs can be distinguished from fertile eggs by their translucent appearance on a seed. Indeed, the eggs of *C. maculatus* appear translucent white after laying, and if they hatch, they become milky. According to Beck & Blumer (2014) [5], laid eggs are approximately 0.75 mm long and are ovoid or fusiform, with a glossy appearance, and attached to the surface of the pods. The eggs are attached to the outer surface of the seeds or pods (Beck & Blumer, 2011) [4] by a gelatinous substance (Alzouma, 1981; Delobel & Tran, 1993) [6, 7]. The boxes are then kept on the lab bench at room temperature until the adults emerge.

Upon the first emergence, the date is recorded and monitoring of the adults begins. From that date onward, and for each box, the emerged adults are removed and identified by sex. These adults are then counted. This monitoring of emergences lasted two weeks. This process allowed us to determine the number of males and females and, consequently, the total number of adults that emerged.

The following parameters were determined:

- **The average number of eggs laid per female (N)** corresponds to the total number of eggs a female can lay over her entire lifetime. This number is obtained by dividing the sum of all eggs laid by all females in a batch (for example, a total of ten females per batch) by the number of females, that is to say, on 10.

$$N = \frac{\text{Total number of eggs laid by all females}}{\text{Number of females}}$$

- **Fertility rate (FR):** This is the percentage of fertile eggs relative to the total number of eggs laid. It is calculated by dividing the number of fertile eggs (multiplied by 100) by the total number of eggs laid.

$$FR (\%) = \frac{\text{Number of fertile eggs} \times 100}{\text{Total number of eggs laid}}$$

- **Larval survival rate (LSR):** This is the percentage of emerged individuals relative to the total number of fertile eggs.

$$LSR (\%) = \frac{\text{Number of emerged individuals} \times 100}{\text{Total number of fertile eggs}}$$

- **Emergence rate (ER):** This is the percentage of individuals that have emerged relative to the total number of eggs laid;

$$ER (\%) = \frac{\text{Number of individuals that have emerged} \times 100}{\text{Total number of eggs laid}}$$

4. Statistical Analysis

An Excel spreadsheet was used to compile all the results into a workbook and to calculate the value of each parameter described.

Statistical analyses were performed using R software version 4.3.1 at a 5% significance level (alpha = 0.05) with the Shapiro test, the Kruskal-Wallis test, the Student's t-test, and ANOVA.

Results

1. Variation in the number of eggs laid per female *C. maculatus*

Figure 1 shows the average number of eggs per female as a function of the type of laying substrate. Females from the Barkedji population (lot 1) had the highest average number of eggs per female, at 55.60 ± 19.144 eggs per female. Lot 2, consisting of females from the Fouta population, therefore has the lowest average number of eggs per female, at 45 ± 18.577 eggs per female.

The ANOVA test shows a p-value of 0.498, which is not significant. This means that the differences or variations in the average number of eggs observed between the groups are not significant.

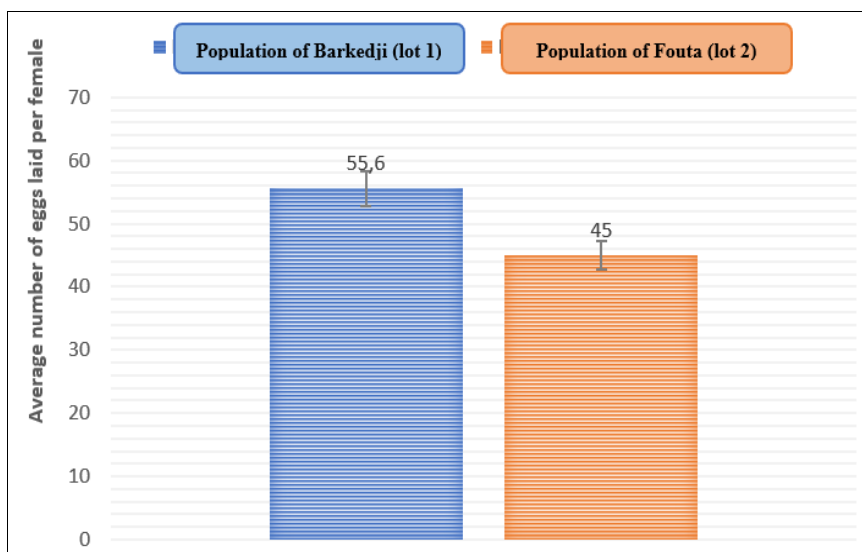


Fig 1: Average number of eggs laid per female, depending on the type of laying substrate

2. Study of Embryonic Development

2.1. Egg Fertility

The egg fertility rate appears to vary depending on the seeds. We found that regardless of the type of seeds considered, the fertility rate exceeds 60% (Figure 2). It is lower for seeds from Barkedji (lot 1) or the Barkedji population (62.589%) and higher for seeds from Fouta (lot 2) or the Fouta population (72.666%).

Nevertheless, these differences are not statistically

significant based on the statistical tests conducted. The Shapiro-Wilk test, with a non-significant p-value of 0.1238, allowed us to fail to reject the null hypothesis H_0 , i.e., that the variable follows a normal distribution. This allowed us to perform the ANOVA test, which yielded a non-significant p-value (p-value = 0.148). This means that the differences observed between the various egg fertility rates are not significant. In other words, the rates obtained can be considered equal.

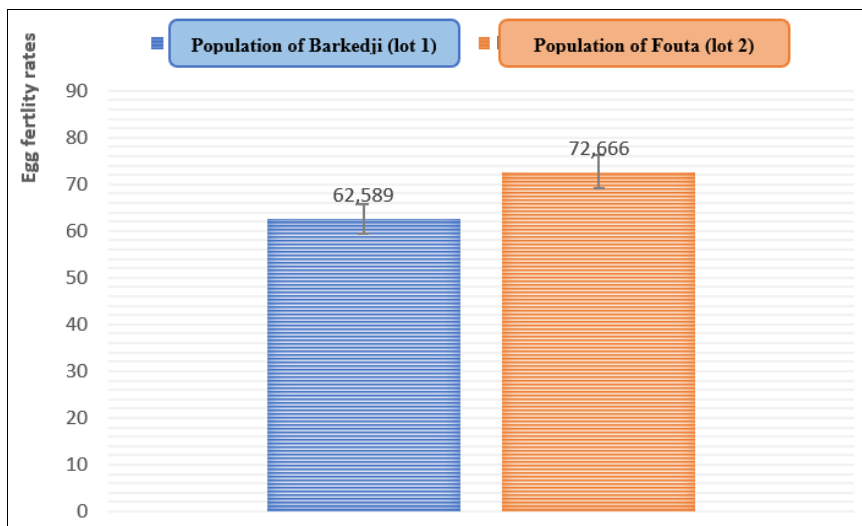


Fig 2: Fertility rates of *C. maculatus* eggs by seed type

2.2. Larval survival

The results shown in Figure 3 indicate that, regardless of the seeds used, 75% of the eggs hatched. Furthermore, we also observed that the larval survival rate obtained from Barkedji

seeds or batch 1 (78.448%) is lower than that of Fouta seeds or batch 2 (84.403%).

The Kruskal-Wallis test shows no significant difference between these rates (p-value of 0.5428).

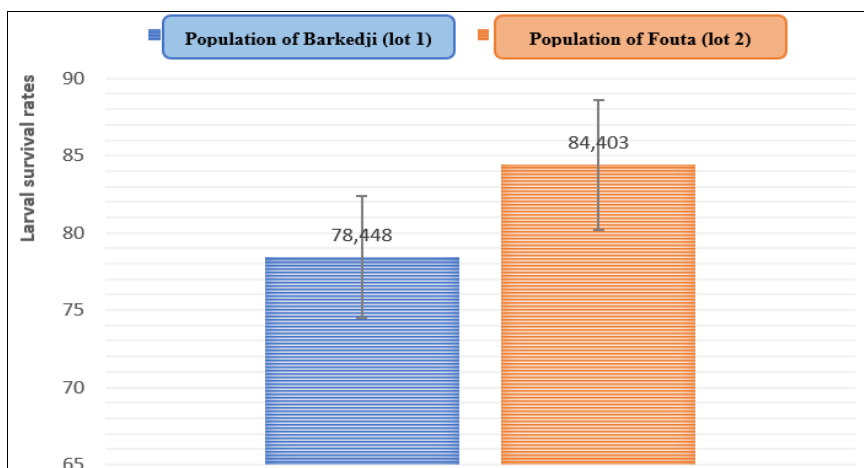


Fig 3: Larval survival rates by seed type

2.3. Adult Emergence

Figure 4 shows variations in adult emergence rates based on the types of seeds used. We found that the emergence rate for Barkedji seeds (49%) was lower than that for Fouta

seeds (61%).

The ANOVA test (p-value = 0.364) indicates that the differences observed between the calculated rates are not significant.

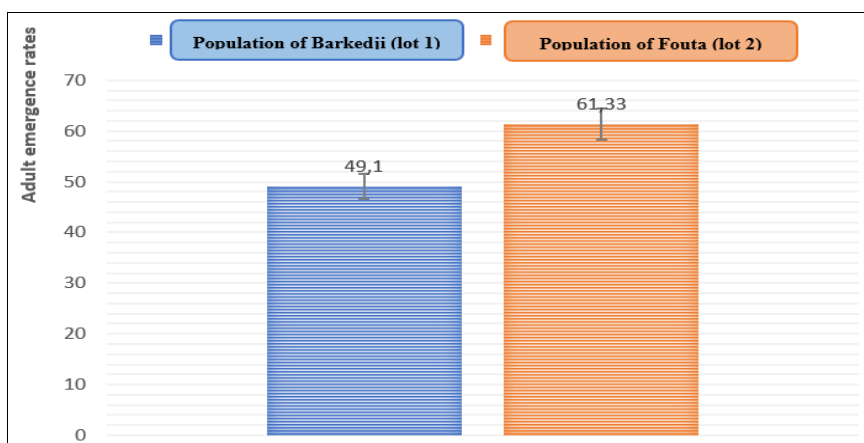


Fig 4: Hatching rates of *C. maculatus* adults by seed type

Discussion

Cowpea seeds provide a favorable egg-laying substrate for female *C. maculatus*. However, according to Olajire *et al.*, (2011) [8], none of the cowpea varieties, with the exception of TVu 2027, are fully resistant to this pest of stored cowpea seeds. Consequently, *C. maculatus* can infest the vast majority of cowpea varieties and produce a new generation of adults, thereby increasing its population. Populations of *C. maculatus* in storage often vary in size. The size of a population may depend on several factors, including the nature of the seeds serving as an egg-laying substrate for females and as food for larvae. This is the focus of this article.

A study of the egg-laying activity of *C. maculatus* shows that the average number of eggs laid by females varies depending on the origin and type of seeds used. We found that the average number of eggs laid is higher for Barkedji seeds (Lot 1, GBMBFeB) and lower for Fouta seeds (Lot 2, GFMMFeF). Specifically, we observed 55.60 and 45.50 eggs, respectively. The nature of the seeds appears to be a

factor influencing fertility. Indeed, since Barkedji seeds are larger, the female has more surface area available for laying eggs. This may explain the higher number of eggs obtained when using these seeds. Cope & Fox (2003) [9] demonstrated that when several seeds of the same size are presented to female *C. maculatus*, they tend to distribute their eggs evenly across the seeds. However, when the seeds differ in size, they lay their eggs in a way that maximizes the available resource for their offspring and thus preferentially lay on the larger seeds. This is consistent with Lale and Makoshi, 2000 [10]; Edd and Amatobi, 2003; Messina and Jones, 2009 [12], who state that factors such as texture, the chemical characteristics of the seed coat, and nutritional quality can also strongly influence egg-laying.

Thus, females can more accurately assess the relative amount of resources available inside a seed by evaluating the difference in size between seeds (Kébé, 2013) [13]. Seed color can also influence whether females lay a large or small number of eggs: white is the best color for camouflaging *C. maculatus* eggs; this would explain the higher egg-laying

observed on Barkedji seeds, which are white. According to Nicole (2002) ^[14], the typical behaviors of insects in their search for oviposition and feeding sites can also be influenced by color perception, the shape of the host plant, spectral intensity, or reflectivity.

However, since the observed differences were not statistically significant, our results are more in line with those of Somta *et al.*, (2006 and 2008) ^[15, 16], who, on the other hand, showed that seed size plays no role in the differences in infestation observed among different varieties of *Vigna radiata*, a wild relative of cowpea.

The number of eggs is comparable to that reported by Doumma *et al.*, (2011) ^[17], who obtained an average of 60 eggs over 5 days while studying varietal resistance.

However, the fertility of all our females on Barkedji and Fouta seeds appears to be low compared to the results obtained in the laboratory by Sanon (1995) ^[18], which show that between May and June, the fertility per female of *Callosobruchus maculatus* ranges between 76 and 95 eggs. This can be explained by the temperature and humidity conditions prevailing between July and October (temperatures between 29 and 36°C and relative humidity between 70 and 75%), the period during which we conducted our experiments. These conditions tend to favor the less fertile winged form, which can therefore lead to a decrease in female fertility. According to Sanon (1995) ^[18], the low fertility of *C. maculatus* is linked to the relatively high temperatures that prevail between May and July in the Sahel.

However, regardless of the type of seed, the egg fertility rate is high, exceeding 60%. These rates are similar to those reported by Kellouche *et al.*, (2004) ^[19]. However, females from Fouta showed the highest rate, with 72.40% of eggs being fertile. This may be due to the nature of the seed. Indeed, since the seeds used are of different varieties, they may have different textures or chemical compositions. According to Kellouche *et al.*, (2004) ^[19], seed texture can influence the egg-laying behavior of females.

Larval survival rates are higher with the Fouta substrate. These rates appear to be influenced by the nature of the food substrate used. More likely, however, this is due to the availability of the resource, which is linked to the females' egg-laying activity. Increased egg-laying leads to larval competition and, consequently, a decrease in egg fertility. The relatively high larval survival rates obtained (78% on average) may be due to the fact that the females were able to distribute the eggs evenly across the seeds, which may result in low larval density within the seeds and thus ensure the availability of the food substrate. Indeed, according to the work of Zannou (2000) ^[20], Booker (1967) ^[21], and Howe and Curie (1964) ^[22], as intra-seed larval density increases, the larval mortality rate also increases; however, the emergence rate decreases.

The emergence rate of adults from Fouta females is higher than that of Barkedji females. The nature of the seed also influences emergence, as the highest emergence rates are obtained with seeds from Fouta. This could be due to high fertility rates. Indeed, the egg-laying substrate may limit the female from depositing multiple eggs on a single seed. A limited number of eggs on the seed thus prevents an increase in larval density inside the seed and thereby avoids larval competition. This increases the adult emergence rate. Nyamador (2009) ^[23] states that high larval survival and emergence rates are favored by climatic conditions during

larval development and the availability of egg-laying substrate. These high emergence rates could also be influenced by climatic conditions affecting larval development or by the genetic characteristics of the strains used.

However, according to Somta *et al.*, (2008) ^[16], the fact that weevil larvae are able to penetrate the seed coats of resistant seeds and reach the cotyledons of *Vigna radiata* suggests that the seed coats of resistant seeds do not play an effective role in protecting the seeds against weevils. The low emergence rates observed with Barkedji seeds can be explained by high larval mortality in the cotyledons. This mortality is likely due to the presence of biochemical compounds that some larvae were unable to detoxify (Kébé, 2013) ^[13], whereas Edde & Amatobi (2003) ^[11] argue the opposite. For them, this mortality is due to antibiosis at the cotyledon stage. According to Souza *et al.*, 2011 ^[24], a very large number of proteins and peptides found in the cotyledons of cowpea (*Vigna unguiculata*) seeds have been associated with the resistance of some seeds against insects. It may also be due to the low density of the seed, which implies a lack of nutrients and thus larval competition.

Conclusion

The results obtained from the study of the biodemographic parameters of these two populations—namely, the Barkedji population and the Fouta population (lot 1 and lot 2, respectively) — revealed a possible influence of seed characteristics. It is this influence that is believed to account for the differences observed following the determination of the parameters of the two populations. This influence may depend more on the organoleptic quantity than on the organoleptic quality of the seed. The quantity of available organic matter plays an important role in the insect's larval development. The more food is available, the higher the number of eggs that hatch and emerge.

It is therefore important, in the search for effective control methods, to include this demographic parameter of *C. maculatus* populations, which can be affected by the conditions of their developmental environment.

However, the use of genetically identical strains will provide a clearer picture.

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