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Kodjo Adaba Tano Thierry
University Nangui Abrogoua, UFR-Natural Sciences, Agricultural Entomology Research Unit, Abidjan, Côte d'Ivoire

Kwadjo Koffi Eric
University Nangui Abrogoua, UFR-Natural Sciences, Agricultural Entomology Research Unit, Abidjan, Côte d'Ivoire

Doumbia Mamadou
University Nangui Abrogoua, UFR-Natural Sciences, Agricultural Entomology Research Unit, Abidjan, Côte d'Ivoire

Kra Kouadio Dagobert
University Nangui Abrogoua, UFR-Natural Sciences, Agricultural Entomology Research Unit, Abidjan, Côte d'Ivoire

Corresponding Author:
Kodjo Adaba Tano Thierry
University Nangui Abrogoua, UFR-Natural Sciences, Agricultural Entomology Research Unit, Abidjan, Côte d'Ivoire

Life history in *Nedotepa curta* (Homoptera: Cicadellidae) carrying the phytoplasma responsible for coconut lethal yellowing disease in Côte d'Ivoire

Kodjo Adaba Tano Thierry, Kwadjo Koffi Eric, Doumbia Mamadou and Kra Kouadio Dagobert

Abstract
Coconut (*Cocos nucifera*) cultivation, the main coastal crop in Côte d'Ivoire, is compromised by the coconut lethal yellowing disease. *Nedotepa curta* (Homoptera: Cicadellidae) carries the phytoplasma responsible for this disease. This study focused on the life history of this potential vector and the seasonal impact on some of its biological parameters in Grand-Lahou. Individuals of *N. curta* were placed in rearing units made by mosquito nets, set on some coconut palms leaflets and followed until the females laid egg. Emerged larvae were followed until adult stage, at each season of the year. As a result, the average number of eggs laid was 26.95 ± 1.20 eggs per batch. Eggs hatch on average 9.65 ± 0.67 days after laying. The total duration of larval development, which occurs in five stages, was 23.41 ± 2.72 days on average, with the duration of stage 1 and stage 5, respectively the shortest (3.75 ± 0.79 days) and the longest (6.65 ± 0.78 days). The larval survival rate is higher than 90% for all stages of development. Dry seasons induce reduction of embryonic and larval development, while promoting an increase in the hatching rate.

Keywords: *Nedotepa curta*, life history, coconut lethal yellowing disease, Côte d'Ivoire

1. Introduction
The coconut tree (*Cocos nucifera* L.), native to the Indo-Atlantic and Pacific basins is a perennial oleaginous plant grown in more than 90 countries. It plays a vital socio-economic role throughout the intertropical wetland, providing cash income to millions of small farmers. It is also known for its water (liquid albumen) and its milk with unique nutritional characteristics [1]. Large farms are located in Southeast Asia (Indonesia, Malaysia), West Africa (Côte d'Ivoire, Ghana) and East Africa (Mozambique) [2]. In Côte d'Ivoire, the coconut palm is an important coastal crop [3]. According to statistics provided by the [4], village and industrial plantations occupy respectively 60% and 40% of coconut cultivated areas in Côte d'Ivoire. Unfortunately, coconut growing in Côte d'Ivoire is threatened by the lethal yellowing disease [5].

This disease has devastated more than 4,000 ha of coconut orchards in Côte d'Ivoire and threatens more than 7,000 ha of village coconut plantations, a source of income for 85,000 small coconut farmers in Grand-Lahou [6]. The lethal yellowing disease of the coconut is a systemic disease associated with phytoplasmas belonging to group IV, whose propagation is generally done by phloem feeding insects of the family Cicadellidae (Homoptera) [7,8,9,10]. The molecular analysis of some Cicadellidae individuals, collected on infested coconut tree in Grand-Lahou, made it possible to identify *Nedotepa curta* Dmitriev as a carrier of the phytoplasme responsible Côte d'Ivoire lethal yellowing disease (CILY) of the coconut and therefore suspected to be a probable vector of this disease [11]. Previous work on this insect focused on the morphological description of the adult [12]. However, the knowledge of its biology is a prerequisite to understand its role in the dissemination of CILY. Thus, the present work, which is part of the preliminary studies to facilitate the understanding of the epidemiology of this coconut disease, aims to study the biology of *N. curta*. The data obtained is also intended to increase knowledge and provide baseline data for future studies on control of this Cicadellidae.

2. Materials and Methods

2.1. Study site

The study was carried out on Avikam Island, in Grand-Lahou Department (5°08'07 north latitude, -5°01'26 west longitude). Grand-Lahou belongs to the Great Bridges region, in the south of Côte d'Ivoire and is located at 140 km from Abidjan. It is a coastal town at the mouth of the Bandama River (Fig. 1). The climate, of equatorial type, is characterized by four seasons: a great rainy season (April to mid-July); a short dry season (mid-July to September); a small rainy season (October to November) and a long dry season (December to March) [13].

The vegetation is marked by a great diversity dominated by three vegetation formations that are mangroves, forest and coconut orchards [14].

2.2. Rearing of *N. curta*

The establishment of the insect rearing began with an observation phase of hatching and the monitoring of larval development in nature. Adults from that first step were captured with a pear-like aspirator (Fig. 2) and stored in screw cap tubes (Fig. 3).

About fifteen individuals, mostly females, were introduced into each rearing unit for observations under natural conditions. The rearing unit consisted of a cylindrical whitish sleeve (20 cm of diameter) made with mosquito net (Fig. 4). Both ends were left open to allow the passage of the preselected and cleaned palm leaflets. Both ends were subsequently closed with a rubber band. An opening is made on the lateral face of the cylindrical sleeve for observations and closed with velcro. The observations were made daily, from 7:00 am to 9:00 am and from 4:00 pm to 6:00 pm, using a pocket magnifier (Fig. 5).

This study was conducted for a whole year, under natural conditions, with five repetitions each season of the year (annual temperature between 26 °C and 32 °C with an average of 28 °C; annual relative humidity varying from 80% to 91% with an average of 85%). A dozen of heaps of eggs were observed each season. The average temperature and relative humidity of each season were assessed from records made at each observation, using a thermo-hygrometer. These climatic data made it possible to assess the influence of the seasons on embryonic and post-embryonic development in *N. curta*.

2.3. Development cycle and some biological parameters in *N. curta*

The following biological parameters of *N. curta* were determined: the average number of eggs per batch (fecundity), the hatching rate of the eggs (fertility), the incubation time of eggs, the duration of development of larval stages, the survival rate of the different larval stages, the sex ratio.

2.3.1. Average number of eggs per batch, egg incubation time, and average hatching rate (fertility)

The average number of eggs per batch (Ne) is the average number of eggs constituting the batches. It was obtained by dividing the total number of eggs by the number of batch.

$$Ne = \frac{\sum n_i}{a}$$

n_i: number of eggs for a given batch i; α: number of observed egg batches

Egg laying date (Ld) and egg hatching date (Hd) were recorded for each batch. The incubation time (I_i) of the eggs for a given batch i, expressed in days, was determined from the formula:

$$I_i = Ld - Hd$$

The mean incubation time (It) was also determined from the following formula:

$$It = \frac{\sum I_i}{a}$$

I_i: incubation time for a given batch i; α: number of egg batches observed.

The number of eggs laid and the number of eggs hatched were recorded. The hatching rate (Hr) was obtained according to the following formula:

$$Hr = \frac{The\ number\ of\ eggs\ hatched\ from\ the\ batch}{number\ of\ eggs\ in\ the\ batch} \times 100$$

2.3.2. Duration of different larval stages and the development cycle

Larvae from egg hatch were followed to adult stage. The date of each moult was recorded and the duration of each larval stage (Dls) was calculated and expressed in days.

$$Dls = dm_x - dm_{x-1}$$

dm_x: day of moulting for a given stage x; dm_{x-1}: day of the previous moult

The duration of the larval development cycle, which is the period from the emergence of the larva from the egg to the emergence of the adult after the fledging, has been determined.

2.3.3. Larval survival rate and sex ratio

The larval survival rate (Ls) was obtained from the number of survivors after each stage.

$$Ls = \frac{n_x}{n_{x-1}} \times 100$$

n_x: number of individuals at stage x; n_{x-1}: number of individuals at stage before stage x.

As for the sex ratio, it was obtained according to the formula:

$$Sex - ratio = \frac{number\ of\ males}{number\ of\ females}$$

2.4. Statistical analysis

The data processing was carried out using the STATISTICA software version 7.1. The Levene test allowed verifying the homogeneity of the variances. Then, means were compared through a one-way analysis of variance (ANOVA 1) followed by the Fisher test at 5% threshold for the homogeneity of the variants.

3. Results

3.1. Average number of eggs per batch, egg incubation time, and average egg hatch rate of a batch

Females laid an average of 26.95 ± 1.20 eggs per batch. The

average number of eggs hatched per batch is 22.84 ± 5.49, for an overall hatching rate of 84.09 ± 6.83%. Egg incubation lasted on average 9.65 ± 0.67 days (Table I).

3.2. Duration of larval stages and development cycle

The development cycle observed has involved 5 successive larval stages. The duration of the development cycle (stage 1 at the emergence of the adult) is 23.41 ± 2.72 days. The fifth stage is significantly longer (6.65 ± 0.78 days) and the shortest are the first two stages (3.75 ± 0.79 days for stage 1 and 4.01 ± 0.72 days for stage 2) (Table 1).

3.3. Larval survival rate and sex ratio in *N. curta*

The larval survival rate in *N. curta* ranged from 93.03 ± 2.22% (stage 5) to 97.14 ± 1.69% (stage 3). From 491 individuals in the first stage, 387 were able to reach the adult stage, representing an overall survival rate of 78.82 ± 3.97% (Table 2). The adults obtained are divided into 173 males and 214 females, a sex ratio of 0.79 for females.

3.4. Seasonal incidence on incubation duration and egg outbreak rate in *N. curta*

The seasons have had a direct effect on both the embryonic development time and the hatching rate. The hatching rate of the egg during the dry seasons was higher than that observed during the rainy seasons. It was higher and significantly different in the long dry season (94.63 ± 2.52%). The embryonic development was shortest during the long dry season (9.15 ± 0.45 days) while it was significantly longer during the long rainy season (10.75 ± 0.34 days) (Table 3).

3.5. Seasonal impact on post-embryonic development in *N. curta*

The duration of each stage during the four seasons allowed appreciating the influence of the different seasons on the post-embryonic development in *N. curta*. During the long dry season, the duration of the first larval stage at emergence of the adult was the shortest with an average of 21.39 ± 1.14 days. On the other hand, the duration of the first larval stage at the emergence of the longest adult is observed during the long rainy season with an average of 26.43 ± 1.82 days. In all seasons, the fifth stage was significantly longer than the others and the shortest stages were the first and second. Dry seasons with high temperatures generally result in a decrease in the larval development time and thus in the complete life cycle (Table 4).

4. Discussion

The study focused on the biology of *Nedotepa curta* under semi-controlled natural conditions. The life cycle took place in three stages of development, namely the egg, the nymph and the adult. The eggs were laid in batches, with an average 26.95 ± 1.20 eggs per batch. The embryonic development lasted on average 9.65 ± 0.67 days with an average hatching rate of 84.09 ± 6.83%. Various authors who have worked on Cicadellidae have noted a trend similar to what was observed in this study. In fact, the Cicadellidae species *Homalodisca coagulata* (Say) and *Scaphoideus titanus* Ball, that lay around 20 eggs per batch, have a hatching rate above 80% [15-17]. The Cicadellidae *Empoasca vitis* (Göthe) has a maximum embryonic development of 10 days [18].

Observations made during post-embryonic development in *N. curta* have shown that this is a heterometabolic development. Indeed, from hatching to adults, we observed successively five exuviae that are evidence of the passage from one stage to another and a gradual and incomplete metamorphosis between the nymph and the adult. Thus, *N. curta* develops in 5 larval stages whose shortest duration is the first stage and the longest is the fifth stage. Different species of Cicadellidae also presented five larval stages [19] with the fifth as the longest stage [20, 21]. The long duration of the last larval stage could be the result of morphological, anatomical and physiological transformations leading to the imago. This argumentation was also proposed by [22], who reported that differentiation of internal reproductive organs and completion of wing development would be the reasons for the long duration of the 5th instar in Hemiptera. The larval development time obtained averages 23.41 ± 2.72 days, which shows that larval development is relatively short which could explain the polyvoltinous nature of the insect allowing several generations to overlap [23].

The larval stage survival rate is greater than 90% in *N. curta*. Its increasing evolution from the first stage could be due to the aggregative behavior of the larvae. In fact, the larvae are grouped together when they emerge from the egg and this offers them advantages such as the modification of micro environmental conditions. Some authors have shown that in high density conditions, Diptera and Lepidoptera larvae benefit from better regulation of their body temperature and water loss than isolated larvae, which increases the survival rate [24, 25]. This aggregative behavior of the larvae is also a more effective exploitation strategy to counteract the toxins or non-nutritive compounds contained in the punctured sap [26], which are fatal for the early instar larvae, with a low ability to metabolize these elements contained in the sap [27]. The high mortality observed after the third stage is linked to the large number of larvae in the same area, which would lead to intraspecific competition [28]. This competition would be unfavorable to some larvae, because the survival of the larvae of a stage would depend on the energy accumulated by the previous stage [27].

The sex ratio obtained in *N. curta* (0.79) was in favor of females. This would reflect the high reproductive potential of this insect. This trend has also been reported in the Cicadellidae *Zygnidia sohrab* Zachvatkin [30]. Abiotic factors play a vital role in the development, survival and reproduction of insects [31, 32]. In fact, temperature and relative humidity appeared as determining factors, the variations of which lead to important changes in developmental parameters in *N. curta*. This study revealed that fecundity, embryonic development, egg hatch rate, and postembryonic development appear to be affected by temperature and humidity changes in different seasons. [33] also state that temperature plays an important role in several phases of the insect life cycle. Thus, embryonic and postembryonic developments are reduced during dry seasons when temperatures are high. In addition, fertility and hatching rate increase under these same conditions. In fact, insects are ectothermic organisms (poikilotherms) and thus have a very weak capacity to regulate their body temperature, so that ambient temperature determines all biological activities [34]. This results in significant changes in their development, survival, reproduction, and behavior [35-37]. In addition, [38] has shown that a rise in temperature leads to an increase in metabolic activity, which would result in an increase in reproduction and in some cases in the number of generations per year. As for hygrometry, the embryonic, larval and cycle durations seem to be reduced to low humidities. This reduction could be due to an acceleration of physio-

biochemical processes during embryonic and larval development. Similarly, low hygrometry values could favor the moulting phenomenon, allowing the insect's integument to release more easily under body pressure [39]. Temperature and hygrometry being inverse functions in nature, these two parameters could influence each other in coconut orchards, preventing the dissection of eggs.

Table 1: Average duration of different stages of development in *Nedotepa curta*

Stages of development	Average duration ± standard deviation (days)	p
Incubation	9,65 ± 0,67	
Stage 1	3,75 ± 0,79a	
Stage 2	4,01 ± 0,72a	
Stage 3	4,72 ± 0,55b	0,001
Stage 4	4,28 ± 0,51ab	
Stage 5	6,65 ± 0,78c	
Stage 1 at the emergence of the adult	23,41 ± 2,72	

Values in the same column followed by the same letter do not differ significantly from each other at $P \leq 0.05$ (ANOVA 1 and Fisher's Test)

Table 2: Survival rate of the different larval stages in *Nedotepa curta*

Stages of development	Number of larvae		Larval survival rate ± standard deviation (%)
	Initial	Dead	
Stage 1	491	21	95,72 ± 1,23
Stage 2	470	15	96,81 ± 1,85
Stage 3	455	13	97,14 ± 1,69
Stage 4	442	26	94,12 ± 2,39
Stage 5	416	29	93,02 ± 2,22
Total	491	104	78,82 ± 3,97

Table 3: Embryonic development time and hatching rate of eggs at different seasons in *Nedotepa curta*

Seasons [Temperature °C – Relative humidity %]	Average number of eggs ± standard deviation		Incubation time ± standard deviation (days)	Hatching rate ± standard deviation (%)
	Laid	Hatched		
Long dry season [31 °C- 84%]	166,32 ± 16,87a	160,87 ± 18,85a	9,15 ± 0,45a	94,63 ± 2,52a
Short dry season [30 °C – 86%]	160,67 ± 21,63a	148,68 ± 23,46a	9,33 ± 0,55ab	91,28 ± 3,17b
Short rainy season [29 °C – 87%]	143,81 ± 17,58b	130,36 ± 10,75b	10,02 ± 0,71b	89,51 ± 2,48b
Long rainy season [27 °C – 89%]	132,93 ± 12,25b	80,38 ± 15,34b	10,75 ± 0,34c	60,44 ± 1,93c

Values in the same column followed by the same letter do not differ significantly from each other at $P \leq 0.05$ (ANOVA 1 and Fisher's Test)

Table 4: Average duration of postembryonic development at different seasons at *Nedotepa curta*

Seasons [Temperature °C – Relative humidity %]	Average duration of development of the larval stages ± standard deviation (days)					
	Stage1	Stage2	Stage3	Stage4	Stage5	Stage 1 to adult emergence
Long dry season [31 °C – 84%]	3,10 ± 0,11a	3,58 ± 0,65a	4,57 ± 0,53a	4,02 ± 0,13a	6,12 ± 0,45a	21,39 ± 1,14a
Short dry season [30 °C – 86%]	3,44 ± 0,57a	3,70 ± 0,84ab	4,78 ± 0,45a	4,22 ± 0,44a	6,32 ± 0,58a	22,46 ± 1,81ab
Short rainy season [29 °C – 87%]	4,20 ± 0,84b	4,00 ± 0,71ab	5,02 ± 0,68a	4,60 ± 0,45b	6,78 ± 0,84ab	24,60 ± 2,68bc
Long rainy season [27 °C – 89%]	4,29 ± 0,55b	4,65 ± 0,51a	5,21 ± 0,34a	4,76 ± 0,47b	7,53 ± 0,24b	26,43 ± 1,82c

Values in the same column followed by the same letter do not differ significantly from each other at $P \leq 0.05$ (ANOVA 1 and Fisher's Test)

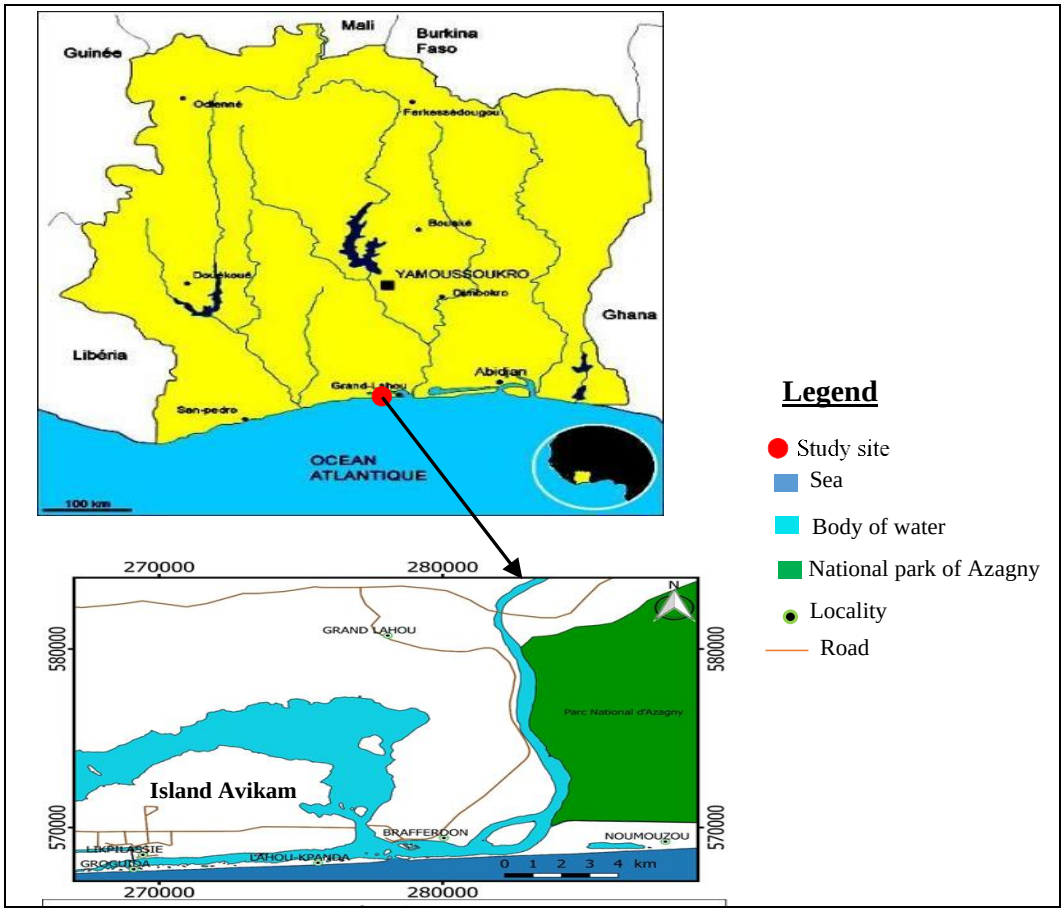

Fig 1: Geographical location of the study site

Fig 2: Pear-like aspirator

Fig 4: Rearing unit for *Nedotepa curta*

Fig 3: Screw cap tube containing individuals of *Nedotepa curta*

Fig 5: Pocket magnifier

5. Conclusion

The study of biological parameters showed that *N. curta* female lays an average of 26.95 ± 1.20 eggs per batch. The hatching rate is 84.09 ± 6.83%, after an embryonic development of 9.45 ± 0.67 days. The Cicadellidae *N. curta* is a heterometabolic insect involving 5 larval stages and showing high rates of larval survival. Dry seasons reduce embryonic and larval development by increasing fecundity and hatching rate. These new data on this pest must be taken into account in the development of control methods.

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