Influence of substrates on the rearing success of *Rhynchophorus phoenicis* (Fabricius)

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ABSTRACT

Entomophagy (consumption of insects) is more and more regarded as a possibility to solve food problems of deficiencies or malnutrition. Our objective was to analyze the possibility of rearing the *Rhynchophorus phoenicis* weevil on side products resulting from agriculture in order to improve its availability at an acceptable cost by the local populations. All experiments were done under basic local conditions and using local products. To this end, we carried out the breeding of *R. phoenicis* on five types of substrates (old and young palm trunk split in two parts and reassembled or not, sugar canes and two artificial diets) by using either eggs, or first stage larvae to start the production. The young palm trunk gave a much better production when not split, with shorter development duration (15 days against at least 35 days) and heavier last stage larvae (6, 95 g *vs* 3.4g) on this substrate than on all the others. However, pieces of sugar cane and artificial diet based on plantain-sweet potato pasta added with cowpea gave encouraging results. Larval infestation worked better than eggs infestation. This work underlined the potential of *R. phoenicis* rearing under the conditions of poor developing countries.

Keywords: Entomophagy, Substrates of rearing, *Rhynchophorus phoenicis*, Artificial diet.

INTRODUCTION

Insects are more and more considered as a source of food and proteins (Rumpold and Schlüter, 2013). Several companies like Micronutris (France), Yunnan Insect Biotechnologies (China) already sell insects and the Universities of Wageningen (Netherlands), of Liège Agro-Bio Tech (Belgium) and of Wisconsin (USA) have poles of research entirely devoted to entomophagy (Lavalette, 2013). This list is not exhaustive.

Consumed from immemorial time, insects can be reared in high quantity using little space, less energy and less food requirement than vertebrates. Nearly 1500 to 2000 species are known to be edible throughout the world (MacEvelly, 2000; Malaisse, 2004; Mitsuhashi 2008; Nonaka, 2009 ; Shorkley & Dossey, 2014). The nutritional quality of insects and the possibility of producing them at low prices could be a solution to solve many problems of protein deficiencies (Hardouin, 2003). In that context, the larvae of *Rhynchophorus phoenicis* constitute a well-known and appreciated resource in tropical Africa (Bahuchet, 1975; Dounias, 1993; Thies, 1995; Malaisse, 1997 ; Van Huis, 2003). For numerous insect species, it is already known that the quality of the host plant used to rear the larvae significantly influences the main fitness traits such as the morphology of adult insect (Albert and Bauce 1994; Dodds et al.,1996; Tammaru 1998). Several works on *Rhynchophorus* sp. and particularly on *R. ferrugineus* already tried to develop artificial diets (Rananavare et al.,1975; Giblin et al., 1989; Zagatti et al., 1993) and substrates for laying and breeding (Rahalkar et al., 1978; Kalleshwaraswamy and Jagadish, 2005; Karmataka, 2009; Shahina et al., 2009). However these studies did not aimed to produce *Rhynchophorus ferrugineus* for food consumption but to test chemical control methods, as this species is a pest for palm tree culture. In consequence,

Figure 1 : Picture of a standing trap. A : open wound. B : folded wound. C individuals harvesting

they use antibiotics to avoid bacterial contamination in their diet, (Walid Kaakhek et al. 2005; El-Shafie *et al.* 2013). In our case, the objective was to produce larvae of *Rhynchophorus* spp*.* for human consumption excluding the use of antibiotics. Moreover, to mimic the conditions encountered in a poor country, we worked with simple elements readily available on the local market, and without sophisticated climate chamber. The objective of the current work was to produce the last larval stage of *R. phoenicis* for human consumption by using five rearing substrates. These rearing substrates weremade with agricultural products or residues to promote the insects rearing and to develop the available local resources and to fight malnutrition.

MATERIALS AND METHODS

Adults trapping

The *R. phoenicis* were collected in the forest of Akodale, Congo, (034' Northern latitude and to 025, 08' longitude) at 450 m of altitude from 28th October to11th December 2010. Standing traps were used to capture individuals. This type of trap (figure 1) is constituted by a young palm oil pruned to keep only ten internal leaves that form a crown. This crown is cut at its base and folded to prevent water flooding the wound; the rest of the crown is sectioned at 1.3 m from the base. Harvesting was done by lifting the crown to recover the adults inside.

Production of eggs and first stage larvae of *Rhynchophorus phoenicis*

This work was done under ambient conditions of temperature, photoperiod and relative humidity to correspond to the true conditions of rearing in poor countries with no climatic facilities. The collected insects were placed at a sex ratio of 1:1, in plastic box (2.0 to 2.4 dm³) with holes to allow ventilation. The density of individuals was the same in each box. Pieces of split sugarcane of 5-10 cm were added to be used as food and laying substrate and renewed every three days.

Extraction of eggs and larvae

Eggs and first stage larvae were retrieved from pieces of sugar cane remained in the box for three and six days respectively. To do that, pieces of sugar cane were carefully torn off with a knife and individuals (eggs or first stage larvae) were collected using a brush. Once collected, eggs and larvae were then placed in Petri dishes and used to infest various substrates.

Tested substrates

Five substrates were tested and infested with eggs or first stage larvae. The number of eggs and larvae used for infestation was chosen according to the size of the substrate: (1) young palm trunk split or not (30 to 50 cm long and 20-40 cm in diameter) infested with 30 larvae or 40 eggs ; (2) old palm oil trunk split or not (40 cm long and 30 cm in diameter) infested with 35 larvae or 60 eggs ; (3) 25 pieces of sugar cane (30 cm long) placed in a jar and infested with 50 larvae or 100 eggs ; (4) an artificial medium composed of a paste made of ripe plantain, sweet potato for the energy component (40 %) and cowpea for the protein component; (5) an other artificial medium where palm cake replaced cowpeas as a protein component (60 %) and lemon juice was used to prevent mold. These two artificial diets were prepared after boiling cowpeas, bananas and sweet potatoes. They were then crushed to form the paste. Two kilograms of medium diet is composed of: 1,2 kg of palm cake/cowpea, 0,4 kg of ripe plantain/sweet potato and the juice of one lemon. Five replicates were made for each medium and each type of infestation (eggs and first stage larvae). The eggs and first stage larvae were placed individually in small holes dug at the top of the substrate. The holes were then locked with a piece of feather grass or sugar cane. The holes were distant from each other (10 cm) to reduce competition

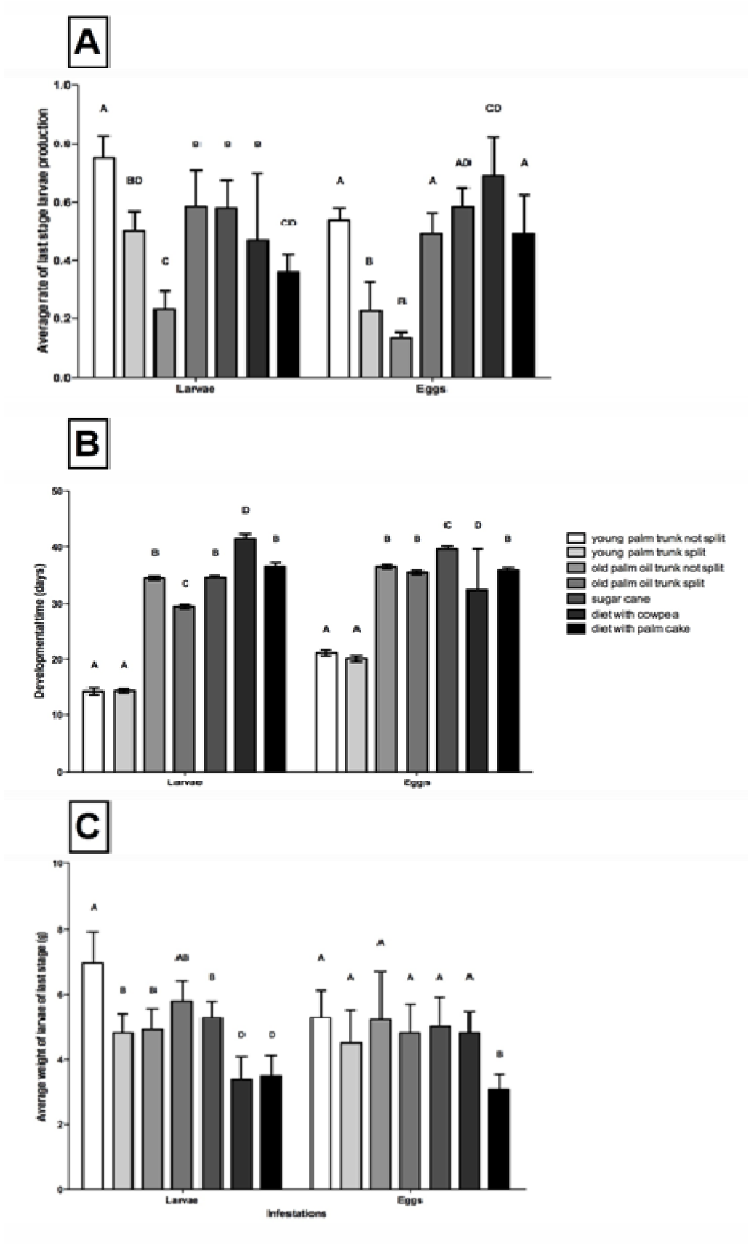

Figure 2. Average production rate (A), developmental time (B), and weight of last stage larvae of *R. phoenicis* according to the rearing substrate and the type of infestation. Error bars represent confidence interval (95%). Different letters indicate significant differences at a threshold of 0.05.

between individuals. The infested substrates were stored at room temperature until the last larval stage. The pieces of sugar cane and the artificial diet used as breeding substrates were renewed each week.

Collection of the last stage larvae

For the trunk of palm oil tree, it is difficult to note the presence of the larvae during the first ten days of their existence. After this time, they produce a chewing noise similar to a washing machine. As the larvae grow, the noises increase in intensity and the frequency of the vibrations decreases. With maturity, the frequency of vibration is approximately ten per minute; and it is the time to harvest the larvae (Mignon, 2002). For the sugar cane and the artificial diet, this state is noticed by the brown-yellowish coloring of the larva .

Table 1. Student's t-values of the Bonferroni's multiple comparison test between each substrate and a same infestation (eggs or larvae) for the production rate, the developmental time and the final weight.

	Average rate of last larvae produced		Developmental time		Weight	
	Larvae infestation	*Eggs infestation*	*Larvae infestation*	*Eggs infestation*	*Larvae infestation*	*Eggs infestation*
S1 Vs. S2	4.06***	5.10***	0.09	0.90	4.18***	1.55
S1 Vs. S3	8.42***	6.58***	18.14***	13.90***	3.98***	0.19
S1 Vs. S4	2.65*	0.82	13.63***	13.00***	2.23	0.97
S1 Vs. S5	2.75*	0.73	18.23***	16.70***	3.20**	0.58
S1 Vs. S6	4.46***	2.35*	23.95***	9.97***	6.89***	0.97
S1 Vs. S7	6.31***	0.81	19.94***	12.99***	6.70***	4.27***
S2 Vs. S3	4.36***	1.49	18.05***	14.80***	0.19	1.36
S2 Vs. S4	1.42	4.28***	13.54***	13.90***	1.94	0.58
S2 Vs. S5	1.31	5.82***	18.14***	17.60***	0.97	0.97
S2 Vs. S6	0.51	7.31***	23.86***	10.84***	2.72*	0.58
S2 Vs. S7	2.25	4.15***	19.85***	13.87***	2.52*	2.72*
S3 Vs. S4	5.77***	5.76***	4.51***	0.90	1.75	0.78
S3 Vs. S5	5.67***	7.31***	0.09	2.80*	0.78	0.39
S3 Vs. S6	3.73***	8.75***	6.30***	3.56**	2.91**	0.78
S3 Vs. S7	2.10	5.60***	1.81	0.54	2.72*	4.08***
S4 Vs. S5	0.10	1.55	4.60***	3.70***	0.97	0.39
S4 Vs. S6	1.89	3.14**	10.69***	2.68*	4.66***	0.00
S4 Vs. S7	3.67***	0.01	6.32***	0.34	4.47***	3.30**
S5 Vs. S6	1.79	1.64	6.21***	6.29***	3.69***	0.39
S5 Vs. S7	3.56**	1.51	1.72	3.26**	3.50**	3.69***
S6 Vs. S7	1.68	3.07**	4.54***	2.95**	0.19	3.30**

Measured parameters

Once the last stage larvae are collected, several parameters were measured/calculated : 1) the production of last stage larvae (number produced/ number introduced), 2) the developmental time in days (time between the introduction and the collection), and 3) the average weight of last stage larvae.

Statistical analysis

Two-ways ANOVA and Bonferroni post hoc tests were performed to test the impact of the kind of substrate and the type of infestation on the three tested parameters: the production of last stage larvae, the developmental time and the average weight of last stage larvae. Tests and graph were performed with the software Graph Pad prism5 (Graph Pad Software, San Diego, California, the USA). The level of significance was fixed at 5 %.

RESULTS

Production of last stage larvae

The two-way ANOVA showed that both type of infestation ($F = 4.56$ dfn = 1, dfd = 123, $p < 0.05$), and type of

Table 2. Indicators of success of *Rhynchophorus* spp breeding

Authors and years	Weight of last stage larvae (g)	development time (days)	rearing substrate	species
Berthier, 1986	9-11	120	Artificial	*R. palmarum*
Rochat, 1991	1,5 – 2,5		Artificial	*R. ferrugineus*
Salama et Abdel-Razek, 2002	3,99±0,52,	58	Artificial,	*R. ferrugineus,*
	4,57±0,20,	58	Banana,	*R. ferrugineus,*
	4,26±0,27	58	Sugar cane	*R. ferrugineus.*
Al-Ayedh, 2011	2,73±0,08	35	date palm	*R. ferrugineus.*
Karmataka, 2009	4,95	55,69	Sugar cane	*R. ferrugineus.*
Monzenga, 2014	5,80 ± 0,61	30	Old trunk split	*R. phoenicis.*
Monzenga, 2014	6,95 ± 0,98	15	Young palm trunk	*R. phoenicis.*
Monzenga, 2014	5,30 ± 0,49	34	Sugar cane	*R. phoenicis.*
Monzenga, 2014	3,40 ± 0,68	42	palm cake media	*R. phoenicis.*

substrate ($F = 27.17$ dfn = 6, dfd = 123, $p < 0.001$) have a significant effect on the mean production of last stage larvae produce (Figure 2). This finding was, however, rendered ambiguous by a significant interaction ($F = 7.87$ dfn = 6, dfd = 123, $p < 0.001$) between the two tested parameters. We, therefore, used the Bonferroni procedure to detect differences in the average rate of last stage larvae produced for the same type of infestation on different substrates (Table 1).

When starting with young larvae, the average production of last larval stage was significantly higher with the young palm trunks not split (75%) than with the split ones, the diet with palm cake, the diet with cowpea and the old trunk not split (50%) (Figure 2, Table 1). Furthermore, the old trunk not split and the sugar cane present the lowest production of last stage larvae (35%) (Figure 2, Table 1) For the infestation with eggs, the cowpea diet arrived first for the production of last stage larvae (70%), followed by the young palm trunk not split, old palm oil trunk split, the sugar cane and the diet with palm cake (55%) (Figure 2, Table 1). The young palm trunk split and old palm oil trunk not split present the lowest production of last stage larvae for this type of infestation (15%) (Figure 2, Table 1).

Developmental time

The two-ways ANOVA showed that the type of substrate ($F = 259.90$, dfn = 6, dfd = 123, $p > 0.01$) and the type of infestation ($F = 27.19$, dfn = 1, dfn = 123, $p < 0.001$) significantly influenced the developmental time (Figure 2). However, the significant interaction between the two parameters rendered difficult to interpret this result ($F = 24.17$, dfn = 6, dfd = 123, $p < 0.001$). We, therefore, used the Bonferroni procedure to detect differences in the mean developmental time for the same type of infestation on different substrates (Table 1).

The shortest development time was obtained for the young palm trunk, split or not for both eggs and larvae (15 and 20 days respectively) (Figure 2, Table 1). For all other substrates the developmental time took more than 30 days for the two infestations (Figure 2, Table 1).

Weight

Our results show that the substrate significantly influences the final weight of the larvae ($F = 12.45$, dfn = 6, dfd = 123, $p < 0.001$). On the contrary the type of infestation seems to not have any influence on the final weight ($F = 2.05$, dfn = 1, dfd = 123, $p > 0.05$).

However, this observation was rendered ambiguous by a significant interaction between the two tested parameters ($F = 3.850$ dfn = 6, dfd = 123, $p < 0.001$). Concerning the larval infestation, the best result was obtained with the young palm trunk not split with a mean weight of about 7g and the worse results were obtained with the two artificial diets and 3g (Figure 2, Table 1).

For the eggs infestations, almost all the substrates gave the same quality of larvae with a weight of about 5g, except the diet with palm cake with which we obtained larvae of about 3g (Figure 2, Table 1).

DISCUSSION

Our results highlight that the young palm trunk allows a faster growth of the larvae, a higher rate of success and produced larvae of bigger size than all the other substrates we used in our experiments. It is obviously the substrate that has best food qualities for *R. phoenicis*. For the young palm trunk, the split reduced the production rate and the final weight while the developmental time stay the same compared to the non-split. It is possible that this handling affects food quality for example by oxidation or by facilitating the penetration of molds or pathogenic agents. On the contrary, making a split in the old trunk improves the production success. It is possible that,once split the young palm trunk is more easily colonized by bacteria, fungi and other microorganism than old split palm trunk,affecting the development of *Rhynchophorus phoenicis* larvae.

On artificial diet, the time of development is doubled compared to the young palm trunk. The weights obtained at the end of the experiments are also smaller. This shows that the diets still need to be improved. The good results obtained with the sugar cane could be a solution to improve these artificial diets. Indeed, by identifying the nutrient compounds present in the sugar cane, we will then be able to add it in the artificial diet to improve the results we obtained on this substrate.

Globally, the infestations with eggs have less success than infestations with larvae. It could be due to the fact that it is very difficult to detect sterile and dead eggs. The larvae of last stage of our breeding are similar in weight or even a little heavier than those produced by other systems of breeding using natural hosts, artificial diets or sugar canes and those collected in nature with regard to the weight (1,5 - 9 g) and the incubation duration (Rochat, 1991).

The table 2 compared the results of the literature concerning *Rhynchophorus* spp using various media and our present data. Although, these results concern different species, the comparison remains possible because they belong to the same genus and they all attack the same family of plant, the Arecaceae.If we consider the developmental time, our results present lower durations than what it is presented in the literature (old and young palm trunk; sugar cane),except for Al-Ayedh, (2011) who obtained a shorter developmental time than our rearing on palm cake diet. It is necessary of course to take into account the specific differences and conditions of these studies (T: 25, 1 – 28, 7 °C and H.R 70 - 90, 5 %.) .

For the future, it would be interesting to analyze the availability of the young palm trunk, in particular within the framework of the replacement of the oils palm plantations. Another idea would also be to improve the results obtained on artificial diets.

CONCLUSIONS

The originality of this study was to work with simple and readily available elements, without sophisticated climate chambers, that is to say under very close conditions to what could be a production of larvae on artificial diets in a poor country. It appeared that it is in their natural habitat (the young trunk of palm oil) that *Rhynchophorus phoenicis* larvae showed the highest production rate, the shortest time of development and the highest weight in the last larval stage. The sugar cane can also be an interesting substrate of rearing, but it is necessary to be armed with patience for the renewal of the substrate each week.

However, these results on artificial media are encouraging particularly on sugar cane but also on the diets produced from plantain and sweet potato with a complement of cowpea or palm cake.

We noted an almost doubling of development time on these diets and reduced larval weight, especially in the absence of cowpea. By improving these artificial diets, it would be possible to obtain a production of larvae without using the trunk of palm. Mixing sugar canes and plantain plus sweet potato pasta could be a satisfactory solution. Two questions remain to be addressed: firstly the formation of cocoons which will probably require the addition of fibers in the artificial diet and secondly the elimination of fungal and bacterial infestations. In this context, the lemon juice is an interesting alternative that should be investigated as it contains the natural ascorbic acid that could reduce the microorganism action. The infestation process using young larvae is globally the best suited, death or sterile eggs being difficult to detect.

The development of insect rearing in developing countries could usefully replace harvests and gatherings in nature to ensure a permanent provisioning of the markets and to avoid a pressure on biodiversity in forest around densely populated areas. There remains nevertheless a series of practical difficulties to solve. The next steps will be to analyze the adult fecundity and the nutritive quality of larvae produced under our rearing conditions.

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