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Potential Reproductive Activity of *Callosobruchus subinnotatus* Pic. (Coleoptera: Bruchinae), Bambara Groundnut (*Vigna subterranea* Verd.) Depredator

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Abstract: *Callosobruchus subinnotatus* Pic. (Coleoptera: Bruchinae) is the most important depredator of Bambara groundnut seeds (*Vigna subterranea* Verd.) in West Africa. In view of an efficient control of its populations, some of the biological parameters of *C. subinnotatus* were determined under laboratory conditions (30 ± 2 °C; 72 ± 2% HR; 12h: 12h LD). The results have shown that the mean lifespan of females (11.36 ± 1.85 days) is shorter than that of males (15.14 ± 2.4 days). The female lays 80.83% eggs within the first 6 days after her emergence with a mean fecundity of 121.34 ± 27.62 eggs. The means egg fertility, survival and adult emergence rates are respectively 96.19 ± 1.45%, 97.72 ± 1.08% and 94.01 ± 2.14%. The sex ratio is in favor of males with a mean of 0.845 ± 0.08. The monitoring of adult emergence in connection with their reproductive status showed that the offspring of *C. subinnotatus* consists of two physiotypes, a reproductive type that appears during the first 8 days of emergence with a short development time (28.35 ± 3.36 days) and a non reproductive type that appears from the 9th day of emergence with a longer development time (37.92 ± 3.92 days).

Key words: *Callosobruchus subinnotatus*, *Vigna subterranea,* biological parameters, physiotypes.

1. Introduction

Callosobruchus subinnotatus Pic. (Coleoptera: Bruchinae) is the main pest of stocks of Bambara groundnut (*Vigna subterranea* Verd.) in Togo [1, 2]. Bambara groundnut is third in terms of production among food legumes. West Africa alone provides 45 to 50% of this production [3]. It is mainly cultivated for its seeds, which are an important source of protein in the diet of West Africans. Bambara groundnut seeds are also rich in fats, carbohydrates, minerals and vitamins [3-5]. Contamination by *C. subinnotatus* starts in the field before harvest. Females lay their eggs on dry pods that are near the soil surface. It continues in stocks after harvest [6]. The weevil populations of this species persist in stocks as long as Bambara groundnut seeds are available. This can cause damage in terms of weight loss [7]; viability and the market value of seeds [8, 9]. Weight losses after 5 months of storage were estimated at 40% in Nigeria by Mbata [10]. These substantial losses are related to the multivoltine character of this species of beetle. Studies of its bioecology revealed that in one year, *C. subinnotatus* was capable of producing about ten generations on Bambara groundnut [1, 9]. Unfortunately, these losses are magnified by *Callosobruchus maculatus* F., the sympatric species [11].

C. maculatus develops preferentially on cowpea (*Vigna unguiculata* Walp.). On this food source, it produces sixteen generations in a year when seeds are available [12] and causes losses estimated between 800 and 900 g/kg in different parts of West Africa [13].

Three reproductive types of *C. maculatus* are distinguished: the sexually active individuals, the quiescent and the diapausing [14]. Similarly, *Bruchidius atrolineatus* Pic and *Callosobruchus rhodesianus*, two weevil species of economic

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importance in West Africa, a reproductive diapause has been reported [15, 16]. The presence of quiescent or diapausing individuals within populations of weevils can be manifested by morphotypes that allow to distinguish non reproductive adults from those that are sexually active, by the pigmentation of the elytra and abdomen [9]. In *C. subinnotatus* populations, two adult morphs which differ in their reproductive behavior are observed [1, 2, 9].

This work was conducted to evaluate the reproductive capacity of *C. subinnotatus* through the study of the influence of the quantity of spawning substrate and the number of congeneric females on the reproductive potential of this bruchid species in order to define the conditions that promote optimal oviposition in the female of this weevil species.

2. Materials and Methods

2.1. Insect Rearing

Adult *C. subinnotatus* used in this study come from the Applied Entomology Laboratory strain (LEA) of the Faculty of Science of the University of Lome. This strain was made from the offspring of adults collected in nature. The rearing was done by the method of Dick and Credland [17] in Plexiglas boxes in the laboratory at 30 ± 2 °C; 72 ± 2% RH and 12 h: 12 h LD. The temperature and relative humidity were recorded using a handheld thermohygrometer "Pen-Type". The photoperiod is maintained using a programmable timer. Adults of the sexually active form of *C. subinnotatus* aged 0 to 24 h from the culture were used to estimate reproductive potential.

Seeds of the local variety of Bambara groundnut characterized by a cream ivory integument and black hilum were used for *C. subinnotatus* rearing in different experiments. They were bought in markets in the city of Lome and stored in a freezer at 0 °C for at least two weeks to remove any previous infestation. They were subsequently dried at room temperature.

2.2 Study of Some Biological Parameters of C. subinnotatus

2.2.1 Determination of Reproductive Parameters of *C. subinnotatus*

Fifty couples of *C. subinnotatus* were formed. Each couple was placed in a petri dish with 10 healthy Bambara groundnut seeds for 24 h. Ten (10) seeds were renewed every day until the death of the couple. Fifty couples were monitored simultaneously. The seeds were collected daily and arranged per couple and per day of spawning then kept for five (5) days. They were subsequently observed under a binocular magnifying glass to count the eggs laid per female per day and the number of embryonated eggs. Egg fertility rate (ratio of the number of embryonated eggs to the total number of eggs laid by the females) was then determined. The lifespan of each sex was determined.

All seeds with eggs laid by all 50 females were pooled and divided into 2 groups (A and B). Each batch was monitored until adult emergence to determine the insect development time.

Adult males and females emerging from batch A were recovered daily and put on healthy Bambara groundnut seeds for two (2) days to stimulate reproduction. They were then dissected under a binocular magnifying glass to examine the level of ovariole development in females and the testes and accessory glands in males. Adults from batch B were recovered at emergence and received no Bambara groundnut seeds before dissection under a binocular magnifying glass for the same period to examine the status of the reproductive system as done before. Both experiments were conducted to assess the influence of the presence or absence of Bambara groundnut seeds on the reproductive status of female *C. subinnotatus*. These dissections were also used to link adult morphotypes to their physiotypes.

At the end of the experiments, the following parameters were also determined:

- the survival rate (ratio of the total number of larvae to the total number of individuals emerged at the end of post-embryonic development);
- the emergence rate (ratio of total number emerging to the total number of eggs);
- sex ratio (ratio of the total number of emerged females to the total number of emerged males).

2.2.2 Duration of Development of Different Pre-imaginal Stages of *C. subinnotatus*

One hundred (100) of *C. subinnotatus* couples were set up to lay eggs in plexiglass boxes each containing Bambara groundnut seeds, for twelve (12) h. Five (5) boxes were thus formed in order to have a sufficient quantity of egg-carrying seeds for the remainder of the experiment. Seeds with 1 or 2 eggs were sorted and divided into 30 batches of 50 seeds in order to monitor the embryonic and post-embryonic development of the weevil under laboratory conditions. Each batch of 50 seeds was introduced into a petri dish for monitoring. The eggs were observed until the formation of the hatched larvae allowing to determine the duration of embryonic development and fertility rate. Different batches of 50 seeds in 30 petri dishes were dissected sequentially every 24 h to determine larval development stage, and the average duration of post-embryonic development.

2.2.3 Determination of *C. subinnotatus* Optimum Oviposition Conditions

The reproductive potential of the sexually active form of *C. subinnotatus* was evaluated by varying, in a first experiment, the number of females in the presence of a fixed mass of seeds and in a second experiment, the seed mass in the presence of a fixed number of females. In both experiments, the proportions of the different morphotypes and their appearance period were determined.

2.2.3.1 Influence of Variation in the Number of Congeneric Females on Oviposition of C. subinnotatus

Five (5) boxes, each containing 100 g of Bambara groundnut seeds received respectively 10, 20, 30, 40 or 50 couples of *C. subinnotatus*. The couples were removed from the boxes after the death of the females. All seeds in each box were observed under a binocular magnifying glass and the eggs laid were counted to determine the average number of eggs laid per female. The seeds were then monitored until emergence of the adult.

2.2.3.2 Influence of Seed Quantity on the Reproductive Activity of C. subinnotatus

In each of four (4) rearing boxes containing respectively 100, 200, 300 and 400 g of Bambara groundnut seeds, 20 couples of *C. subinnotatus* were introduced and left until they died. On the death of the females, the couples were removed from the boxes and all the seeds in the 100 grams of Bambara groundnut seed box were observed under a magnifying glass to count the eggs laid. In the other three seed batches (200, 300 and 400 g), after homogenizing the whole batch, a sample of 100 g of seeds was randomly taken and observed under a magnifying glass to count the eggs laid. The average number of eggs laid per female was then determined for each batch by extrapolation. The seeds are then monitored as before until adult emergence.

2.3 Statistical Analysis

The various tests were repeated four times. The results were expressed as mean ± SD. Means were compared by the analysis of variance (ANOVA). Means were discriminated by Newman Keuls test at 5% level using the STATISTICA software, version 5.1 (1998).

3. Results

3.1 Biological Parameters of C. subinnotatus Adults

3.1.1 Male and Female Adult Lifespan

Male mean longevity is 15.14 ± 2.4 days and that of the female is 11.36 ± 1.85 days. There is a statistically significant difference between the longevity of both sexes ($F = 77.185$; $df = 1$; $P = 0.0000$). The males live longer than females (Fig. 1).

However, survival of males and females is almost the same during the seven (7) days following their

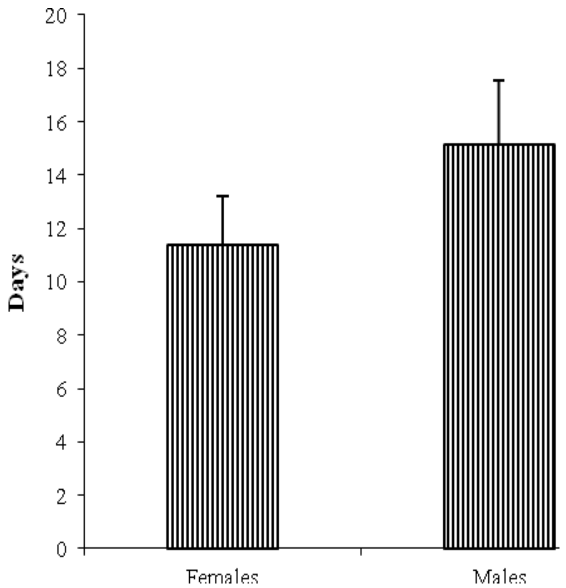

Fig. 1 Mean lifespan (X ± SD) of *C. subinnotatus* males and females.
(F = 77.185; df = 1; *P* = 0.0000)

emergence from seeds (Fig. 2). It was from the 8th day after emergences that the mortality rate in females is higher than in males. The longest lifespan observed in our study conditions is 18 days for females and 22 days for males (Fig. 2).

3.1.2 Fecundity

Daily monitoring of female *C. subinnotatus* in the presence of the same number of Bambara groundnut seeds shows that they lay on the 1st day. The number of eggs increases from the 2nd day and spreads over 11 days with 80.83% of the eggs deposited in 6 days. Two spawning peaks are obtained, one on day 3 and the other on day 5. The highest peak is observed on the 3rd day with an average of 20.72 ± 6.08 eggs (Fig. 3). The mean number of eggs laid per female per day decreases after the second peak until the 11th day after which no more egg is laid until the death of all females. The total number of eggs laid by the female during her lifetime ranges from 35 to 165 with an average of 121.34 ± 27.62.

When the number of *C. subinnotatus* females is varied in the presence of a fixed number of Bambara groundnut seeds, the mean number of eggs laid per female varies from 63.78 ± 8.01 in the presence of 40 congeneric females to 82.65 ± 7.83 eggs in the presence of 20 females (Fig. 4). However, there are no statistically significant differences between the mean numbers of eggs laid (F = 0.796, df = 4, *P* = 0.554).

When the number of seeds is varied in the presence of a fixed number of females, the mean fecundity of *C. subinnotatus* is between 92.38 ± 8.62 eggs in the presence of 100 g of seeds and 135.13 ± 11.11 eggs in the presence of 400 g of seeds (Fig. 5). Statistical analysis performed at the 5% threshold, however, reveals that the mean number of eggs laid per female does not vary significantly when Bambara groundnut seed quantity is considered.

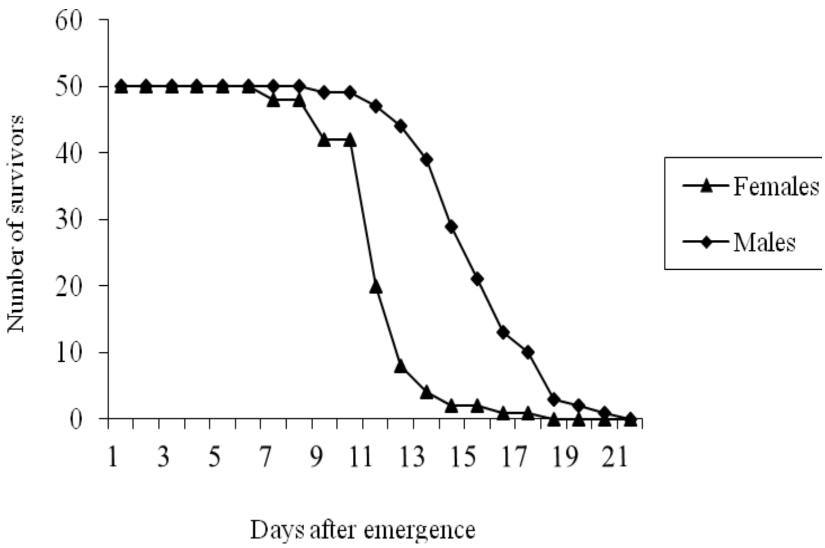

Fig. 2 Survival of *C. subinnotatus* in the presence of spawning substrate.

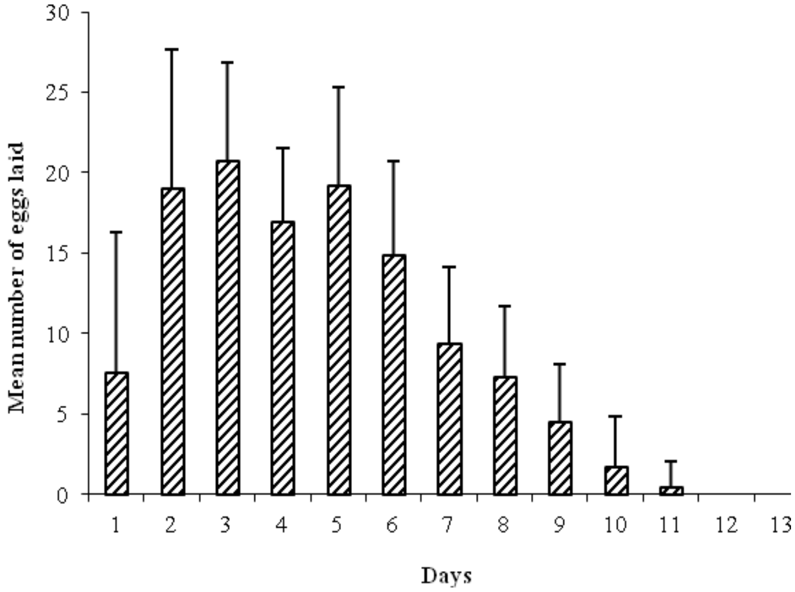

Fig. 3 Mean number (X ± SD) of eggs laid per day by reproductive females of *C. subinnotatus* (n = 50 females).

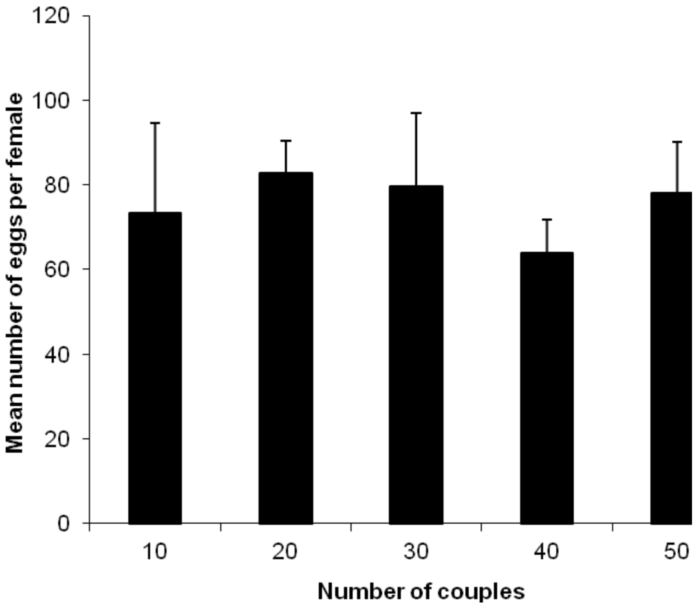

Fig. 4 Variation in *C. subinnotatus* female fecundity (X ± SD) depending on the number of couples (F = 0.796; df = 4, *P* = 0.554).

3.1.3 Embryonic and Post-embryonic Development

The average fertility rate of eggs was 96.19 ± 1.45%, the average rate of larval survival is estimated at 97.72 ± 1.08% and the average rate of adult emergence is 94.01 ± 2.14% (Table 1).

Embryogenesis lasts on average 3.11 ± 0.32 days. Four larval stages (L_1, L_2, L_3 and L_4) were identified with respective mean development duration of 2.78 ± 1.98 days, 8.14 ± 0.87 days, 11.47 ± 1.29 days, 15.14 ± 2.06 days. Pupation lasts on average 19.71 ± 1.71 days (Table 2).

The sex ratio is slightly in favor of males and averages 0.845 ± 0.08.

3.1.4 Status of the Reproductive Systems of Males and Females

Examination of the reproductive organs of *C. subinnotatus* adults revealed that the offspring of this beetle species consists of two types of individuals that

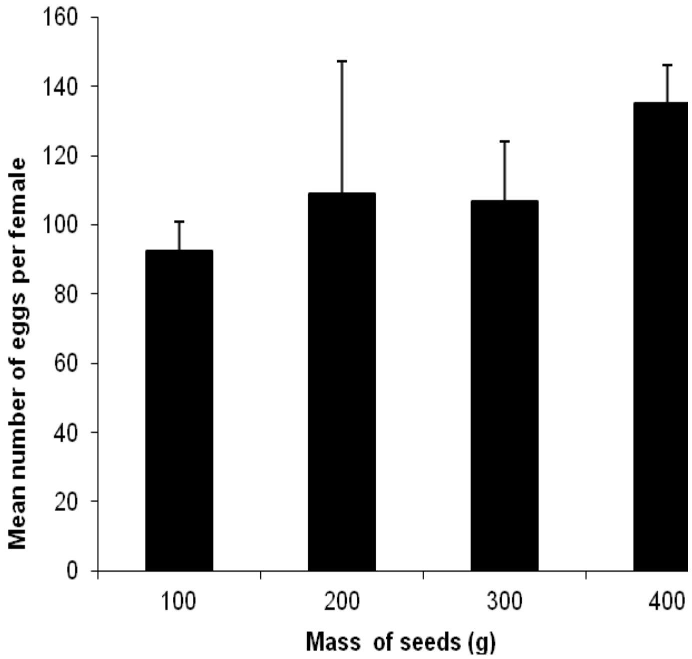

Fig. 5 Variation in *C. subinnotatus* female fecondity (X ± SD) depending on seed availability (F = 1.083; df = 3, *P* = 0.410).

differ in the status of their reproductive system. We distinguish two types of adults. Some are qualified as "Reproductive type (RT)" and the others, called "Non Reproductive Types (NRT)". In the RT females, the reproductive system is formed, at the emergence, of a pair of ovaries, each comprising six well developed ovarioles. Ovarioles contain all the ovarian follicles containing very visible oocytes whose size and maturity increase as we descend to the base of the vitellarium. In males, the reproductive system consists of two pairs of well developed testes and four pairs of accessory glands, two middle pairs and two side pairs, all filled with a whitish secretion. The NRT adults have reduced reproductive system. In females of this physiotype, the reproductive tract consists of a pair of ovaries with ovarioles which are reduced to the germarium. In males of this type, the reproductive system is very small with poorly developed testes and the accessory glands are very small, transparent and devoid of secretions.

In batch A (adults that stayed with the Bambara groundnut seeds), the reproductive type adults represent 94.34% of the offspring while non-reproductive type adults represent 5.54% (Table 3). Similarly, in batch B (adults that were not in contact with Bambara groundnut seeds), the RT adults represent 87.28% of the offspring while NRT adults represent only 12.71%.

The mean development period varies depending on the physiotype considered in adults of *C. subinnotatus* emerging from both batches (Table 3). In NRT, the mean development period is respectively 37.92 ± 3.92 days and 37.25 ± 5.54 days for the males, and 37.19 ± 3.87 days and 38.11 ± 6.02 days for females on the other hand. This development duration is not significantly different in both sexes regardless of the batch considered (F = 0.049, df = 1, p = 0.845). For RT, the mean development period is respectively 28.35 ± 3.36 and 28.55 ± 4.25 days for males; and for females, it is 28.15 ± 3.26 and 28.45 ± 4.1 days, respectively (Table 3). This period is also identical in males and females of both batches (F = 5; df = 1; *P* = 0.155). The average duration of development is however longer in NRT (37.58 ± 2.47) than in RT (28.25 ± 2.14) in group A (F = 712.966, df = 1, *P* = 0.001) as in group B where this period is 37.68 ± 2.61 for NRT and 28.50 ± 2.07 for RT (F = 449.693, df = 1, *P* = 0.002) respectively.

Table 1 Reproductive parameters of *C. subinnotatus* (X ± SD) (n = 50).

Number of embryonated eggs/♀	Mean Fertility rate (%)	Mean number of emerged adults/♀	Mean rate of emergence (%)	Mean larval survival rate (%)
116.96 ± 27.54	96.19 ± 1.45	114.5 ± 27.38	94.01 ± 2.14	97.72 ± 1.08

Table 2 Development time of *C. subinnotatus* premature stages (X ± SD).

Premature stages	Embryogenesis	Instars				Nymph
		L_1	L_2	L_3	L_4	
Duration (days)	3.11 ± 0.32	2.78 ± 1.98	8.14 ± 0.87	11.47 ± 1.29	15.14 ± 2.06	19.71 ± 1.71

Table 3 Mean Development time of *C. subinnotatus* adults' different types and the sex ratio of this species in two different batches.

Batch	Number emerging		Sex ratio	Reproductive status (%)				Mean Development time (X ± SD) (days)			
				DRS		RRS		RT		NRT	
	♂	♀		♂	♀	♂	♀	♂	♀	♂	♀
A	2,231	1,989	0.899	48.85	45.49	3.95	1.59	28.35 ± 3.36	28.15 ± 3.26	37.92 ± 3.92	37.19 ± 3.87
(Presence of Bambara groundnut)				T_1 = 94.34		T_2 = 5.54		X = 28.25 ± 2.14 Y= 37.58 ± 2.47 F = 712.966; df = 1 ; P = 0.001			
B	1,357	1,074	0.790	45.29	41.99	10.53	2.18	28.55 ± 4.25	28.45 ± 4.1	37.25 ± 5.54	38.11 ± 6.02
(Absence of Bambara groundnut)				T'_1 = 87.28		T'_2 = 12.71		X' = 28.50 ± 2.07 Y'= 37.68 ± 2.61 F = 449.693 ; df = 1 ; P = 0.002			

DRS: Developed Reproductive System; RRS: Reduced Reproductive System.
RT: Reproductive Type; NRT: Non Reproductive Type;
X and X': Mean Duration of development of RT in both sexes.
Y and Y': Mean Duration of development of NRT in both sexes.
T1 and T'1: Total percentage of adults in developed reproductive system.
T2 and T'2: Total percentage of adults in reduced reproductive system.

Figure 6 shows the evolution of the number of adults emerging from Bambara groundnut seeds within a generation. Monitoring the emergence of adults in connection with their reproductive status shows that the offspring consists only of reproductive adults between the first and eighth day of emergence (Fig. 6). From the 9th day, appear Non Reproductive adults only (100%) emerging in the last 5 days (Fig. 6).

Taking as indicator, the status of the reproductive system, it is noted that the offspring of *C. subinnotatus* is heterogeneous. *C. subinnotatus* therefore presents an imaginal sexual dimorphism. Observation of the two physiological types indicates indeed that they differ in the two sexes by the coloration of the wing covers and pygidium.

- In "reproductive" type, males have elytral and abdominal segments of light brown color; the light brown pygidium also has a white median line, pubescent, while females have each of their wings colored in black, a whitish ring and the pygidium is also black with a white median line.
- In Non Reproductive individuals, males appear dark or dull brown at their elytra, while females appear gray because the pubescence of the elytra is more diffuse. In these individuals, there is no white median line on the pygidium.

4. Discussion

The study of some biological parameters of *C. subinnotatus* to characterize this beetle species which develops preferentially on Bambara groundnut has permitted to note that oviposition is optimal when spawning substrate is available. Indeed, when spawning substrate (10 seeds per day) is renewed every day, the mean number of eggs laid by the female is similar to that obtained when the quantity of spawning substrate

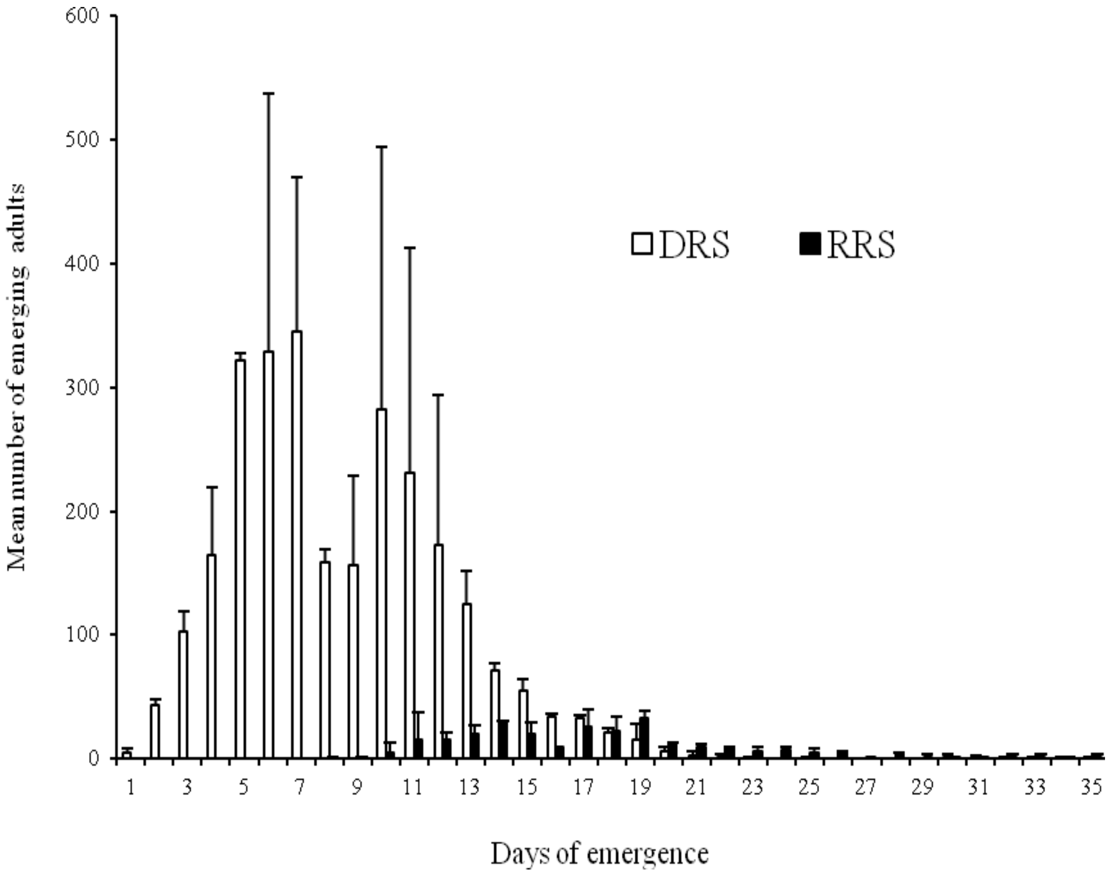

Fig. 6 Distribution of *C. subinnotatus* emerging adults depending on their reproductive system status within a generation.
DRS: Developed Reproductive System RRS: Reduced Reproductive System.

is varied for 20 females. This mean fecundity obtained in the female of *C. subinnonatus* in our laboratory conditions is similar to that obtained by Mbata [18] and Appleby and Credland [9]. But, it is greater than that obtained by Ketoh et al. [1]. The low fecundity obtained by the latter author is probably due to the small quantity of seeds offered to females. The variability in *C. subinnotatus* fecundity compared to data in the literature is also probably due to climatic conditions [19] and photoperiod that influence female oviposition and larval development with optimum spawning and development observed at 30 °C. It is also linked to the strain used as observed in *C. maculatus* [17, 20]. The influence of climatic conditions on the fecundity of weevils was also reported in the sympatric species *C. maculatus* [19, 21]. Under our experimental conditions, the female of *C. subinnotatus* lays her eggs from the 1st day of its emergence; indicating that there is no preoviposition period in the latter. She lays most of its eggs (80.83%) in 6 days with a peak on the 3rd day during an average lifespan of 11.36 ± 1.85 days. After this period, no more egg is laid until the death of all the females. This indicates that in the female of *C. subinnotatus*, there appears to be a post-oviposition period of 1 to 7 days. Ketoh et al. [1] and Mbata [18] observed that the female of this beetle species laid 75% of its eggs in 8 days and 4 days respectively. Similarly, the egg-laying peak obtained differs from that obtained by Appleby and Credland [9] and Ketoh et al. [1] who observed a peak respectively on the 2nd and 4th days with a spreading of spawns over 15 days. These differences from previous work would be related to our experimental conditions.

Spawning activity causes a significant reduction of the lifespan of the female compared to the male. Indeed, in our conditions, males live longer than females; this is generally observed in insects because the female expends energy using reserves accumulated in the form of fat for the maturation of oocytes during vitellogenesis. According to Tatar et al. [22], coupling significantly affects the life of a female. According to Ketoh [23], there exists within populations of *C. maculatus*, individuals that live longer than others, and the physiological significance of this biological variation is very important because adults that live longer are potentially resistant the control methods used in the fight against pests in stocks.

Fertility, larval survival and emergence rates are very high (95% each) in *C. subinnotatus* in our conditions. These high rates are favored by the climatic conditions of larval development and availability of spawning substrate; because, when Bambara groundnut seeds are renewed every day, females of *C. subinnotatus* lay one to two eggs per seed thus limiting intraspecific competition among larvae. Indeed, it has been proven in *C. maculatus* [21] and in *B. atrolineatus* [24] that if the intra-grain larval density increases, the rate of larval mortality also increases resulting in a decrease in the rate of emergence.

In *C. subinnotatus*, sex ratio (0.845 ± 0.08) is slightly in favor of males in our conditions while Ketoh [25] obtained a sex ratio close to 1 (0.99 ± 0.307).

In *C. subinnotatus*, an imaginal polymorphism previously reported in *C. maculatus* [26, 27], *Callosobruchus chinensis* L. [28] and in *Zabrotes subfasciatus* Boh. [29] was noted. The monitoring of emergence showed that the population of *C. subinnotatus* consists of two sub-populations whose morphotypes are distinguished by their morphology and the physiology of their reproductive system at emergence. Biologically, the presence of a reduced reproductive system in *C. subinnotatus* individuals emerging belatedly, permit to qualify them as Non Reproductive and, those that emerge first, with a developed reproductive system, are qualified as Reproductive. This heterogeneity observed in the offspring of *C. subinnotatus* even in the conditions of availability of spawning substrate, was also reported by Ketoh et al. [1] and Appleby and Credland [9]. According to these authors, both *C. subinnotatus* adults' physiotypes are distinct by their morph and their reproductive activity. This difference in reproductive activity related to the difference in the size of the reproductive organs is also manifested according to Ketoh et al. [1] by the pigmentation of the integument thereby determining the morphs described by Ketoh [25] and Appleby and Credland [9]. Such heterogeneity of the population of *C. subinnotatus* was also reported by Haines [30] who distinguished a sexually inactive form of the species which he associated with the abnormal form observed in *C. maculatus*.

The emergence of the non-reproductive form in *C. subinnotatus* is systematically observed in every generation unlike *C. maculatus* in which the appearance of this form is a response to worsening conditions of development [27]. In *B. atrolineatus*, the appearance of diapausing individuals is naturally programmed from the 2nd generation in storage conditions.

The observation of the anatomical structure of the reproductive system of *C. subinnotatus* individuals of non reproductive morph suggests that they may exist in this species of beetle, a reproductive diapause as described in various species [14, 31-33]. However, further studies in histology and biochemistry like those done by several authors [33-35] on weevils are needed to determine the nature of this physiological behavior. But the characterization of the nature of the proteins involved is needed to confirm or refute an imaginal diapause in *C. subinnotatus*.

The multivoltinisme observed in these two sympatric species (*C. subinnotatus* and *C. maculatus*) is a systematic biological adaptation when development conditions are favorable. In other weevil species living in sympatric with *C. maculatus* on cowpeas, some authors have reported biological aptitudes that are observed when developing conditions become unfavorable. These biological adaptations are reproductive quiescence.

During the development of *C. subinnotatus* there also appear two distinct forms of individuals based on morphological, physiological and behavioral criteria [1, 2, 9, 36] as has been observed in the sympatric *C. maculatus* [27] and in two other species of Bruchinae beetle *C. chinensis* [28] and *Z. subfasciatus* [29].

5. Conclusions

The study of some biological parameters of *C. subinnotatus*, under laboratory conditions revealed that mean fecundity and fertility rates are very high. Monitoring of adult emergence and dissection showed that the population of *C. subinnotatus* consists of two physiotypes: Reproductive adults (whose females lay eggs and have a normal reproductive system) that appear on the first day of emergence and non reproductive adults (with a reduced reproductive system) that appear from the 9th day of emergence. The systematic appearance of the two physiotypes in each generation regardless of substrate availability is probably related to genetic programming, it would be necessary to determine the condition of inductions and type of physiological adaptation.

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