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ORIGINAL RESEARCH ARTICLE

Evaluation of different larval substrates on selected parameters of african palm weevil (*Rhynchophorus phoenicis*) (F.) (Coleoptera: Curculionidae)

Joshua Kpakpo Hermann[1] · Shadrack Kwaku Debrah[1] · Jacob Paarechuga Anankware[1] · Daniel Obeng-Ofori[2]

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Abstract

Proteins and carbohydrate contents of a substrate are two important macronutrients that gives organisms the energy and necessary amino acids that affect their fertility, growth, and development. However, it is often assumed that larval food stress reduces lifetime fitness regardless of the conditions. It is imperative to understand how substrates affects *R. phoenicis* larval development and adult reproductive performance. To mitigate these challenges that hinder the development and reproductive performance, three different diets were evaluated to assess the effect of different larval substrates on some parameters of *Rhynchophorus phoenicis.* Larvae were reared on diet A (500 g of rice husk, coconut coir, mango waste and water), B (500 g of rice husk, coconut coir, cocoa pod, and water), and C (500 g of rice husk, coconut coir, palm yolk, and water) and subsequent life history parameters were assessed. Cocooning rate, cocooning duration, and percentage of male and female adults were also evaluated. The emerged adults were sexed based on the presence of a setae on the male's rostrum which are absent on the female's rostrum The larval development on the different diets indicated a steady increase in weight gain from day 3 to day 21. The weight gain of the larvae declined after day 24. Survival rate of the larvae was significantly different ($P < 0.05$) among the substrates with diet C recording 78%. Cocooning and emergence rate were significantly different ($P < 0.05$) among the substrates. Percentage of male and female adults varied significantly ($P < 0.05$) among the different diets. Adult that emerged from diet C had a higher fecundity than those that emerged from the other substrates. It is therefore recommended that diets with similar characteristics of the natural host plant (oil palm yolk) should be adopted for the production of *R. phoenicis* larvae.

Keywords African palm weevil · Diet · Nutrition · Rearing

Introduction

Rhynchophorus phoenicis larvae are widely used as food and are easy to rear on the natural host plant (Debrah et al. 2022a). This species has a long life cycle, and the nutrients required for their survival and reproduction are supplied from the natural host plant (Quaye et al. 2018). During each stage of their development, various tissues require a specific quantity and blend of nutrients for body function (Okamoto and Yamanaka 2015). This necessity is true for all insects as well, whose functions and effects change based on the stage of development, growth, and fecundity (Wehry et al. 2022). It can also differ significantly depending on the stage and sex, with larvae and adult females and males making highly distinct demands for protein and carbohydrates (Scala et al. 2020). On a single diet, an insect cannot maximize both longevity and egg production rate (Bava et al. 2019). A diet that has high amount carbohydrate and low in protein has the potential to increase the lifespan of both male and female insects (Fowles and Nansen 2020). Furthermore, comparing the dietary mix required to maximize a female's longevity

✉ Joshua Kpakpo Hermann
hermannjoshua40@yahoo.com

Shadrack Kwaku Debrah
shadrackdebrah@gmail.com

[1] Department of Horticulture and Crop production, School of Agriculture and Technology, University of Energy and Natural Resources, P.O. Box 214, Sunyani, Ghana

[2] Catholic University of Ghana, P.O. Box 363, Fiapre-Sunyani, Ghana

with the mix required to achieve the best possible female reproductive function can frequently result in two ratios that are very different from one another (Chapman and Partridge 1996). There is a sexual variation in the trade-off between lifespan and reproduction in males and females; males need low-protein, high-lipid diets to create energy stores to support them in their search for females, whereas females need high levels of protein for reproduction (Chapman and Partridge 1996). Debrah et al. (2022a) reported that, female fecundity differed significantly on different substrates. The female fecundity recorded on oil palm yolk, peeled sugarcane, sliced false yam, and elephant ear corm outperformed those recorded on banana corm, cocoyam cake, and spoiled onion bulb (Debrah et al. 2022b). Quaye et al. (2018) reported that fecundity and hatchability recorded on palm yolk was superior to fruit waste. Debrah et al. (2022a) further reported significant weight per larva and larval survival on elephant ear corm and oil palm yolk whilst sugarcane and coconut coir were good substitute to palm frond for cocooning and eclosion.

However, obtaining the necessary hosts is one of the major challenges for *R. phoenicis* production (Debrah 2016).

Theoretical and empirical data point to the importance of stored glycogen and protein in influencing egg number, emergence ratio, and duration to adult emergence depending on the qualitative and quantitative combinations of food supply (Wathes et al. 2007). Egg number and sex ratio are crucial for an effective mass production system (Debrah 2016), and research with artificial diets in improving insect fecundity has frequently proved a success (El-Shafie et al. 2013). Furthermore, the availability of nutrients in the larval diet may have an impact on either maintenance or development activities, such as tissue repair or organ growth (El-Shafie et al. 2013). In a variety of taxa, it has been demonstrated that reducing food quantity during developmental stages has long-term impacts on adult metabolism (Okamoto and Yamanaka 2015). This finding suggests that insect species may be able to adapt to food scarcity in the future (Lencion 2004).

Rhynchophorus species have been reared effectively on different substrates (Debrah et al. 2022a). Sugarcane stem has proved to be a useful replacement for rearing *R. cruentatus* in the lab according to Giblin-Davis et al. (1989). Also, *R. cruentatus* was effectively reared using pineapple syncarpium (Giblin-Davis et al. 1989). According to Monzenga et al. (2017) sugarcane can be a good substitute for palm yolk when added to the agar diet as a supplement (Rananavare et al. 1975). The fertility, hatchability, and developmental rate of *R. phoenicis* larvae had previously been evaluated with softer part of Raphia and oil palm yolk (Debrah 2016). However, none of these authors reported on how the evaluated larval substrates affects other life history parameters.

For example, oil and raphia palm substrates, Atuahene et al. (2017) reported crude protein levels of 3.2% and 3.5%, respectively, while Okai et al. (2005) found that millet residual meal had high crude 70% protein levels ranging from 10.51 to 21.86%. Debrah et al. (2022a) confirmed that the nutritional component of these substrates is vital during growth, egg maturation, and oviposition.

Insects frequently face scarcity of a specific nutrient, such as protein or carbohydrate in these substrates, as opposed to a complete absence of food, and may be obliged to provide their young ones with unsatisfactory and unbalanced diet (Joern et al. 2012). However, there are a few direct studies on how substrates affects *R. phoenicis* larval development and adult reproductive performance (Debrah 2016; Quaye et al. 2018; Debrah et al. 2022a). In view of this, this study was designed to evaluate the effect of different larval substrates on some selected parameters of *R. phoenicis*.

Materials and methods

Substrate sourcing and preparation

Rice husks were milled into fine powder to make it accessible to the larva during feeding. Cocoa pod, mango waste, and palm yolk were sourced from the farmers' farm. The palm yolk was cut into 5 cm with a knife. Coconut coir was sourced from the coconut sellers in the Goaso Municipality and washed with water to prevent mold infestation.

Experimental conditions

The containers were kept at a constant temperature of 26 ± 2 °C and $70 \pm 5\%$ RH with a photoperiod of 12:12 (L:D) h in a controlled room. The different experimental setups were laid out using completely randomised design.

Larval feeding

Larvae were reared on diet A (500 g of rice husk, coconut coir, mango waste and water), B (500 17 g of rice husk, coconut coir, cocoa pod, and water), and C (500 g of rice husk, coconut coir, palm yolk, and water) in different containers measuring 4 cm × 8 cm (width × height). 500 ml of water was added to each container to enhance the moisture content of the substrates (Debrah et al. 2022a). These substrates were selected because they are readily available in most communities in Ghana. To study the larval development, 20 neonates with a mean weight of 0.5 ± 0.1 g were added to each diet and the containers were covered with a nylon cloth. The larvae from each diet were monitored and weighed every three days using a digital balance. The larval duration experiment lasted for 30 days and each experimental setup was done in 5 replicates. Larval weight and survival rate of the larvae were recorded. The weight of the larvae was weighed using a digital scale (Fuzion Digital Gram Scale, Fuzion Global Corp., Concord, ON, USA) during the duration of setup. Larval duration was calculated using the days elapsed from inoculation to the day of maturity. Larval survival rate was calculated as the number of larvae at harvest divided by the number of larvae at used at beginning multiplied by a hundred.

Cocooning and adult emergence

Twenty (20) eighth instar individual larva were selected randomly from the larval development study weighing 5 ± 0. 1 g were placed in different containers measuring 5 cm × 6 cm (width × height) and labelled with the diets that they were fed on. Fifteen (15) pieces of sugarcane weighing 2 kg were kept in different containers at 26 ± 2 °C and 70 ± 5% RH. Sugarcane was used for the cocooning because it is readily available in most communities. The containers were covered with a thin cloth. Each substrate was replicated five times. The adults' emergence from each diet was monitored for 21 days. The emerged adults were sexed based on the presence of a setae on the male's rostrum which are absent on the female's rostrum (Kaakeh et al. 2001) (Fig. 1). Percentage cocooning, cocooning duration, and percentage emergence of male and female adults were recorded on each treatment period. Percentage cocooning was calculated as the number of cocoons woven divided by the number of larvae inoculated multiplied by a hundred. Cocooning duration was calculated using the days elapses from inoculation to the day the cocoons were woven. Emergence rate was computed as the number of emerged adults divided by the number of larvae inoculated multiplied by a hundred.

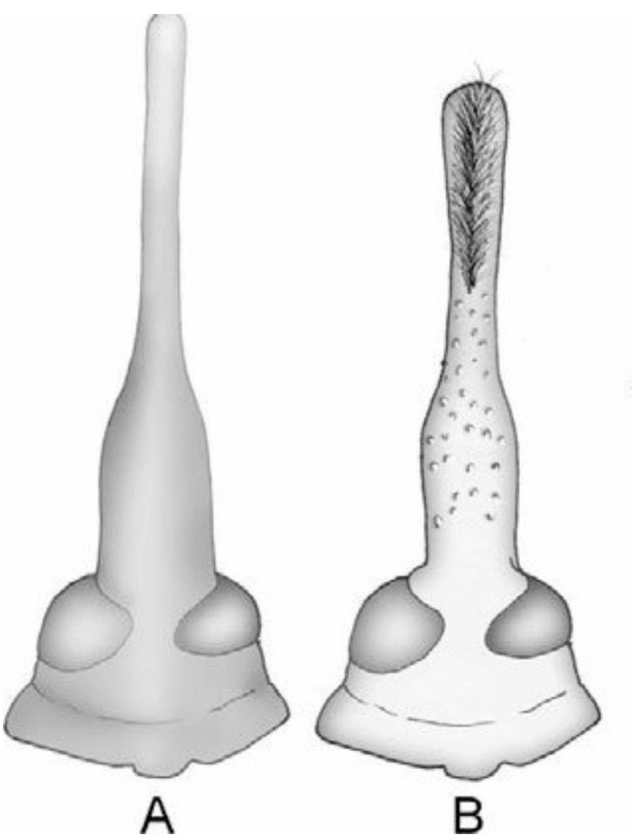

Fig. 1 **A**- Female and **B**- Male

Adult oviposition

One pair of adults from each diet (A, B, and C) were kept in different containers. Peeled sugarcane of 5 cm was added and covered with perforated lids. The adults were allowed to mate and lay their eggs for a period of 30 days at 26 ± 2 °C and relative humidity (RH) of 70 ± 5%. The oviposited substrate was removed from the container every day and new substrate was replaced for continuous egg-laying. The substrate was split with a knife and the eggs were counted using camel hair brush. The setup was replicated 10 times.

Statistical analysis

The data obtained from the different parameters were analysed using Microsoft excel and Analysis of Variance (ANOVA) Genstat Discovery (12th Edition). Treatment means were separated using Fisher's test at 95% confidence level and the standard deviation of the different means were reported.

Results

Larval weight gain

The development of the larvae reared on the different diets indicated a steady increase in weight gain from day 3 to day 21. The weight gained declined after day 24. The larvae reared on diet C had a mean weight of 5.8 g at day 21 whilst 3.2 g was recorded at day 18 on diet B and 3.0 g at day 24 on diet C (Fig. 2).

Larval duration and survival

Larval survival recorded varied significantly ($P < 0.05$) among the different diets. Percentage larval survival recorded on diet C was 78 ± 4.2 compared to diet B (55 ± 5.6), and A (36 ± 2.4) (Table 1). The longest larval duration was

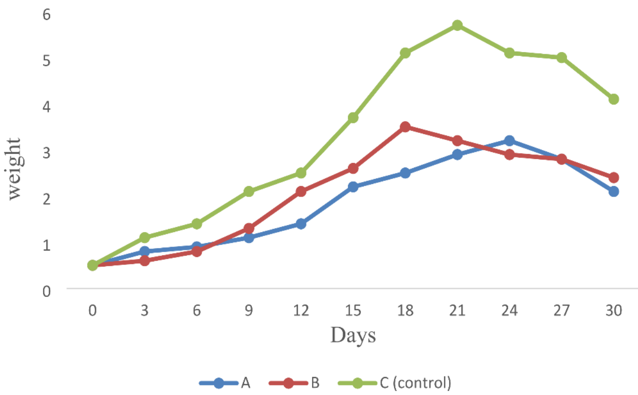

Fig. 2 Larval developmental rates of different diets

Table 1 Larval duration and survival rate

Substrates	larval duration (days)	% larval survival
A	31 ± 2.4[a]	36 ± 2.4[a]
B	28 ± 3.4[b]	55 ± 5.6[b]
C (control)	20 ± 2.6[c]	78 ± 4.2[c]

Means with different superscript letters in the column showed a significant (P < 0.05) difference between the treatments

Table 2 Cocooning and adult emergence

Substrates	% cocoons	% Adult Emergence		
		Emerged adults	Males	Females
A	66 [a]±2.0	51[a] ± 4.7	50 [a]±4.0	50 [a]±4.0
B	74 [b]±1.0	62 [b]±3.6	42 [b]±3.2	58 [b]±4.0
C (control)	87 [c]±4.0	71 [c]±2.8	38 [c]±2.4	72 [c]±3.4

Means with different superscript letters in the column showed a significant (P < 0.05) difference between the treatments

Table 3 Mean pre-oviposition, oviposition, and fecundity of adults

Treatments	Pre-oviposition duration	Oviposition duration	Fecundity
A	2.33[a] ± 0.8	3.33[a] ± 1.0	29.00[a] ± 8.1
B	3.00[a] ± 0.7	3.33[a] ± 1.0	36.67[b] ± 6.2
C (control)	3.00[b] ± 0.4	4.00[b] ± 0.7	82.00[c] ± 4.2

Means with different superscript letters in the column showed a significant (P < 0.05) difference between the treatments

recorded on diet A (31 ± 2.2), followed by (28 ± 3.4), and (20 ± 2.6) (Table 2).

Cocooning and adult emergence

The results showed that cocooning rate and adult emergence were significantly different ($P < 0.05$) on the diet fed at the larval stage. Furthermore, the percentage of male and female adults varied significantly ($P < 0.05$) among the different diets (Table 3). The emerged adults from larvae reared on diet C recorded 38 ± 2.4 males and 72 ± 3.4 females. Diet B had 42 ± 3.2 males and 58.40 ± 4.0 females whilst diet A recorded 50 ± 4.0 males and 50 ± 4.0 females.

Reproductive performance of adults from larvae

The pre-ovipossition, oviposition, and the fecundity of adults from larvae fed on the different diets at the larval stage varied significantly ($P < 0.05$). The adults from diet A had a lower mean ovipositional duration of 2.33 ± 0.8 compared to those reared on diet B and C. Furthermore, adults from diet C had a superior fecundity (82.00 ± 4.2), followed by B (36.67 ± 6.2) and A (29.00 ± 8.1).

Discussion

The larval development had a significant difference in weight gain among the different diets. The apparent differences could be attributed to the nutritional component of the diets. Diet C had palm yolk as it main active ingredient which has been reported to be a good source of protein. This palm yolk in diet C is also a natural host plant of the *R. phoenicis* larvae which may exhibit certain superior nutrient in the diet compared to the others. Akpanabiatu et al. (2001) reported that mineral analysis revealed high concentrations of magnesium, manganese, iron, copper, zinc, phosphorus, sodium, and potassium in palm trunks. Furthermore, Matsumoto et al. (1998) reported that the proportion of manganese (Mn) and zinc (Zn) in sago palm leaves may have an impact on the composition of these nutrients in the larvae. Avand-Faghih (1996) revealed that sago has limited nutrients as it contains only pure carbohydrates and little protein for larval growth. This protein deficiency in the sago diet may prolong the development of the larvae reared on this diet. Avand-Faghih (1996) reported that sago contains 84.6% carbohydrate and 15.4% of moisture, fibre, ash, protein, and fat.

Bong et al. (2008) studied the developmental rate of *R. vulneratus* on meridic diet and revealed that sago flour diet was the most suitable diet compared with other diets. However, the study did not investigate the number of larvae that survived until last instar. The quality of host plant plays a significant role on the larval survival, development, and weight gain (Al-Ayedh 2008). On the other hand, Liu et al. (2020) found that adding about 30% of fresh plant material to wheat bran did not significantly change mealworm survival or proximate composition, but it did increase the size of the mealworm and its growth rate. Lundy and Parrella (2015) concluded that the nitrogen concentration, N-to-acid detergent fibre concentration ratio, and crude fat concentration accounted for the majority of the variations among feed treatments used.

According to Guazzugli and Campadell (1976), larvae fed on a diet with no cholesterol developed slowly in the first generation. The current study showed that the development and survival of *R. phoenicis* were influenced by the diets fed to the larvae. This result supports the views of Chandel et al. (2003), Birah (2008), and Kulkarni et al. (2012) who stated that a thorough biological examination is required to assess artificial diet alterations. Diet C had a significant effect on larval duration compare to the others. Larval duration was prolonged on the diet A, containing mango waste as its main active ingredient: however, it was reduced on C which had palm yolk as it main active ingredient. According to Asomah et al. (2023) and Debrah (2016) palm yolk which is the natural host plant provides a good larval survival rates when used as a rearing substrate. This could be as a result of the ability of palm yolk to provide all the necessary nutrients required by the larvae for their survival. The larval growth and survival percentage on diet A was shorter than what was recorded on the other diets. This could be a result of the larvae being unable to ingest dry food during the first week of feeding, which may be connected to the quality and digestibility of the dry food or a lack of digestive enzymes in the first feeding.

The results revealed a significant difference ($P<0.05$) in the cocooning and emergence of male and female *R. phoenicis* among the different diets. The larvae fed on diet C had a superior cocooning percentage compared to the other diets. This could be attributed to the fact that palm yolk has carbohydrate content, which gave the larvae enough energy to weave the sugarcane fiber around themselves. This corroborates with Debrah (2016) who reported that larvae weighing above 8 g lack the required energy needed to weave cocoons. The percentage of emerged adults varied significantly ($P<0.05$) among the different diets. This could be attributed to the fact that larvae fed on rich protein diet produces more females than males. This agrees with the findings of Coskun et al. (2009) who reported a variation between male and female adults of *P. turionellae* across generations on artificial diets.

The pre-oviposition duration ranged from 2.33 days on diet A to 3.00 days on both diet B, and C. The delay in pre-oviposition on both diet B and C could be caused by the slow pace of the assimilated nutrients to stimulate early egg maturation. Contrarily, Kaakeh (2005) reported pre-oviposition duration of *R. ferrugienus* ranging from 3.1 days on potato and pineapple diets to 3.6 days on palm leaf base diet whilst the oviposition period on the different diets ranged from 3.2 days on potato, pineapple, oat-potato diets to 3.8 days on palm yolk diet. There is similarity in both experiments in term of the diets that was used, the diets fed to the larvae before they emerged as an adult might have contributed to the variation in the results. The fecundity of the adults from larvae that were reared on diet C was high compared to other diets. Rich diets used at the larval stage play a key role on the nutritional potential of the larvae which influences the reproductive performance of the female adult. A diet or substrate that harbor all the characteristic that are similar to oil palm yolk content should be used for the production of *R. phoenicis* larvae to enhance the fecundity of the adults that may emerged from these larvae. This assumption agrees with Kaakeh (2005) who reported that larval nutrition could affect the fecundity of the females depending on the larval diet type used.

Conclusion

The nutrient assimilated by the larvae at the larval stage has the capacity to influence the reproductive performance of the adults. In this research, it was found that the developmental response to low larval nutrition resulted in low adult reproductive performance, longer larval duration, and low survival percentage whereas, those feed high larval diet produced an adult with high fecundity, high survival percentage, and shorter larval duration. It is therefore recommended that diets that provide protein, crude fat, crude fiber, and carbohydrate required for insect development should be used for the production of *R. phoenicis* larvae while further research on the effect of nutritional component of other agricultural by-products on the reproductive performance of the adults should be conducted.

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Authors' contribution Joshua Kpakpo Hermann conducted experiments and collected data. Shadrack Kwaku Debrah planned the experiments. Joshua KpaKpo Hermann and Shadrack Kwaku Debrah wrote the manuscript and Jacobe Paarechuga Anankware and Daniel Obeng Ofori refined the concept and edited the final manuscript prior to submission.

Data availability Data will be available upon request.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Competing interests There is no conflict of interest among the authors.

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