AGRONOMY (AGRONOMIA)

Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.20, n.1, e3295, 2025
Recife, PE, UFRPE. www.agraria.pro.br
DOI: 10.5039/agraria.v20n1e3295 - Protocol 3295
Subm. 30/05/2023 • App. 05/12/2024 • Pub. 29/04/2025

Maintenance and mass rearing of the coffee berry borer on new artificial diets

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ABSTRACT: Studies on the biology and methods of control of the coffee berry borer require the development of a low-cost laboratory technique for mass rearing of the individuals so that there are sufficient numbers available for experimentation throughout the year. Therefore, the aim of this study was to develop a methodology for the mass rearing and maintenance of *Hypothenemus hampei* individuals on artificial diets in the laboratory. First, bored coffee berries were collected in the field and placed in a container to collect the insects, which were then transferred to a modified version of the artificial diet of Portilla (1999) for rearing under suitable environmental conditions. Once enough adult females were obtained, they were used to evaluate the performances of four modified artificial diets: two of Portilla (1999) (diets 1 and 2) and two of Villacorta and Barrera (1993) (diets 3 and 4). The modifications (removal or replacement of specific ingredients) were made for simplifying the diets. After 60 and 80 days, the offspring obtained were counted. The total numbers of offspring obtained on days 60 and 80 were 246 and 376 on diet 1, 237 and 293 on diet 2, 488 and 622 on diet 3, and 413 and 644 on diet 4, respectively, indicating that the modified diets of Villacorta, Barrera (1993) had resulted in more *H. hampei* individuals. The mass rearing and maintenance of *H. hampei* on these new artificial diets are possible, with the ones based on the Villacorta, Barrera (1993) diet resulting in more individuals.

Key words: bioassay; *Coffea arabica*; *Hypothenemus hampei*; insect rearing

Manutenção e criação massal da broca-do-café em novas dietas artificiais

RESUMO: Para estudos da biologia e de possíveis métodos de controle da broca-do-café, faz-se necessário um método de criação massal em laboratório que permita obtenção de insetos em quantidade suficiente e em qualquer época do ano, com qualidade e de baixo custo. Dessa forma, objetivou-se neste trabalho estabelecer uma metodologia para criação massal e manutenção da *Hypothenemus hampei* em dietas artificiais em laboratório. Inicialmente, frutos brocados foram coletados no campo e colocados em recipiente para coleta dos insetos. Após isso, as brocas-do-café foram transferidas para dieta artificial de Portilla (1999) com modificações em ambiente climatizado. Quando atingido um número suficiente de fêmeas adultas avaliou-se o desempenho de quatro dietas artificias, duas de Portilla (1999) com modificações (dietas 1 e 2) e duas de Villacorta e Barrera (1993) com modificações (dietas 3 e 4). Essas alterações nas dietas foram realizadas com objetivo de simplificá-las, retirando ou substituindo alguns de seus componentes. Após 60 e 80 dias foram quantificados os indivíduos obtidos. Obtendo-se uma média de 246 e 376; 237 e 293; 488 e 622; 413 e 644 indivíduos totais nas dietas 1, 2, 3 e 4, respectivamente. As dietas 3 e 4 de Villacorta e Barrera (1993) modificada foram superiores ao número de indivíduos de *H. hampei*. Assim, conclui-se que é possível a criação massal e manutenção de *H. hampei* tanto nas dietas artificiais modificadas de Portilla (1999) quanto de Villacorta e Barrera (1993), com a última proporcionando maior número de indivíduos.

Palavras-chave: bioensaio; *Coffea arabica*; *Hypothenemus hampei*; criação de insetos

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Associate Editor: Jorge Braz Torres

Introduction

Studies on the biology and possible methods of control of the coffee berry borer (*Hypothenemus hampei* (Ferrari); Coleoptera: Curculionidae, Scolytinae) require the development of a mass rearing method so that sufficient insect individuals can be obtained for experimentation at any time of the year (Lagos Giraldo 2009). Additionally, it is essential to develop rearing techniques that yield insects of good quality. Despite the importance of the coffee berry borer, current rearing techniques have shortcomings that hinder the mass rearing of this crop pest (Celestino et al. 2016).

The rearing of *H. hampei* on berries or grains is difficult because the crop products are not available throughout the year, as harvesting occurs annually. Although processed berries and grains can be stored in a cool place, they quickly lose moisture when used, which affects the development of the immature stages of the insect (Benassi & Benassi 2000). Additionally, the mass rearing of *H. hampei* on coffee berries or parchment coffee is hampered by the growth of opportunistic saprophytic fungi, such as *Aspergillus* spp. and *Penicillium* spp., and the difficulty of handling insects that remain inside the parchment coffee.

The method developed by Hirose & Neves (2002) for the mass rearing of *H. hampei* requires regular monitoring of the health of newly infected berries as well as spraying them with water to manually control and maintain their moisture content. According to Celestino et al. (2016), *H. hampei* can be reared on Robusta coffee (*Coffea canephora*) beans without the need for aseptic procedures and stored in a freezer at –20°C for use in the off-season. However, these beans still lose moisture quickly during use. Given these shortcomings, there is a need to produce artificial diets that are easy to prepare in the laboratory and consist of readily available ingredients. Jaramillo & Parra (2018) found that the modified diet of Portilla (1999) did not alter the development of *H. hampei* compared with that on the natural diet, and the mean time from the egg to the adult stages was 24.1 days and the sex ratio was 0.82. According to those authors, the diet was comparable to the natural diet and also inexpensive and easy to prepare.

The ability to rear *H. hampei* on an artificial diet would greatly facilitate the obtainment of enough individuals for all laboratory tests and treatments and allow a closer determination of the insect's age. However, current methodologies do not address the procedures that are important for rearing *H. hampei* on this type of diet, such as maintaining the relative humidity of the external environment and the relative moisture content of the diet, resting the diet to allow the initial dissolution of preservatives, and using a container that facilitates handling of the insects. Therefore, the aim of this study was to develop a methodology for the mass rearing and maintenance of *H. hampei* on artificial diets in the laboratory.

Materials and Methods

Mass rearing of *H. hampei*

The study was conducted from March 2021 to July 2022 at NOOA Ciência e Tecnologia Agrícola, a company located in the rural area of the municipality of Patos de Minas, Minas Gerais, Brazil.

Collection of the initial insect population

Bored berries of the Arabica coffee cultivar Catuaí 144 were collected from a commercial cultivation with no insecticides applied in the last six months. To minimize contamination, the berries were surface sterilize by immersion in 70° INPM alcohol for 1 min followed by 5% sodium hypochlorite (NaClO) for 3 min and then washed under running water. To remove excess moisture, the berries were dried with paper towels and left in the shade for 24 h.

Subsequently, the cleaned berries were transferred to containers for collection of the *H. hampei* individuals. The collection containers were made by cutting off the bottom of a rectangular 3.5-liter plastic container (27.5 × 17.0 × 9.5 cm) and replacing it with a mosquito net, which was attached with contact adhesive. Another container of the same size was placed underneath to collect the insects. To facilitate gas exchange, part of the lid was cut off and replaced with a piece of voile fabric, which was attached with contact adhesive (Figure 1).

The volume of berries placed was 1.75 L, which was half the capacity of the container. On every 3 days, borers that had fallen to the bottom of the container were transferred to the artificial diet using a No. 00 brush. The containers were kept under a 12 h photophase in a room that was regulated to a temperature of 25 ± 3°C (using an air heater and a cooler) and humidity of 70 ± 15% (through periodic use of a humidifier).

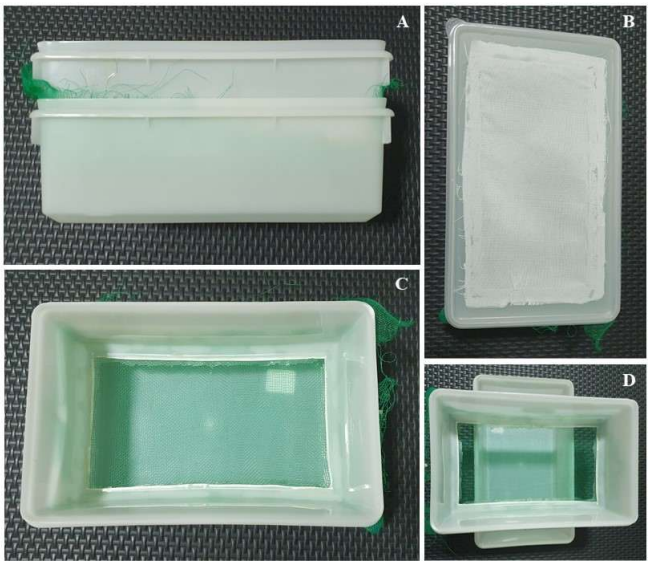

Figure 1. Coffee berry borer collection container. (A) Side view of the trap; (B) Cut lid with voile fabric; (C) Top view with the bottom attached; and (D) Top view with the bottom detached.

Rearing of the *H. hampei* individuals

A modified version of the artificial diet of Portilla (1999), designated as diet 1 (Table 1), was used for rearing the collected insects. Processed green coffee beans were ground in a simple centrifugal mill (Vieira MCS 280 - 05 cv) with a 0.2 mm sieve. Then, the green coffee and agar (mixed with water) were sterilized in separate containers for 30 min. Subsequently, the warm sterilized ingredients and other solid ingredients were homogenized in a blender for 1 min before the agar could solidify. The liquid ingredients were then added, and the mixture was homogenized in the blender for another minute. The diet mixture was then poured into a plastic container (16 cm diameter × 8.8 cm height) that had been previously irradiated with UV light for 20 min and dried in an oven at 55°C for approximately 8 h (Figure 2 A).

During this time, the moisture content of the gelled diet mixture was checked periodically using a Gehaka IV3100 moisture analyzer until it was approximately 63–68%, whereupon the drying process was stopped. Then, the gel was cut into cubes of approximately 1 cm^3 (or 1 cm at the edge) and the moisture was checked again. If the cubes did not have the correct moisture content, they were placed in the oven at 55°C for another 30 min. Subsequently, the cubes were irradiated with UV light for 20 min in a laminar flow chamber and then placed in the same plastic container with a lid at ambient temperature. After a rest period of at least 5 days, during which the preservatives were dissolved, 7–10 diet cubes (approximately 10 g) were placed in 30 mL plastic pots with lids (Figure 2 B). No further diet was added during rearing of the insects.

Twenty adult females were placed in each diet-containing plastic pot, which was then incubated for 60 days in the dark in a room with a temperature of 25 ± 3°C and humidity of 70 ± 15%. After 60 days, 20 adult females were picked from the pots and transferred to new diet-containing pots for insect maintenance (Figure 2 C).

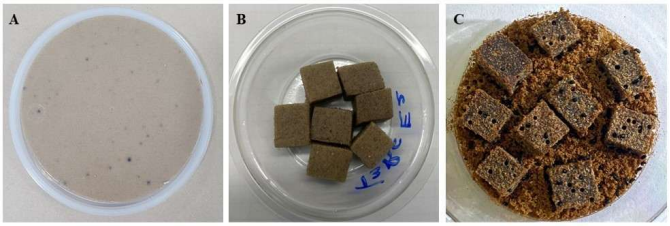

Figure 2. (A) Solid diet; (B) Plastic pot with diet cubes; and (C) Coffee berry borers on the artificial diet after 60 days of incubation.

Evaluation of artificial diets for rearing *H. hampei*

Four artificial diets with different compositions were prepared (Table 1). Torula yeast was replaced with Brewer's yeast in all diets because it is more commercially available. The amounts of agar and coffee in the modified diets of Portilla (1999) (designated diets 1 and 2) were changed according to the final texture of the diet, and the moisture content was also changed. Diets 1 and 2 were nutritionally enriched with salts and vitamins. By contrast, of the two modified diets of Villacorta & Barrera (1993), diet 3 was a simpler one without the addition of coffee, yeasts, salts, and vitamins, whereas diet 4 had the same ingredients as diet 3 but with the addition of salts and vitamins. The commercial brand of the ingredients and the cost of each diet are listed in Table 2, where diet 3 is shown as having the lowest cost (R$ 19,83).

To determine the number of *H. hampei* offspring that would be obtained on these diets, 10 g of cubed diet and 10 adults were placed in 30 mL plastic pots with lids. Adult females were collected from the various pots after 30 days

Table 1. Composition of the artificial diets of Portilla (1999) and Villacorta and Barrera (1993) and the four modified diets used in the bioassays.

Ingredients (weight/volume)	Amount					
	Portilla (1999)	Diet 1	Diet 2	Villacorta & Barrera (1993)	Diet 3	Diet 4
Water (L)	1.0	1.0	1.0	0.7	1.0	1.0
Agar (g)	10	13	13	27	18	18
Ground green coffee (g)	150	180	100	100	154	154
Roasted ground coffee (g)	-	-	80	-	-	-
Sucrose (g)	10	10	10	14	9.0	9.0
Casein (g)	15	15	15	20	15	15
Candida utilis yeast (g)	15	-	-	-	-	-
Torula yeast (g)	-	-	-	20	-	-
Brewer's yeast (g)	-	15	15	-	15	15
Ethanol (mL)	10	10	10	10	16	16
Benzoic acid (g)	1.0	1.0	1.0	-	-	1.0
Vanderzant vitamins (g)	0.5	0.5	0.5	-	-	0.5
Wesson salts (g)	0.8	0.8	0.8	2	-	0.8
Formaldehyde 37% (mL)	1.0	3.2	3.2	2	3.2	3.2
Benomyl (g)	1.5	-	-	-	-	-
Nipagin (g)	-	1.6	1.6	1	1.6	1.6
Sodium benzoate (g)	-	-	-	0.8	-	-
Potassium sorbate (g)	-	-	-	-	1.6	1.6

Table 2. Ingredients, commercial brand, and cost of each diet.

Ingredients	Commercial brand	Cost (R$ L^{-1})			
		Diet 1	Diet 2	Diet 3	Diet 4
Agar	Dinâmica	6.02	6.02	8.53	8.53
Ground green coffee	Catuaí 144	3.99	2.22	3.41	3.41
Roasted ground coffee	Bençavó	-	3.18	-	-
Sucrose	Delta	0.04	0.04	0.04	0.04
Casein	Synth	6.66	6.66	6.84	6.84
Brewer's yeast	Quimicenter	0.30	0.30	0.31	0.31
Ethanol	Dinâmica	0.20	0.20	0.31	0.31
Benzoic acid	Synth	0.10	0.10	-	0.10
Vanderzant vitamins	Sigma-Aldrich	2.28	2.28	-	2.28
Wesson salts	Scharlau	2.30	2.30	-	2.30
Formaldehyde 37%	Dinâmica	0.04	0.04	0.04	0.04
Nipagin	Dinâmica	0.25	0.25	0.25	0.25
Potassium sorbate	Synth	-	-	0.10	0.10
Total (R$ L^{-1}):		22.18	23.59	19.83	24.51

of incubation and combined before distribution in each experiment. After 60 and 80 days of incubation in a dark environment with a temperature of 25 ± 3°C and relative humidity of 70 ± 15%, the resultant offspring were separated into those egg, larval, pupal, and adult stages, with the adults also divided according to male or female sex. The total numbers in each developmental stage (eggs, larvae, pupae, adult females, and adult males) were then counted. The experiment was conducted in a completely randomized design with four treatments and six replications. Each plastic pot containing 10 adults represented one plot.

Outliers were identified by plotting the externally studentized residuals (RStudent) against the predicted values (Y variable). RStudent values that fell outside the range of –2 to 2 were considered outliers, and the corresponding observations were removed from the data analysis. The data obtained were tested for normality using the Shapiro-Wilk test, for homoscedasticity with the Hartley test, and for independence of the residuals through graphical analysis. Once assumptions were met, the data were subjected to analysis of variance with the F-test ($p \leq 0.05$). The treatment effects of all samples were compared with the Tukey test, with statistical significance considered at the 5% probability level ($p \leq 0.05$). All statistical analyses were performed using Statistica 7.0 software (Statsoft, 2007).

Results

Rearing and maintenance of *H. hampei* on an artificial diet

Diet 1 was used for rearing *H. hampei* under a temperature of 25 ± 3°C and humidity of 70 ± 15%, where it took an average of 12 days for larvae to hatch (Figure 3 C), 16 days for pupation, and 8 days for adults to emerge (Figure 3 D), totaling approximately 36 days. The longevity of females on the diet was 190 days and that of males was 45 days. However, more studies are needed to verify these parameters.

A minimum oviposition period of 7 days, which could extend to approximately 20 days depending on the moisture

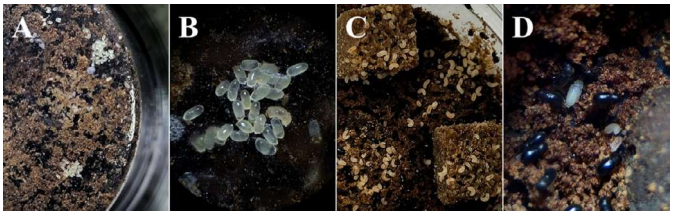

Figure 3. Developmental stages of *Hypothenemus hampei* on an artificial diet. (A) Egg mass outside the diet; (B) Egg mass; (C) Larvae; and (D) Pupae and adults.

content of the diet, was observed for both the *H. hampei* adults that were collected in the field and subjected to the artificial diet and the adults that resulted from this rearing. Oviposition occurred outside the diet, closer to the wall of the plastic pots, separately or in groups (Figure 3 A, B). Portilla (1999) reported that the time before *H. hampei* oviposition on the Cenibroca diet was 3–10 days, and egg laying occurred on the surface of the diet. Portilla & Streett (2008) reared the coffee berry borer on the modified Cenibroca diet for 70 generations with no effect on insect activity, fecundity, weight, or size.

One of the difficulties encountered in the mass rearing of *H. hampei* has been the selection of suitable containers, as the insects can easily perforate plastic. Therefore, we chose reinforced plastic containers in this study (Figure 2 B). Unlike glass test tubes that make handling of the insects difficult, small glass jars with lids can be used to rear and maintain these insects on an artificial diet. Large pieces of diet are also problematic because recovery of the insects is difficult once they bore into the diet. By contrast, small blocks with an edge of approximately 1 cm help not only in insect handling but also in standardizing the moisture content of the diet.

Villacorta (1985) reared more than 15 generations of *H. hampei* on an artificial diet based on cottonseed and reported the difficulties of rearing them in test tubes. According to Brun et al. (1993), the number of *H. hampei* offspring obtained from rearing on an artificial diet was probably limited by the size of the plastic containers used (sealed with stoppers), which restricted the movement of

females to new sites for oviposition during the 70 days of evaluation.

Another important issue in the rearing of *H. hampei* is the moisture of the artificial diet, where a moisture content of above 70% or below 60% makes it practically impossible for insects to reproduce. Therefore, control of the humidity in the rearing environment is also very important to avoid rapid drying and hence frequent replacements or exchanges of the diet. Another important aspect is the need to let the diet rest after preparation, to allow for dissolution of some of the preservatives, since some of the *H. hampei* individuals from the field died approximately 24 h after contact with the freshly prepared diet.

The Cenibroca diet of Portilla (1999) is easy to prepare, contains few ingredients, has good nutritional and physical properties, retains moisture longer, and has a low contamination rate. Jaramillo (2016) tested the Portilla (1999) diet for the rearing and maintenance of *H. hampei* and found it to be comparable to the natural diet (parchment coffee) in terms of the average duration of the egg, larval, and prepupal growth stages, total cycle (from egg to adult), and sex ratio.

Evaluation of artificial diets for rearing *H. hampei*

The rearing of *H. hampei* on the four different artificial diets for 60 days had significant effects on the numbers of females, males, eggs, and pupae, and total number of offspring (Table 3). With regard to the number of females, diets 3 and 4 resulted in 126 and 84 more individuals than diet 1 and 125 and 83 more than diet 2, respectively. In terms of the males, diets 3 and 4 resulted in 10 and 11 more individuals than diet 1 and 8 and 9 more than diet 2, respectively. Diet 3 resulted in 13 more pupae than diets 1 and 2. Regarding the eggs, diets 3 and 4 resulted in 85 and 55 more than diet 1 and 101 and 71 more than diet 2, respectively. By contrast, there was no significant difference in the number of larvae among the diets. In terms of the total number of offspring, diet 3 yielded 242, 251, and 75 more individuals compared with diets 1, 2 and 4, corresponding to 99%, 107%, and 18% increases, respectively (Table 3). In this experiment, the total number of offspring per female adult after 60 days of incubation on diets 1, 2, 3, and 4 was 24.6, 23.7, 48.8, and 41.3, respectively.

A similar effect was observed after 80 days of rearing, where diets 3 and 4 again stood out in most of the parameters evaluated. Additionally, the effects for the numbers of females, males, eggs, larvae, and pupae, and total number of individuals were all statistically significant (Table 4).

Diet 4 resulted in 129 and 131 more females than diets 1 and 2, respectively. Diets 3 and 4 resulted in 12 and 13 more males than diet 2, respectively. Diet 4 resulted in 29 more pupae than diet 1 as well as 38 more pupae and 83 and 106 more larvae than diets 2 and 3, respectively. Diet 3 yielded 268 and 182 more eggs than diets 2 and 4, respectively. With regard to the total number of offspring, diets 3 and 4 resulted in 246 and 268 more individuals (corresponding to 65% and 71% increases) than diet 1 and 329 and 351 more (corresponding to 112% and 120% increases) than diet 2, respectively. In this experiment, the total number of offspring per female adult after 80 days of incubation on diets 1, 2, 3, and 4 was 37.6, 29.3, 62.2, and 64.4 individuals, respectively. Diets 3 and 4 retained more moisture than diets 1 and 2, which may have contributed to this result.

Discussion

Several techniques for rearing *H. hampei* on natural and artificial diets can be found in the literature. Celestino et al. (2016) tested rearing of the insect on a natural diet with different treatments: Arabica coffee beans, Arabica parchment coffee, and Robusta coffee beans, with or without asepsis and storage. The best was Robusta coffee beans without asepsis, which can be stored in a freezer at –20°C to be used in the off-season. With this treatment, the

Table 3. Number of *Hypothenemus hampei* (coffee berry borer) offspring after 60 days of rearing on different artificial diets.

Treatment		Adults		Pupae	Larvae	Eggs	Total
		Females	Males				
1	Diet 1	51 b[1]	7 b	30 b	96 ns	62 b	246 c
2	Diet 2	52 b	9 b	30 b	100	46 b	237 c
3	Diet 3	177 a	17 a	43 a	104	147 a	488 a
4	Diet 4	135 a	18 a	40 ab	103	117 a	413 b
CV (%)		12.5	36.9	23.8	19.4	9.3	5.6

[1] Means followed by the same letters in the same column do not differ from each other by the Tukey test at the 5% probability level. ns: not significant using the F-test.

Table 4. Number of *Hypothenemus hampei* (coffee berry borer) offspring after 80 days of rearing on different artificial diets.

Treatment		Adults		Pupae	Larvae	Eggs	Total
		Females	Males				
1	Diet 1	148 b[1]	16 ab	31 b	110 ab	71 bc	376 b
2	Diet 2	146 b	9 b	22 b	81 b	35 c	293 b
3	Diet 3	218 ab	21 a	22 b	58 b	303 a	622 a
4	Diet 4	277 a	22 a	60 a	164 a	121b	644 a
CV (%)		37.0	35.7	46.7	45.9	18.5	20.7

[1] Means followed by the same letters do not differ from each other by the Tukey test at the 5% probability level.

total numbers of offspring with and without storage were 464 and 387 individuals, respectively. In that study, the maximum number of offspring after 70 days of rearing was 11 individuals per female adult. By contrast, in another study by Benassi (2000), the average number of *H. hampei* offspring was 22 individuals (varying from 17 to 36 individuals) per female in *C. canephora* berries after 28 days of rearing.

As reported by the studies cited above, the number of *H. hampei* offspring resulting from artificial diets was greater than the number resulting from natural diets. The various generations of *H. hampei* reared on artificial diets tended to overlap because the females born were fertilized and soon began oviposition. Thus, individuals in different developmental stages were found (eggs, larvae, prepupae, pupae, and adults) as long as the diet provided the necessary nutrient and moisture conditions for insect development (Villacorta et al., 2000)

Villacorta et al. (2000) tested the rearing of *H. hampei* on the Villacorta & Barrera (1993) diet by replacing the bacteriological agar with less expensive industrial agar (reduction of R$ 2.95). They obtained an average of 153 individuals on the modified diet after 66 days of incubation. According to the authors, the experiment was conducted with individuals collected in the field. When the experiment is conducted with offspring resulting from an artificial diet, the number of adults obtained is likely to be higher because the population members on the same diet are of a more similar age and under the same climatic and feeding conditions compared with the natural population collected in the field.

Brun et al. (1993) obtained an average of 16 *H. hampei* offspring after 70 days of rearing on an artificial diet (consisting of water, agar, wheat germ, sucrose, ground coffee, ascorbic acid, *p*-hydroxymethyl benzoate, sodium propionate, penicillin, streptomycin, and Vanderzant vitamins) under temperature and humidity conditions similar to those of the present study (25°C and 85%). Villacorta & Barrera (1993) obtained a population of up to 79 *H. hampei* individuals on their original diet. Portilla & Streett (2008) evaluated the fecundity of *H. hampei* on a modified Ceniborca diet and obtained up to 84.2 individuals per borer after 70 days of incubation. The data obtained in the present study are consistent with those obtained by these authors, as up to 66 individuals were obtained after 80 days of rearing.

According to the data obtained for the parameters evaluated in this study, diets 3 and 4 were the best sources of nutrients, being more efficient than diets 1 and 2 in rearing the coffee berry borer. Further studies are needed to evaluate the longevity of *H. hampei* adults on each of these diets as well as the number of generations that can be obtained over time.

Conclusions

The mass rearing and maintenance of *H. hampei* on the evaluated diets were successfully achieved, with the modified diets of Villacorta & Barrera (1993) (diets 3 and 4) resulting in the highest number of offspring.

Acknowledgements

We are grateful to Nooa Ciência e Tecnologia Agrícola and FAPEMIG for providing financial support fot this research.

Compliance with Ethical Standards

Author contributions: Conceptualization: VA; CAGF, CMSS; Data curation: JSF, TSS; Formal Analysis: CAGF; Funding acquisition: CAGF, CMSS; Investigation: JSF, CAGF, TSS; Methodology: JSF, VA, CAGF, CMSS; Project administration: JSF, VA; Resources: JSF, CAGF, TSS, CMSS; Supervision: JSF, VA, CAGF, ; Validation: JSF, VA, CAGF, CMSS; Visualization: JSF; Writing – original draft: JSF, CAGF; Writing – review and editing: JSF, VA, CAGF, TSS, CMSS.

Conflict of interest: There is no conflict of interest.

Funding source: The Universidade Federal de Uberlândia.

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