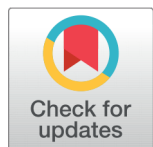


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Study of the Oothecae During the Laying Period in the Stinking Locust, *Zonocerus variegatus* (Linnaeus, 1758) (Orthoptera: Pyrgomorphidae) in La Mé Region (Southeast, Côte d'Ivoire)

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Abstract

Background: This work aims to control certain egg-laying parameters in the stinking locust *Zonocerus variegatus* (L.) in order to determine the favorable times for a more effective management of this pest. **Methods:** Indeed, following rearing in cages of nymphs fed on leaves of *Chromolaena odorata* (C. *odorata*) (L.) RM King & H. Robinson and *Manihot esculenta* Crantz, adults were obtained and placed in pairs in tanks in order to have oothecae after mating. **Findings:** In total, 446 oothecae, which provided 13050 eggs, were collected. The egg laying parameters including the length, width, circumference and mass of the ootheca as well as the number of eggs per ootheca and the number of egg-layings per female showed significant differences depending on the month. Furthermore, the results revealed that females lay on average 48.33 ± 19.16 eggs per ootheca and the number of laid eggs per female is 1.65 ± 0.68 with a maximum of 4 eggs laying during the reproduction period. In addition, it is during the month of February that females lay more eggs per ootheca with 55.66 ± 21.24 eggs, compared to 37.51 ± 11.15 in June. However, the peak of ootheca laying is in June unlike in February where laying is lower. Finally, these results made it possible to deduce that the more eggs a female lays per ootheca, the fewer ootheca it can lay during its life. **Novelty and applications:** Thus, the period from January to June represents the ideal time to effectively combat this pest in the Côte d'Ivoire region.

Keywords: *Zonocerus variegatus*; *Chromolaena odorata*; Ootheca; La Mé region

1 Introduction

Zonocerus variegatus, also called stinking locust, is a chronic pest and defoliator of very variable importance. It constitutes a formidable species, because it is polyphagous and causes significant damage to a large number of crops⁽¹⁾.

The study of the biological cycle of *Z. variegatus* has been the subject of several works that have given somewhat contradictory results such that there is much to do to more fully understand the dynamics of this species in various ecological areas⁽²⁾.

However, at the level of certain reproduction parameters such as the number of egg-laying of females and the number of eggs per ootheca, opinions are also divided. Thus, egg laying can vary from 2 to 4, sometimes from 3 to 6 and often with an average of little more than unity^(3–7). In addition, Vuillaume who worked in lower Ivory Coast suggests that laying begins in February and continues in March to end in April⁽⁸⁾. Chiffaud and Mestre believe that this set of data reveals the lack of interest in studying the reproduction of *Z. variegatus* in order to implement means of combating the eggs of this pest⁽²⁾. This is a gap that deserves to be filled, especially since it does not pose any particular technical study problems. Thus, to act on the eggs of the stinking locust, a mechanical control which consists of the collection or manual destruction and weeding of the laying sites was proposed. Weeding should be done about ten centimeters deep. It allows the ootheca of *Z. variegatus* to be destroyed by exposing them to the surface of the soil where they will dry out or be attacked by natural enemies^(2,9,10). This method was strongly recommended by Modder and has been used in southern Nigeria by Chiffaud and Mestre with success^(2,10). However, the lack of effectiveness of this technique is linked to poor knowledge of the stinking locust's laying periods⁽¹¹⁾. It is to fill part of this gap that this study was carried out in la Mé region in the south-east of Ivory Coast. Indeed, this work aims to control certain egg-laying parameters in the stinking locust in order to determine the favorable times for a more effective fight against this pest. Thus, specifically, the aim is to identify the main egg-laying periods for *Z. variegatus* in the La Mé region and to know the characteristics linked to egg-laying in this pest.

2 Material and methods

2.1 Study site and sampling

Z. variegatus were collected between January and June in the la Mé region, near Agou, a locality located in the southeast of Ivory Coast, 14 km from Adzopé. The average annual temperature is 27.60 °C with an average annual rainfall of 1514 mm. The soil is ferrallitic type on shale and on moderately leached and rich granite. This work was carried out in the laboratory of an establishment in the said locality.

2.2 Monitoring of captured nymphs until the adult stage

After capture, the nymphs were reared in six cages of dimensions 0.5 × 0.5 × 1.0 meters (m). They were fed daily with *Chromolaena odorata* and cassava leaves until the adult stage. After the imaginal molt, the imagoes were transferred to another cage where the different couples were formed which made it possible to obtain the egg laying.

2.3 Females laying eggs

Each couple was isolated in a cylindrical tank measuring 30 × 19 × 4.5 centimeters (cm) covered with a small mesh net and then fed exclusively with fresh leaves of *Chromolaena odorata* and cassava. During the trials, individuals that died before mating or laying eggs were immediately replaced by other adults from the storage cages.

Of the 300 pairs of *Z. variegatus* that were formed, 270 laid eggs. In each of the tanks, a cylindrical nest, measuring 16 × 10 × 4 cm, containing sterilized coconut soil moistened to 10%, was introduced to collect the ootheca. In addition, those laid outside the nests, a razor blade was used to delicately detach them to avoid destruction of the eggs. After laying, certain parameters such as mass, length, width and circumference of the oothecae were measured. Next, the number of eggs per ootheca was determined. Females in each tank were followed until natural death to determine the number of egg-laying per female.

2.4 Data analysis

During this study, 300 pairs of *Z. variegatus* were obtained of which 270 produced 446 oothecae with 13050 eggs. For data analysis, Statistica version 7.1 and Excel version 2013 software were used. An analysis of variance (ANOVA) was used to bring up the paramount differences. The significance level was estimated at $p < 0.05$. Furthermore, the correlation indices between the different laying parameters were determined. Also, Component Analysis (PCA) and a dendrogram were performed in order to have a good representation of the different groups within the sample groups.

3 Results

3.1 Description of egg laying in the laboratory

As egg laying approaches, the female's abdomen lengthens considerably. Laying can occur on the same day of mating or after 1 to 2 days (Figure 1A). They always begin with the emission of a foamy substance having the appearance of whitish foam at the abdominal end of the female. The egg-laying ends as it began with the emission of the same foamy substance. (Figure 1B, 1C and 1D). At times, females die during or after laying a large ootheca and the abdominal end of the female remains stuck to the ootheca thanks to the solidified foamy substance (Figure 1E).

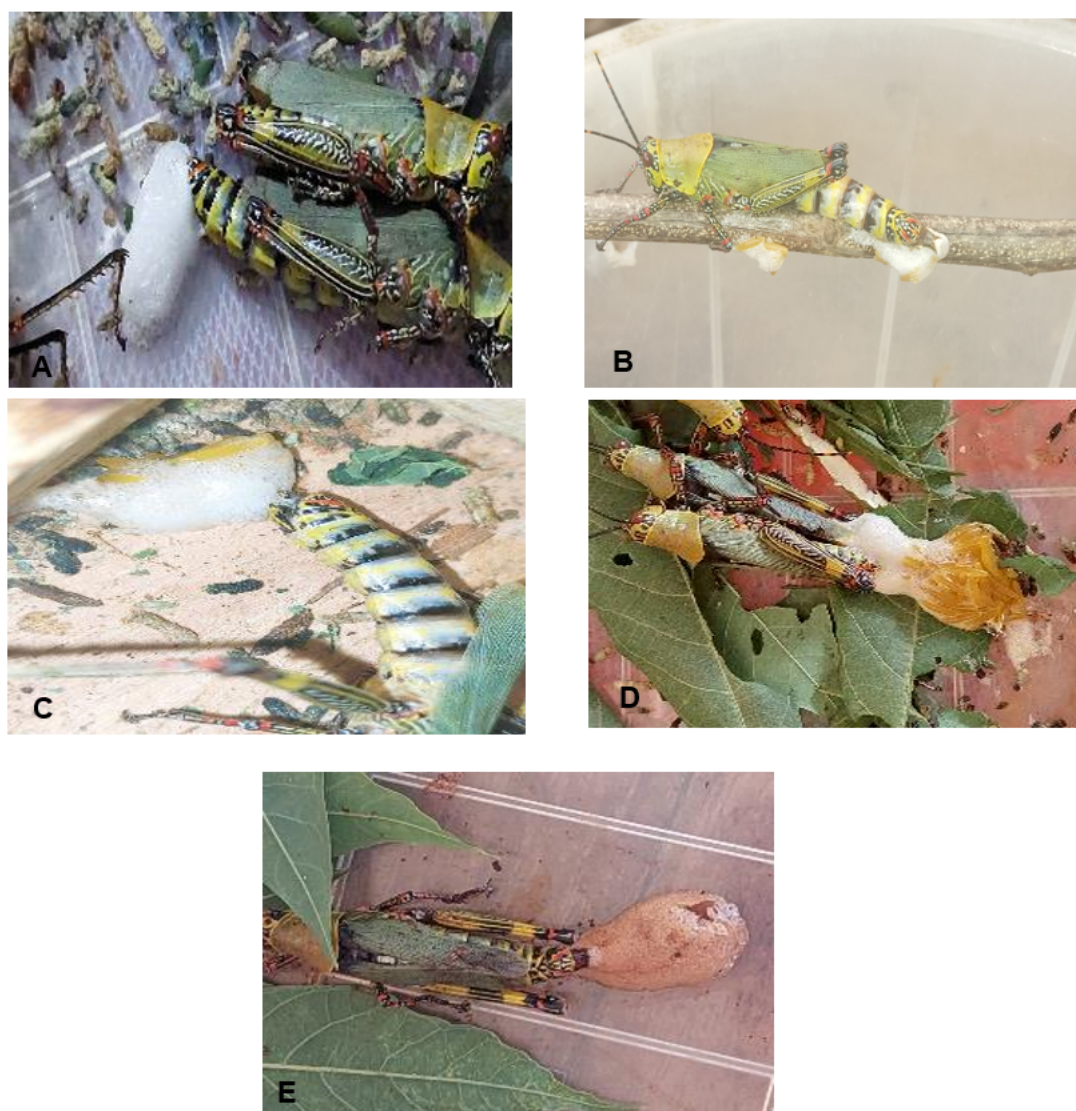


Fig 1. Egg-laying steps in *Zonocerus variegatus*. A: emission of a foamy substance before the egg-laying, B: laying of the first eggs, C: beginning of the egg-laying, D: egg laying female with a male on its back, E: dead female after laying oothecae

3.2 Description of the oothecae

The oothecae of *Z. variegatus* come in different shapes which can be rounded, oval or elongated. They are often characterized by a swollen part containing the eggs and a thinned part consisting of foamy material (Figure 2A, 2B). This foamy material,

sticky at first, tends to harden and change color depending on its duration of exposure. The foam is first whitish and then, turns brown and finally blackish (Figure 2C, 2D, 2E, 2F, 2G).

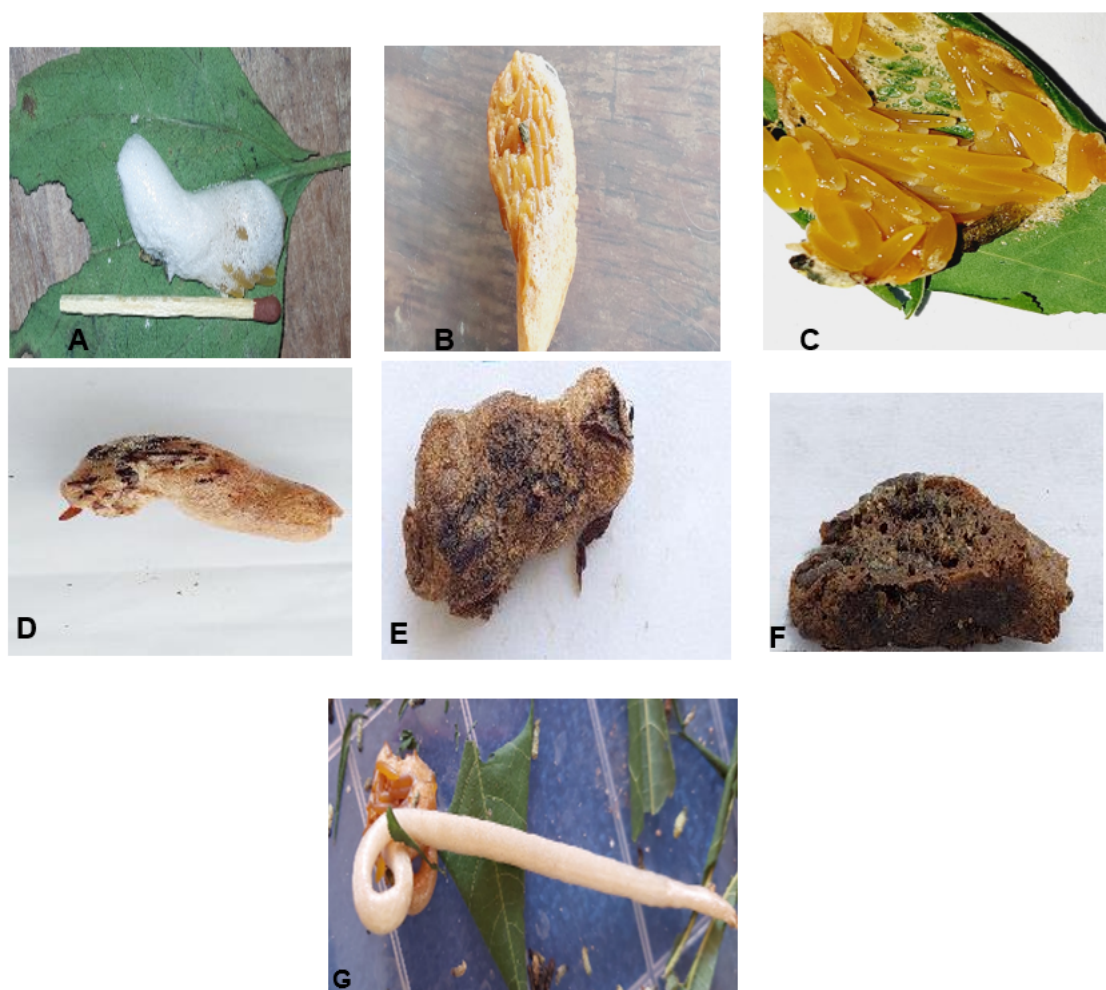


Fig 2. Oothecae of *Zonocerus variegatus*. A: Oothecae covered with foamy substance, B: Mace like new oothecae, C: Oothecae showing the disposition of eggs, D: One day oothecae, E: several tens of days oothecae, F: several weeks Oothecae, G: lengthy oothecae

3.3 Description of eggs

During laying, the female's eggs come out one by one from the end of her abdomen according to the lengthwise position. Newly laid eggs have a bright light-yellow color due to the yolk they contain (Figure 3A, 3B, 3C). The eggs change color and harden over time, their previously smooth surface becoming rough a few days later with small spines (Figure 3D, 3E, 3F). Observations have also shown that $\pm$ the size of the eggs of the same female can be different from one ootheca to another regardless of the order in which its oothecae were laid.

3.4 Counting oothecae and eggs

In total, 446 oothecae were collected: 80 in January (45 females), 66 in February (46 females), 64 in March (46 females), 75 in April (46 females), 77 in May (46 females) and 84 in June (46 females) (Figure 4). Of the total of 13,050 eggs laid, the number laid per month ranged from 2347 in March to 1688 in June (Figure 5). The number of eggs per ootheca was highest (>50) in February and March and lowest (<45) in January and June (Figure 6).

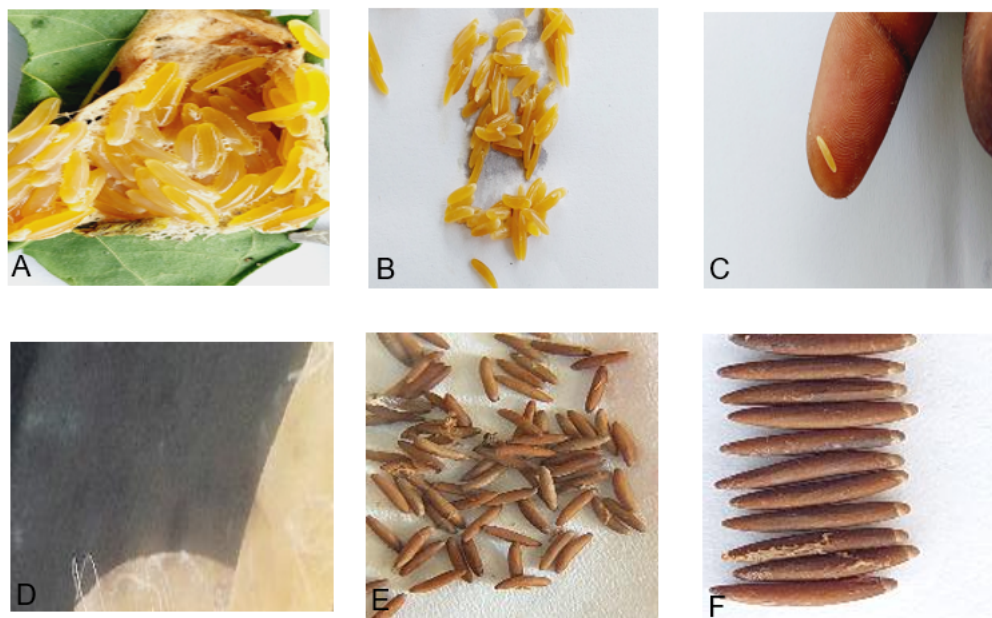


Fig 3. Pictures of eggs laid by *Zonocerus variegatus*. A: Eggs in an oothecae, B: Isolated eggs, C: Eggs on a finger, D: Eggs from binocular lens view, E: One day eggs, F: Eggs of several days

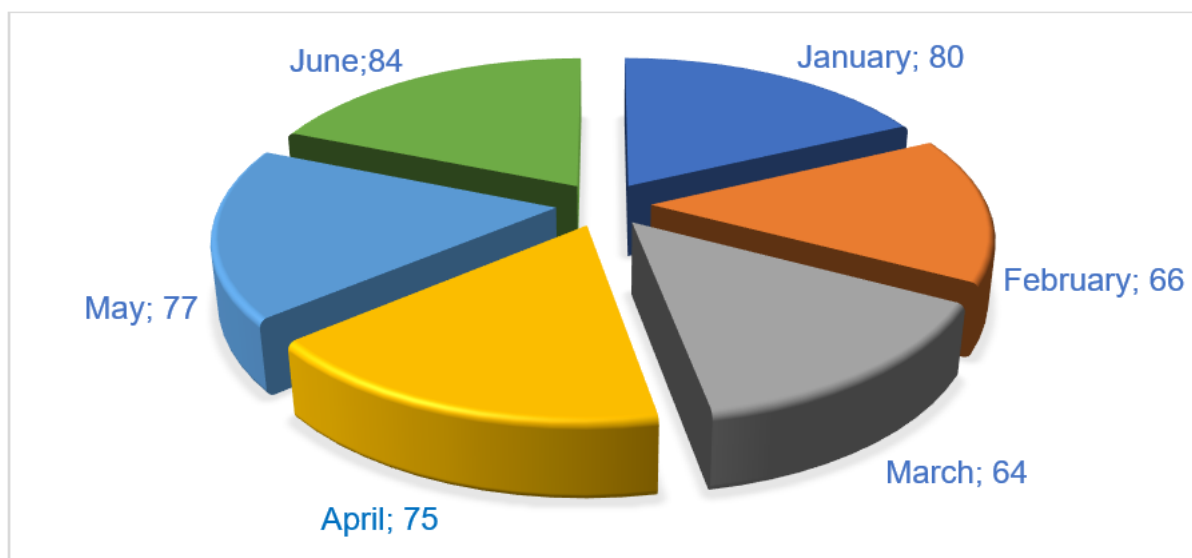


Fig 4. Number of oothecae according to egg-laying months

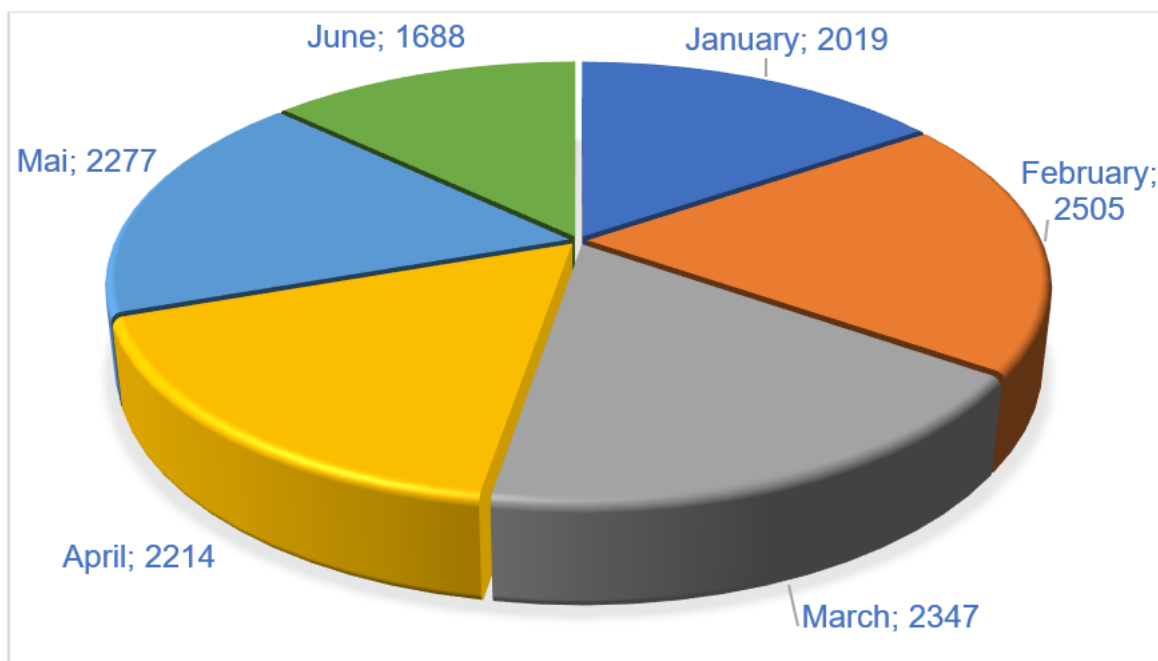


Fig 5. Number of eggs laid according to egg-laying months

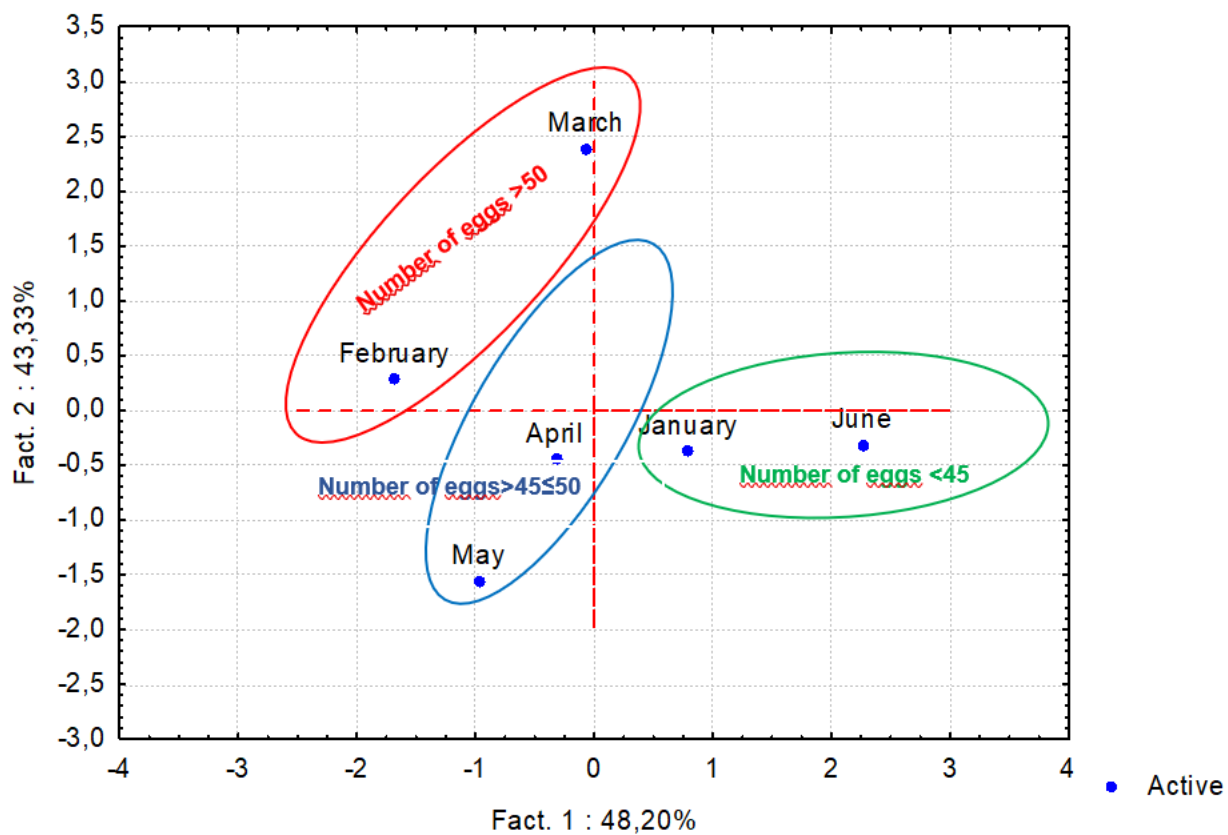


Fig 6. PCA of the egg-laying parameters according to months

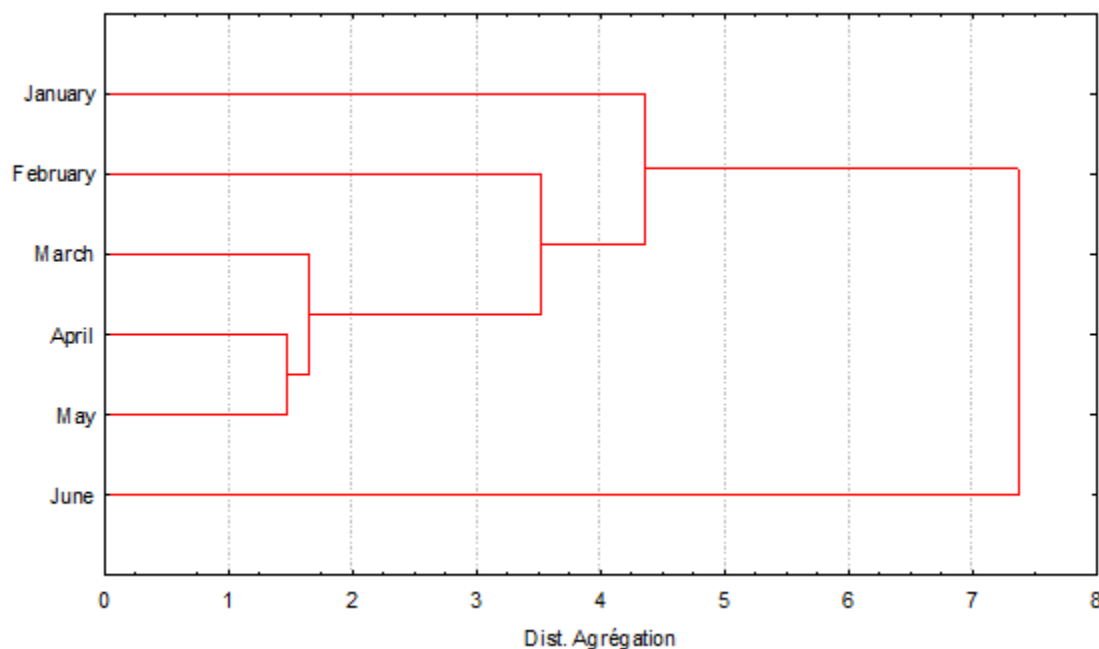


Fig 7. Classification of oothecae according to egg-laying parameters

3.5 Study of egg-laying parameters in *Zonocerus variegatus*

In la Mé region, during the six (6) main months of laying (January to June), the average length, width and circumference of the stinking locust oothecae are respectively 3.06 ± 0.85 cm, 1.13 ± 0.23 cm and 2.97 ± 0.5 cm and they weighed on average 0.66 ± 0.34 g. The average number of eggs per ootheca is 48.33 ± 19.16 with a maximum of 102 eggs and a minimum of 5 and the average number of egg-layings per female is 1.65 ± 0.68 which can reach 4 egg-layings (Table 1).

Table 1. Average egg-laying parameters

Oothecae Parameters	Average	Maximum	Minimum
Length (cm)	3.06 ± 0.85	7.2	1.4
Width (cm)	1.13 ± 0.23	2	0.6
Girth (cm)	2.97 ± 0.5	4.5	1.8
Weight (g)	0.66 ± 0.34	2.5	0.11
Number of eggs/ootheca	48.33 ± 19.16	102	5
Number oothecae/female	1.65 ± 0.68	4	1

3.5.1. Characteristics of egg laying according to months of laying

The oothecae laid in March/April were the longest, being 3.28 ± 0.80 cm long in March and 3.28 ± 0.93 cm long in April, while those in June were shortest at 2.71 ± 0.74 cm. Analysis of the results show that there is a highly significant difference at the 5% threshold ($p = 0.0$) (Table 2). In terms of the width of the ootheca, the months of February and April have respectively 1.3 ± 0.31 cm and 1.16 ± 0.27 cm. The smallest width was obtained in May with 1.02 ± 0.18 cm. There is a highly significant difference at the 5% threshold ($p < 0.05$). The oothecae of February have circumferences of 3.19 ± 0.59 cm compared to those of the other months which are lower. A highly significant difference exists between the month of February and the other months at the 5% threshold ($p < 0.05$). The oothecae harvested in March had an average mass of 0.78 ± 0.47 g greater than those of other months. As for the month of January, it presents the lightest ootheca with 0.43 ± 0.2 g (Table 2). The results indicate a highly significant difference between months for this parameter at the 5% threshold ($p < 0.05$). The oothecae laid in February contain more eggs with 55.66 ± 21.24 eggs compared to the months of March (52.15 ± 17.25), May (50.6 ± 22.62), April (49.2

± 16.2), January (44.87 ± 19.72) and June (37.51 ± 11.15) (Table 2). These results show a highly significant difference at the 5% threshold ($p < 0.05$) for this parameter between the month of June and all other months. *Z. variegatus* females lay more oothecae in June with 1.87 ± 0.5 and few in March (1.42 ± 0.62) (Table 2). For this parameter, the analyzes show a significant difference between months at the 5% threshold ($p < 0.05$).

Table 2. Laying parameters according to months

Months	Length (cm)	Width (cm)	Girth (cm)	Weight (g)	Number of eggs/oothecae	Number oothecae /female
January	3.06 ± 0.64^{ab}	1.07 ± 0.15^b	2.83 ± 0.41^b	0.43 ± 0.2^b	44.87 ± 19.7^{ab}	1.78 ± 0.76^{ab}
February	3.17 ± 1^{ab}	1.3 ± 0.31^a	3.19 ± 0.59^a	0.64 ± 0.23^a	55.66 ± 21.24^a	1.46 ± 0.66^{ab}
March	3.28 ± 0.8^a	1.14 ± 0.22^b	3.02 ± 0.51^b	0.78 ± 0.47^a	52.15 ± 17.25^a	1.42 ± 0.62^b
April	3.28 ± 0.93^a	1.16 ± 0.27^{ab}	2.99 ± 0.49^b	0.76 ± 0.44^a	49.2 ± 16.2^a	1.66 ± 0.67^{ab}
May	2.89 ± 0.82^{ab}	1.02 ± 0.18^b	2.8 ± 0.5^b	0.68 ± 0.29^a	50.6 ± 22.62^a	1.71 ± 0.75^{ab}
June	2.71 ± 0.74^b	1.05 ± 0.13^b	2.95 ± 0.4^b	0.66 ± 0.26^a	37.51 ± 11.15^b	1.87 ± 0.5^a
p	0.0	0.0	0.0	0.0	0.0	0.01

The averages affected having the same letters in the same row are not significantly different with a threshold of 5% from Student Newman Keuls method.

3.5.2. Relationship between laying parameters and time

The correlation indices between laying parameters and laying months are very weak, ranging from -0.15 to +0.17 (Table 3). The strongest correlation of -0.74 was between the total number of layings per female and the number of eggs per ootheca (Table 3). This result reflects the fact that a female has only so many eggs, and if there are many eggs per ootheca, there are fewer layings. Various parameters regarding the size the eggs had high correlations including width and circumference (0.61) weight and length (0.34) and weight and circumference (0.42) (Table 3). The number of eggs/ootheca had strong correlations with length, width, circumference and weight of eggs (Table 3). Concerning the correlations between the egg-laying parameters, the analyzes showed that there is a very strong correlation (-0.74) between the number of eggs per ootheca and the number of eggs per female and also that (0.61) existing between the width and the circumference. Then, a strong correlation of 0.51 exists between the mass of the ootheca and the number of eggs per ootheca. At the level of acceptable correlation indices, there is that (-0.44) between the mass of the ootheca and the number of eggs per female, the index (0.42) between the circumference and the mass and finally that (0.39) between the length of the ootheca and the number of eggs per ootheca (Figure 2, 3). These analyzes above show that the number of egg-laying per female depends on the number of eggs laid per ootheca. Which means that the more eggs a female lays per ootheca, the fewer ootheca it will be able to produce. These results also reveal that the higher the mass of an ootheca, the more eggs it contains. The mass of the oothecae is correlated with the number of eggs laid (0.51) and the width is correlated with the circumference (0.61) (Table 3).

Table 3. Indices of correlations between laying parameters and time

	Months	Length (cm)	Width (cm)	Circumference (cm)	Weight (g)	Number of eggs/ oothecae	Number of layings/female
Months	1	-0.15	-0.19	-0.05	0.17	-0.14	0.10
Length (cm)	-0.15	1	0.20	0.16	0.34	0.39	-0.36
Width (cm)	-0.19	0.20	1	0.61	0.19	0.31	-0.30
Circum-ference (cm)	-0.05	0.16	0.61	1	0.42	0.3	-0.32
Weight (g)	0.17	0.34	0.19	0.42	1	0.51	-0.44
Number of eggs/oothecae	-0.14	0.39	0.31	0.30	0.51	1	-0.74
Number of /female	0.10	-0.36	-0.30	-0.32	-0.44	-0.74	1

4 Discussion

According to this work, the egg-laying period in *Z. variegatus* in la Mé region, extends over six (6) months, that is to say from January to June. This period is longer than that obtained by Vuillaume in 1954 in Lower Ivory Coast which presents a shorter period of three (3) main months of laying⁽⁸⁾. In fact, he estimates that egg laying begins in February and continues in March and April, period which begins with the last two months of the dry season and ends with the first two months of the rainy season. However, some authors such as Jerath in 1965 obtained a longer laying period of eight (8) months in Nigeria which extends from March to October that is, during the entire rainy season⁽⁴⁾. Laying by *Zonocerus* occurs during the rainy season and these differences in the time of laying could be explained by differences in the rainy seasons in the countries of the Guinean and forest zone of West Africa. Since the life cycle of *Z. variegatus* varies depending on the regions, the insect's distribution area timing varies with the different climates to which these regions are subjected⁽¹²⁾.

The observations during this study made it possible to describe other aspects of the oviposition process in *Z. variegatus* as well as those of the oothecae and eggs. These descriptions present similarities with those already stated by other authors such as Vuillaume and Chiffaud and Mestre^(2,8).

In this study, the average length of the stinking locust's oothecae was 3.06 cm with a maximum of 7.2 cm. These results are close to those obtained by Vuillaume, Jerath, Toye and Popov who described the oothecae and eggs of the stinking locust and obtained oothecae measuring between 3 and 7 cm^(4,8,13,14).

Concerning the number of eggs laid per female and the number of oothecae per female, the results revealed that the females of *Z. variegatus* in this region of the Ivory Coast lay an average of 48.33 eggs with a maximum of 102 eggs per ootheca. These results agree with the ranges of egg numbers collected by some authors. Thus, Vuillaume in another part of Ivory Coast described ootheca containing 30 to 120 eggs, while Kpindou the number of eggs varies from 28 to 85 per ootheca with a maximum number of 272 eggs in Benin^(7,8,15). For De Gregorio the means of eggs per ootheca fluctuates between 23 and 83⁽¹⁾.

In addition, this work showed that the average number of laid eggs per female is estimated at 1.65 with a number varying from 1 to 4 eggs laying per female. These results are close to those of Kpindou whose number of ootheca laid varies from 1 to 5 per female in southern Benin and Coulibaly who reported an average slightly higher than unity^(5,7). Analyses have also shown that the more eggs that a female lays, the fewer the number of ootheca produced. These results are confirmed by Muse who demonstrated that mating and oviposition are two necessary processes that consume energy and influence the longevity and development of adult insects, including *Z. variegatus*⁽¹⁶⁾. The copulation and the laying are of the activities which exhaust the nutrients of the fabrics of adults of this locust⁽¹⁷⁾. In other words, these authors worked on the average concentration in organic substances (proteins, glucose and lipids) and in inorganic substances (sodium: Na⁺; potassium: K⁺; calcium: Ca²⁺ and chloride: Cl⁻). They showed that there is a significant decrease in the concentration of body metabolites during copulation, which in females, decreases further during laying. This situation influences the longevity of females, and their ability of carrying out further egg-laying.

5 Conclusion

At the end of this study, it appears that egg laying of *Z. variegatus* extends from January to June in this region of Ivory Coast. During this reproduction period, there was substantial variation in number of eggs laid and number of oothecae produced. The month of peak numbers of each criterion was as follows: the peak was in June for the number of oothecae (84), while the peak was in February for the number of eggs per ootheca and total number of eggs (2505). The lowest were in March for the number of oothecae (64) and in June for the total number of eggs (1688). However, apart from the diet which would influence reproduction in this locust, we could look at the abiotic factors which could influence the egg-laying parameters of this pest in this region of Ivory Coast.

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