Reproductive Parameters of *Diastocera trifasciata* (Fabricius, 1775) (Coleoptera: Cerambycidae: Lamiinae), Cashew Branches Girdler in Côte d'Ivoire, Under Semi-Natural Conditions

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DOI: 10.29322/IJSRP.8.9.2018.p8193
http://dx.doi.org/10.29322/IJSRP.8.9.2018.p8193

Abstract- In Côte d'Ivoire, the cashew nut still known as "brown gold" represents a hope for the populations of the producing zones and contributes to the international influence of the country. However, it is facing attacks from multiple insects including *Diastocera trifasciata* formerly *Analeptes trifasciata*. The lack of data on the reproductive parameters of the species motivated this study. The objective of this study was to provide knowledge related on the reproductive parameters of *Diastocera trifasciata*. The study was conducted under semi-natural conditions at a temperature of 26.83 ± 2.36 ° C and a relative humidity of 75.37 ± 15.09%. Forty-five pairs of adults were reared in insectarium to study the biology of the species. Data collected were analyzed with Statistica version 7.1 software. The study revealed a pre-mating period of 123.33 ± 8.45 days (mean ± SE) and pre-oviposition period 1.83 ± 0.79 days. Oviposition period was 64.26 ± 8.88 days with lifetime fecundity of 174.3 ± 18.77 eggs and fertility rate 82 ± 9.38%. In females, body length was 39.71 ± 2.74 mm and longevity was 196.97 ± 6.43 days. There is a positive correlation between longevity, lifetime fecundity, female size and fertility. In males, body length was 42.23 ± 3.03 mm and longevity 184.56 ± 7.47 days. Sex ratio was 0.73 in favor of females. Incubation period of eggs was 11.81 ± 1.22 days. Development duration are 198.2 ± 16.22 days for the larval stage and 18.69 ± 0.63 days for pupal stage. With a total life cycle 211.81 ± 18.87 days, *D. trifasciata* is univoltine species. The reproductive parameters of *D. trifasciata* were obtained. Knowledge of these parameters could be used to support the development of effective control strategies.

Index Terms- *Diastocera trifasciata*, branch girdler, reproductive cycle, longevity, lifetime fecundity, fertility.

I. INTRODUCTION

Diastocera trifasciata (Fabricius, 1775), formerly *Analeptes trifasciata*, is a wood-boring insect that attacks cashew trees (*Anacardium occidentale*, Anacardiaceae) in several areas of West Africa [1, 2]. Its damage has been observed and described for the first in 1964 in the forest station Kokondekro to Bamoro and many smallholdings around Bouake in central Côte d'Ivoire [3]. Since the work of these authors, no other study has made a case of the biology and ecology of this species to determine effective means of control. In 2014, *D. trifasciata* has been identified as one of the main four major insect pests of cashew in the country [2]. This insect girdle and cuts a large number of branches and trunks causing up to 55% cashew nut yield loss [4]. It represents a serious threat on the farmers' yields and the country's economy [5].

The control technique used until now by producers in Côte d'Ivoire is to capture manually adults during periods of occurrence and killing them with machetes or grilling. This practice does not make reach all individuals and proves to be very inefficient with regard to the spread of growing outbreaks. In addition, this practice is not apply in the duration and the periods of application are ill-chosen.

Since the work investigated by [6] as well as those conducted by [3] which focused on the morphological description of this pest and this damage, no recent study has made any reference to the biological parameters of this species. The knowledge of biology and especially the control of reproduction is essential for the development of an effective control strategy.

The objective of this work is to know the reproductive parameters of *D. trifasciata*. Specifically, it is to determine the egg incubation period, the duration of the development cycle, female lifetime fecundity, fertility, duration of sexual maturation of adult, emergence rate, sex ratio and adult longevity.

II. Materials and Methods

A. Study site

The study was conducted in the locality of Brobo located 20 km from Bouake on the axis Bouaké-M'Bahiakro, in the center of Côte d'Ivoire (latitude: 07°38.18N, longitude: 004°49.29W). The vegetation is herbaceous savannah, shrubby or wooded with forest islets mainly in the lowlands [7]. The study period extended from October 2015 to September 2017 in semi-natural conditions.

B. Material

B1. Biological material

The plant material used was branches and leaves of cashew obtained from the orchards of Brobo. These organs serve both as food for the individuals and as a female's egg-laying support. The adult used come from the branches of cashew trees containing eggs of *D. trifasciata* kept in rearing in the insectarium.

B2. Breeding device

Thirty wooden cages in the form of a parallelepiped of dimensions 80 cm x 60 cm x 60 cm were used for the rearing of adults. The four (04) lateral sides are in a metal screen of fine mesh of 2 mm for ventilation of cages. The base and the roof are plywood. One of the four lateral side serves as the door for cleaning droppings and dead individuals. The plywood of the roof is free to facilitate the renewal of the branches inside the cage. The temperature inside the insectarium was 26.83 ± 2.36 ° C (mean ± SE) ranged from 22.6 - 32 ° C. The relative humidity was 75.37 ± 15.09% (mean ± SE) (range 40.94 – 83). These data were recorded by a digital thermohygrometer MEDISANA. The shots were taken with a NIKON B700 digital camera. The measure of every instar of the insect were recorded with the help of a vernier slide caliper.

C. Study of reproductive parameters

C1. Adult pre-mating, mating and egg-laying

Forty-five pairs of adults (45 females and 45 males) newly emerged from the branches collected in different fields were observed in fifteen (15) cages at the rate of three pairs per cage. The body length of each adult was measured from the top of the head to the apex of the elytra using a vernier slide caliper. The date of emergence is noted. Individuals emerged from the same branch and from the same orchard were never put in the same cages to avoid consanguinity [8]. In each cage, small fresh branches of 10 to 15 mm in diameter and dried leaves of cashew trees are provided to insects as food. These branches are renewed every two days until observation of the first mating. The date of the first mating is noted. **Pre-mating period,** which is time elapsed between the adults' date of emergence and that of the first mating of each pair was recorded.

For mating and egg-laying experiments, as soon as the first mating are observed, the pair is isolated in another cage. In each cage, a branch of larger size whose circumference is between 15 and 25 cm is made available to them. This branch serves both food for the pair and female support for laying. Daily, branches are removed from cages and inspected to note the presence or not of laying. Oviposition pit is marked by the presence of gelatinous substance at the entrance of the holes [8, 9]. Egg counting is based on the presence of this substance. In case of death of the male before the female, it is replaced by another and observations continue. The dates and times of mating and laying are observed and noted until the death of the female. The date of female death is noted.

The **Preoviposition period** (elapsed time between first mating and first oviposition) the **oviposition period** which is elapsed time between first and last oviposition were determined.

The number of eggs deposited per female in the branches was recorded daily. The experiment continued until the death of the female. **Lifetime fecundity** (total number of eggs laid by a female during its life) was determined.

After the laying, the oviposition pit are inspected from the outside every day until the observation of wood dust coming out of these holes. Wood dust is powder from wood rejected by the larva during its feeding activity in the branch. As soon as detection of this wood dust, the pit is then opened to observe first instar larvae. Number of hatched and unhatched eggs was counted and **egg fertility rate** (percentage of the number of eggs hatched to the total number of eggs laid) was determined. **The longevity** (elapsed time between date of emergence and the date of death) was determined**.**

C2. Emergence rate and sex ratio

The emergence rate was studied on 76 fresh branches of average length 149.31 ± 53.34 cm collected in different orchards. These branches contain eggs newly laid. The number of eggs laid and laying date are recorded on each branch with permanent marker. They are then brought back to an insectarium to be stored on tables. They are followed until the emergence of adults. The number of emerged adults was noted. **Emergence rate** and **sex ratio** (number of males emerged on number of females emerged) were calculated.

C3. Incubation period, larval and pupal development of D. trifasciata

During attacks, 100 freshly girdled branches containing eggs were collected from different orchard of Brobo. These selected branches are those cut the day before the visits. They are recognized by the fresh state of the substance covering the oviposition pit. They were divided into 50 lots of 02 branches. The eggs are observable by superficial opening of the oviposition pit with a knife. On each branch, laying date and the number of notch containing the egg are recorded in the indelible marker. According to [10], the time between egg-laying and hatching of Cerambycidae eggs in general ranges from 3 - 7 days but can be up to 25 days. A batch of two (02) branches is dissected every three (03) days during the first month following the laying date to observe the evolution of the eggs. The number of hatched eggs was noted at each observation and the date of hatching was also on each branch. **Incubation period** (time elapsed between egg laying and hatching) has been recorded.

The determination of larval and pupal duration was made from the 50 lots of branches formed. After hatching, the branches are dissected every seven (07) days for seven (07) months to see the evolution of the larvae until observation of pupae and adult emergence. After dissection of the branches, the shape, the dimensions and the color of the observed stages are described. The following parameters were recorded: **duration of larval development** (elapsed time between the date of hatching of the eggs of the moulting pupal), **duration of pupation** (time that covers the process that allows the passage of larvae of last stage to the pupa), **duration of pupal development** (elapsed time between the date of the pupal moulting and that of obtaining the adult), **duration of development cycle (**elapsed time between the date of egg laying and obtaining of the adult stage).

D. Data Analysis

Data processing was done using Statistica software version 7.1 StatSoft [22]. A one-way analysis of variance (ANOVA) followed by the Newman and Keuls test at the 5% threshold allowed the averages to be compared. Linear regression analyses (Pearson's correlation) was used to test the correlation between some reproductive variables in females (lifetime fecundity, fertility, longevity, oviposition period, and female length) and in males (pre-mating period, longevity and body length).

III. RESULTS

A. Pre-mating and pre-oviposition periods of adults

The individuals made the first mating between the 104th and 138th days after emergence, pre-mating period of 123.33 ± 8.45 days (mean ± SE). Mating takes place during the day. The first oviposition was observed between the 1st and 4th day after mating, pre-oviposition period of 1.83 ± 0.79 days (mean ± SE). Pre-mating and pre-oviposition periods are not correlated with any of the biological parameters studied in the female in this study (Table 1).

Table 1: Linear regression analyses (Pearson's R correlation matrix) between the different parameters of the female of *D. trifasciata* in semi-natural conditions (temperature 26.83 ± 2.36 °C and relative humidity 75.37 ± 15.09 %)

Parameters	Pre-mating period (days)	Longevity (days)	Oviposition period (days)	Pre-oviposition period (days)	Lifetime fecundity	Body length (mm)
Pre-mating period (days)	**1**					
Longevity (days)	0.356 ns	**1**				
Oviposition period (days)	-0.058 ns	0.315 ns	**1**			
Pre-oviposition period (days)	-0.058 ns	0.114 ns	-0.087 ns	**1**		
Lifetime fecundity	0.291 ns	**0.958***	0.265 ns	0.181 ns	**1**	
Body length (mm)	0.270 ns	**0.813***	0.155 ns	0.259 ns	**0.875***	**1**
Fertility	0.076 ns	**0.477****	**0.608***	-0.035 ns	**0.518****	**0.424*****

Significance of P-values: ns: non-significant: $^{*}P < 0.0001$; $^{**}P < 0.01$; $^{***}P < 0.05$

B. Oviposition period, lifetime fecundity, fertility rate and longevity of adults

Egg-laying begin at dusk and end at dawn. Oviposition period was 64.26 ± 8.88 days (mean ± SE). Female lifetime fecundity was 174.3 ± 18.77 eggs, ranged from 126 - 200 eggs. On 174.3 ± 18.77 eggs laid per female, 143.46 ± 22.92 hatched, fertility rate was 82 ± 9.38% (mean ± SE). Males of *D. trifasciata* emerge firstly but a longevity was 184.56 ± 7.47 days (range 168 – 201). In females, longevity was 196.97 ± 6.43 days (range 178 - 208) (Table 2). The linear correlation test shows that there is a positive correlation between female lifetime fecundity, longevity and egg fertility (Figure 1) (Table 1).There are significant difference between the longevity in males and females ($F = 47.43$, ddl = 1, $P < 0.05$).

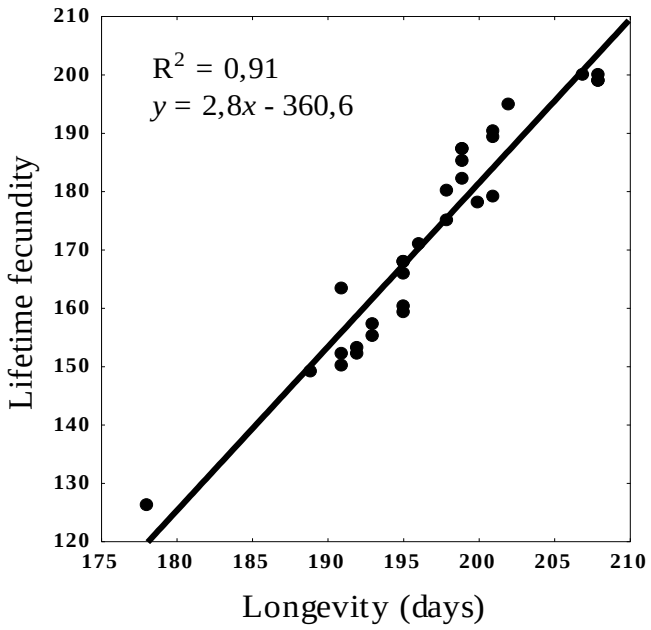

Figure 1: Correlation between female longevity and lifetime fecundity

C. Correlation between adult length, fecundity and longevity

In female, body length was 39.71 ± 2.74 mm (means ± SE), ranged 33.10 - 43.50. The linear correlation test between female length and lifetime fecundity shows that large females lay more eggs ($R = 0.87$, $P < 0.05$) (Figure 2). Female length is also positively correlated with longevity ($R = 0.81$, $P < 0.05$) (Figure 3). There were mating between some females who lost their male partners and newcomers. Females were therefore polyandrous and males were polygynous. In males, body length was 42.23 ± 3.03 mm (range 31.49 - 46.20). Males are larger in size than females. Analysis of variance at the 5% threshold showed that there is a significant difference between the body length in males and females ($F = 11.38$, ddl = 1, $P < 0.05$). The sexual dimorphism is marked by the length of the antennae which is 59.72 ± 4.88 mm in the males against 47.98 ± 6.77 mm in the females (Table 2). Male length correlated neither with longevity ($P > 0.05$, $R^2 = 0.002$) nor with the pre-copulation period ($P > 0.05$, $R^2 = 0.001$).

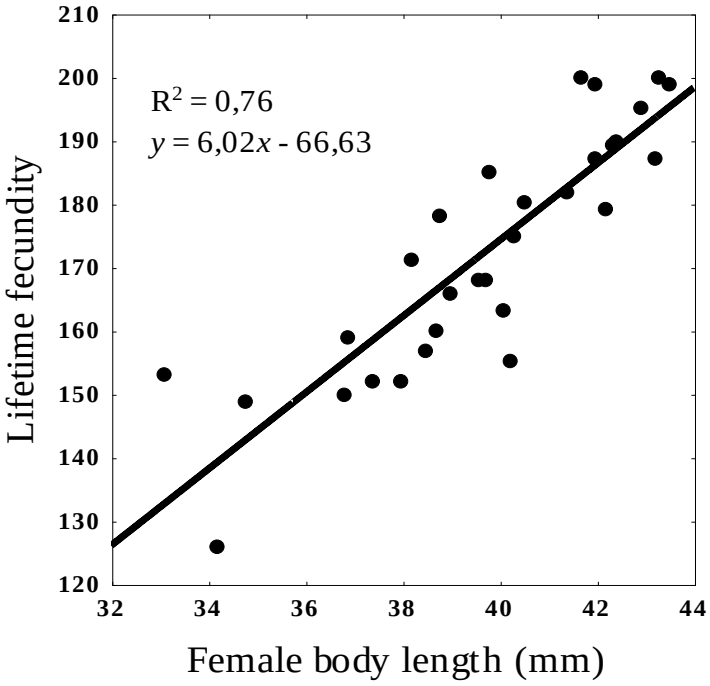

Figure 2: Correlation between female body length and lifetime fecundity

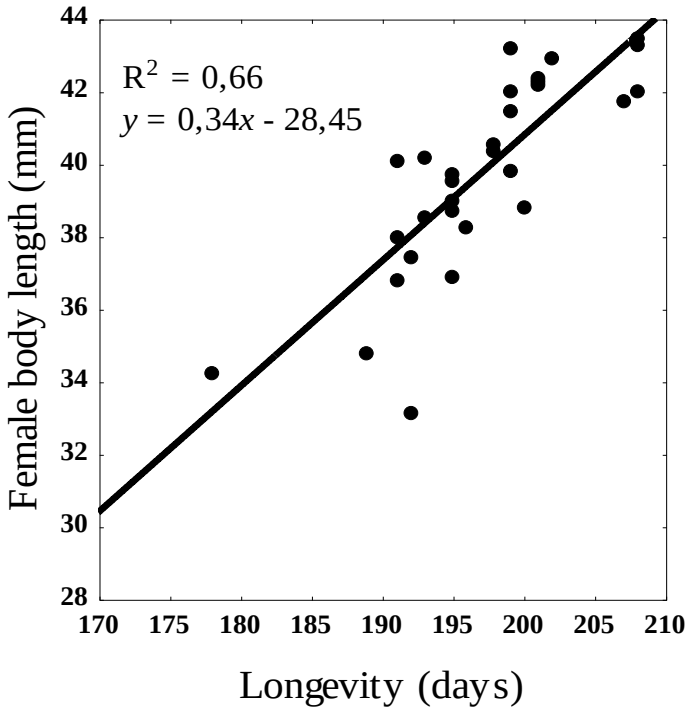

Figure 3: Correlation between longevity and female body length

Table 2: Some morphological characteristics of adults of *D. trifasciata*

Sex	Body length (mm)	Antennae length (mm)	Longevity (days)
Males	42,23 ± 3,03 (31,49 - 46,20	59,72 ± 4,88 (50,8 - 69,95)	184,56 ± 7,47 (168 - 201)
Females	39, 71 ± 2,74 (33,10 - 43,50)	47,98 ± 6,77 (38,18 - 59,41)	196,97 ± 6,43 (178 - 208)

n = 45 in each sex; Mean ± SE (Standard Error of the mean); (minimum – maximum values)

D. Emergence rate and sex ratio of *D. trifasciata*

Out of a total of 2953 eggs monitored, only 247 adult individuals have emerged, an average emergence or rate of 8.36%. The emerged adults are divided into 142 females and 105 males, a sex ratio of 0.73 in favor of females.

E. Egg of *D. trifasciata* and incubation period

The laying were recorded between 18h and 07h in the morning. The eggs are laid in notches made in the bark by the females only. Each notch of eggs contains only one egg disposed parallel to the longitudinal axis of the branch or stem cut (Figure 4a-b). The newly laid of *D. trifasciata* egg is white, oval and similar to a rice grains (Figure 4 b). Eggs length were 5.52 ± 0.23 mm (mean ± SE), ranging from 5.04 - 6.1 mm. The width of the eggs ranged 1.05 - 1.71 mm with a mean of 1.28 ± 0.11 mm. The incubation period of the eggs was 11.81 ± 1.22 days (range 9 – 15).

(a) **(b)**

Figure 4: Laying site and fresh eggs of *D. trifasciata*
(a) Laying notch (red encircled); (b) egg (red encircled) still inlaid in the branch

F. Larval and pupal development of *D. trifasciata*

The first instar larvae of *D. trifasciata* are translucent rosy white color. The body length of larvae (mean ± SE) was 6.39 ± 1.20 mm (range 4.80 - 8.70). The body width was 1.34 ± 0.16 mm (range 1.01 - 1.77). The length of late-instar larvae was 36.45 ± 4.56 mm (range 27.60 - 48.12). The width of late-instar larvae was 7.72 ± 1.2 mm (range 5.8 - 10.60). They are cream-white tinged with yellow and it is located under the bark. The duration of larval development was 198.2 ± 16.22 days (range 169 - 226).

Before the end of their development, the larvae reduce their gallery in the wood to construct a pupal chambers. At the ends of this chamber are piled woody debris undigested that look like sawdust. Pupation and pupal development occurs at the end of the larval development in this pupal chamber. The length of the pupal chamber was 89.26 ± 13.01 mm (range 55.1 – 135). Its width was 24.95 ± 3.36 mm (range 15.7 - 34.9). The pupation duration was (mean ± SE) 9.02 ± 2.13 days.

The pupa is white or black according on whether it is at beginning or end of its development. The head is inflected toward the ventral side and carries the antennae which are elongated dorsally along the body before wrapping in a spiral at the end on the legs. The wing cases are concealed under the folded legs on the ventral side. The length of the pupae was (mean ± SE) 38.64 ± 2.99 mm (range 32.5 - 46.9). The width was 10.66 ± 1.11 mm (range 8.1 - 14.7).

Pupal development occurred between days 16 and 19 after pupation, for an average duration of 18.69 ± 0.63 days. The young imago who comes out, the wings and the whole body were soft. The head, legs and abdomen have a grey and black color at the joints. The bands of the wings have a grey coloration. The average duration of pigmentation is 3.68 ± 1.03 days (mean ± SE).

G. Duration of the development cycle of *D. trifasciata*

The duration of the life cycle was 211.81 ± 18.87 days (range 167 – 240). *D. trifasciata* is therefore a species univoltine. The adult, once the pigmentation is complete, emerges through a circular hole made with his mandibles in the bark covering pupal chamber. The diameter of these exit hole was 17.63 ± 2.43 mm (mean ± SE) (range 11.4 - 28.1) (Figure 5).

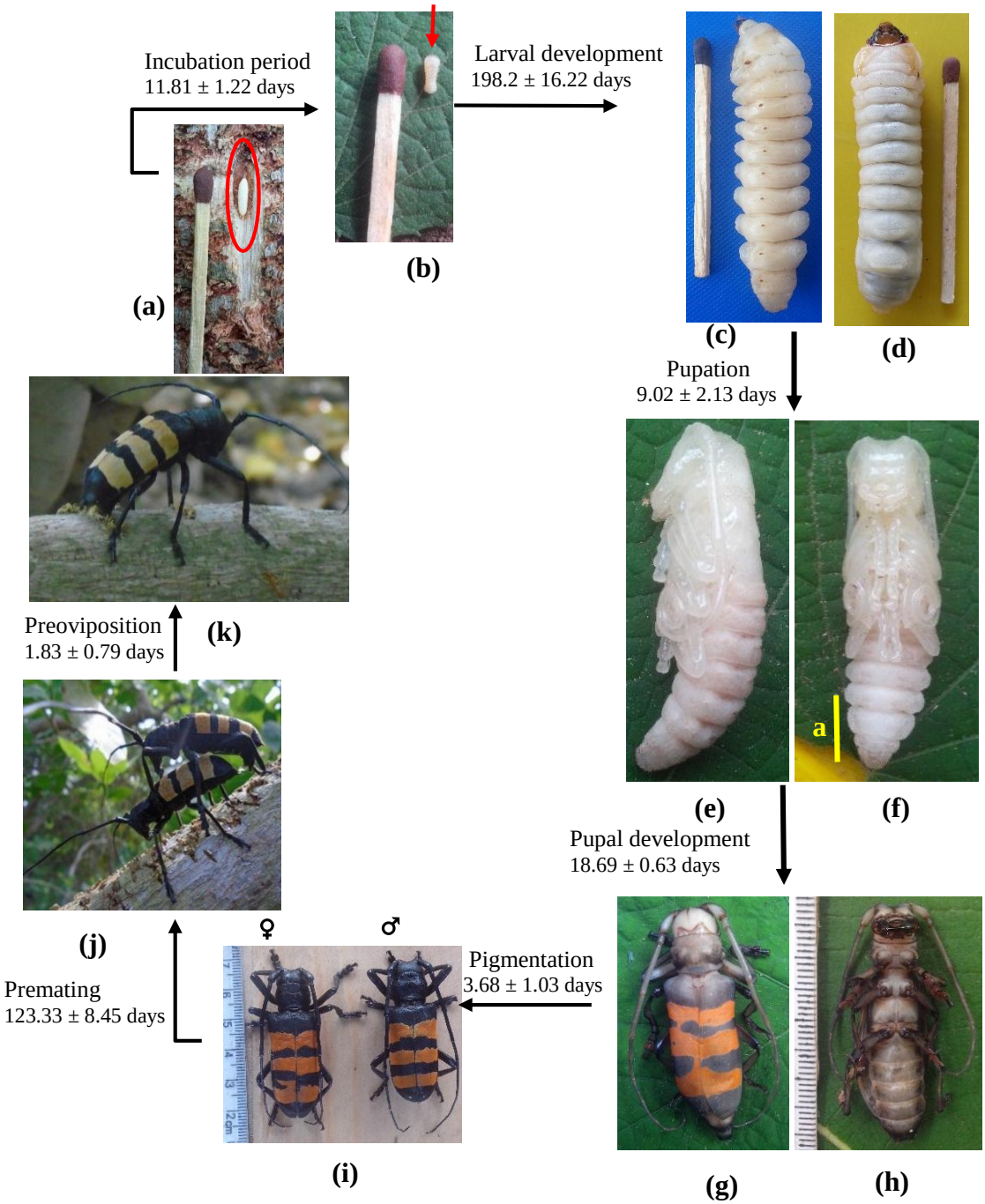

Figure 5. Life cycle of *Diastocera trifasciata* (Coleoptera: Cerambycidae: Lamiinae) under semi-natural conditions (temperature 26.83 ± 2.36 ° C and relative humidity 75.37 ± 15.09%.

(a): egg (red encircled) observed after opening of a notch; (b): first instar larva (red arrowed); (c) : late instar larva lateral view; (d): late instar larva ventral view; (e): pupa lateral view; (f): pupa ventral view; (g): young imago (not emerged from branch) dorsal view; (h): young imago (not emerged from branch) ventral view; (i): adults emerged; (j): mating on cashew branch; (k): female laying in a cashew branch.
Scale a: 0.79 cm

IV. DISCUSSION

In this study, the results revealed that the pre-mating period was 123.33 ± 8.45 days. This period is very high compared to that of Cerambycidae of the Lamiinae subfamily, whose adults feed from a few days to three weeks before becoming sexually mature [8, 12]. Among Cerambycidae *Anoplophora glabripennis* (Motschulsky), adult females become sexually mature about 9 to 17 days after emergence. The pre-mating period of *D. trifasciata* is explained by the fact that Cerambycidae Lamiinae are synovigenic pattern that do not mate immediately after emergence. Adults emerge with immature sexual organs. They must feed on young shoots and dry leaves for a period ranging from several days to weeks before reaching sexual maturity [8, 13]. The duration of this period may also depend on the phenology of the host plant *Anacardium occidentale*. Indeed, the mating period begins when the plant begins its push vegetative pre-flowering and extends until flowering. There would be a synchronization between the reproductive period of *D. trifasciata* and that of the host plant. According to [14], this phenomenon of synchronization of the cycle of insects and their host plant is frequent and determines the quantity and the quality of the resources available for reproduction.

The pre-oviposition period obtained in this study (1.83 ± 0.79 days) is close to that obtained in Cerambycidae *Cerambyx welensii* which is 2.8 ± 0.2 days, investigated by [15].

The oviposition period of *D. trifasciata* recorded was 64 ± 8.88 days. These observations are close to those of Cerambycidae Lamiinae that have an oviposition period of 60 ± 7 days [14]. On the other hand, in the species of the subfamily Cerambycinae, the oviposition period was 41 ± 17 days [15].

Regarding female's lifetime fecundity (174.3 ± 18.77 eggs) in breeding condition is superior to the lifetime fecundity in Cerambycidae Lamiinae which is 155 ± 30 eggs [14]. [16] showed a higher fecundity of 284 ± 27 eggs in the female Cerambycinae *Phoracantha recurva*. The positive correlation between fecundity and female size shows that larger females lay more eggs than smaller females. According to [17], female size is usually a good indicator of potential fecundity.

The phenomena of polyandry and polygyny were observed respectively in females and males of *D. trifasciata* as *in Cerambyx cerdo* [18]. The egg fertility rate recorded in *D. trifasciata* was 82.37 ± 9.38%. This result is close to those of Cerambycidae such as *Anoplophora glabripennis* which is 83 ± 2% [19], *Cerambyx cerdo* 78 ± 1% [17]. On the other hand, other authors have recorded a fertility rate ranging from 90 to 98%, in *Monochamus galloprovincialis* and *Phoracantha semipunctata* [16, 20]. Rates of between 48-66% were in *Anoplophora glabripennis* [21].

Adult males measure 42.23 ± 3.03 mm and live 184.56 ± 7.47 days while females measure 39.71 ± 2.74 mm and live 196.97 ± 6.43 days. This difference in longevity has been reported by various authors. In Cerambycidae, females generally live longer (54 ± 6 days) than males (42 ± 6 days) for a given species [15, 22]. Adults' longevity of *D. trifasciata* could be influenced by the long period of nutrition that precedes reproduction. According to [23], the adult phase of Cerambycidae is entirely devoted to reproduction. Female longevity was positively correlated with lifetime fecundity, egg fertility. Similar results have been obtained in other Cerambycidae [15, 24]. Longevity can also be influenced by body size, although factors such as temperature, diet, and overall health of an individual may play a larger role [25].

The emergence rate obtained in this study was 8.36%. These results are quite similar to those of [26] who recorded an emergence rate of 12% in adults of Cerambycidae *Monochamus carolinensis*. These findings could be justified by competitions within the branches at the time of larval life. Indeed, various studies have shown that competition for food resources in cases of high larval density increases the mortality rate in Cerambycids and / or decreases the body weight of adults. The cannibalism in the larval stage may occur when competition is intense [10, 27].

The sex ratio obtained in *D. trifasciata* was 0.73 in favor of females. But in Cerambycidae *Monochamus galloprovincialis*, the sex ratio is in favor of males [8].

The incubation period of *D. trifasciata* obtained was 11.81 ± 1.22 days. These results are similar to those of [10] who reported that the time between egg-laying and hatching of eggs' Cerambycidae varies from 3 to 7 days in general but can go up to 25 days.

In this study, the total duration of larval development of *D. trifasciata* (198.2 ± 16.22 days) is close to that obtained by [28] who reported that the larval period of Cerambycidae *Monochamus leuconotus* was approximately 210 days. In *Anoplophora versteegi* and *Aeolesthes holosericea*, larval development requires respectively 263.0 ± 22.64 days and 516.9 days [29, 30]. The duration of pupal development of *D. trifasciata* was 18.69 ± 0.63 days. It is similar to that recorded in *Anoplophora versteegi* which is 25.76 ± 2.77 days [29]. On the other hand, this duration is 145.16 ± 29.10 days in females of *Plocaederus ferrugenius* [31].

After pupal development, adult stay 3.68 ± 1.03 days in the pupal chamber before emerging from the exile hole. This time is important because many physiological changes occur in these recently emerged adults of the pupal development, including the pigmentation of the exoskeleton. This process may take several days before the young adult emerges from the wood through a circular hole as mentioned by [32] as well as [8].

The complete lifecycle of *D. trifasciata* under rearing conditions was 211.81 ± 18.87 days. These results are close to those obtained on *D. trifasciata* by [3]. Indeed, these authors observed emergence of adults by mid-June from laying at the end of November, about 210 days after laying.

V. CONCLUSION

This study was motivated by the almost total absence of data on the biological parameters of *D. trifasciata*. The study of the development cycle made it possible to determine for the first time the reproductive parameters of this species. The pre-mating and pre-oviposition periods were 123.33 ± 8.45 days and 1.83 ± 0.79 days, respectively. Lifetime fecundity 174.3 ± 18.77 eggs was obtained with a fertility rate 82 ± 9.38%. Longevity of *D. trifasciata* was 184.56 ± 7.47 days in males and 196.97 ± 6.43 days in females. The emergence rate was 8.36% and the sex ratio has been in favor of females. The results revealed that *D. trifasciata* is a univoltine patterns species with a total development cycle duration of 211.81 ± 18.87 days. The knowledge of the reproductive parameters of *D. trifasciata* could help identify the appropriate methods and times of intervention in developing effective control strategies.

ACKNOWLEDGMENT

The authors thank Kouamé Francis Koffi, Mathieu Zogbe, Léon Kouamé and all the producers of the study area for their availability and help with data collection throughout the year. They thank N'guadi Blaise Allou, CSIR-TWAS fellow (FR number: 3240280453) and CSIR-NEIST, Jorhat, India for agreeing to revise the English version of the manuscript. They are also grateful to the institution such as cotton and cashew council, African center of excellence on Climate Change, biodiversity and sustainable agriculture, led by Professor Koné for collaboration and financial support.

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