Biological parameters of *Leptoglossus membranaceus* (Heteroptera: Coreidae), a cucumber pest (*Cucumis sativus* Linné, 1753; Cucurbitacae) of Tokyo F1 and poinsett varieties in offseason in Daloa region (Côte d'Ivoire)

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Summary

L. membranaceus is an insect of Coreidae (Heteroptera.) family. It is a pest of cucumbers, appearing at fruiting stage and damage fruits and young stems. The female lays between 90 and 230 eggs, an average number of 150 \pm 32 eggs with a frequency of 2 to 23 eggs every 2 to 3 days. The period of oviposition was 78.76 \pm 9 days. The incubation time of the eggs is 6.25 \pm 0.45 days. Out of total of 230 \pm 30 eggs laid by a female, 221 \pm 23 eggs emerged, giving a fertility rate of 96.09 \pm 3.22. Only 44 out of 221 larvae have reached stage 5 and adulthood; an average larval and overall survival rate of 19.46%. The sex ratio of 0.77 is in favour of males. The average life span is 85.9 \pm 10.31 days for females and 112.4 \pm 15.20 days for males. The average length of the development cycle was 22.53 \pm 1.90 days. In the fruit, repeated attacks cause a regression in weight, length and circumference, also causing early ripening, deformation and decoration. This work will allow us to control L. membranaceus by controlling the biological cycle and the stage of development that causes the most damage to the cucumber.

Keywords: Cucumber, Daloa, Insects, Pest, Larvae

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I. Introduction

The cucumber (*Cucumis sativus*) is an annual, herbaceous, vegetable plant of Cucurbitaceae family. It grew naturally at the base of the mountain range of Himalayas and was first domesticated in India at least 3.000 years ago. It is cultivated for its pulpy fruits, which are generally eaten raw, and which are also used in pharmacy and perfumery (beauty milk, cosmetics...). The leaves are lobed and the flowers unisexual male or female [1]. The male and female flowers are distinct, but carried by the same foot (monoecious plant). Fruits are elongated and fleshy, with a rough touch, and can reach 30 cm in length and 5 cm in diameter. They are berries containing many seeds. Some varieties of cucumber are parthenocarpic, the flower buds producing seedless fruit. Today, professionals only grow parthenocarpic varietie [2]. Nutritionally, the cucumber consists of 0.46% protein, 96.01% water, 0.11% fat, 0.81% carbohydrates and vitamins A, B1, B2 and C [3]. World production in 2013 is estimated at 71.365.573 tons (FAOSTAT, 2014)[4]. Ivorian production, in the main cities, was estimated at around 30.000 tonnes in 2008 [5]. But, it is unfortunately subject to attacks by insects, including *Leptoglossus membranaceus*. Adults and larvae sting young stems and fruits. The present work is part of preliminary studies to find new control methods, aims to determine the duration of oviposition, the longevity of adults and identify the most voracious stages through the study of growth cycle of *L. membranaceus*.

2.1 MATERIAL

II. Material And Method

- Study site

The study was done from November to January in 2019 and 2020 on a site (06° 52'38" N and 06° 27'00" W) in the town of Daloa (Ivory Coast). The Study Area is characterised by a sub-equatorial, hot and humid climate with two seasons: a rainy season from February to October and a dry season from November to January [6] (Figure 1). During the test (from November to February), the average temperature, rainfall and relative humidity of the region were respectively 27.57°C; 25.66 mm and 68.5%. The average temperature during the year was 2.8°C and the average annual rainfall was 1317 mm.

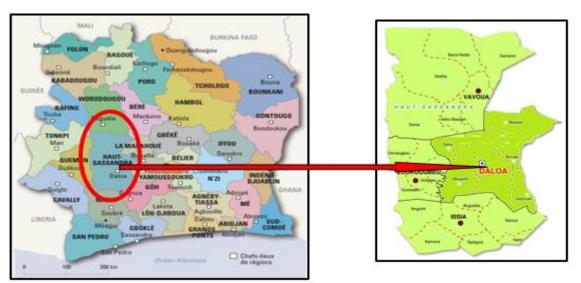


Figure 1: Location of the research area

- Biological material

The biological material is composed, firstly of vegetable material (the Tokyo F1 and Poinsett 76 cucumber varieties) and secondly of animal material (*L. membranaceus*).

- Breeding material

Cages in the shape of a Paver (70 cm wide and 160 cm high), made with No. 6 rebar and white muslin of 0.50 mm mesh (Figure 2) were used. These cages are provided with an opening lined with adhesive strips to prevent the exit of insects rearing on the cucumber stalk. These cages were used as a rearing device for the study of bioecological parameters in semi-natural conditions. Cylindrical boxes with a diameter of 10 cm and a height of 20 cm with a perforated cover were used to transfer the insects from one cage to another one.

2.2 METHOD

2.2.1 Determination of biological parameters

The biological parameters studied are adult lifetime, period, pre-oviposition and oviposition, number of eggs laid per female, egg incubation time, fertility rate, duration of larval stages, survival rate and sex ratio.

2.2.1.1 Pre-oviposition periods

Breeding of *L. membranaceus* was undertaken on the plot. Pairs are formed with adult insects caught on the cucumber plants. They are placed on the plants covered with a muslin cage and observed daily. After egg-laying and hatching, the larvae are placed in other cages containing a cucumber plant and observed daily to monitor their growth and protect them from predators. The cages are also cleaned weekly to avoid contamination of the larvae. The larvae will feed on the sap and grow to adulthood. At this stage, 32 pairs have been formed with these insects (Figure 2).



Figure 2: Experimental design

2.2.1.2. Oviposition sites, frequency and number of eggs layed per female.

32 pairs formed with the insects from the farm will be monitored. As soon as the first egg laying is observed, the eggs are counted and labelled. The trial continued until the death of the female. The sum of the eggs laid per female was made. The date of the last egg laying (Ddp) was recorded and then the average laying period was determined.

Mean oviposition period (days) = $\Sigma pifi / \Sigma fi$ pi = Jdp - Jpp; fi: number of females; Jpp: date of first oviposition

2.2.1. 3. Incubation period and egg fertility rate

The date of egg laying was noted Jp. For each of the 32 females, the number of eggs hatched was recorded and the average fertility rate was calculated.

Average fertility rate (%) = $\frac{\sum tifi}{x \ 100}$

ti= $\frac{Number of eggs hatching}{Number of eggs lays}$

fi : female population

2.2.1.4 Larval survival rate, Development time, Sex ratio and Adult lifespan

Observations on larval survival, development time and sex ratio were made from the descendants of 32 pairs. First instar larvae hatched were isolated in a cage containing a cucumber plant. The cages were monitored daily. The dates of successive moults were recorded. The average survival rate was calculated for each larval stage.

Mean larval survival rate (%) = $\sum sifi$

Si = Number of larvae in the next stage

fi : female population

Number of larvae in the previous stage

The change from one larval stage to the next is marked by a molting followed by the release of an exuvia. The size of this exuvium is always larger than that of the previous stage. The average duration of the transition from one larval stage to the next was calculated and expressed in days. After the imaginal molt, the insects that reached adulthood were grouped by sex. The average total development time, which combines the egg incubation period (Pi) and the duration of the larval period (Dl), was calculated.

Total average development time (days) = $\frac{\sum biki}{\sum ki} X100$

bi = Pi + DI; ki: number of adults

Then the sex ratio was calculated for the offspring of each female, according to the formula

Sex-ratio = Number of female Number of male

Finally, the longevity is the time between the date of egg eclosion (Jo) and the date of death (Jm). The average longevity of the females and males of the 32 pairs was calculated.

Average lifespan (days) = $\frac{\Sigma \text{divi}}{\Sigma \text{vi}}$

di =Jm - Jo; vi: number of males or females

2.2.2 Impact of *L. membranaceus* attacks on cucumber

To evaluate the damage on the vegetative stage, 5 plants were covered with tissue cages and adult insects were introduced in each cage in the following order: 2 insects in cage 1; 4 in cage 2; 6 in cage 3; 8 in cage 4 and 10 in cage 5. 24 hours later these 5 plants are observed. In order to evaluate the damage on the cucumber production, 60 plants were selected on the experimental plot and covered with tissue. Assisted pollination was carried out in order to obtain fruits. 30 of these 60 plants were subjected to repeated attacks by

L. membranaceus and the other 30 remained protected. The number of fruits of each group of plants (attacked and healthy plants) was counted. Pi : Number of plants fi: Number of fruits

Mean number of fruits per plant =		Σfi	
Loss rate (%) =	$X 10(-\Sigma fipia)$ Σpis	Σ Pi	Pia : Number of plants damaged Pis : Number of healthy plants

2. 3 Data analysis

The data obtained were compiled in Excel sheet, version 2013 and were used to obtain the variability of the number of larvae according to the larval stages and to draw the curves of the influence of temperature and relative humidity on the period of larval stages. The mean determination and the statistical analysis of variance ANOVA 1, were carried out using the paleontological software Past.3. Kruskal-Wallis test for equality of means was therefore used to test differences in means observed at the 5% probability level. The means are considered significant at a probability p < 0.05.

3.1. Pre-oviposition periods

The first coupling was observed on the 8th day after the imaginal molt. The first eggs were laid on days 9; 8 and 10 after mating, an average pre-oviposition period of 9 ± 0.66 days (Figure 3).

Results

III.

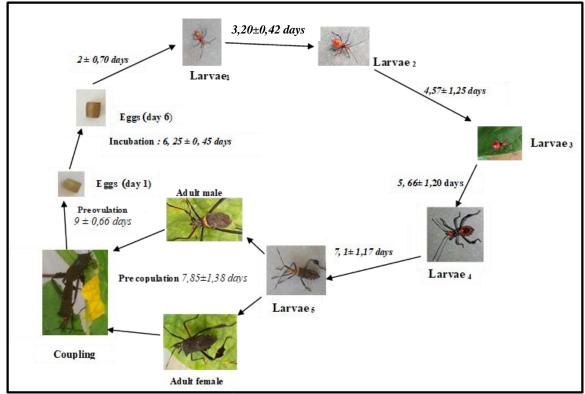


Figure 3: Development cycle of *Leptoglossus membranaceus*

3.2 Oviposition sites, frequency and number of eggs laid per female.

The eggs, usually laying on the undersides of leaves, on tendrils and sometimes on stems, are aligned in a row in the form of a thread. One set corresponds to one egg laying. The number of eggs per clutch varies from 2 to 25. The average is about 13 eggs per clutch (Figure 4). The female lays 90 to 230 eggs, an average of 170 ± 30 eggs with a frequency of 2 to 28 eggs every 2 days. The average duration of the oviposition period was 78.76 ± 13 days. The eggs are brown in colour and turn chocolate a few days before hatching.

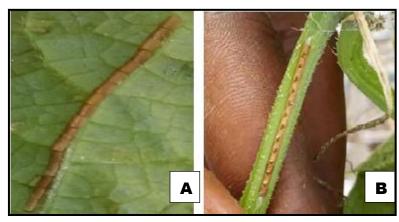


Figure 4: Eggs of *L. membranaceus*. A: One day old eggs, B: 6 day old eggs

3.3 Incubation period and egg fertility rate

Incubation of the eggs lasted 5 to 7 days, with an average period of 6.25 ± 0.45 days. The temperature of the cages varied from 27.4 to 33.1°C with an average of 28.45 ± 0.7 °C. The number of days required for eggs to hatch decreased as the temperature increased. Total of 230 ± 30 eggs laid by one female, 221 ± 23 eggs were hatched, giving an average fertility rate of 96.09 ± 3.22 (Figure 5).



Figure 5: Eclosion of *L. membranaceus* eggs

3.4 Larval survival rate, development time, sex ratio and adult life span

Five larval stages (L1, L2, L3, L4 and L5) were observed. The periods of the larval stages ranged from 2 ± 0.70 days (L1) to 7.1 ± 1.17 days (L5), giving a total larval period of 22.53 ± 1.90 days. Stage 1 larvae were obtained after the eggs had hatched (Figure 6). Larval survival rates varied from 48.83% (L1) to 97.83% (L5). The larva grows from 2 ± 0.5 mm in the L1 stage to 13 ± 1.3 mm in the L5 stage. To change stage, a molt is necessary. The number of larvae decreases from L1 to L3 and then remains almost constant from L3 to L5 (Table). The larvae are attacked by spiders (Arthropods) and several types of ants (Hymenoptera). In addition to these beneficials insects, climatic conditions such as rain and wind also kill the larvae per female, only 44 reached stage 5 and 39 reached adulthood, giving an average larval and overall survival rate of 19.46% (Figure 6). The total development time of L. membranaceus (from egg to adult) was 30.38 ± 1.35 days. The adults obtained were divided into 22 males and 17 females, the sex ratio was 0.77 in favour of the males. The average lifescycle was 85.9 ± 10.31 days for females and 112.4 ± 15.20 days for males.

Larval period (days)	Larvae 1 2,1±0,70	Larvae 2 3,40±0,42	Larvae 3 5,07±1,25	Larvae 4 6,66±1,70	Larvae 5 7,5±1,17
Survival rate (%)	48, 83	66,66	92, 85	92,30	97, 83
Mean length of larvae (mm)	2±0,5	4±0,6	8±0,4	10±0,8	13±1,3

Table: Period length and survival rate of larval stages of *L. membranaceus*.

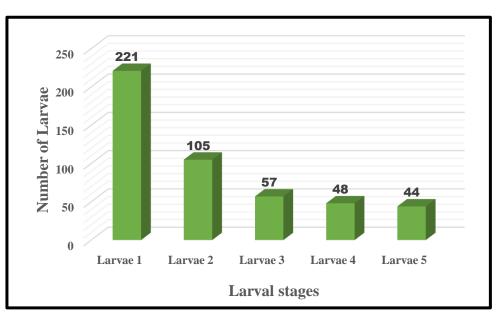


Figure 6: Variation in number of *L. membranaceus* larvae depending on the larval stage

3.5 Impact of L. Membranaceus attacks on cucumber production

The number of fruits of healthy plants is higher than attacked plants. A healthy plant produced an average of 12.33 ± 4.5 fruits compared to 7.93 ± 0.75 for a plant attacked by *L. membranaceus* in the dry season. Analysis of variance showed that there was a significant difference between the number of fruits at the 5% threshold (p=8.431. 10⁻⁰⁹). The loss rate due to repeated attacks by *L. membranaceus* was 64.85%.

IV. Discussion

Our work reveals that the pre-oviposition period is 9 ± 0.66 days. This is different from those observed by [7] in *pseudotheraptus devastans* which is 5.10 ± 0.60 days and by [8] which is 4.10 days obtained in another Coreidae (*Clavigralla tomentosicollis* Stäl.). The female lays an average of 170 ± 30 eggs, which is lower than the rate obtained by [9] with the same insect in the rainy season (180 \pm 35 eggs). The season thus seems to have an influence on the fertility of the females. The average duration of the oviposition period was 78.76 ± 13 days, which is different from 107.56 ± 18 days obtained by [10] in C. lameensis. Eggs are usually laid on the lower surface of leaves and on stems almost as in the female C. lameensis which lays on the lower surface of leaflets [10]). The incubation period of the eggs of L. membranaceus was on average 6.25 ± 0.45 days. This is close to that obtained in the rainy season with the same insect by [9] Similar results were obtained in P. wayi (6 to 9 days) by [11] and in Amblypelta cocophaga (6 to 8 days) by [12]. The average egg fertility rate is 96.09 ± 3.22 in L. membranaceus which is similar to those observed in other Coreidae species such as Leptoglossus gonagra (93.4%) [13] and Veneza stigma (91.66%) [14]. [15] recorded a higher fertility rate in Corecoris dentiventris (99.63%). The lowest larval survival rates were obtained at the 1st and 2nd larval stages. According to [16] the survival of early instar larvae depends on the energy accumulated by the previous stage. For the same authors, the high mortality could be explained by a low capacity to metabolise, for the first time, toxins or non-nutritive compounds contained in the sap collected. Another cause of this mortality could be linked to the high number of L. membranaceus larvae, which could lead to intra-specific competition, following a limitation of trophic resources, as indicated by [17] for Callosobruchus maculatus and [18] for Dinarmus basalis and Eupelmus vuilleti. Concerning the life cycle, five larval stages were recorded in L. membranaceus. The duration of the larval stages varied from 2 ± 0.70 days (L1) to 7.1 ± 1.17 days (L5). Our results agree with those of [11] [8] and [19] who reported respectively that the development of *P. wayi*, *Clavigralla tomentosicollis* and *Amblypelta* bilineata comprises 5 larval stages. In L. membranaceus, the duration of the 5th instar was the longest and the 1st instar the shortest. This observation was made by [20] in the same species and other Coreidae species such as Leptoglossus gonagra [13], Corinocerus sanctus [21], L. zonatus [22], L. fulvicornis [23] and C. tomentosicollis [8] The total duration of larval development of L. membranaceus (from egg to adult) is 30.38 ± 1.35 days. This is within the range defined by [11] for P. wayi (26 to 40 days). The adults obtained were divided into 22 males and 17 females, i.e. a sex ratio of 0.77 in favour of the males. This is contrary to the results obtained by [7] where the sex ratio is 0.87 in favour of females in P. devastans and also contrary to those obtained in Leptoglossus zonatus (0.90) [24], C. dentiventris (0.97) [15] and C. tomentosicollis (0.67) [8] always

in favour of females. Concerning the life span of *L. membranaceus* adults, our observations revealed that males live longer (112.4 \pm 15.20 days) than females (85.9 \pm 10.31 days). These results are in line with those of [7] and [11] who observed a longevity of 136.9 \pm 22.84 days in males; 117.9 \pm 18.42 days in females of *P. devastans* and 84 days in males and 73 days in females of P. wayi respectively. Concerning the impact of *L. membranaceus* attacks on cucumber production, damage was observed at the plant and fruit level. These attacks are responsible for yield losses. These results are similar to those obtained by [25] with sesame where *Asphondylia catalaunalis* was responsible for yield losses ranging from 25 to 100%.

V. Conclusion

The study of the biological parameters showed that *L. membranaceus* is a heterometabolous pest with a developmental cycle of 5 larval stages with low survival rates for stages 1 and 2. Total life cycle duration of this pest was 30.38 ± 1.35 days. Males lived longer than females. A female of L. membranaceus laid an average of 170 ± 30 eggs, under semi-natural conditions. The average egg fertility rate was $96.09 \pm 3.22\%$. The fact that the sex ratio was in favour of males seems to slow down the reproductive potential of the species. The study determined the duration of oviposition, the longevity of the adults and specified that the developmental stages of the insect are all pests of cucumbers but stages 4 and 5 remain the most voracious as the adult. Thus, the knowledge of the development cycle of *L. membranaceus* would allow the implementation of appropriate control methods.

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