NOTA PRÉVIA

ARTIFICIAL DIET ADJUSTMENTS FOR BRAZILIAN STRAIN OF *Hypothenemus hampei* (FERRARI, 1867) (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT: A modified artificial diet based on the "Cenibroca diet" used in Colombia for mass rearing of *Hypothenemus hampei,* was compared with the natural diet presently used to rear this insect. The modified diet was cheaper than Portilla's diet and did not affect insect fitness, developmental time, viability or sex ratio.

Index terms: Artificial diet, *Hypothenemus hampei*, rearing.

AJUSTES DE DIETA ARTIFICIAL PARA "STRAIN" BRASILEIRO DE *Hypothenemus hampei* (FERRARI, 1867) (COLEOPTERA: CURCULIONIDAE)

RESUMO: Uma dieta artificial para *Hypothenemus hampei*, com modificações a partir de uma dieta utilizada para a espécie na Colômbia e denominada Cenibroca, foi avaliada. Ela mostrou-se adequada e comparável à dieta natural utilizada para o inseto, considerando-se o tempo de desenvolvimento, a viabilidade total e a razão sexual da broca do café, sendo mais económica quando comparada com a dieta desenvolvida por Portilla em 1999.

Termos de indexação: Dieta artificial, *Hypothenemus hampei*, técnicas de criação.

The coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Curculionidae) is considered the most damaging insect pest of coffee crops worldwide, attacking the berries and causing weight loss, berry depreciation and quality problems (BENAVIDES et al., 2013; BUSTILLO, 2002; JARAMILLO et al., 2006; ROMERO; CORTINA, 2007). Damage from this insect pest is estimated at US$500 million annually (JARAMILLO et al., 2006), affecting the income of more than 25 million small farmers worldwide (FAIRTRADE FOUNDATION, 2012).

Artificial diets are an efficient option for insect mass rearing and studies, because insect colonies can be continuously maintained in the laboratory under controlled conditions (PARRA et al., 2002). According to Portilla & Streett (2006), the artificial diets presently used in Colombia, USA and elsewhere to rear the coffee borer are adequate to maintain their development, fecundity and sex ratio.

The present study evaluated the performance of a modified of the "Cenibroca" artificial diet (PORTILLA, 1999), originally used in Colombia, as an artificial diet for mass rearing of Brazilian populations of the coffee berry borer. The diet tested included modifications in the type of agar, yeast, vitamin composition and antifungal (Table 1) and calculated the cost (in dollars) of producing one liter of diet using the components of Portilla´s recipe (1999) as well as the modified diet .

Females of the coffee berry borer (CBB) were obtained from a stock colony established in July 2010 with beetle-infested coffee berries collected from a coffee plantation in Piracicaba, São Paulo, Brazil (22°42'51.0366" S, 047°37'41.556" W). The stock colony is maintained at the Laboratory of Insect Biology of the Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ-USP), Piracicaba, where the insects are reared on ca. 150-day-old coffee berries (*Coffea arabica* var. Obatã) kept at room temperature (25±2 °C), 65±10% relative humidity [RH], and 0:24 h (L:D) photoperiod. In this study, the insects were obtained from infested berries that are kept in square plastic containers 50x50x20 cm (height x width x depth) with perforated lids (55 mm diameter) covered with voile.

Some components of the Cenibroca artificial diet were modified (Table 1). The study evaluated whether these changes in diet affected

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TABLE 1 - Composition of artificial diets for *Hypothenemus hampei* rearing.

Component	D1[a]	D2[b]
Agar	10 g	–
Carregeenan	–	10 g
Water	993 ml	993 ml
Coffee	150 g	150 g
Sucrose	10 g	10 g
Casein	15 g	15 g
Torula yeast	15 g	–
Brewer´s yeast	–	15 g
Ethanol	10 ml	–
Benzoic acid	1 g	1 g
Vanderzant´s Vitamin solution	7.4 ml	–
Vitamin solution[c]	–	7.4 ml
Wesson salts	0.8 g	0.8 g
Formaldehyde 37%	2650 µL	2650 µL
Benomyl®	1.33 g	–
Methyl parahydroxybenzoate (Nipagin®)	–	1.33 g
Cost/L	US$ 16.06	US$ 8.73

[a] Portilla (1999)

[b] Portilla (1999), modified

[c] Niacinamide: 1.00 g; calcium pantothenate: 1.00 g; Riboflavin: 0.50 g; Thiamin: 0.25 g; Pyridoxine: 0.25 g; folic acid: 0.02 mg; Biotin: 0.02 mg; Vitamin B_{12} (1,000 mg/ml): 2.00 ml.

the development of *H. hampei*.

To prepare the diet, agar (carrageenan) was dissolved in distilled water at a temperature of 65 °C and mixed with the other ingredients (Table 1) with the aid of a blender. The diet was dried in a Marconi® MA 033 oven at 45 ± 5 °C for 12–15 h to a final water content of 55 ± 10%. The diet was offered to the insects in a 24-well culture plate (Techno Plastic Products® 920024) with 3–4 ml of diet/well. Two 24-h-old eggs from the stock colony were transferred.

This new modified diet was compared with the "natural" diet of *C. arabica* parchment coffee to 50% ± 10% water content, at a temperature of 25 ± 2 °C, RH of 65 ± 10% and 0:24 (L:D) photoperiod. The method of Romero & Cortina (2007) was used to prepare the coffee berries: a hole 1 mm in diameter and 7 mm deep was bored into each dried berry, and two 24-h-old eggs were placed in the hole. The parchment coffee were placed individually in the same type of culture plates used for the artificial diet.

The development of *H. hampei* on the artificial and natural diets was evaluated with 400 replications. The numbers of CBB life stages (eggs, larvae, prepupae, pupae and adults) were assessed daily for 30 days. Each day, 10 samples of both parchment coffee berries and diet were removed from the plates and dissected under a Zeiss® Stemi SV¨6 stereomicroscope and the immature stages and emerged females and males were counted.

Data Analysis: Data were analyzed by generalized linear models (GLMs) (NELDER; WEDDERBURN, 1972), using a binomial model for viability and sex-ratio data, and a Poisson model for duration. The data were evaluated using a standard half-probability graph with simulated envelope (DEMETRIO; HINDE, 1997;, HINDE; DEMETRIO, 1998). In case of significant differences, the Tukey multiple comparisons test was applied at 5% significance, using the glht

function of the multicomp package with adjusted P.

We calculated the cost (in dollars) of producing one liter of diet using the components of Portilla´s recipe (1999) as well as the modified diet in this work

Compared with the natural diet, the modified Cenibroca artificial diet did not affect the egg-adult development period of *H. hampei*, which averaged 24.1 days on both. Nor were the total viability or the sex ratio significantly different (Table 2).

The artificial diet proved to be adequate with the proposed modifications. This diet provided the necessary nutrients for egg-adult development, with viability and sex ratio comparable to the original Cenibroca diet, which according to Portilla (1999), supports the development of these insects for 15 generations and with development similar to that obtained with the natural diet.

The cost of producing one liter of diet using Portilla´s recipe was US $ 16.06 while the cost of the modified diet was US $ 8.73.

The results obtained here allow us to conclude that this modified artificial diet can be used to maintain the breeding stock of *H. hampei* from Brazil, to obtain eggs and females for experimental use. This diet is comparable to the natural diet, in addition to being low-cost and easy to prepare.

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TABLE 2 - Effects of two different diets on *Hypothenemus hampei*: duration of developmental stages (mean days ± SE), egg to adult viability (mean % ± SE) and sex ratio. Temp.: 25 ± 2 °C, RH: 65 ± 10% and photophase: 0h.

Diet	Duration (days)[1]					Viability (%)[1]	Sex ratio[2]
	Egg	Larva	Pre-pupae	Pupae	Egg-adult		
Parchment coffee berries	4.3±0.05 a	8.6 ±0.17 a	5.1±0.12 a	5.2 ±0.12 a	24.5±0.9 a	83±2.9 a	0.85±0.01 a
Portilla modified	4.1± 0.04 a	8.4 ± 0.20 a	4.8± 0.13 a	5.9±0.09 a	24.1±0.7 a	85±3.5 a	0.82±0.07 a
F. X^2	4.19	1.71	5.17	1.87	3.69	1.19	2.50
P	0.04	0.19	0.02	0.17	0.05	0.317	0.02

[1] Means followed by the same letter in the column do not differ significantly (GLM with quasi-binomial distribution, followed by post-hoc Tukey test). df=1 for all comparisons.

[2] No difference in sex ratio (X^2, $P<0.05$)

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