

## CROP PROTECTION

### Feeding Substrates to Black Coconut Bunch Weevil, *Homalinotus coriaceus* (Gyllenhal) (Coleoptera: Curculionidae), Rearing in Laboratory

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Substratos de Alimentação Para Criação da Broca do Cacho do Coqueiro, (*Homalinotus coriaceus* (Gyllenhal), Coleoptera: Curculionidae) em Laboratório

RESUMO - A broca do cacho do coqueiro, *Homalinotus coriaceus* (Gyllenhal), é uma praga limitante da produção de coco no Brasil, sendo que tanto as larvas como os adultos provocam a queda das flores femininas e dos frutos imaturos, pela interceptação do fluxo de seiva ou pela alimentação direta nas estruturas reprodutivas. Em virtude da escassez de informações sobre sua biologia, realizou-se esse trabalho com o objetivo de desenvolver uma metodologia mais adequada para a criação da praga em laboratório. Foram utilizados os parâmetros biológicos para avaliação e comparação dos sistemas de criação estudados. Toletes de cana-de-açúcar foram utilizados como substrato para alimentação dos adultos coletados no campo e obtenção dos ovos. As larvas foram criadas em três substratos alimentares no Laboratório de Entomologia da Embrapa-CPATC (Centro de Pesquisa Agropecuária dos Tabuleiros Costeiros), em Aracaju, SE. Os substratos alimentares estudados foram: o mesocarpo do coco, dieta para criação da broca dos citros e dieta para criação da broca do olho do coqueiro, sendo esta a que proporcionou o melhor desenvolvimento larval num menor tempo, com boa viabilidade, maior facilidade no preparo e manutenção.

PALAVRAS-CHAVE: Insecta, coco, dieta artificial

ABSTRACT - The black coconut bunch weevil, *Homalinotus coriaceus* (Gyllenhal), is an important coconut palm-tree pest in Brazil, making the female flowers and immature fruits fall down. This paper aimed to get information about its biology and laboratory rearing methods. Pieces of sugar cane were used as nourishment for the adults and oviposition substrate. The larvae were reared in three different diets at temperature of 25 ± 2°C, 70 ± 10% RH and 12h photophase, at the Laboratório de Entomologia of EMBRAPA (Centro de Pesquisa Agropecuária dos Tabuleiros Costeiros), Aracaju County, SE, Brazil. The three feeding substrates were: the coconut mesocarp, the citrus stem borer diet and the coconut american palm weevil diet. The citrus stem borer diet provided the best larval development.

KEY WORDS: Insect, pest, artificial diet

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Many areas where coconut palm-trees are grown (*Cocos nucifera* L.) are located in the tropical zone, between the parallels of latitude 20° (Cuenca 1989). These areas contain the most important perennial crops that can be explored in an auto-sustainable system by the almost 86 countries

located at the inter-tropical regions. For some Asian countries the coconut palm-tree crops mean source of money, protein and calories for the population (Cuenca 1997).

The most important worldwide coconut producers are

Philippines, Indonesia and India; being Brazil the ninth of the rank (Cuenca 1997). Until 1996, Brazil produced about 980 millions of coconut fruits from cv 'giant', aiming to obtain shredded coconut and 125 million liters of coconut milk from cv 'green dwarf' (Viglio 1997). This large scale production has favored the occurrence of many pests (Ferreira et al. 1997).

According to Fonseca (1962) the coconut palm-trees grown in the littoral or some interior areas at altitudes below 800 m are susceptible to the attack of the black coconut bunch weevil, *Homalinotus coriaceus* (Gyllenhal), one of the most typical pests of South America, specially in Brazil and Argentina (Ferreira et al. 1998).

This insect can reduce 50% of the coconut production (Gomes 1992). The larvae drill the floral peduncles and block sap circulation, leading the female flowers to abortion, dropping the immature fruits and causing the loss of the bunches (Ferreira et al. 2002a). Besides larval damage, also the adults use flowers and new fruits as food supply, ingesting the sap and making the bunch dry. For this reason, this insect species is considered one of the most important coconut pests in Brazil (Ferreira et al. 2002b).

The study of the biology and behavior of this pest is important to develop an adequate strategy of control. However, its behavior and feeding characteristics make difficult the insects rearing at laboratory conditions. The objective of this study was to develop a rearing methodology of *H. coriaceus* at laboratory conditions, using feeding substrates that permit the larval development, with all the features necessary for a borer insect to grow.

## Material and Methods

The study was conducted at the Entomology Laboratory of EMBRAPA - CPATC (Centro de Pesquisa Agropecuária dos Tabuleiros Costeiros), Aracajú County, SE, Brazil, at  $25 \pm 2^\circ\text{C}$  temperature, 70% RH and 12h photophase.

Adults of *H. coriaceus* were collected from coconut trees cv 'green dwarf' in Saquarema County, State of Rio de Janeiro and kept on pieces of sugar-cane as substrate for feeding and oviposition. The eggs collected in the inner part of the sugar-cane pieces were placed, individually, in petri dishes lined with moisturized filter paper to avoid dehydration.

Newly hatched larvae were reared on three different feeding substrates. The first was coconut fruit mesocarp (diet A); the second was the artificial diet developed by Machado & Berti Filho (1999) to rear *Diploschema rotundicollis* (Serville) (Coleoptera: Cerambycidae), the citrus stem borer (diet B); the third was the artificial diet developed by Nadarajan (1986) to rear the coconut american palm weevil *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae) with some modifications (diet C) (Table 1).

To establish the rearing for both artificial diets (diet B and C), newly hatched larvae were placed in plastic cups of 50 ml, containing a piece of the diet. Each cup received only one larva to avoid possible cannibalism. The cups, with diet and larva, were covered with a foam disk and kept on a rack.

Measurements of larval head capsules were taken every five days, when the diet was replaced to avoid contamination. As soon as the larvae were getting bigger, they were removed for larger plastic cups (100 ml) until reaching the pupal stage.

Table 1. Diets used to rear the black coconut bunch weevil (*H. coriaceus*) under laboratory conditions.

Ingredients	Diet B	Diet C
	(Machado & Berti Filho 1999)	(Nadarajan 1986)
Casein	-	50 g
Soybean flour	-	60 g
Sucrose	-	125 g
Corn flour	150 g	60 g
Wheat germ	35 g	48 g
Yeast of beer	40 g	48 g
Wesson's salts	-	20 g
Cholesterol	-	4 g
Coconut fiber powder	-	100 g
Methylparabenzoate	01 g	3 g
Benzoic acid	01 g	3 g
Formaldehyde 40%	1.5 ml	4 ml
Vitamins for child	-	10 ml
Áscorbic acid	05 g	4.8 g
Agar	30 g	32 g
Distilled water	800 ml	800 ml
Ampicilin e chloranphenicol	-	1 tab

To establish the rearing on the natural diet (diet A), newly hatched larvae were placed individually in petri dishes (3 cm x 1 cm). According to their development, the larvae were placed in larger petri dishes (8.5 cm x 1.8 cm), where they were fed with little pieces of coconut mesocarp. While the food supply was changed every other day to avoid fungal contamination, the measurements were taken every five days.

The data of larvae that originated males and females were analyzed separately. The number of larval instars was determined through direct observations of each larva and confirmed through the frequency curve developed by Taylor (1930 and 1931) and reviewed by Parra e Haddad (1989). For the larvae fed with diets B and C, the number of instars was determined through direct observation of the exuviae easily found in the diets.

To determine the number of instars, the larvae had the head capsule measured: 265 larvae on diet A, 243 larvae on diet B and 146 larvae on diet C, using a Somet® paquimeter with 0,001 mm of precision. Larval survival and the minimum, maximum and mean duration of larval stage were determined. The growth ratio was determined for each ecdyse, calculating the mean growth ratio.

As soon as the insects reached the pre-pupal stage, they were removed from the feeding container and placed on petri dishes lined with moisturized filter paper. The petri dishes were kept in dark boxes to simulate the natural environmental conditions. Sex and pupal weigh were determined 24h after the metamorphosis.

The assessed biological parameters were: period of egg incubation, number of larval instars, head capsule width, growth ratio, duration of larval stage, larval survival, duration of pre-pupa stage, pupa weigh after 24h, duration of pupal

stage, pupal survival and body size.

The body and rostrum length and width of the recent emerged imagoes were taken with a Somet® paquimeter. The body length was considered as the distance between the anterior part of rostrum until the further abdomen and the body width as the distance between the extreme parts of elytron wings at resting position.

All the observed parameters were recorded and the mean values with each standard error were calculated according to the number (n) of insects obtained from each stage of development after submitted to ANOVA. The means were compared by Tukey test at 5% of probability.

## Results and Discussion

The incubation period was observed on eggs obtained from adults collected in the field, because of the long period require to complete the total life cycle. The incubation period, with no influence of the food supply used for larval development, was 10 days (ranging from 6 to 14). So, the average period of egg incubation was considered 10 days for the total life cycle determination in the three diets.

Larval development lasted from five to seven instars. Despite the same number of instars obtained from the three diets, the insects reared on coconut mesocarp showed better growth ratio when compared to those reared on the other two artificial diets (Tables 2, 3 and 4). The growth ratio followed the Dyar's rule (Taylor 1931), however, its value was maintained in 1.4 only during the initial instars, decreasing during final ones. This reduction was greater in the artificial diet than in the natural ones and it interfered in the mean growth ratio of the insects.

Table 2. Mean width (mm) ( $\pm$  SE) of male and female head capsules, with their respective coefficient of variation and growth ratio for each larval instar of *H. coriaceus* on diet A. (Temperature:  $25 \pm 2^\circ\text{C}$ , RH: 70% and photophase: 12h)

Instars	Male		Female		n
	Mean width of the head capsule	Growth ratio	Mean width of the head capsule	Growth ratio	
I	1.3 $\pm$ 0.01 (1.04 - 1.48)	1.4 $\pm$ 0.08	1.3 $\pm$ 0.01 (0.93 - 1.45)	1.4 $\pm$ 0.09	265
II	1.8 $\pm$ 0.02 (1.43 - 2.02)	1.4 $\pm$ 0.09	1.8 $\pm$ 0.02 (1.42 - 2.22)	1.4 $\pm$ 0.10	230
III	2.6 $\pm$ 0.03 (2.02 - 3.14)	1.4 $\pm$ 0.09	2.5 $\pm$ 0.03 (1.63 - 2.94)	1.4 $\pm$ 0.08	204
IV	3.6 $\pm$ 0.04 (2.91 - 4.68)	1.4 $\pm$ 0.06	3.4 $\pm$ 0.05 (2.37 - 4.10)	1.3 $\pm$ 0.07	193
V	5.5 $\pm$ 0.06 (4.17 - 6.21)	1.3 $\pm$ 0.08	4.4 $\pm$ 0.06 (3.17 - 5.51)	1.2 $\pm$ 0.07	185
VI	5.8 $\pm$ 0.05 (5.00 - 6.38)	1.2 $\pm$ 0.00	5.4 $\pm$ 0.07 (4.14 - 6.32)	1.2 $\pm$ 0.06	143
VII	6.2 $\pm$ 0.00 (6.16)		5.9 $\pm$ 0.09 (5.11 - 6.59)		41
Mean growth ratio		1.35		1.32	

Table 3. Mean width (mm) ( $\pm$  SE) of male and female head capsules, with their respective coefficient of variation and growth ratio for each larval instar of *H. coriaceus* on diet B. (Temperature:  $25 \pm 2^\circ\text{C}$ , RH: 70% and photophase: 12h)

Instars	Male		Female		n
	Mean width of the head capsule	Growth ratio	Mean width of the head capsule	Growth ratio	
I	1.2 $\pm$ 0.01 (0.96 - 1.40)	1.4 $\pm$ 0.08	1.3 $\pm$ 0.01 (1.08 - 1.39)	1.4 $\pm$ 0.09	243
II	1.7 $\pm$ 0.01 (1.30 - 2.02)	1.4 $\pm$ 0.09	1.8 $\pm$ 0.01 (1.48 - 2.17)	1.4 $\pm$ 0.10	190
III	2.4 $\pm$ 0.01 (1.94 - 2.84)	1.3 $\pm$ 0.09	2.4 $\pm$ 0.02 (1.88 - 3.14)	1.3 $\pm$ 0.08	185
IV	3.2 $\pm$ 0.02 (2.69 - 3.98)	1.3 $\pm$ 0.06	3.3 $\pm$ 0.02 (2.62 - 4.00)	1.3 $\pm$ 0.07	179
V	4.1 $\pm$ 0.03 (3.54 - 5.01)	1.2 $\pm$ 0.08	4.2 $\pm$ 0.03 (3.49 - 5.04)	1.2 $\pm$ 0.07	171
VI	4.9 $\pm$ 0.03 (4.32 - 5.62)		5.0 $\pm$ 0.04 (4.33 - 5.58)		149
VII	5.2 $\pm$ 0.04 (4.92 - 5.63)		5.3 $\pm$ 0.06 (4.74 - 5.89)		44
Mean growth ratio		1.10		1.10	

The mean duration of larval stage was 147.5 days for males and 140.8 days for females on diet A, 80.2 days for males and 81.0 days for females on diet B and 89.8 days for males and 90.0 days for females on diet C (Table 5). The larval survival was 64.9% on diet A, 55.1% on diet B and 54.2% on diet C. The shortness of the larval stage can be

considered as a favorable characteristic for diets aiming insects rearing (Parra 2000).

At the end of larval stage, the insects started the pre-pupal phase that lasted 6.9 days to the larvae fed on diet A, 4.9 days on diet B and 6.2 days on diet C. During this stage, the development period on diet established by Machado & Berti

Table 4. Mean width ( $\pm$  SE) of male and female head capsules, with their respective coefficient of variation and growth ratio for each larval instar of *H. coriaceus* on diet C. (Temperature:  $25 \pm 2^\circ\text{C}$ , RH: 70% and photophase: 12h)

Instars	Male		Female		n
	Mean width of the head capsule	Growth ratio	Mean width of the head capsule	Growth ratio	
I	1.3 $\pm$ 0.01 (1.21 - 1.41)	1.4 $\pm$ 0.08	1.3 $\pm$ 0.01 (1.12 - 1.39)	1.4 $\pm$ 0.09	146
II	1.8 $\pm$ 0.01 (1.64 - 2.33)	1.4 $\pm$ 0.09	1.8 $\pm$ 0.01 (1.68 - 1.96)	1.4 $\pm$ 0.10	123
III	2.4 $\pm$ 0.02 (2.03 - 3.00)	1.3 $\pm$ 0.09	2.4 $\pm$ 0.01 (2.03 - 2.67)	1.3 $\pm$ 0.08	116
IV	3.1 $\pm$ 0.02 (2.58 - 3.71)	1.3 $\pm$ 0.06	3.0 $\pm$ 0.03 (2.50 - 3.69)	1.3 $\pm$ 0.07	109
V	4.0 $\pm$ 0.03 (3.46 - 4.69)	1.2 $\pm$ 0.08	4.0 $\pm$ 0.04 (3.38 - 4.73)	1.2 $\pm$ 0.07	94
VI	4.9 $\pm$ 0.03 (4.51 - 5.56)	1.1 $\pm$ 0.00	4.7 $\pm$ 0.03 (4.10 - 5.11)	1.1 $\pm$ 0.06	80
VII	5.2 $\pm$ 0.02 (5.10 - 5.36)		5.2 $\pm$ 0.02 (5.04 - 5.36)		48
Mean growth ratio		1.10		1.10	

Table 5. Mean duration period ( $\pm$  SE) of the different development stages and pupal weight of *H. coriaceous*, in three feeding substrates at laboratory conditions (temperature:  $25 \pm 2^\circ\text{C}$ , RH: 70% and photophase: 12h).

	Duration of the development stages (days)				Pupal weight (mg)
	Egg	Larva	Pupae	Total	
Diet A		140.8 $\pm$ 2.63 b (n = 62)	31.0 $\pm$ 0.66 b (n = 53)	181.9	1330 $\pm$ 37.10 (n = 50)
Diet B		81.0 $\pm$ 2.39 a (n = 57)	27.4 $\pm$ 0.34 a (n = 70)	128.4	1311 $\pm$ 36.30 (n = 50)
Diet C		90.0 $\pm$ 4.45 a (n = 14)	30.4 $\pm$ 0.93 b (n = 21)	119.4	1336 $\pm$ 62.04 (n = 14)
S	10.1 $\pm$ 0.17 (n = 100)	19.15	3.89		256.19
Cv		18.43	13.12		19.32
Dms		12.08	2.15		145.26
Diet A		147.5 $\pm$ 3.20 b (n = 75)	30.6 $\pm$ 0.53 b (n = 79)	188.5	1405 $\pm$ 41.20 (n = 50)
Diet B		80.2 $\pm$ 2.56 a (n = 43)	28.3 $\pm$ 0.41 a (n = 53)	136.3	1393 $\pm$ 45.10 (n = 43)
Diet C		89.8 $\pm$ 1.78 a (n = 24)	30.8 $\pm$ 0.68 b (n = 32)	126.8	1413 $\pm$ 53.23 (n = 24)
s	10.1 $\pm$ 0.17 (n = 100)	22.52	4.07		292.24 <sup>n.s.</sup>
Cv		21.26	13.63		20.81
Dms		12.26	1.99		165.69

The letters indicate similarity among the means in columns, for males and females separately, according to the Tukey test at 5% probability.

Filho (1999) for citrus stem borer was reduced.

The exarate pupae of *H. coriaceous* is white-yellowish and becomes darker next to adult emergence. The mean period of pupal phase was 29.7 days, but the pupae obtained from diet B showed a mean period of pupal phase about 27.8 days shorter than the others.

There were no significant differences in pupal weight after 24h (Table 5) on the various substrates studied, thus indicating that the artificial diets tested were able to show faster development without interfering in the weight gain. Therefore, these diets seem nutritionally adequate to *H. coriaceous* rearing. The pupal survival of insects reared was 94% for males and 89% for females on diet A, 86% for males and 91% for females on diet B and 70% for males and 65% for females on diet C.

The body size of *H. coriaceous* adults obtained on the three diets were compared to the body size of adults obtained from the field, look for differences that could assess the quality of the diets. No significant differences were observed among the insects reared on the diets, but these insects were significantly shorter than those obtained in the field (Table 6). The same was verified for rostrum length and width (Table 7).

The total life cycle of the insects reared was 188.5 days for males and 181.9 days for females on diet A, 136.3 days for males and 128.4 days for females on diet B and 126.8 days for males and 119.4 for females on diet C. So, the egg-adult cycle of insects reared on diets B and C was reduced in about 2.8 months for males and 1.9 months for females, in comparison with the insects reared on natural substrate (diet A) (Table 5).

Besides the mesocarp coconut diet have shown the best growth ratio that could result in bigger insects, similar to the insects collected in the field, the frequent manipulation during the changes of substrates, could have caused modifications during the larval and pupal phase, that resulted in a significant late in development or gain of weigh. The insects reared on artificial diets were not as manipulated as the others and were left to perform their borer behavior.

Based on these data, it was possible to verify that *H. coriaceous* can be reared in laboratory, on the artificial diet developed by Machado & Berti Filho (1999) for citrus stem borer, which favored the adequate insect development in a shorter time, the lowest cost, with good survival and easy to prepare.

Table 6. Mean body size (cm) ( $\pm$  SE) and respective variation intervals of *H. coriaceus* adults collected in the field and obtained from diets A, B and C (temperature:  $25 \pm 2^\circ\text{C}$ , RH: 70% and photophase: 12h).

Origin	Mean body length		General mean	Mean body width		General mean
	Male	Female		Male	Female	
Field	3.12 $\pm$ 0.04 b (2.57 - 3.85) (n = 50)	3.05 $\pm$ 0.03 b (2.20 - 3.91) (n = 50)	3.1	1.15 $\pm$ 0.01 b (0.92 - 1.42) (n = 50)	1.12 $\pm$ 0.01 b (0.97 - 1.30) (n = 50)	1.1
Natural (diet A)	2.86 $\pm$ 0.03 a (2.49 - 3.31) (n = 50)	2.90 $\pm$ 0.03 a (2.44 - 3.35) (n = 50)	2.9	1.05 $\pm$ 0.01 a (0.91 - 1.21) (n = 50)	1.09 $\pm$ 0.01 a (0.89 - 1.22) (n = 50)	1.1
Citrus (diet B)	2.85 $\pm$ 0.02 a (2.48 - 3.59) (n = 43)	2.87 $\pm$ 0.01 a (2.55 - 3.70) (n = 50)	2.9	1.04 $\pm$ 0.01 a (0.88 - 1.27) (n = 43)	1.08 $\pm$ 0.01 a (0.94 - 1.27) (n = 50)	1.1
Fiber (diet C)	2.86 $\pm$ 0.04 a (2.56 - 3.23) (n = 24)	2.83 $\pm$ 0.03 a (2.59 - 3.00) (n = 14)	2.8	1.04 $\pm$ 0.01 a (0.92 - 1.20) (n = 24)	1.06 $\pm$ 0.02 a (0.92 - 1.18) (n = 14)	1.0
General mean	2.9	2.9		1.1	1.1	
s	0.23	0.22		0.08	0.07	
Cv	7.92	7.56		7.86	6.71	
Dms	0.12	0.13		0.04	0.04	

The letters indicate similarity among the means in columns, for males and females separately, according to the Tukey test at 5% probability.

Table 7. Mean rostrum size (mm) ( $\pm$  SE) and their respective variation interval of *H. coriaceus* adults collected in the field and obtained from diets A, B and C (Temperature:  $25 \pm 2^\circ\text{C}$ , RH: 70% and photophase: 12h).

Origin	Mean rostrum length		General mean	Mean rostrum width		General mean
	Male	Female		Male	Female	
Field	8.08 $\pm$ 0.10 b (6.36 - 9.92) (n = 50)	7.43 $\pm$ 0.08 a (6.38 - 8.90) (n = 50)	7.76	1.71 $\pm$ 0.02 b (1.20 - 2.20) (n = 50)	1.65 $\pm$ 0.02 b (1.20 - 1.90) (n = 50)	1.68
Natural (diet A)	7.14 $\pm$ 0.07 a (6.34 - 8.78) (n = 50)	7.16 $\pm$ 0.08 a (6.46 - 8.94) (n = 50)	7.15	1.41 $\pm$ 0.02 a (1.20 - 1.80) (n = 50)	1.54 $\pm$ 0.02 a (1.30 - 1.90) (n = 50)	1.47
Citrus (diet B)	7.29 $\pm$ 0.10 a (6.24 - 8.94) (n = 43)	7.32 $\pm$ 0.07 a (6.36 - 9.28) (n = 50)	7.30	1.50 $\pm$ 0.03 a (1.10 - 1.90) (n = 43)	1.60 $\pm$ 0.02 ab (1.20 - 2.10) (n = 50)	1.55
Fiber (diet C)	7.30 $\pm$ 0.13 a (6.36 - 8.82) (n = 24)	7.22 $\pm$ 0.16 a (6.62 - 8.14) (n = 14)	7.41	1.40 $\pm$ 0.04 a (1.20 - 1.90) (n = 24)	1.60 $\pm$ 0.04 ab (1.40 - 1.80) (n = 14)	1.50
General mean	7.48	7.30		1.52	1.59	
s	0.62	0.64		0.18	0.16	
Cv	8.45	8.85		12.14	10.39	
Dms	0.34	0.35		0.10	0.09	

The letters indicate similarity among the means in columns, for males and females separately, according to the Tukey test at 5% probability.

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