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Study of the Biodemographic Parameters of Two Genetically Distinct Strains of *Callosobruchus maculatus* from Two agroecological Zones of Senegal

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ABSTRACT: During this study, we determined different biological parameters of *Callosobruchus maculatus* populations from two agro-ecological zones of Senegal (the ZSP and the ZVFS). To do this, strains of *C. maculatus* were collected in two localities, each representing an agro-ecological zone. The analysis of the results obtained shows that fecundity is greater for the strain coming from the silvopastoral zone (55.60 ± 19.144 eggs per female) than for that coming from the Senegal River valley zone (45 ± 18.577 eggs per female.). Furthermore, the evolution of egg laying shows that whatever the strain or population, more than 95% of eggs are laid during the first four days of the infestation with a peak on the second. In addition, the strains are characterized by an egg fertility rate exceeding 65% with a larval survival rate of over 75% and the Fouta strain has the highest rates. However, the emergence rate of the strain from the silvopastoral zone is lower at 49%. The longevity of adults in the Senegal River valley zone is significantly longer (11.035 ± 1.527 days) than that of adults in the silvopastoral zone. The sex ratio is in favor of males for both populations. The total development time and the oviposition-emergence time are relatively the same in the two strains with an average of 31 days and 21 days respectively.

KEY WORDS: agroecological zones, Biological parameters, Cowpea (*Vigna unguiculata*), *Callosobruchus maculatus*, Senegal.

INTRODUCTION

Callosobruchus maculatus is a cosmopolitan insect, pest of cowpea stocks. It is found in all agro-ecological zones of Senegal as in many other countries. The genetic characteristics of *C. maculatus* may differ from one area to another following adaptability phenomena. From a general point of view, knowledge of diversity and genetic structure is a crucial issue for the species we wish to study. Since this genetic structure is shaped by multiple factors. Genetic studies relating to the characterization of *C. maculatus* populations according to the agro-ecological zones of Senegal have demonstrated that populations from the silvopastoral zone (ZSP) and those from the Senegal River valley zone (ZVFS) are the most distant or genetically distant (Faye *et al.,* 2023).

We then asked ourselves the question of whether genetic characteristics do not have an effect on the biodemographic parameters of the insect?

This is why we wanted to determine the biodemographic parameters of populations from these two zones, namely the populations of the silvopastoral zone and that of the Senegal River valley zone. To do this, we carried out matings between individuals from the silvopastoral zone on the one hand and between individuals from the Senegal River valley zone on the other hand to determine these parameters. The new generation resulting from crosses (F1) and with the parents made it possible to determine the biodemographic parameters such as the total duration of the cycle, the lifespan of the adult or adult longevity, the spawning-emergence duration, the sex- ratio, emergence rate, larval survival rate, fertility rate and average number of eggs laid per female.

1. MATERIAL AND METHOD

1. 1. Sampling of cowpea seeds and mass rearing

To carry out the experiments, cowpea seeds were taken from the localities of Barkédji and Fouta belonging respectively to the silvopastoral zone and that of the Senegal River valley. The choice of these localities was based on the fact that their populations are genetically distant (Faye *et al.,* 2023). Seeds from these localities were taken from producers or from the market. Part was kept in the freezer for testing and the other part was put in jars for mass rearing.

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These samples were kept in the laboratory in aerated jars with a volume of approximately 1 liter until emergence. The breeding of each strain was carried out on cowpeas coming from the same locality. The breedings were carried out in the entomology and acarology laboratory of the Faculty of Sciences and Technology of the Cheikh Anta Diop University of Dakar (UCAD) under ambient temperature and humidity. Permanent monitoring of the farms was carried out until a fairly large number of insects was obtained. These adults allowed us to carry out mating without being between insects of the same generation following the determination of the sex of each of them.

At the imaginal stage, *C. maculatus* measures 3.5 to 5 mm. The black to red adult has crenate antennae from the 5th article, the last articles are sometimes darkened (Delobel and Tran, 1993). Females are larger than males. The most distinctive feature is the coloring of the plaque covering the pygidium (last abdominal segment). In females, the plaque is enlarged and dark in color on both lateral sides. Males have a reduced pygidium without any coloration (Beck and Blumer, 2014).

1. 2. Experimental protocol

1. 2. 1. Choice of crossed individuals

Mass breeding has allowed us to have a lot of insects. Thus, at the last emergence considered, the contents of the boxes were sifted so as to eliminate all the adults who had emerged. Six (06) hours later, the insects which have just emerged are isolated. This process allowed us to obtain adults aged less than 12 hours. The latter which emerged from the jars and were less than 12 hours old were isolated individually, identified according to sex and locality and are used in mating tests.

1. 2. 2. Experimental conditions

The experiments were carried out under natural climatic conditions in the entomology and acarology laboratory of the Faculty of Sciences and Technology of UCAD. They were carried out between August 15 and October 15. During this period, average temperatures were between 29 and 36°C and relative humidity between 70 and 75%. Sterilized cowpea seeds from the localities of Fouta and Barkédji were used as substrate for the tests. Sterilization involves first sorting seeds that appear uninfested to the naked eye. These seeds are then placed in the freezer at zero degree temperatures for 15 days to eliminate any probable infestation. Before use, the sterilized seeds are returned to room temperature for at least 24 hours to warm up.

1. 2. 3. Mating tests

Mating tests were carried out. To do this, we took, for each locality, males and females aged at most 6 hours. These insects were isolated individually and then used to form pairs.

The mating tests were carried out as follows: each male from a locality is mated with a female from the same locality in a box containing 25 sterile seeds. More explicitly we took for example a male from Barkédji crossed with a female from Barkédji and a male from Fouta crossed with a female from Fouta. For each locality and therefore for each agroecological zone, a batch of ten (10) couples was constituted. The two lots obtained, with their code name, are as follows:

- ✓ Lot 1: Barkédji seeds; Barkédji males X Barkédji females (GBMBFeB) or Barkédji population.
- ✓ Lot 2: Fouta seeds; Fouta males X Fouta females (GFMFFeF) or Fouta population.

The fertilized females of each batch constitute a population whose biodemographic parameters are studied here. Which means that we have a total of two populations (each batch constituting a population). These populations are of the first generation.

The boxes used are plastic and have a volume of approximately 85 ml with a diameter of 05 cm on average. The lids of these boxes, which close tightly, have been finely perforated for ventilation.

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

1. 2. 4 Determining the biodemographic parameters of *C. maculatus*

Determination of the biodemographic parameters studied here begins on the first day of infestation. Every day for 08 to 09 days, seeds infested by females are removed and replaced by new sterile seeds. The removed seeds are placed in another box bearing the same label or code name, and the eggs laid are counted using a magnifying glass. This operation enabled the daily egg-laying rate of each female to be determined. Once the eggs had been counted, the well-referenced boxes were placed on a bench.

Between days seven (D7) and ten (D10) from the start of the experiments, sterile or infertile eggs and fertile eggs were counted using a magnifying glass or even the naked eye. Sterile eggs can be distinguished from fertile eggs by their translucent appearance on a seed. The boxes are then kept on the laboratory bench at room temperature until the adults emerge.

At the first emergence, the date is noted and monitoring of adults begins. From this date, and for each box, the emerged adults are removed, identified according to sex. These adults are then counted. This monitoring of emergences lasted two weeks. This process allowed us to know the number of males and females and therefore the total number of adults that emerged. Among adults, a batch of 10 males and a batch of 10 females are made up of up to 10 repetitions per population. A total of 100 male individuals and 100 female individuals were obtained for each batch initially constituted during the mating tests. Once batches of 10 are made up, the insects are monitored and each day the number of deaths is counted down to the last one. This allowed us to calculate the longevity of males and females.

The following parameters were determined:

- ✓ **The average number of eggs laid per female** corresponds to the total number of eggs that a female can lay during her entire life. This number is obtained by adding up all the eggs laid by all the females in a batch for example (a total of ten females per batch) over the number of females, i.e. over 10.
- ✓ **The fertility rate:** it is the percentage of fertile eggs compared to the total number of eggs laid. It is the ratio between the number of fertile eggs multiplied by one hundred and the total number of eggs laid.
- ✓ **The larval survival rate:** it is the percentage of individuals emerged in relation to the total number of fertile eggs.
- ✓ **The emergence rate**: it is the percentage of individuals emerged in relation to the total number of eggs laid;
- ✓ **The sex ratio** corresponds to the numerical ratio between males and females of the offspring.
- ✓ **The duration of development or spawning-emergence duration:** this is the time which separates the release of an egg on a seed and the emergence of the adult;
- ✓ **The lifespan of the adult or adult longevity**: this is the time interval between the emergence of the insect and its death;
- ✓ **The total duration of the cycle**: it is the time interval between the emission of an egg and the death of the adult which emerges there. It encompasses the developmental time and longevity of adults.

1. 3. Statistical analysis

The Excel spreadsheet made it possible to list all the results obtained in a workbook and to calculate the value of each parameter described.

Statistical analyzes were carried out using R software version 4.3.1 at the 5% threshold (alpha = 0.05) with the Shapiro, Kruskal-Wallis, Student's test and ANOVA tests.

2. RESULTS

2. 1. Study of laying activity

2. 1. 1. Variation in the number of eggs laid by female *C. maculatus*

Figure 1 shows the average number of eggs per female depending on batches or populations. Females from the Barkédji population (lot 1) have the highest average number of eggs per female with 55.60 ± 19.144 eggs per female. Lot 2, made up of females from the Fouta population, therefore have the lowest average number of eggs per female with 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 batches are not significant.

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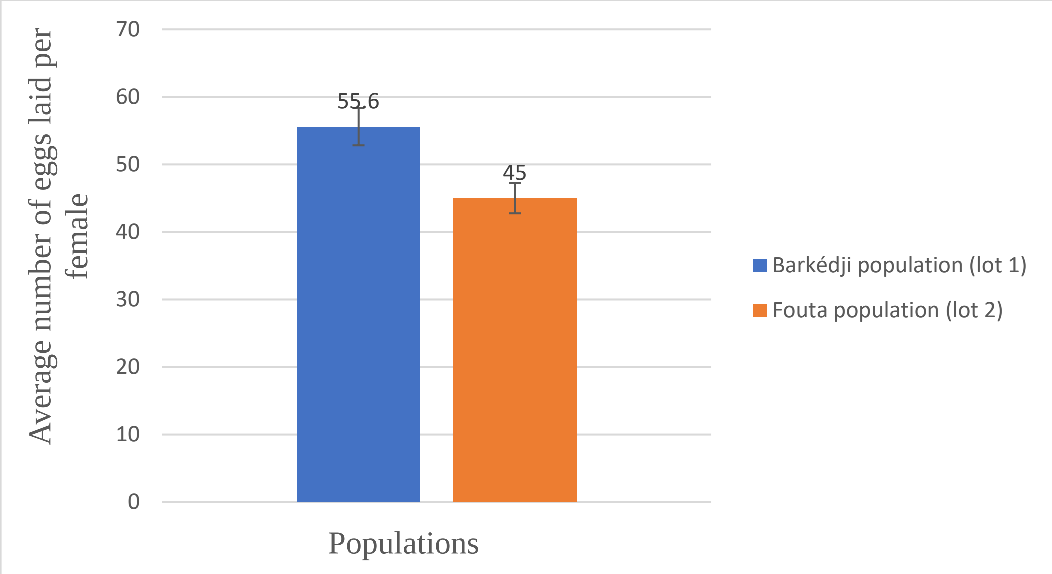

Figure 1: Average number of eggs laid per female according to batches or populations

2. 1. 2. Evolution of laying activity

The laying activity began on the first day of the females' life with more than 20% of eggs laid. This daily spawning peaked on the 2nd day for the two batches or populations (with 33.453% for batch 1 or Barkédji population and 38% for batch 2 or Fouta population). This egg-laying activity decreases as the age of the females increases. This drop was considerable after the peak and became very weak or even zero after four days of female survival. The results thus presented are recorded in **Figure 2**.

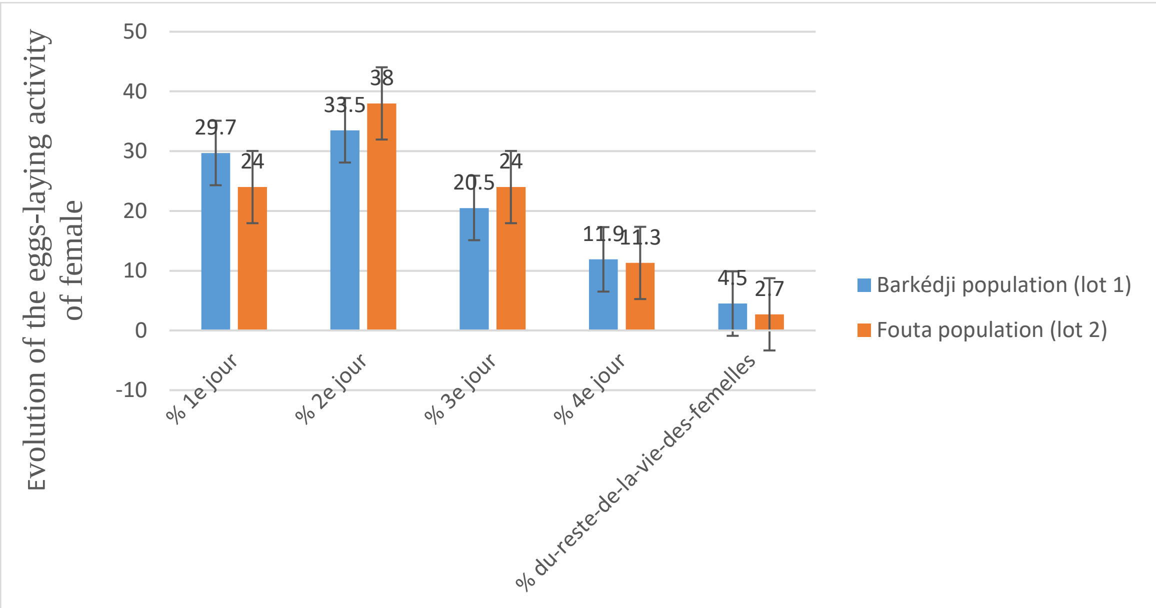

Figure 2: Evolution of the egg-laying activity of female *C. maculatus*

Regarding the cumulative laying percentages, **Figure 3** shows that more than 90% of eggs are laid by *C. maculatus* females during the first four days of their existence. Note also that the females from Barkédji (lot 1) laid more than 95% of their eggs after 4 days of laying while those from Fouta (lot 2) laid more than 97%.

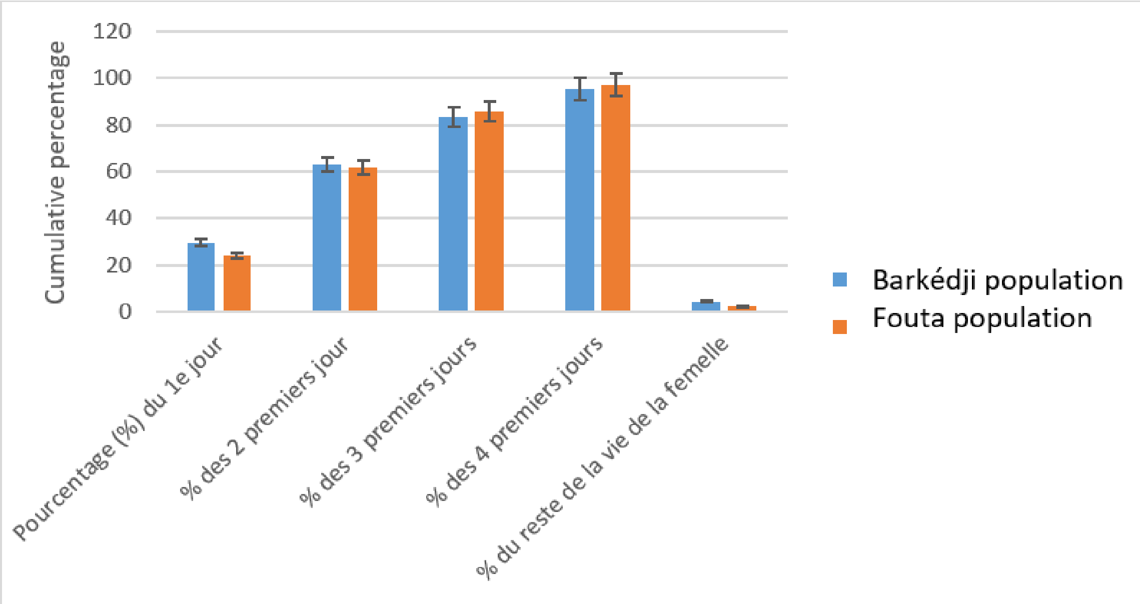

Figure 3: Cumulative percentage of eggs laid during the life of the female per batch

2. 2. Study of post-embryonic development

2. 2. 1. Egg fertility

The fertility rate of the eggs seems to vary depending on the origin of the females. We found that whatever the population considered, the fertility rate is above 60% (**see Figure 4**). It is lower with females from Barkédji (lot 1) or Barkédji population (62.589%) and is higher with females from Fouta (lot 2) or Fouta population (72.666%).

However, these differences are not significant based on the statistical tests carried out. The Shapiro Wilk test, with a non-significant p-value of 0.1238, made it possible to not reject the null hypothesis H0, i.e. that the variable follows the normal distribution law. This made it possible to carry out the ANOVA test, the p-value of which is not significant (p-value = 0.148). Which means that the differences observed between the different egg fertility rates are not significant. In other words, the rates obtained can be considered equal.

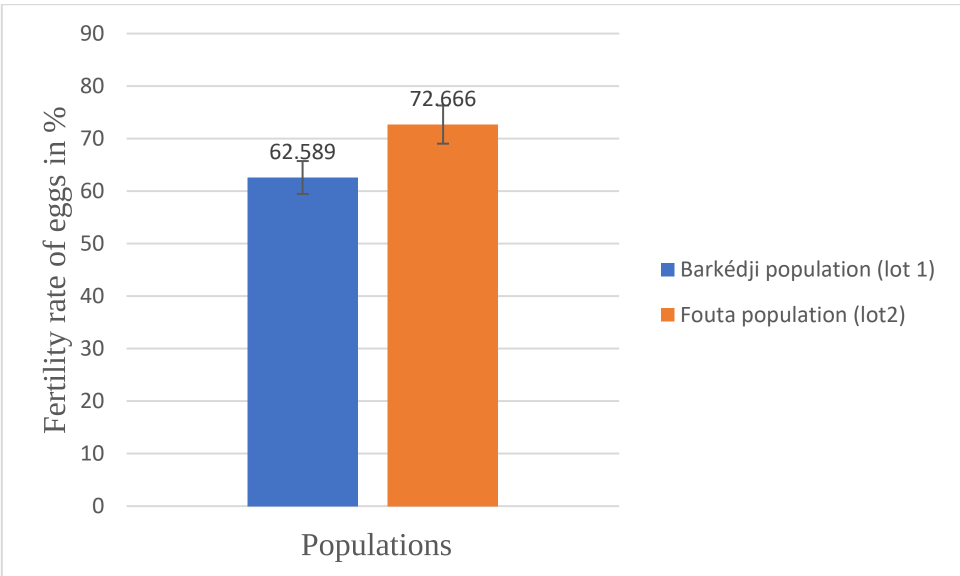

Figure 4: Fertility rate of *C. maculatus* eggs according to populations

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2. 2. 2. Larval survival

The results recorded in Figure 5 show that, whatever the population considered, we have more than 75% larval survival rate. Furthermore, we also noted that the larval survival rate of females from the Barkédji population or lot 1 (78.448%) is lower than that of females from the Fouta population or lot 2 (84.403%).

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

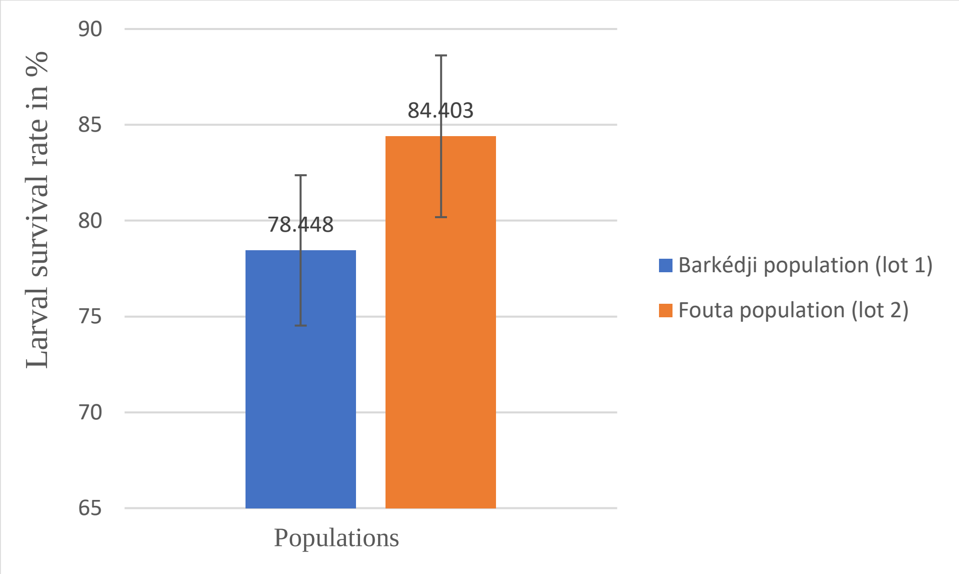

Figure 5: Larval survival rate depending on populations

2. 2. 3. The emergence of adults

The variation in larval survival depending on the population results in a variation in adult emergence rates (**figure 6**). In fact, the emergence rate varies depending on the batches and therefore depending on the populations or the origin of the females.

We found that the emergence rate of females from Barkédji (49%) is lower than that of females from Fouta (61%), with whom they are genetically distinct.

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

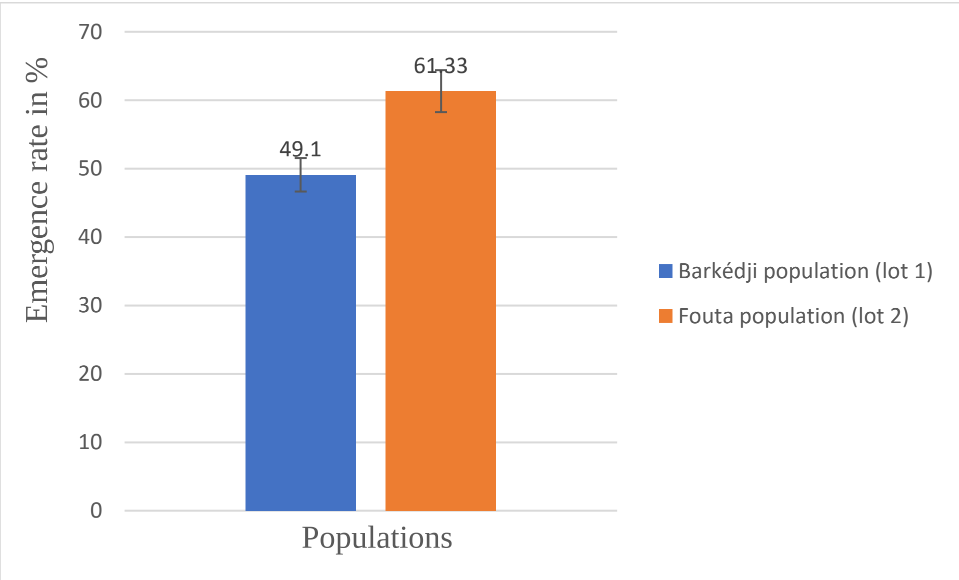

Figure 6: Emergence rate of adults of *C. maculatus* depending on the populations

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There is not a significant difference between these rates.

2. 3. The sex ratio

The sex ratio, which is the ratio between male offspring and female offspring (**Table 1**), did not vary from one batch to another or from one population to another. The analysis of the sex ratio in F1 (first generation) obtained shows that it is in favor of males for the two batches or populations which are genetically distinct. It is 1.109 for lot 6 (population of Fouta) and 1.022 for lot 3 (population of Barkédji).

Table 1: Variation in the sex ratio (R) of *C. maculatus* depending on the batches

Lots or populations and code name	*Lot 1 or population of Barkédji (GBMBFeB)*	*Lot 2 or population of Fouta (GFMFFeF)*
Sex-ratio	01,022	01,109

2. 4. Study of the development cycle of *C. maculatus*

2. 4. 1. The spawning-emergence duration

Table 2 shows that the duration of embryonic development (laying-emergence duration) hardly varies depending on the batches or origin of the *C. maculatus* females used.

This duration is on average 21 days. It is longer for lot 3 or population of Barkédji (21.576 ± 1.711 days) and smaller for lot 6 or population of Fouta (20.382 ± 1.094 days). However, this difference is not significant. The Kruskal Wallis test confirms this with a non-significant p-value of 0.3534, suggesting equality between the determined durations.

Table 2: Laying-emergence duration depending on batches or populations

Lots or populations and code name	*Laying-emergence duration in days*
Lot 1 ; Population of Barkédji (GBMBFeB)	21,576 ± 1,711 **a**
Lot 2 ; Population Fouta (GFMFFeF)	20,382 ± 1,094 **a**

The presence of the letter "a" only indicates that there is not a significant difference between the calculated spawning-emergence times.

2. 4. 2. Adult lifespan or adult longevity

The results recorded in Table 3 show that the lifespan of *C. maculatus* adults varies depending on the sex and the geographic origin of the females who fathered them.

For each batch or population considered, we found that the lifespan of females is longer than that of males.

We also noted that adults from the Barkédji population (lot 1: GBMBFeB) have a lower average lifespan (9.904 ± 0.733 days) than that of adults from the Fouta population (lot 2: GFMFFeF) which is 11.035 ± 1.527 days. But the difference observed is not significant. Depending on the sex, we found that the males of the Barkédji population (lot 1) have an average lifespan (07.845 ± 0.483 days) which is significantly lower than that of the males of Fouta (lot 2), which have a longevity of 09.758 ± 1.846 days. In females the difference between average longevity is not significant.

Table 3: Variation in the average lifespan of *C. maculatus* according to sex and populations

Lots or populations and code name	*Average lifespan of males in days*	*Average lifespan of females in days*	*Average adult lifespan in days*
Lot 1 or Population of Barkédji (GBMBFeB)	07,845 ± 0,483 **a**	12,000 ± 1,327 **b**	09,904 ± 0,733 **a.c**
Lot 2 or Population of Fouta (GFMFFeF)	09,758 ± 1,846 **b**	12,313 ± 1,930 **b**	11,035 ± 1,527 **a**

Lots with the same letter (a, b or c) do not show a significant difference between them. However, those with different letters are significantly different.

2. 4. 3. The total cycle duration of *C. maculatus*

The total duration of the cycle also varies according to the batches or according to the two populations studied (**See table 4**).

We found that the Barkédji population (lot 1) has a total cycle duration of 31.480 ± 1.863 days, substantially equal to that of the Fouta population (lot 2) which is 31.427 ± 2.357 days.

These two populations, which are genetically distinct, do not present a significant difference between their total development cycle.

Table 4: Variation in the total development time of *C. maculatus* depending on the batches

Lots or populations and code name	***Total cycle duration in days***
Lot 1 or Population of Barkédji (GBMBFeB)	31,480 ± 1,864 **a**
Lot 2 or Population of Fouta (GFMFFeF)	31,427 ± 2,357 **a**

The presence of the letter "a" only indicates that there is not a significant difference between the calculated spawning-emergence times.

3. DISCUSSION

The demographic parameter cannot be forgotten in the quest for effective control methods. A control method whatever its nature, its effectiveness can only be the control of insect populations where they wreak havoc. These populations often vary in size. The size of populations could therefore rather depend more on genetic parameters than on climatic and geographical parameters. Several studies on the effect of agro-ecological zones on the demographic parameters of *Callosobruchus maculatus* have been carried out. According to Moumouni *et al.,* (2013), the geographical origin of the strains seems to influence the biological parameters of *C. maculatus*.

Thus the study of the laying activity of *C. maculatus* shows that the average number of eggs laid by females varies depending on the origin of the females and therefore depending on the agroecological zones concerned. We noted that the average number of eggs laid is greater for the population of Barkédji or lot 1 (GBMBFeB) than for that of Fouta or lot 2 (GFMFFeF, with 55.60 eggs. The geographical origin of the females seems to be a factor acting on fertility However, the results of females from Barkédji are comparable to those obtained by Doumma *et al.,* (2011) who obtained an average number of 60 eggs in 5 days by studying the varietal resistance. females of these two agroecological zones (ZSP and ZVFS) seems to be low compared to the results obtained in the laboratory by Sanon (1995) which show that between May and June, the fecundity per female of *Callosobruchus maculatus* varies between 76 and 95 eggs. can be explained by the temperature and humidity conditions which prevail between July and October (temperatures between 29 and 36°C and relative humidity between 70 and 75%), when we carried out our experiments. These conditions are rather. appear the sailboat form less fertile. This can therefore cause a drop in female fertility. According to Sanon (1995), the low fecundity of *C. maculatus* is linked to the relatively hot temperatures which occur between May and July in the Sahel.

However, whatever the batch considered or the population considered, the egg fertility rate is high and is greater than 60%. These rates are close to those obtained by Kellouche *et al.,* (2004). However, females from Fouta presented the best rate with 72.40% fertile eggs. The fertility of the eggs is therefore influenced by the geographical origin of the strains of *C. maculatus* or even by their genetic aspect knowing that the two populations used here are the most distant from each other from the populations of all the agroecological zones of Senegal (Faye *et al.,* 2023).

The larval survival rate is higher with the Fouta substrate. These rates seem to be induced by the origin of the females or by the nature of the food substrate used. 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 well on the seeds, which can make it possible to have a low larval density in the seeds and thus availability of the food substrate. Indeed, according to the work of Zannou (2000), Booker (1967), Howe and Curie (1964), when the intra-granary larval density increases, the larval mortality rate also increases; but the emergence rate is decreasing.

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The emergence rate of adults from Fouta females is higher than that of Barkédji females. The origin of seeds or females could be a factor influencing the emergence of adults. The nature of the seed also influences emergence because the highest emergence rates are obtained with Fouta seeds. However, it could be said that high fertility rates made it possible to have such high emergence rates. The oviposition substrate can help limit the deposit of several eggs on the same seed by the female. A limited number of eggs on the seed thus avoids an increase in larval density inside the seed and therefore makes it possible to avoid larval competition. This increases the rate of adult emergence. According to Nyamador (2009), states that the high rates of larval survival and emergence are favored by the climatic conditions of larval development and the availability of the spawning substrate. These high emergence rates could also be favored by the climatic conditions which influence the development of the larvae or by the genetic characteristics of the strains used.

The sex ratio does not vary depending on the two populations studied. He is in favor of males for the population of Barkédji as well as for that of Fouta. The genetic difference noted between the strains has no effect on the sex ratio which seems to depend on one or other factors. The sex ratio could therefore depend on climatic and environmental factors.

The spawning-emergence duration varies slightly between batches (less than a day). This duration is approximately equal to 21 days in the two populations studied. The fact that these two populations are genetically distinct has no effect on the embryonic development of *Callosobruchus maculatus*.

However, the total duration of the cycle is relatively long for the two populations (the population of Barkédji and the population of Fouta). It is 31 days in these two populations.

The lifespan of females, which is relatively long, is explained by the fact that females, by being isolated, escape mating. A mating that requires a lot of energy and is highly risky on the part of the female. Indeed, mating in *Callosobruchus* is very atrocious. Males cause enormous damage to the genital tracts of females through their spiny copulatory organ during mating. Which means that for females, escaping this physical aggression from males would increase their chances of living even longer, hence the greater longevity of females compared to that of males. According to Tatar *et al.,* (1993), mating significantly affects the lifespan of a female. Hence the concept of "cost of reproduction" used by Williams (1966) to link the effort of reproduction to the other functions of the insect. The lifespan of males is shorter due to the fact that, being isolated, they are restless and active almost all the time. Which exhausts them after a few days and ends up dying a little earlier.

The lifespan of Barkédji males is shorter than that of Fouta males. It is this difference which has induced a variation in the average lifespan of adults.

CONCLUSION

The results obtained from the study of the biodemographic parameters of these two populations, namely the population of Barkédji and that of Fouta (lot 1 and lot 2 respectively), revealed a possible influence of the geographical origin of the insects, the nature of the seeds and therefore climatic and environmental factors. Agro-ecological zones therefore have an influence on *C. maculatus* biodemographic parameters. It is this influence which would be at the origin of the differences observed following the determination of the parameters, especially in terms of the average lifespan of adults from the two populations which present a significant difference in longevity between them. 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 development environment.

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