

Study of the lifetime of the Stinking Locust, *Zonocerus variegatus* (Linnaeus, 1758) (Orthoptera: Pyrgomorphidae) fed on cassava (*Manihot esculenta* Crantz) in the La Mé region (southeast, Côte d'Ivoire)

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World Journal of Advanced Research and Reviews, 2025, 27(02), 1616-1624

Publication history: Received on 09 July 2025; revised on 20 August 2025; accepted on 22 August 2025

Article DOI: <https://doi.org/10.30574/wjarr.2025.27.2.2986>

Abstract

Zonocerus variegatus is a crop pest whose larvae and adults cause damage to leaves and stems, resulting in huge losses in agricultural yield. The objective of this study was to determine the duration of the different stages of the stink bug on cassava (*Manihot esculenta* Crantz) in the La Mé region with a view to implementing alternative methods of controlling it. Larvae captured in the wild were used to obtain adults, whose eggs were incubated in sterilized coconut compost. After hatching, the larvae, which were fed exclusively on cassava leaves, were monitored from stage 1 to the adult stage in order to determine the number and duration of the different stages. When *Zonocerus variegatus* is fed cassava leaves, the incubation period in the laboratory is 160 ± 9.95 days (5.33 months). It has seven stages in captivity, including six larval stages and one adult stage. Stage 1 is the longest larval stage, lasting 32.26 ± 2.35 days, and stage 2 is the shortest, lasting 22.59 ± 1.49 days. The larval stages last 161.29 ± 3.69 days. The adult stage lasts an average 47.01 ± 6.59 days. Among adults, females have a lifespan of 51.01 ± 5.59 days and males 43.01 ± 4.33 days, with a significant difference. The post-hatching lifespan of stink bugs fed on cassava leaves in the La Mé region is 208.29 ± 4.69 days, and the lifespan of the bug from egg to adult is estimated at 368.3 ± 9.5 days, or a total of approximately one year.

Keywords: *Zonocerus variegatus*; Cassava; Ootheca; Eggs

1. Introduction

Zonocerus variegatus, also known as the stink bug, is a chronic pest and defoliator of varying importance. It is a formidable species because it is polyphagous and causes significant damage to a large number of crops [1]. The biological cycle of *Z. variegatus* has been the subject of several studies and hypotheses that are more or less contradictory. To date, much remains to be done to fully understand the variability of this cycle across different ecological zones [2]. However, opinions are also divided on certain parameters such as the incubation period of the eggs and the lifespan of the locust. In Guinean and forest areas, information on the incubation of eggs from the wet season "population" (laid from June to November) is much scarcer. Jerath [3], in breeding, provided values ranging from 98 to 141 days, with eggs laid in August incubating for only two months. Anya [4] mentions 5 to 6 months, and De Grégorio [5,6] indicates 4.5 to 6 months, noting that in the laboratory, regardless of the origin of the oothecae, the incubation period is never less than 4 months. Coulibaly et al. [7] give a value of 162 days for eggs laid in October and November, and Page [8] mentions a duration of 4 months (eggs laid in September) in only two cases [2]. As regards longevity, this aspect of imaginal biology is poorly documented. Most field observations provide the dates of appearance and disappearance of the imago, but as there are no details on the numbers involved over time, it is generally not possible to estimate the average imaginal longevity. In the laboratory, Vuillaume [9] indicates longevity of 2 to 3 months but notes that it appears to be shorter in the field. Still in breeding, Jerath [3] obtains values of 117 to 147 days for females. In the field, Vilardebo [10] estimates the lifespan of imagoes to be 2.5 to 3 months, but this is certainly an overestimate,

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and Duranton et al. [11] give an average of around 40 days (maximum 123 days). According to the figures from De Grégorio [6], we can suggest a figure of around 2 months for longevity in the field [2]. Chiffaud and Mestre [1] believe that this data set reveals the lack of interest in studying the reproduction of *Z. variegatus* in order to develop methods of controlling the eggs of this pest. This is a gap that should be filled, especially since it does not pose any particular technical problems for study. It is to fill part of this gap that this study was conducted in the La Mé region in the south-east of Côte d'Ivoire.

The aim of this work is to understand the life cycle of the stink bug in order to determine the best times for more effective control of this pest in the La Mé region. Specifically, the aim is to determine the number of stages and the duration of each stage of this pest in this region of Côte d'Ivoire.

2. Materials and methods

2.1. Study site and sampling

This study was conducted in the La Mé region, specifically in Agou, a town located in southeastern Côte d'Ivoire, 14 km from Adzopé. The average annual temperature is 27.60°C with an average annual rainfall of 1.514 mm. The soil is ferrallitic on schist and moderately leached, rich granite. This work was carried out in the laboratory of an establishment in the aforementioned locality.

2.2. Monitoring of captured larvae until adulthood

After capture, larvae in stages 1 to 6 were raised in six (6) cages measuring 0.5 m long, 0.5 m wide, and 1 m high. They were fed daily with *Chromolaena odorata* leaves and cassava until they reached the adult stage. After imaginal moulting, the imagoes were transferred to another cage where different pairs were formed, which allowed eggs to be laid.

2.3. Preparation of the incubation substrate

For this work, the substrate chosen for incubating *Zonocerus variegatus* eggs in the laboratory is coconut coir (*Cocos nucifera*). To prepare this incubation medium, bleach granules were added to tap water at a ratio of ten (10) grams per two (2) liters of water, resulting in a concentration of 0.5 g/L. The purpose of this solution was to eliminate any potential parasite germs contained in the substrate. This bleached water was used to heat the incubation substrate at a ratio of one (1) liter per five (5) kilograms of coconut coir until the solution had completely evaporated from the medium.

2.4. Egg laying by females

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

A total of 300 pairs of *Z. variegatus* were formed. However, only 270 pairs produced eggs. In each container, a cylindrical egg-laying box measuring 16×10×4 cm and containing sterilized, moistened coconut soil was introduced to collect the egg cases. In addition, for egg cases laid outside the nesting boxes, a razor blade was used to gently detach them to avoid destroying the eggs.

2.5. Egg incubation

After each laying, the eggs were incubated in coconut coir and watered daily using a hand sprayer containing distilled water. The soil was watered with a total of four (4) sprays from this sprayer. To promote aeration of the incubation medium, the coconut soil was stirred weekly until hatching.

2.6. Rearing method

After hatching, *Z. variegatus* larvae were isolated in cages and rearing tanks according to their respective hatching dates. They were then fed daily on a diet consisting exclusively of fresh cassava leaves (*Manihot esculenta* Crantz). After each moult, individuals from the same rearing tank that had moulted were moved to another tank in order to record the exact duration of the stages.

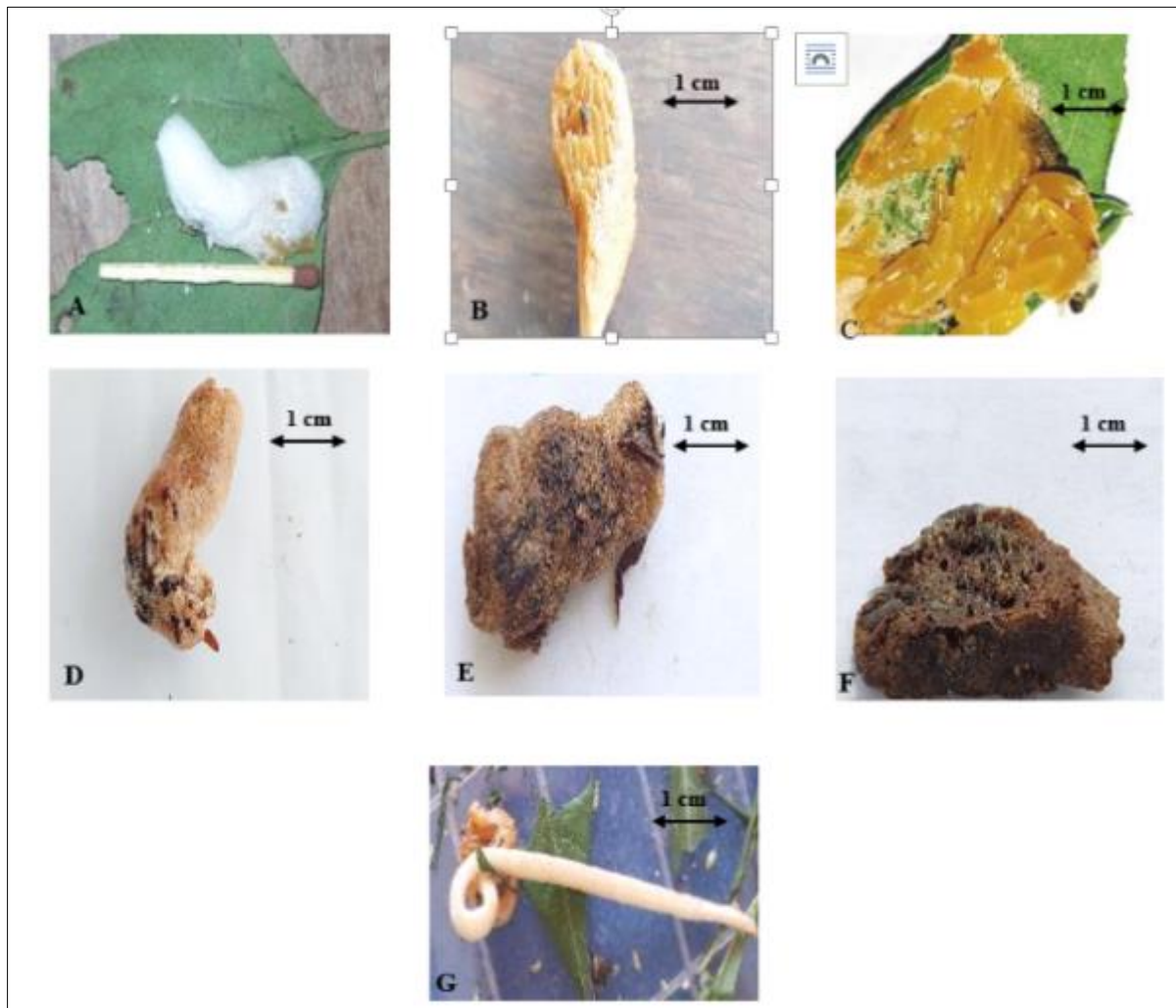
2.7. Data analysis

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 $p < 0.05$.

3. Results

3.1. Description of 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 thinner part consisting of foamy material (Figures 1A, 1B). This foamy material, which is sticky at first, tends to harden and change color depending on how long it is exposed. The foam is initially whitish, and then turns brown, and finally blackish (Figures 1C, 1D, 1E, 1F, 1G).

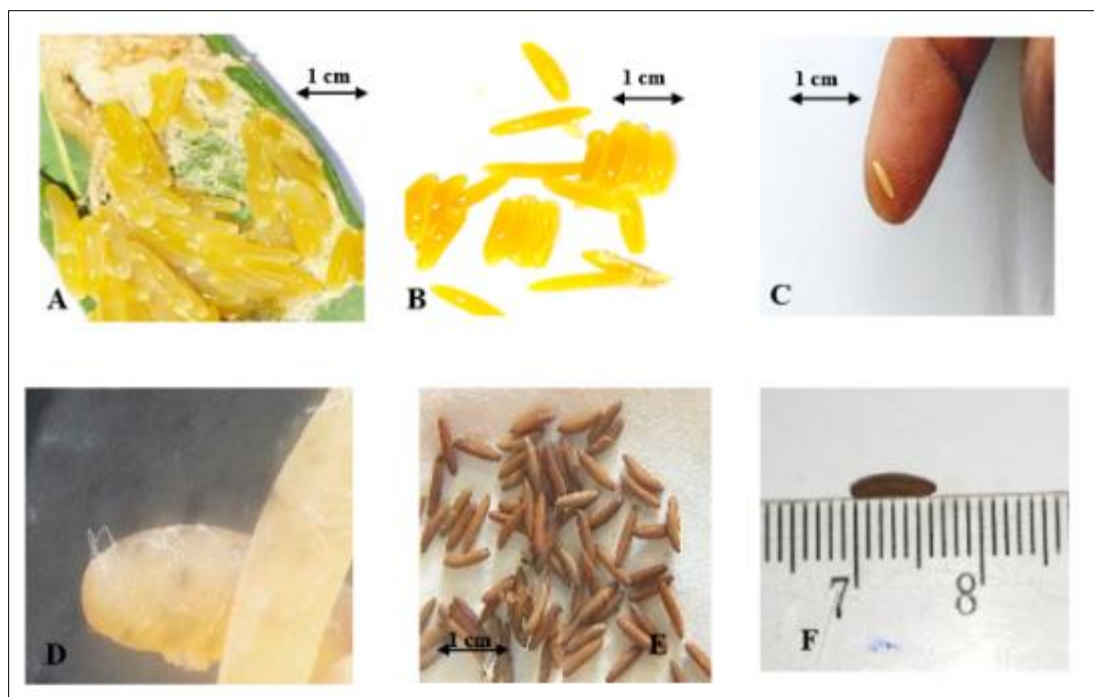


A: Egg sac covered with foamy moss; B: New club-shaped egg sac; C: Egg sac showing the arrangement of the eggs; D: Egg sac one day old; E: Egg sac several dozen days old; F: Egg sac several weeks old; G: Long-shaped egg sac

Figure 1 Some oothecae from *Zonocerus variegatus*

3.1.1. Description of eggs

During egg laying, the female's eggs emerge one by one from the abdominal end of her body, depending on their position lengthwise. Newly laid eggs have a bright yellow color due to the vitellus they contain (Figures 2A, 2B, 2C). The eggs change color and harden over time, their previously smooth surface becoming rough a few days later with small spines (Figure 2D, 2E, 2F). Observations have also shown that the size of eggs from the same female can vary from one ootheca to another, regardless of the order in which the oothecae are laid.



A: Eggs in an oothecae; B: Isolated eggs; C: Egg on a finger; D: Eggs from binocular lens view; E: One day eggs; F: Eggs of several days

Figure 2 Pictures of eggs laid by *Zonocerus variegatus*

3.2. Incubation period and hatching of *Zonocerus variegatus* eggs

During this study, 300 pairs of *Z. variegatus* were obtained, of which 270 produced 446 oothecae with 13.050 eggs which were incubated. In the laboratory, the average incubation period for eggs of the stink bug in the La Mé region is 160 ± 9.95 days (5.33 months), with a minimum of 142 days (4.73 months) and a maximum of 194 days (6.46 months) (Table I and Figure 3).

Table 1 Incubation period of *Zonocerus variegatus* eggs in the La Mé region

Mean (Day)	Minimum	Maximum
160 ± 9.95	142	194

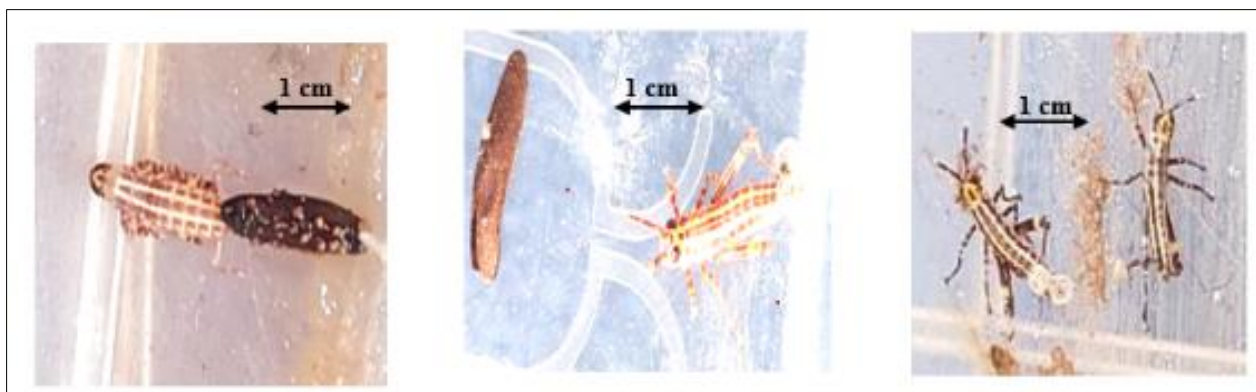


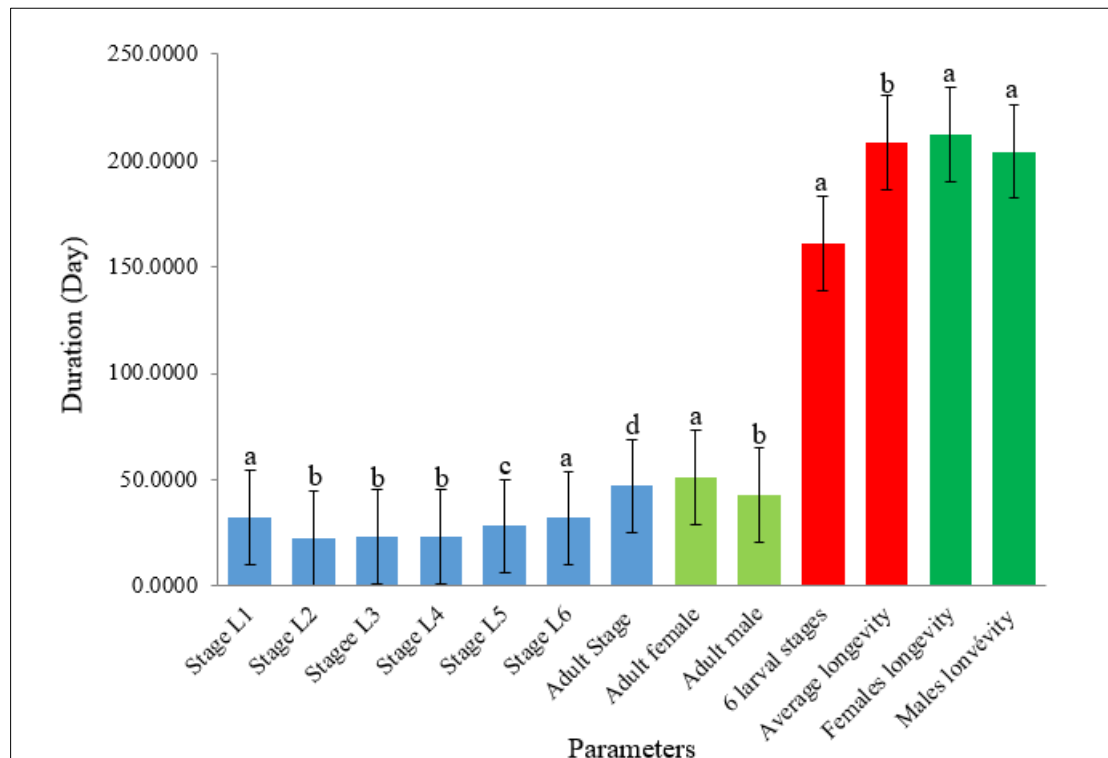
Figure 3 Hatching of *Zonocerus variegatus* eggs

3.2.1. Number of stages in *Zonocerus variegatus* in La Mé

Monitoring of the larvae showed that in the La Mé region, the stink bug develops through seven (7) stages in the laboratory, with six (6) larval stages and one adult stage constituting the seventh stage.

3.2.2. Duration of stages

When *Z. variegatus* is fed cassava leaves, stage L1 lasts 32.26 ± 2.35 days, stage L2 lasts 22.59 ± 1.49 days, stage L3 lasts 23.02 ± 1.32 days, stage L4 is 23.17 ± 1.28 days, stage L5 is 28.26 ± 1.04 days, stage L6 is 31.94 ± 1.17 days, and the adult stage is 47.01 ± 6.59 days. For this parameter, the analyses showed that there is no significant difference in the duration of stages L2, L3, and L4, nor between stages L1 and L6 at the 5% threshold. For this same diet, the larval stages last a total of 161.29 ± 3.69 days, with a minimum of 152 days and a maximum of 171 days. Also, the duration of the adult stage in females is 51.01 ± 5.59 days and that of males is 43.01 ± 4.33 days, with a statistical difference according to the student's t-test at 5% ($p=0.00$ $df=156$). The results also showed a longevity of 212.29 ± 7.96 days for females and 204.29 ± 5.76 days for males, with a significant difference between these values ($p=0.00$ $df=156$). The average lifespan of stink bugs fed *Manihot esculenta* leaves is estimated at 208.29 ± 4.69 days (Figures 4 and 5).



The bands assigned the same letter in the same color are not significantly different at the 5% threshold by the Student Newman Keuls method.

Figure 4 Duration of the stages of *Z. variegatus* fed only cassava leaves

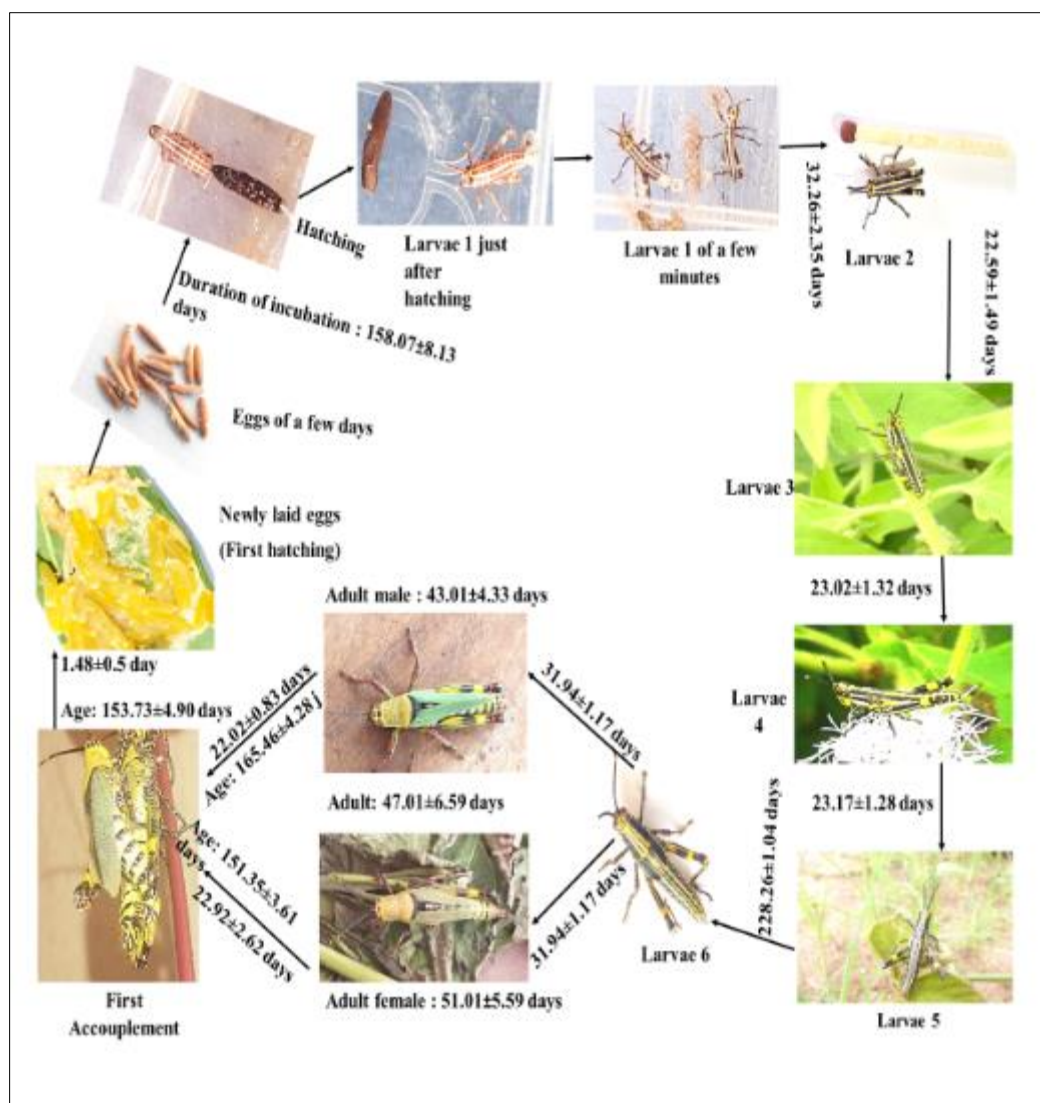


Figure 5 Development cycle of *Zonocerus variegatus* fed on cassava

3.2.3. Duration of the pre-ovulation period in females and pre-copulation period in males

During this study, the pre-oviposition periods in females and pre-copulation periods in males were evaluated. In addition, during reproduction in *Z. variegatus*, two (2) modalities of the pre-oviposition period were identified and studied. These are the duration between the imaginal moult of young females and their first mating (pre-oviposition period 1) and the duration between mating and the first egg-laying (pre-oviposition period 2). Thus, the duration of pre-oviposition period 1 for females fed cassava leaves is 22.92±2.62 days. The duration of pre-oviposition period 2 is 1.48±0.5 days. In *Zonocerus variegatus*, the pre-copulation period for males is 22.02±0.83 days (Table II).

Table 2 Duration of pre-ovulation and pre-copulation periods in the Stinking Locust

Pre-ovulation period 1 (days)	Pre-ovulation period 2 (days)	Pre-copulation period (days)
22.92±2.62 ^a	1.48±0.5 ^b	22.02±0.83 ^c
p	0.84	0.41

Means assigned the same letter on the same line are not significantly different at the 5% threshold by the Student Newman Keuls method.

3.2.4. Total life cycle duration of *Zonocerus variegatus* in the laboratory

Taking into account the incubation period of the eggs, the duration of the larval stages, and the duration of the adult stage, the life cycle of the stink bug fed on cassava leaves in the La Mé region is estimated at 368.3 ± 9.5 days, or a total of approximately one year.

4. Discussion

During this study, the incubation period of *Zonocerus variegatus* eggs in coconut soil was 160 ± 9.95 days, or 5.33 months, with a minimum of 142 days (4.73 months) and a maximum of 194 days (6.46 months). This duration is consistent with those obtained by Anya [4], who reported 5 to 6 months, and De Grégorio [5,6], who reported 4.5 to 6 months, noting that in the laboratory, regardless of the origin of the oothecae, the incubation period is never less than 4 months. Contrary to the previous authors, Vuillaume [9] obtained a longer incubation period of 230 days (7.33 months) in Lower Côte d'Ivoire. This difference could be explained by the type of substrate used for egg incubation, as this author incubated the eggs in sand rather than coconut soil, which promoted better humidification of the environment, resulting in rapid embryonic development. According to Vuillaume [9], oothecae do not survive more than 15 days in completely dry soil, but water content (by weight) of 1.25% is sufficient to allow normal egg development. The soil was moistened to at least 10% of its weight every two (2) days until hatching. When *Z. variegatus* was reared in the laboratory and fed on leaves in the La Mé region, the duration of the L1 stage was 32.26 ± 2.35 days. These data are similar to those obtained in Côte d'Ivoire by Coulibaly et al. [7] with 30 days and by Kpindou et al. [12] with an average duration ranging from 20 to 37 days in Benin when individuals have cassava leaves in their diet. Vuillaume [9] reported a lifespan of 18 to 22 days for L1 in Lower Côte d'Ivoire. Other authors have reported shorter L1 durations, such as Kaufmann [13] with 12 to 21 days, Jerath [3] with 10 to 29 days, and Iheagwam [12] with 16 days in the dry season and 18 days in the wet season. This study showed a stage 2 duration of 22.59 ± 1.49 days. This result is close to that obtained by Kpindou et al. [11], with a stage 2 duration of 12 to 20 days for locusts fed exclusively on cassava leaves in Benin. Vuillaume [9] obtained a shorter stage 2 duration interval of 15 to 17 days in the laboratory with the same diet. Stage 2 also shows great diversity in breeding among other authors, such as Kaufmann [13] with 12 to 15 days, Jerath [3] with 8 to 22 days, Chapman et al. [15] with 29 days, Iheagwam [14] 13.3 days in the dry season and 15.5 days in the wet season, and Coulibaly et al. [7] 16 days. At stage 3, it lasted 23.02 ± 1.32 days. This duration of L3 is within the range obtained by Kpindou et al. [12], which is 20 to 29 days. During this study, the average duration of the fourth larval stage was 23.17 ± 1.28 days, which is close to that obtained by Chapman et al. [13], who reported a duration of 18 to 22 days in the field. Kpindou [12] and Jerath [3] reported a duration of 25 days. The duration of stage 5 was 28.26 ± 1.04 days. This lifespan of L5 is longer than the durations obtained by authors such as Vuillaume [9] with a duration of 15 to 21 days, Kpindou et al. [12] 24 days, Kaufmann [13] 16 days, Jerath [3] 8 to 19 days, Chapman et al. [15] 18 to 22 days in the field and 17 days in captivity, Iheagwam [14] 13.4 days in captivity in the dry season and 20.6 days in the wet season, and Coulibaly et al. [7] 19 days. This difference could be explained by the rearing conditions, as most of these authors carried out their work in cages placed outdoors on plots of land, unlike the rearing in this study, which was carried out exclusively in the laboratory. As for stage 6 larvae, they lived for 31.94 ± 1.17 days during this study. This result is confirmed by Kpindou et al. [12] with an L6 duration of 26 to 38 days during breeding in Benin. However, Vuillaume [9] obtained a shorter L6 duration of 18 to 22 days in Lower Côte d'Ivoire, and this difference could be explained by climate change over the past decades. The duration of the adult stage was 47.01 ± 6.59 days during this rearing. This result is similar to that obtained by several authors, including Chapman [15], Vilardebo [10], De Grégorio [6], Le Gall and Mestre [16], and Couturier et al. [17], who observed adults from late January to February and from June to July, for approximately one and a half to two months. Kpindou et al. [12] observed adults from February to March and from July to August when they were fed cassava leaves in Benin. Similarly, in Lower Côte d'Ivoire, Vuillaume [9] observed adults in this region living for two to three months before dying. The duration of the larval stages in the laboratory in the La Mé region was 161.29 ± 3.69 days when the stink bugs were fed cassava leaves, which is close to the life span obtained by Kpindou et al. [12], whose larvae become adults in 130 to 170 days when they consume *C. odorata* and cassava leaves. However, Vuillaume [9] argued that the larval stages last from 3.5 months (110 to 120 days) to 4 months in Lower Côte d'Ivoire. This difference could be explained by the fact that Vuillaume's assertion is based on observations made on several plots in this region of Côte d'Ivoire. The post-hatching longevity of *Zonocerus variegatus* during this study averaged 208.29 ± 4.69 days overall, 212.29 ± 7.96 days for females, and 204.29 ± 5.76 days for males. These results are consistent with those obtained by Vuillaume [9], which range from 170 to 210 days when the durations of the different phases of the stink bug's development cycle are added together (egg incubation, larval stages, and adult stage). This finding is also consistent with those of other authors such as Kaufmann [13], Jerath [3], Chapman et al. [15], Iheagwam [14], and Coulibaly et al. [7], whose longevity after hatching varies from 190 to 210 days in captivity.

5. Conclusion

The total duration of the development cycle of *Zonocerus variegatus* fed on cassava leaves in the La Mé region is 368.3 ± 9.5 days. This cycle has seven (7) stages with imagoes mating about 20 days after the imaginal molt. In this pest, females live longer than males. Thus, knowledge of the development cycle of the stinking grasshopper will make it possible to put in place methods for better control of this locust.

Compliance with ethical standards

Acknowledgments

We say a big thank you to the Almighty God who gave us the strength to accomplish this work. We also thank all our supervisors who, through their technical contributions and advice, made this work possible. I thank my family and especially my wife for their unwavering support during this work. I thank the Jean Lorougnon Guédé University of Daloa in Ivory Coast who kindly accepted me as a student to carry out this work.

Disclosure of conflict of interest

No conflict-of-interest to be disclosed.

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