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Effect of egg laying substrate on reproduction of rhynchophorus phoenicis fabricius, 1801 (coleoptera: dryophthoridae), oil palm weevil, an insect of food interest in Côte D'ivoire

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Abstract
For the domestication of *Rhynchophorus phoenicis* to increase its availability, the objective of this study is to find alternative and accessible substrates for its rearing. In the laboratory, under 27.86 ± 0.5 °C and 74.15 ± 2.2% relative humidity, upon emergence, the imagos were put on palm trunk, sugarcane and banana stem. The development cycle was followed until their death. In no-choice experiments, fertility was 87, 91, and 56% respectively on palm trunk, sugarcane and banana stem. In choice experiments, fertility was 89 ± 1.2% on sugarcane, 83 ± 1.4% on palm trunk and 30 ± 0.9% on banana stem. The larval development duration was 19.38 ± 1.46 days on the palm trunk, 24.86 ± 0.7 days on sugarcane and 44.52 ± 1.01 days on the banana stem. The total cycle time was 119.12 ± 3.1 days on the banana stem, 107 ± 2.41 days on the sugarcane and 104.31 ± 2 days on the palm trunk. A significant difference was observed in the differents value ($P < 0.05$). Apart from the palm trunk, sugarcane is alternative substrate for the breeding of *R. phoenicis* although the banana stem also gives satisfying results.

Keywords: *R. phoenicis*, rearing, palm trunk, sugarcane stem, banana stem

Introduction
According to the United Nations Food and Agriculture Organization (FAO), the world's population is expected to reach nine billion by 2050, which will require twice as much food as today to meet on demand (FAO, 2014) [4] protein of animal origin (Yen, 2009) [18]. FAO has designated entomophagy (insect consumption) as part of the solution to this problem (Lavalette, 2013) [6]. In fact, insect farming, which consumes less energy and is more respectful of the environment, appears is many away as an alternative to traditional farms, while also providing an excellent source of protein (Van Huis, 2013) [17]. Thus, the potential of industrial insect farming on a global scale is proving to be most interesting. Entomo Farms is currently the largest insect farm in North America with annual production of 900 million (Medhavi, 2017) [8]. In Côte d'Ivoire, the larvae of the weevil are very popular with the population. However, consumers are faced with problems of availability due to the scarcity of palms that host them, but also to the use of pesticides that reduce the amount of larvae expected in the trunk of these plants. All of these factors result in high prices of larvae in the markets. To answer these difficulties, one of the solutions would be the establishment of a system of breeding on alternative substrates less expensive and available locally. The general objective of this study is to analyze the breeding possibilities of *Rhynchophorus phoenicis* by using the stem of sugarcane and banana. The ultimate goal is the mass production of *R. phoenicis* for food and feed. It is a form of valorization of biodiversity, but also of food production with less economical and ecological cost.

2. Material and Methods
• Animal and plant material
The animal material is the palm weevil *R. phoenicis*, Fabricius (1801) (Coleoptera, Dryophthoridae). Its larvae are commonly called palm caterpillars in Côte d'Ivoire. The plant material consists of three species of plants grown in Côte d'Ivoire (Figure 1): the oil palm *Elaeis guineensis* (Arecales: Arecaceae) which is the main host plant of the caterpillar palm, the stalk of sugarcane *Saccharum officinarum* (Poales: Poaceae) and the banana stem *Musa paradisiaca* (Zingiberales: Musaceae). The choice of these plant is justified by of good breeding support in Ghana and Kisangani on species of the genus *Rhynchophorus* (Oladiopo, 1996; Monzenga, 2015) [13, 9].

Fig 1: Plant biological material

A: slice of palm stipe B: slice of sugarcane; C: plantain banana stem.

▪ Methods
In the laboratory, the cocoons were individually incubated in cylindrical and parallelepipedic, plastic boxes of dimensions 18 cm x 8 cm x 12 cm which were transparent, perforated for aeration. A reference number assigned to each box containing the cocoons, making it possible to note the release date of the adults. Upon emergence, adults were separated by sex according to the external characteristics of the rostrum. They then paired and fed with palm trunk, sugarcane stem, and banana stem. Two kind of experiments tested: no-choice and choice experiments. In the first test, only one substrate or plant was given to the pair of *R. phoencis*. While in the second test, the couples were put in

the presence of the three substrates at the same time. The parameters studied fertility, the duration of development of the egg to the adult, and the life span of adults. The plastic boxes were kept under laboratory conditions at 27.86 ± 0.5 °C and 74.15 ± 2.2% relative humidity.

No-choice experiment

Newly emerged adults were introduced in pairs (one male and one female) into 279 x 170 x 15 cm dishes containing three 10 cm x 5 cm portions of the same substrate. For this test, thirty repetitions were performed. The pairs remained in contact with the substrates for 48 hours to allow the female to lay the maximum number of eggs. Then, these were removed and the eggs laid on each support were counted.

Choice experiment

The procedure was the same as in the no-choice experiment, except that the three different substrates were arranged in the same box.

The parameters studied in both tests were: fecundity, fertility, larval development time, emergence rate, sex ratio, longevity and the duration of the development cycle. They were determined as follows:

Fecundity: forty-eight hours after laying, each pair is transferred to a new box with the same characteristics as the first. The total number of eggs deposited per female on each substrate has been determined and the fertility (F) which defines the average number of eggs laid by a female during her lifetime has been calculated according to the formula:

$$\mathbf{F} = \frac{\sum \text{number of eggs laid}}{\sum \text{number of females}}$$

Fertility: The eggs laid on the various substrates are left in incubation for 3 to 6 days in transparent boxes with perforated lid, useful for the renewal of the area and to avoid molds. These boxes were monitored daily until the egg hatch was observed. The number of hatched eggs per day was noted until there was no hatching. Then, the average fertility, which is the ratio expressed as a percentage of the number of eggs hatched to the total number of eggs laid, was determined:

$$\text{Average fertility rate} = \frac{\sum(\text{number of eggs hatched})\text{fi}}{\sum(\text{number of eggs laid})\ \text{fi}} \text{x } 100$$

fi: effective females

Larval development time is the time from hatching of eggs to that of the last inster larva. To determine this duration, three batches of thirty stage 1- and 24-hour old larvae were trained. The first batch of larva was fed with palm trunk, the second batch with sugarcane and the third with banana stem. For each batch, the larvae were placed in 18 x 24 x 16 cm transparent plastic boxes with a perforated lid. These boxes were followed until the last instar larva was obtained. Then the mean larval development time was determined for the larvae of each lot. The food source was renewed every two days.

$$\text{DL} = \frac{\sum Je - Jds}{\sum Hi}$$

Je: hatching day; Jds: date of last instar larva; Hi: number of late instar larvae.

Larval survival (Si): the boxes containing the different batches of larvae were monitored daily to record the moulting date of each larva by observing the exuviae. An enumeration of the larvae from one stage to the next was done to determine the larval survival rate (Si). The measurements of the cephalic capsule and the size of each larval stage were made using calipers.

$$\text{Si} = \frac{\sum number\ of\ larvae\ of\ the\ next\ stage)\ fi}{\sum number\ of\ larvae\ of\ the\ previous\ stage)\ fi)} \times 100$$

fi: number of females

Emergence rate (Te), which represents the number of imagos emerged (ni) on the average number of eggs laid (no) as well as the sex ratio, which represents the percentage of emerged males on that of emerged females, were determined. For the descendants of each female, according to the formulas:

$$\text{Te } (\%) = \frac{\sum \text{nifi}}{\sum \text{nofi}} \times 100$$

$$\text{Sex} - \text{ratio } (\%) = \frac{\sum \text{number of males}}{\sum \text{number of females}} \times 100$$

Longevity of adults: is translated as the time between the date of the emergence (Je) of the imago and that of its death (Jm). The average lifespan of females and males from emergence to death used to monitor fertility were calculated.

$$\text{Average adult longevity (days)} = \frac{\sum(\text{Jm} - \text{Je})\text{vi}}{\sum \text{vi}} \times 100$$

Vi: number of males or females

Average length of development cycle: is defined as the time between Oviposition of eggs in the adult stage. It groups the incubation time of eggs (Di), larval development time (Dl) and pupal development (N). To determine the average duration of the cycle, the hatched eggs were followed until the emergence of the imago.

$$\text{Average duration of the development cycle (days)} = \frac{\sum JiKi}{\sum Ki} \times 100$$

Ji = Di + Dl + Dn; Ki: number of adults

Data analysis

The analysed variables for each susbtrate, reported as mean values (± SEM), were singly compared by one-way analysis of variance (ANOVA). Then, Pearson's correlation test was used to check for relationships between longevity and fecundity of females, regardless of three kind of substrate used. All statistical analysis were performed with Statistica 7.1 software at the 5 % threshold

II-Results

Fecundity and fertility of *R. phoenicis* in no-choice experiment

In no-choice experiment, the average pre-mating period was 2.56 ± 1.32; 3.08 ± 0.5 and 3.35 ± 0.77 days respectively on

the palm trunk, sugarcane, and banana stem. No significant difference was observed between the periods of precopulation (P> 0.05) on the three rearing supports (Table I). A similar observation was made between the periods of preoviposition of females (P > 0.05). These were respectively 3.2 ± 0.4 ; 4 ± 0.8 and 3.6 ± 0.9 days on palm trunk, sugarcane and banana stem. The average number of eggs laid per day and per female was 12.4 ± 0.9 eggs on the palm trunk. On the sugarcane stem, the daily Oviposition rate was 14.89 ± 0.4 eggs / day / female. On the banana stem, this rate was 11.4 ± 0.1 eggs / day / female. Analysis of variance revealed a relationship (P < 0.05) between the type of substrate and the number of eggs deposited per day by females.

Table 1: Pre-mating, pre-oviposition duration and daily fecundity in no-choice experiment

Substrat	Pre-mating (days)	Pre-oviposition (days)	Daily fecundity (eggs)
Palm stipe	2,56 ± 1,32	3,2 ± 0,4	12,4 ± 0,9
Sugarcane	3,08 ± 0,5	3,6 ± 0,9	14,89 ± 0,4
Banana stem	3,35 ± 0,77	4 ± 0,8	11,2 ± 1,85
P	0,0612	0,4500	0,00234

In no-choice experiment, 761.66 ± 9 eggs were laid on all the substrates tested. Females of *R. phoenicis* lay more eggs (289.6 ± 2.8 eggs) on sugarcane than on palm trunk (250.3 ± 3.2 eggs) and banana stem (187 ± 1.41 eggs); respectively 40,36 and 24 % of the total eggs laid on the three substrates (Figure 2). Egg hatch rates for no-choice eggs were 87 ± 1,4; 91 ± 1,2 % and 56 ± 0,9 respectively on palm trunk, sugarcane, and banana stem. Significant differences were noted between hatching rate of eggs on all three substrates (F = 48, 25, P < 0.05) (Figure 3).

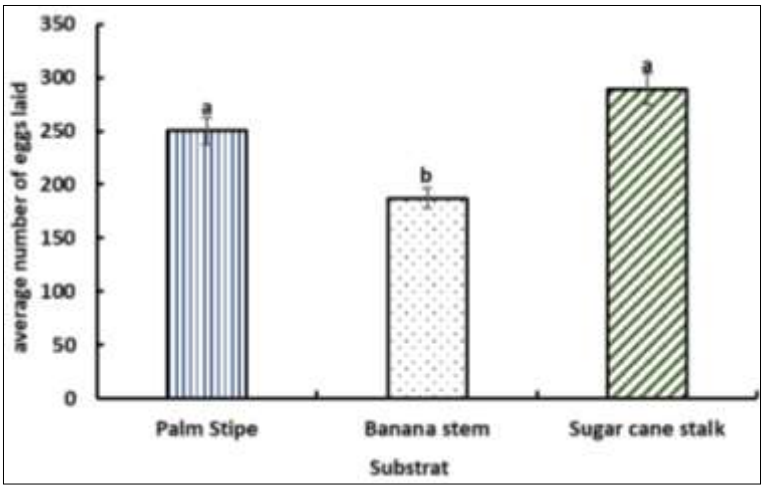

Fig 2: Fecundity of females on the three substrates in no-choice experiment

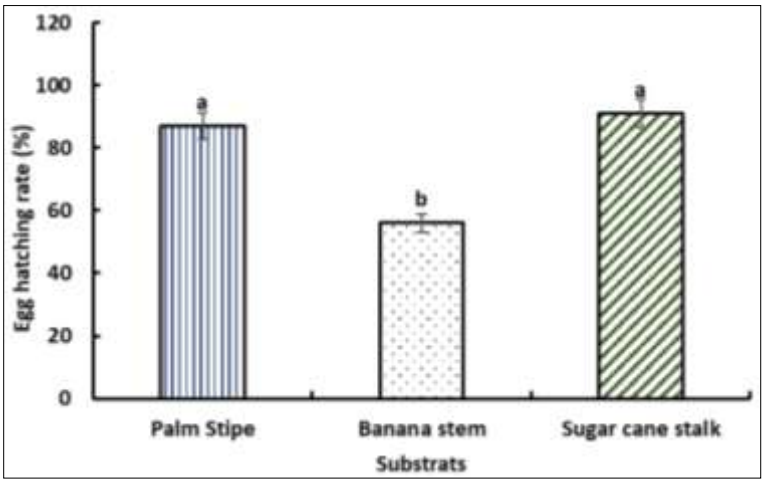

Fig 3: Egg hatching rate on each substrate in no-choice experiment

Fecundity and fertility of *R. phoenicis* in choice experiment

In this test, first mating were observed between the second and fourth day on the banana stem, an average of 3.2 ± 0.8 days. On the sugarcane stem and on the palm trunk, the insects mated from day 1 to day 4, with mean periods of 2.79 ± 0.9 and 2.06 ± 0.58 days, respectively (Table II). The first eggs were laid between the second and third day on the palm trunk, an average pre-oviposition period of 3.42 ± 0.2 days. On the banana and sugarcane stems, these durations were respectively 5.51 ± 0.61 and 4.31 ± 0.55 days. Significant differences (p = 0.00234, P <0.05) were observed between mating time and females oviposition on each substrate. On the palm trunk, females laid an average

of 15.11 ± 3.64 eggs per day. On the banana stem, daily fecundity was 10.22 ± 2.83 eggs. On sugarcane stem, the daily fecundity was 12.05 ± 2.89 eggs (Table II). Daily fecundity of each female significantly varied with the kind of the substrate (F = 245.21, P <0.05).

Table 2: Pre-mating, pre-oviposition duration and daily fecundity in choice experiment

Substrats	Pre-mating (days)	Pre-oviposition (days)	Daily fecundity (eggs)
Palm Stipe	2,06 ± 0,58	3,42 ± 0,2	15,11 ± 3,64
Sugarcane stem	2,79 ± 0,9	4,31 ± 0,55	10,22 ± 2,83
Banana stem	3,2 ± 0,8	5,51 ± 0,61	12,05 ± 2,89
P	0,1329	0,0735	0,0321

A total of 1525 ± 2.21 eggs were laid by *R. phoenicis* females under substrate selection conditions. Egg distribution varied significantly with plant species (P <0.05). In choice experiments, *R. phoenicis* females deposited 581 ± 1.33% of total eggs on the sugarcane stem and 510 ± 1.76 % of the eggs on the palm trunk (Figure 4). The second trend is observed on the banana stem, which received 412 ± 2.52 eggs. As for egg fertility, it was 83 ± 1.4%, 89 ± 1.2%, and 30 ± 0.9 % respectively on palm trunk sugarcane, and banana stem (Figure 5). These levels were significantly different (F = 67.15, P <0.001) on the three substrates. In a no-choice experiment, females showed a lower laying than those with the possibility of choosing their nesting site.

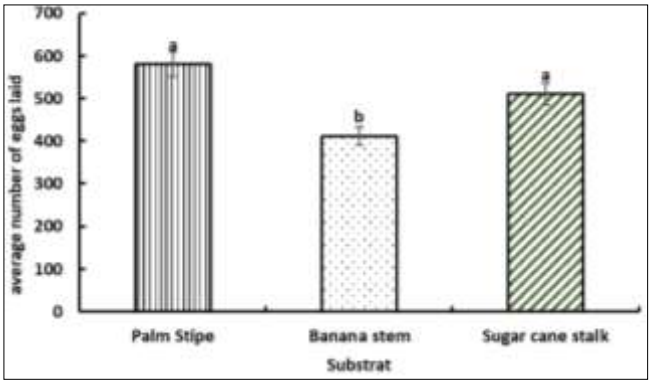

Fig 4: Fecundity of R. phoenicis females in a choice experiment

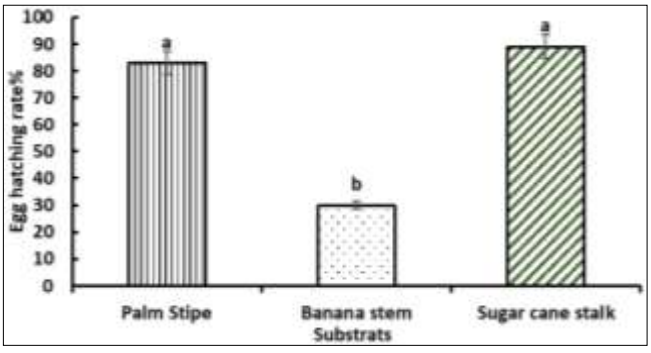

Fig 5: Egg hatching rate on each substrate in a choice experiment

Development time and longevity of R. phoenicis

On the banana stem, larval development was longer than on the other two substrates (44.52 ± 1.01 days). Regarding the duration of pupal development, they were 18.51 ± 1.3; 20.08 ± 0.9 and 32.12 ± 0.8 days respectively on palm trunk, sugarcane and banana stem (Table III). The average first-to-sixth instar survival rates for each substrate ranged from 35% to 67 % for larvae fed on banana stems. As for those grown on palm trunk and sugarcane stems, these values ranged between 47% and 85% and between 50 and 88% respectively (Table IV). Mean survival rates were statistically different from one substrate to another (P < 0.05).

Table 3: Larval and pupal developmental duration of *R. phoenicis* on different substrates

Substrates	Larvalduration (day)	Pupal duration (day)
Palm stipe	19,38 ± 1,46	18,51±1,3
Sugarcane	24,86 ±0,7	20,08 ± 0,9
Banana stem	44,52 ± 1,01	32,12 ± 0,8
F	195,3784	38,4001
P	P < 0,001	P < 0,001

Table 4: Larval monitoring rates on different rearing substrates

	Survival rate (%) per substrate		
Larval stage	Palm stipe	Banana stem	Sugarcane
L1	78,48	35,6	78,46
L2	58,23	49,15	64,6
L3	46,72	44,6	49,15
L4	69,57	56,32	68,2
L5	80,1	65,4	82,4
L6	85	66,32	87,5

L1: first instar larval; L2: second instar larval; L3: third instar larval; L4: fourth instar larval; L5: fifth instar larval; L6: six instars larval

Significant difference was observed in the emergence rate (F = 58.14, p <0.05) of adults on all three substrates. The emergence rates were in increasing order of 53.4 ± 1.2% (on the palm trunk), 59.4 ± 0 58% (on sugarcane) and 45.18 ± 0.41% (on the banana stem,). The emergence rate of males was 42, ± 0.78%, 39.4 ± 0.66% and 31 ± 0.88% respectively on the sugarcane, the palm trunk and the banana stem. Significant difference (F = 78.12, P <0.05) was observed in the longevity of males and females in the presence of the three substrates. The life span of males fed on palm trunk averaged 69.01 ± 1.8 days. On the sugarcane, males achieved an average life of 67.12 ± 3.1 days. The banana stem recorded average lifetimes of 70 ± 2.4 days. As for females, their average life span was 69 ± 2.4 days on sugarcane. For females reared on the banana stem, this duration averaged 63 ± 2.1 days. For those grown on palm trunk, longevity was 55.8 ± 1.6 days. The duration of the development cycle was 119.12 ± 3.1 days on the banana stem, 107 ± 2.41 days on the sugarcane and 104.31 ± 2 days on the palm trunk. The emergence rate was higher on sugarcane than on the other two substrates.

Oviposition duration of *R. phoenicis* according to the age of the females

Pearson's linear correlation tests showed a negative correlation between longevity and fecundity of females,

regardless of the type of substrate used (Figure 6). Longevity of females was strongly influenced by the number of eggs laid. The higher the number of eggs laid, the shorter the life of the female. It appears that oviposition on the stipe begins around the first and second day of emergence of the female (Figure 6). The laying rate peaks between the seventh and eighth days. From the ninth day, it drops to zero at the 22nd day. Oviposition is much lower on the banana stem with a maximum around the sixth day. It drops and vanishes on the 18th day. On sugarcane, oviposition begins as early as for palm trunk, then increases to a first peak on the eighth day. It begins a decline between the 9th and the 11th day. Then, from the 12th day a second peak is observed then the laying stops at the 26th day. There is a statistical relationship between female oviposition durations and substrate type ($P < 0.05$). On palm trunks, banana stems and sugarcane, the average oviposition time is 29 ± 3.1 days, 19 ± 2.1 days and 25 ± 2.8 days, respectively. This average duration was longer on the palm trunk than on the banana stem and sugarcane. However, comparing the banana stem with females' egg-laying time was longer on the sugarcane than on the banana stem (Figure 7).

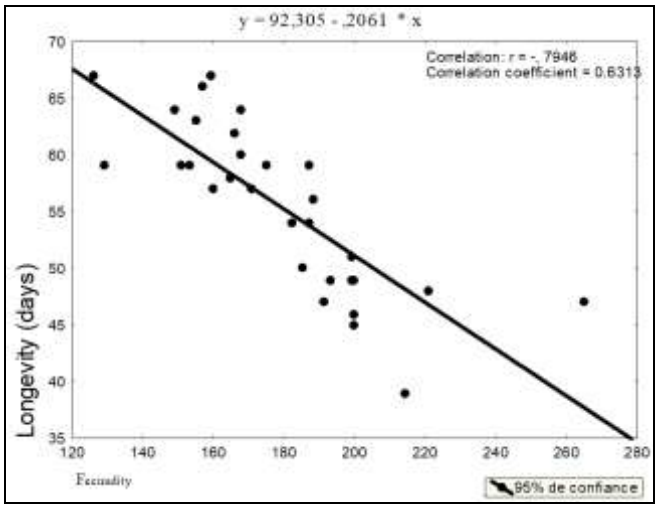

Fig 6: Correlation between fecundity and longevity of female

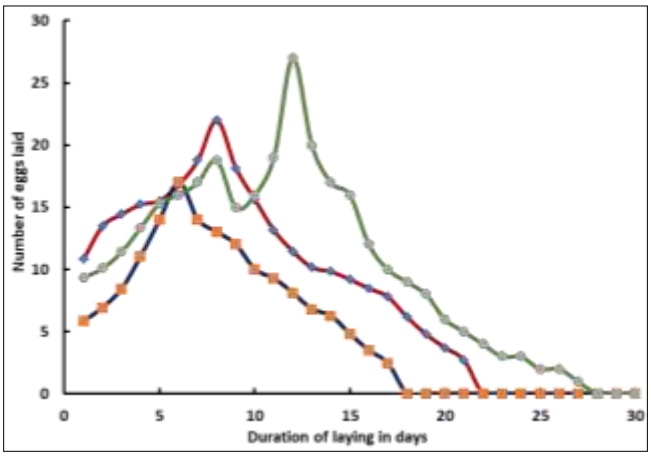

Fig 7: Evolution of R. phoenicis oviposition on each substrate

Discussion

The method of using banana stover and sugarcane for the breeding of *R. phoenicis* has given satisfactory results. Indeed, whatever the nature of the substrate, the time between the emergence of adults and their mating varies between 2 and 4 days. Similarly, the preoviposition periods on all substrates varied from 3 to 4 days. These two periods were longer on the banana stem than on the sugarcane and the palm trunk in both breeding tests. These results are consistent with those obtained by EL-Ezaby (1997) [3] and Aldhafer *et a*l. (1998) [1] in the breeding of *R. ferrugineus*. However, Monzenga (2015) [9] obtained in Kisangani, at *R. phoenicis*, a longer preoviposition period (9.40 ± 1.36 days) on sugarcane than on palm oil rachi (3 ± 1.44 days). With regard to daily Oviposition, the highest rate was observed on oil palm stipe and sugarcane compared to banana stems. This difference could be explained by the fact that initially, the females had not yet become familiar with these two last substrates. Salama *et al*. (2009) [15] found in *R. ferrugineus* a daily Oviposition rate of between 6.12 and 7.92 eggs on banana stem and oil palm stipe. Females used for the no-choice test had fewer eggs than in the choice test. Indeed, *R. phoenicis* are laid on all the substrates made available to insects. Females in good condition emitted more eggs on sugarcane (39 %) and palm trunk (34 %) than on banana stems (27 %). For Delobel *et al*. (2000) [2], the choice of females for egg-laying depends on environmental factors such as stimuli from the host plant such as odor, texture or moisture. According to Ndiaye (1991) [11] and Robert (1984) [14], the choice of Oviposition in insects is determined by hereditarily transmitted factors. The different averages obtained for the duration of laying on the three substrates are due to their quality. Indeed, the sugarcane is juicy, retains its freshness for a relatively long time, while the banana stem dries more quickly. Norzainih *et al.* (2015) [12] reported that sugarcane is more resistant to rot, so able to provide a good quality of food for a much longer period of *R. ferrugineus*. On the other hand, if the palm trunk is juicy and fresh at the beginning of the experiment, it is unable to keep its freshness and is very sensitive to microorganisms. The faster the spoilage, the less favorable the substrate is for Oviposition because females do not lay eggs in fresh substrates. With regard to fertility in breeding condition, sugarcane had the highest hatching rate (89 ± 1.2%), followed by palm trunk (83 ± 1.4 %) and the banana stem (68 ± 0.87 %). Kaakeh *et al.* (2001)[5] and Prabhu and Patil (2009) obtained hatching rates of between 75 % and 90 % when females of *R. ferrugineus* are in the presence of sugarcane. On the other hand, according to Salama *et al.* (2009)[15], the fertility rate of *R. ferrugineus* eggs is higher on the banana trunk (85 %) and the oil palm trunk (83 %) than on the sugarcane stem (52 %). Regarding the incubation time of eggs, it was longer on the banana stem (5.60 ± 1.14 days) than on the sugarcane stem (4.53 ± 1 days) and the palm tree (3.14 ± 0.53 days). Similarly, Shahina *et al*. (2009) [16] obtained an incubation period of between four and five days with *R. ferrugineus* cultured on different substrates. Regarding larval development time, it was longer on banana stems compared to palm stems and sugarcane stems. The variation of this duration on the three substrates could be attributed to the substrates losing their nutritional value during the breeding. Moreau and Thiéry (2013) [10] reported that the nutritional content of the host plant positively affects the larval development of an insect. Pupal development time was less important for larvae fed with palm trunk (18.51 ± 1.3 days) and sugarcane stem (20.08 ± 0.9 days). These results are close to the estimates of Aldhafer et al. (1998) [1] according to which the pupal period of *R. ferrugineus* lasts 21.1 days for males and 23.3

days for females. On all three carriers, the lifespan of males is higher than that of females except on the sugarcane stem where the females showed a longer life span. These results differ from those of Mahmound *et al.* (2015) [7] that revealed longevity rates of 86.2 days and 81.5 days, respectively, for males and females of *R. ferrugineus* on sugarcane. In addition, the longevity of females was strongly influenced by the number of eggs laid; the higher the number, the shorter the life of the female. This observation is similar to that made by Yong *et al*. (2015) [19] on the life span of *R. ferrugineus* females raised on sago palm in Malaysia.

Conclusion

The rearing test of *R. phoenicis* on palm trunk, sugarcane stem and banana stem was successful. Females of this species all laid eggs on the three substrates under no-choice and choice conditions. The female fertility rate was influenced by the age of the females and the type of substrate. Larval development was shorter on palm trunk and sugarcane. Sugarcane has been the most popular substrate for females under both conditions. For a mass rearing of *R. phoenicis* and for a better conservation of the biodiversity, the use of the sugarcane stem is conceivable.

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