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Bioecology of the parasitism of *Billaea rhynchophorae* (Blanchard, 1937) (Diptera: Tachinidae) in *Rhynchophorus palmarum* (L., 1758) (Coleoptera: Curculionidae) in Brazil

Bioecologia do parasitismo de *Billaea rhynchophorae* (Blanchard, 1937) (Diptera: Tachinidae) em *Rhynchophorus palmarum* (L., 1758) (Coleoptera: Curculionidae) no Brasil

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We investigated bioecological aspects of *Rhynchophorus palmarum* parasitism by *Billaea rhynchophorae* under field and laboratory conditions. In oil palm plantations, eleven species of spontaneous flowering plants were also collected and identified. In the laboratory, the effect of food resources on some parasitoid parameters was also assessed. The mean parasitism rate in the field was higher than 60%, but no significant correlation existed between the environmental variables and the parameters evaluated. Spontaneous flowering plants identified were *Acmella uliginosa* (Sw.) Cass., *Ageratum conyzoides* L., (Asteraceae), *Borreria verticillata* (L.) G. Mey. (Rubiaceae), *Clidemia hirta* (L.) D. Don (Melastomataceae), *Commelina erecta* L. (Commelinaceae), *Cuphea racemosa* (L.f.) Spreng. (Lythraceae), *Ipomoea* sp. (Convolvulaceae), *Lantana camara* L., *Stachytarpheta* cf. *maximiliani* Schauer (Verbenaceae), *Ludwigia* sp. (Onagraceae) and *Sauvagesia erecta* L. (Ochnaceae). The mean viability of parasitoid pupae from the field was 41.83%, and the mean pupal duration was 45.54 days. Mean parasitoid puparia length, diameter, and weight were 10.61 mm, 4.8 mm, and 0.07 g, respectively. The sex ratio of flies was 0.59, and the mean longevity was 8.61 days. Each female produced an average of 159.79 planidia, with a length of 0.92 mm. Longevity of parasitoids fed with bee pollen, 20% honey, and sugarcane stalks was greater when compared to those fed with *Adonidia merrillii* flowers, chamomile flowers, and 33% sucrose. However, there was no parasitism or viable planidia when adults were fed with the food resources evaluated until 18 days of age.
Keywords: black palm weevil, tachinid, biological control.

Avaliaram-se aspectos bioecológicos do parasitismo de *Rhynchophorus palmarum* por *Billaea rhynchophorae* em campo e laboratório. Em plantações de dendê, onze plantas espontâneas com flores também foram coletadas e identificadas. Em laboratório, também avaliou-se o efeito de fontes alimentares sobre alguns parâmetros do parasitoide. O parasitismo médio em campo foi maior que 60%, mas não houve correlação entre as variáveis ambientais e os parâmetros avaliados. As plantas com flores identificadas foram *Acmella uliginosa* (Sw.) Cass., *Ageratum conyzoides* L., (Asteraceae), *Borreria verticillata* (L.) G. Mey. (Rubiaceae), *Clidemia hirta* (L.) D. Don (Melastomataceae), *Commelina erecta* L. (Commelinaceae), *Cuphea racemosa* (L.f.) Spreng. (Lythraceae), *Ipomoea* sp. (Convolvulaceae), *Lantana camara* L., *Stachytarpheta* cf. *maximiliani* Schauer (Verbenaceae), *Ludwigia* sp. (Onagraceae) e *Sauvagesia erecta* L. (Ochnaceae). A viabilidade pupal média do parasitoide proveniente do campo foi 41,83%, e a duração pupal média foi 45,54 dias. As médias de comprimento, diâmetro e peso dos pupários foram 10,61 mm, 4,8 mm, e 0,07 g, respectivamente. A razão sexual das moscas foi 0,59 e a longevidade média foi 8,61 dias. Cada fêmea produziu, em média, 159,79 planídios, com comprimento de 0,92 mm. A longevidade dos parasitoides alimentados com pólen apícola, mel 20%, e colmos de cana-de-açúcar foi maior que dos alimentados com flores de *Adonidia merrillii*, flores de camomila, e sacarose 33%. Contudo, não houve parasitismo ou planídios viáveis quando os adultos foram alimentados com nenhum dos recursos avaliados, até 18 dias de idade.
Palavras-chave: bicudo-negro-do-coqueiro, taquinídeo, controle biológico.

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1. INTRODUCTION

Palms (Arecaceae) represent one of the largest families of commercially important plants in the tropics and subtropics. Species such as coconut (*Cocos nucifera* L.) and oil palm (*Elaeis guineensis* Jacq.) are widely cultivated [1]. However, an increasing number of pests has threatened the production and international trade of various palms. The black palm weevil, or South American palm weevil, *Rhynchophorus palmarum* (L., 1758) (Coleoptera: Curculionidae), is a pest of great economic importance for these crops since both larval and adult stages can damage the plants, the larvae being responsible for direct damage, while the adults are responsible for transmitting the red ring disease, caused by the nematode *Bursaphelenchus cocophilus* (Cobb, 1919), which leads plants to die [2-5].

Current pest management is based on capturing adults with aggregation pheromone and chemical control [3, 4, 6]. However, the results are unsatisfactory in some regions, with high economic costs and environmental damage [7]. Biological control can contribute to this management by reducing the economic damage of the pest with little or no adverse environmental consequences. The Tachinidae family represents the main group of parasitoids of the order Diptera [8, 9], with great importance in the parasitism of boring beetles [10, 11].

A potential tachinid control agent for *R. palmarum* is *Billaea rhynchophorae* (Blanchard, 1937) (Exoristinae: Dexiini) [12], which has been found parasitizing the pest in the state of Bahia, Northeast region of Brazil, with average parasitism of 40% [13], and also with significant variation in the size of pupae and adult flies [14]. One of the oviposition strategies of this group's females is depositing larvae called planidia close to the potential hosts. These, in turn, can actively seek out the host to initiate parasitism [15].

Although the Tachinidae family is composed exclusively of parasitoid species in their larval stage [10, 11], adults frequently visit flowers and feed on nectar to obtain energy and pollen as a source of protein, lipids, and vitamins. These floral resources increase adult longevity, fecundity, and host-seeking capacity [16]. Thus, knowledge of the plant species whose flowers are visited by a specific tachinid species can become a tool that helps to improve its performance in the field as a biological controller.

Despite the high rates of parasitism of *R. palmarum* by *B. rhynchophorae* observed under natural conditions, more detailed studies on the biology and behavior of the species, as well as on its interactions with the host, have yet to be carried out. Thus, the present study aimed to investigate some of the bioecological aspects of *B. rhynchophorae* on *R. palmarum* under field and laboratory conditions as a subsidy for the establishment of parasitoid laboratory rearing and the development of strategies that could be used to control the pest.

2. MATERIALS AND METHODS

2.1 Parasitoid collection and laboratory keeping

Three insect collections were carried out in September 2019, December 2019, and November 2020 in commercial oil palm plantations located in the municipalities of Jaguaripe (13°00'49.5"S; 38°51'08.3"W) and Taperoá (13°31'30.9"S; 39°07'09.9"W), in the State of Bahia, Northeast region of Brazil. The climate in the area is tropical without dry season (Af in Koppe's classification), with rainfall of 1900–2200 mm and annual mean temperature of 24-26°C [14, 17]. Oil palms that were dead or had red ring disease symptoms were identified and cut down. Its crown was sectioned longitudinally with a chainsaw to collect larvae and pupae of *R. palmarum* (Figure 1A), as well as the immature stages of the parasitoid fly (larvae and puparia) (Figures 1B-C) (permanent license to collect zoological material by Sistema de Autorização e Informação em Biodiversidade – SISBIO, number 64508-1).

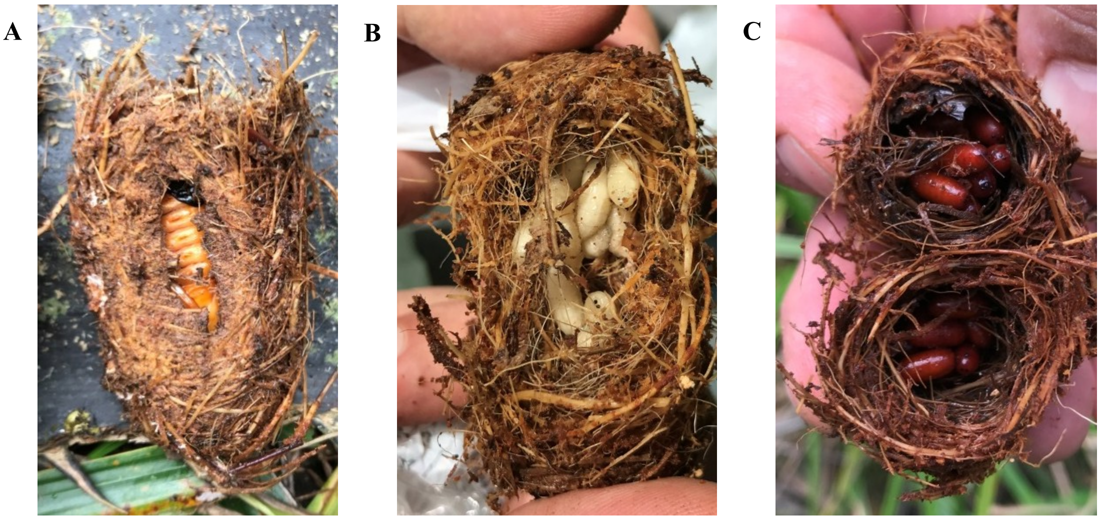

Figure 1. Cocoons of Rhynchophorus palmarum *containing* R. palmarum *pupa (A);* Billaea rhynchophorae *larvae (B); and* B. rhynchophorae *puparia (C).*

In the laboratory, the cocoons of *R. palmarum* containing larvae or pupae of *B. rhynchophorae* were monitored daily until pupation or emergence of the adult parasitoids, respectively. The cocoons containing larvae or pupae of the host that were possibly parasitized were also observed daily for the possible exit of the parasitoid from the host's body. All material was packed in translucent plastic containers (dimensions of 7.9 cm × 7.9 cm and height of 8 cm) and kept in B.O.D. chambers at a temperature of 25.0 ± 1ºC, relative humidity (RH) of 70 ± 10% and a 12-hour photophase. Larvae of *R. palmarum* were kept in sugarcane stalk pieces as a source of food and fibrous material for cocoon formation.

2.2 Spontaneous flowering plant collection

In the surroundings of the oil palms, spontaneous flowering plants were collected, taken to the laboratory, mounted in exsiccatae, and sent to the herbarium of the Instituto do Meio Ambiente do Estado de Alagoas (Herbário MAC) in Maceió, Alagoas, Brazil, for identification of the species.

2.3 Weevil collection and laboratory keeping

Adults of *R. palmarum* were collected in coconut palm plantations in the municipalities of São Miguel dos Milagres (9º13'2.06"S; 35º24'55.65"W) and Coruripe (10º08'25.91"S; 36º11'56.04"W), in the state of Alagoas, Northeast region of Brazil, using bucket-type traps baited with the aggregation pheromone Rincoforol® (Interacta Química, Brazil), and sugarcane stalks as attractants [3, 4]. Adult males were killed and discarded, and females were used to obtain eggs and larvae, according to the methodology described by Löhr (2016) [18], in which adults are confined in ventilated plastic boxes with sugarcane *Saccharum officinarum* L. (Poaceae) stalks as a feeding and oviposition substrate, and kept at room temperature. The newly hatched larvae were transferred to an artificial diet [19] and maintained for approximately six weeks. After this period, the larvae were offered sugarcane stalk until their use in bioassays.

2.4 Biological parameters of parasitoids from the field

After emergence, the adult flies were distributed in cages measuring 30 cm × 30 cm and 70 cm high, lined with voile, and kept in a room at uncontrolled temperature. Food was offered through cotton rolls soaked in distilled water in plastic plates (90 mm in diameter × 15 mm in height), and the introduction of chamomile plants (*Matricaria chamomilla* L.) (Asteraceae) in the flowering stage in the cages for nectar supply. After death, the flies were kept in 99% alcohol under refrigeration and sent to Dr. Ronaldo Toma (Fiocruz, Campo Grande, Mato Grosso do Sul, Brazil) for species identity confirmation [14]. Vouchers were deposited in the Zoological Reference Collection of the Universidade Federal de Mato Grosso do Sul (ZUFMS) in Campo Grande, Mato Grosso do Sul, Brazil.

The following parameters related to the biological aspects of *B. rhynchophorae* were evaluated: viability and duration of the pupal period; weight, length, and diameter of pupae; sex ratio; longevity of adults; and number and size of planidia. The 3265 pupae collected in the field were considered to determine pupal viability. To determine the duration of the pupal period, 139 *B. rhynchophorae* larvae were individualized and monitored from pupation to adult emergence. A total of 266 pupae were used to determine the weight, length, and diameter with a precision analytical balance and a caliper. To determine the sex ratio, the formula SR = NF/(NF+NM) was adopted, where SR= sex ratio, NF= number of females, and NM= number of males. Subsequently, 267 adult fly couples composed of females up to 48 hours old and older males were confined in cages measuring 30 cm × 30 cm × 70 cm in height, lined with voile, containing chamomile plants and water, for determining the longevity of adults. A total of 89 euthanized females were dissected under a stereoscopic microscope. The embryonic sac was removed and placed on a Petri dish with 5% dextrose. The planidia were separated with a fine brush, counted, and measured with the stereoscope's ocular lens scale. Thirty planidia were randomly taken from each female for measurement.

2.5 Food resources and parasitoid longevity

Food resource assessments were conducted with no-choice tests in cages measuring 30 cm × 30 cm × 70 cm in height, covered with voile. Based on the observation of spontaneous plants of the Asteraceae family in *B. rhynchophorae* collection areas, *M. chamomilla* was included as a treatment. The food sources evaluated in the first experiment were: I) *M. chamomilla* flowers; II) palm *Adonidia merrillii* Becc. (Arecaceae) flowers; and III) *S. officinarum* stalks. In the second experiment, it were evaluated: I) 20% honey solution; II) 33% sucrose solution; and III) bee pollen, according to the methodology of Dindo et al. (2019) [20]. The honey and sucrose solutions were offered in soaked cotton pads, and bee pollen (3 g) in pellets. For all treatments, cotton pads soaked in water were also provided.

For each food source, 15 unsexed adult flies, aged between 24 and 48 hours, were inserted. The cages were inspected daily, and the number of dead flies in each was recorded until total mortality. Food sources were replaced with new ones every 2-3 days.

2.6 Food resources and parasitism in the laboratory

Cages (30 cm × 30 cm × 70 cm in height) containing one of the same food sources of the previous essay (*M. chamomilla* flowers, *A. merrillii* flowers, *S. officinarum* stalks, 20% honey solution, 33% sucrose solution, and bee pollen) were used. In each cage, eight *R. palmarum* larvae (4 to 5.3 cm long, cephalic capsule 0.4 to 0.6 cm wide, and weight 6.62 to 10.66 g) were placed individually inside open plastic pots measuring 6 cm × 8 cm × 8 cm in height. In each cage, 15 newly emerged unsexed *B. rhynchophorae* adults were also released. Food sources were replaced by new ones every 2-3 days. Eight female flies from each treatment were sacrificed 8, 10, 12, and 14 days after emergence to verify the presence or absence of active planidia. After the death of all flies, the *R. palmarum* larvae were kept in plastic pots for two weeks and dissected to check for parasitism.

2.7 Experimental design and statistical analysis

Data obtained from the field and the parasitoid biological parameters observed in the laboratory were submitted to descriptive statistics, with the calculation of means and standard error. The influence of the variables diameter at breast height (DBH) of the palm stem, air temperature, and relative humidity on the biological aspects of the pest and the parasitoid was evaluated by determining Pearson's correlation coefficient, whose significance was assessed by Student's t-test ($p < 0.01$), using the software GENES [21, 22]. The DBH was measured at the time of collection with a tape measure, and climate data were obtained from the meteorological station in Valença-BA, the closest station to Jaguaripe and Taperoá available from the Instituto Nacional de Meteorologia – INMET (https://portal.inmet.gov.br/), considering the average of the 30 days before each collection.

Essays on the influence of food resources were conducted in a completely randomized design, with three treatments, each food resource being considered as a treatment. In experiment 1, 45 replications were used and 30 replications in experiment 2, each adult fly being considered as one replication. Means were subjected to analysis of variance and, when necessary, compared to each other by Tukey's test ($p < 0.05$) using the statistical software GENES [21, 22].

3. RESULTS AND DISCUSSION

3.1 Spontaneous flowering plant identification

In the oil palm plantations where *B. rhynchophorae* was collected in Bahia, Brazil, the following species of spontaneous flowering plants were identified: *Acmella uliginosa* (Sw.) Cass., *Ageratum conyzoides* L., (Asteraceae), *Borreria verticillata* (L.) G. Mey. (Rubiaceae), *Clidemia hirta* (L.) D. Don (Melastomataceae), *Commelina erecta* L. (Commelinaceae), *Cuphea racemosa* (L.f.) Spreng. (Lythraceae), *Ipomoea* sp. (Convolvulaceae), *Lantana camara* L., *Stachytarpheta* cf. *maximiliani* Schauer (Verbenaceae), *Ludwigia* sp. (Onagraceae) and *Sauvagesia erecta* L. (Ochnaceae). All exsiccatae were deposited in the collection of the herbarium of the Instituto do Meio Ambiente do Estado de Alagoas (Herbário MAC).

The attractiveness of flowers to natural enemies is an important characteristic that must be considered in plant selection, and adapting the nectar supply to the needs of parasitoids can enhance their effectiveness as biological control agents [23]. Topham and Beardslay Jr (1975) [24] observed that the presence of nectariferous plants, such as *Euphorbia hirta* L. (Euphorbiaceae), on the margins of sugarcane fields resulted in an increase in population levels and efficiency of parasitism of *Lixophaga sphenophora* (Villeneuve, 1911) (Diptera: Tachinidae) on the borer *Rhabdoscelus obscurus* Boisduval, 1835 (Coleoptera: Curculionidae). In this sense, the plant diversity of agricultural environments can act indirectly, enabling conservation or increasing the abundance and diversity of natural enemies [25]. Tooker et al. (2006) [26] also state that Asteraceae flowers serve as a food source for Tachinidae, and Sathe et al. (2014) [16] observed that Asteraceae was the family of floral plants most visited by flies from the Tachinidae family in the districts of Hapur and Satara, India. The present work investigated the effect of a plant from the same family, *M. chamomilla*, on the longevity and parasitism of *B. rhynchophorae* in the laboratory. However, it was inefficient in prolonging the adult parasitoids' longevity. Nor was it possible to observe any correlation between other species of flowering plants and parasitoid longevity.

3.2 Biological parameters of parasitoids from the field

In all collections, cocoons of *R. palmarum* were more abundant at the end of the rainy season (September/2019) (12.2 cocoons/palm) than in the warmer months (December/2019 and November/2020) (7.6 and 9.9 cocoons/palm, respectively) (Table 1). This constant presence of *R. palmarum* cocoons, containing different stages of the biological cycle in the field, maybe because this insect has a long lifecycle, high reproductive potential, and dispersal capacity [27, 28].

Table 1. Variables (mean ± SE) of Rhynchophorus palmarum *and parasitism by* Billaea rhynchophorae *recorded in three collections in the field, at Jaguaripe and Taperoá, Bahia, Brazil.*

Variables	**Collections**		
	Sep/2019	**Dec/2019**	**Nov/2020**
Cocoons of *R. palmarum* per palm	12.23 ± 3.79	7.62 ± 1.44	9.90 ± 1.77
Parasitism by *B. rhynchophorae* (%)	30.91 ± 8.89	60.50 ± 7.50	69.11 ± 7.36
Live larvae of *R. palmarum* (%)[1]	12.71 ± 4.09	17.67 ± 6.58	14.24 ± 6.86
Dead larvae of *R. palmarum* (%)[1]	3.06 ± 1.47	5.64 ± 2.34	4.77 ± 1.86
Live pupae of *R. palmarum* (%)[1]	20.25 ± 6.56	6.39 ± 2.58	5.16 ± 2.36
Dead pupae of *R. palmarum* (%)[1]	18.47 ± 4.01	4.71 ± 2.48	5.35 ± 2.26
Adults of *R. palmarum* (%)[1]	14.57 ± 4.53	5.80 ± 3.27	1.36 ± 0.94

[1] Biological forms found inside the collected cocoons, without parasitism.

The presence of the parasitoid *B. rhynchophorae* was also observed in all collections, with parasitism rates that varied from 30.9% at the end of the rainy season (September/2019) to 60.5% and 69.1% in the warmer months (December/2019 and November/2020, respectively) (Table 1). Such results coincide with those obtained by Moura et al. (1993) [29], who found average parasitism of *Billaea menezesi* (Guimaraes, 1977) of 51%, ranging from 33.0% at the beginning of the warm season (October/1990) to 72.7% at the end of the rainy season (August/1991), and by Moura et al. (2006) [13], of *B. rhynchophorae*, ranging from 18% at the peak of the rainy season (July/2001) to 57% at the beginning of the warm season (November/2000), in the same region of the present study.

In addition to the density and the genotypic and phenotypic characteristics of the host and parasitoid, several abiotic factors, such as temperature, photoperiod, and humidity, can interfere with the parasitism rate [30, 31]. Thus, based on the comparison between the study by Moura et al. (2006) [13] and the present, it can be inferred that the biotic (plant/host) and abiotic (climate) factors in the region of the natural occurrence of *B. rhynchophorae* showed some stability and support capacity in maintaining the parasitoid species within this agricultural landscape over the last decades.

The percentages of live larvae (12.7%, 17.7%, and 14.2%) and dead larvae (3.1%, 5.6%, and 4.8%) of *R. palmarum* remained more or less constant over the three collections (September/2019, December/2019, and November/2020, respectively). However, in the collection of September/2019, the percentages of live pupae (20.3%), dead pupae (18.5%), and adults (14.6%) were much higher than in December/2019 (6.4%, 4.7%, and 5.8, respectively) and in November/2020 (5.2%, 5.4%, and 1.7%, respectively).

This reduction in the percentage of live and dead pupae and adults of *R. palmarum* in the last two collections can be directly related to the increase in parasitism, which was approximately twice as high as in the first one. While natural parasitism by *B. rhynchophorae* can be considered a determining factor in the population of *R. palmarum*, the rate of population increase of parasitoids also occurs as a function of host density [32], and the age of the host larvae is also an important factor for the success of parasitism in Tachinidae [12].

Cysne et al. (2013) [33] found no significant differences in the monthly population fluctuation of adults of *R. palmarum* in *E. guineensis* and *Elaeis oleifera* (HBK) Cortes (Arecaceae) in the Brazilian Amazon. However, Ferreira et al. (2003) [34], Takada et al. (2011) [35], and Correia et al. (2015) [36] observed an increase in the adult population of *R. palmarum* during the rainy season in plantations of *C. nucifera* in the Northeast region, of *Musa* sp. in the Southeast region, and of *E. guineensis* in the North region of Brazil, respectively, demonstrating the influence of precipitation and the consequent increase in humidity on the number of captured insects, which corroborates the present study. Nevertheless, it is worth mentioning that in all the studies reported here, adults were captured with traps containing pheromones and food attractants. In the present study, only the newly emerged adults still inside the cocoons were captured, disregarding the adults already free in the environment. All results suggest that the largest population of adults of *R. palmarum* in the field occurs in the rainiest and wettest months of the year, regardless of region and crop.

The correlation matrix calculated among the *R. palmarum* variables in the three field collections (Table 2) and the environmental variables stem diameter at breast height, mean air temperature, and mean relative air humidity revealed significant correlations between stem diameter at breast height and adults (p = 0.017) in the first collection; between air temperature and number of cocoons/palm (p = 0.045) and number of dead pupae/palm (p = 0.05) in the second collection; and between air temperature and number of live larvae/palm (p = 0.033) and dead larvae/palm (p = 0.048), as well as a highly significant correlation between relative air humidity and number of adults/palm (p = 0.01) in the third collection (Table 2). However, correlations were very weak to weak. Although it is known that the abundance of pest populations can be regulated by factors that depend on their trophic interaction and also on physical factors, such as climate variables [31], for the conditions of the present study, the air temperature and relative humidity did not directly interfere with the biological events evaluated.

Table 2. Pearson correlation matrix (p - values) of Rhynchophorus palmarum × *environmental parameters in three collections at Jaguaripe and Taperoá, Bahia, Brazil.*

Environmental parameters	***Rhynchophorus palmarum* parameters**						
	C	**P**	**LL**	**DL**	**LP**	**DP**	**A**
1st collection							
DBH	0.37	0.21	0.36	0.17	0.36	0.29	0.52*
T	0.07	-0.04	0.03	-0.15	0.14	0.19	0.01
RH	0.08	0.29	0.08	0.29	-0.09	-0.02	-0.08
2nd collection							
DBH	-0.19	-0.20	-0.06	-0.07	-0.30	-0.10	-0.04
T	0.33*	0.19	0.28	0.27	0.21	0.32*	0.17
RH	0.11	0.09	0.05	0.18	0.01	0.01	0.07
3rd collection							
DBH	0.07	-0.03	0.05	0.00	0.07	0.09	0.20
T	0.24	0.21	0.28*	0.26*	0.06	0.08	-0.04
RH	0.08	-0.11	-0.07	-0.08	0.19	0.24	0.33**

DBH= stem diameter at breast height; T= mean air temperature; RH= mean relative air humidity; C= number of cocoons/palm; P= percentage of parasitism by *B. rhynchophorae*; LL= live larvae; DL= dead larvae; LP= live pupae; DP= dead pupae; A= adults. *= Significant ($p \leq 0.05$); **= Highly significant ($p \leq 0.01$).

Although there was no significant correlation between parasitism and air temperature, the highest parasitism rates in the field occurred in November/2019 and December/2020 (Table 1), when the average temperature was around 25ºC (24.6ºC and 24.3ºC, respectively), which is considered ideal for insect development [37]. In the collection of September/2019, the average temperature was 21.4ºC. Besides the action on the metabolism of adults, temperature influences several biological insect factors in the egg, larva, and pupa stages and can change the time of a generation and emergence rates [38]. Conversely, the relative air humidity was lower in the months with the highest parasitism rates (82.0% and 84.3% in December/2019 and November/2020, respectively, and higher in the month with the lowest parasitism (87.0% in September/2019). Rodrigues (2004) [36] points out that the favorable humidity range for insects is between 40% and 80% and that low humidity can affect insect physiology, longevity, development, and oviposition [39].

The puparia of *B. rhynchophorae* showed an elliptical shape with a reddish color when newly formed, later going through a progressive darkening and assuming a dark reddish-brown color. The puparia had mean length and diameter of 10.6 and 4.8 mm, respectively, and weight of 0.07 g. The parasitoids emerged to the adult stage 45.54 days after pupation (Table 3). Pupal viability was higher in the collections of November/2020 and December/2019 (45% and 39.7%, respectively) and lower in September/2019 (33.3%) (Table 3), accompanying the percentages of parasitism (Table 1). We checked nine cocoons of *R. palmarum* containing 90 puparia of *B. rhynchophorae* whose adults had already emerged in the field to detect possible hyperparasitism. The average adult *B. rhynchophorae* emergence in the field was 95.56%, and only one puparium (1.11%) showed signs of hyperparasitism (hole of adult hyperparasitoid emergence). Three puparia were dead by unknown causes, which we considered natural mortality during the species' development.

Table 3. Biological parameters (mean ± SE) of Billaea rhynchophorae *in the laboratory.*

Parameter		Mean
Pupal viability (%)	September/2019	33.33
	December/2019	39.72
	November/2020	45.00
Puparium length (mm)		10.61 ± 0.064
Puparium diameter (mm)		4.80 ± 0.04
Puparium weight (g)		0.07 ± 0.002
Pupal period duration (days)		45.54 ± 0.27
Sex ratio		0.59
Adult longevity (days)	Males	8.54 ± 0.28
	Females	8.65 ± 0.24
	Mean	8.61 ± 0.18
Planidia/female		159.79 ± 13.08
Planidium length (mm)		0.92 ± 0.09

There is no information in the literature about the biology of *B. rhynchophorae*. For *Billaea claripalpis* (Wulp, 1896), Aya et al. (2019) [40] obtained pupal viability of 81% to 98% and duration of the pupal period of 23 days on four species of *Diatraea* (Lepidoptera: Crambidae) under laboratory conditions, with little effect of the environment and handling of the specimens. Almeida et al. (1986) [41] obtained a pupal period duration of 17 days for *B. claripalpis* on *Diatraea saccharalis* (Fabricius, 1794), also under laboratory conditions. For *Billaea adelpha* (Loew, 1873) reared on *Xylotrechus sieversi baiocchii* Rapuzzi & Sama, 2018 (Coleoptera: Cerambycidae), Bolu et al. (2021) [42] observed a pupal period of approximately one week.

The sex ratio of *B. rhynchophorae* was 0.59, with mean adult longevity of 8.6 days, 8.5 days for males, and 8.7 days for females. Each female contained an average of 159.79 planidia, with a mean length of 0.92 mm (Table 3). For *B. claripalpis*, Aya et al. (2019) [40] obtained a sex ratio of 0.38 in *D. saccharalis* and 0.6 in *Diatraea tabernella* (Dyar, 1911). A higher proportion of females in populations of natural enemies brings benefits to biological control since it is the females that oviposit or produce the planidia that will parasitize the pest, and a single male can fertilize more than one female [43].

In the first collection (September/2019), the greatest number of adults emerged was observed 25 to 30 days after the collection of cocoons in the field, with greater longevity in those who emerged in the 15th and 23rd-30th days after the collection. Similarly, in the second collection (December/2019), the highest emergence of adults occurred between 23 and 30 days after collection of cocoons. However, the emergence lasted until the 50th day after collection. On the other hand, in the third collection (November/2020), the period with the highest emergence of adults was between 44 and 47 days after the collection of cocoons in the field, again with an emergence that lasted until the 53rd day (Figure 2). In the three collections, it was possible to observe a great variation in the daily emergence of adults, probably due to the biological aspects of the host, such as size, age, etc., and also to the uncertainty of the dates of the parasitism in the field, unlike what would occur in a controlled laboratory rearing, in which the dates of parasitism are known and controlled, providing a better synchronization of biological events.

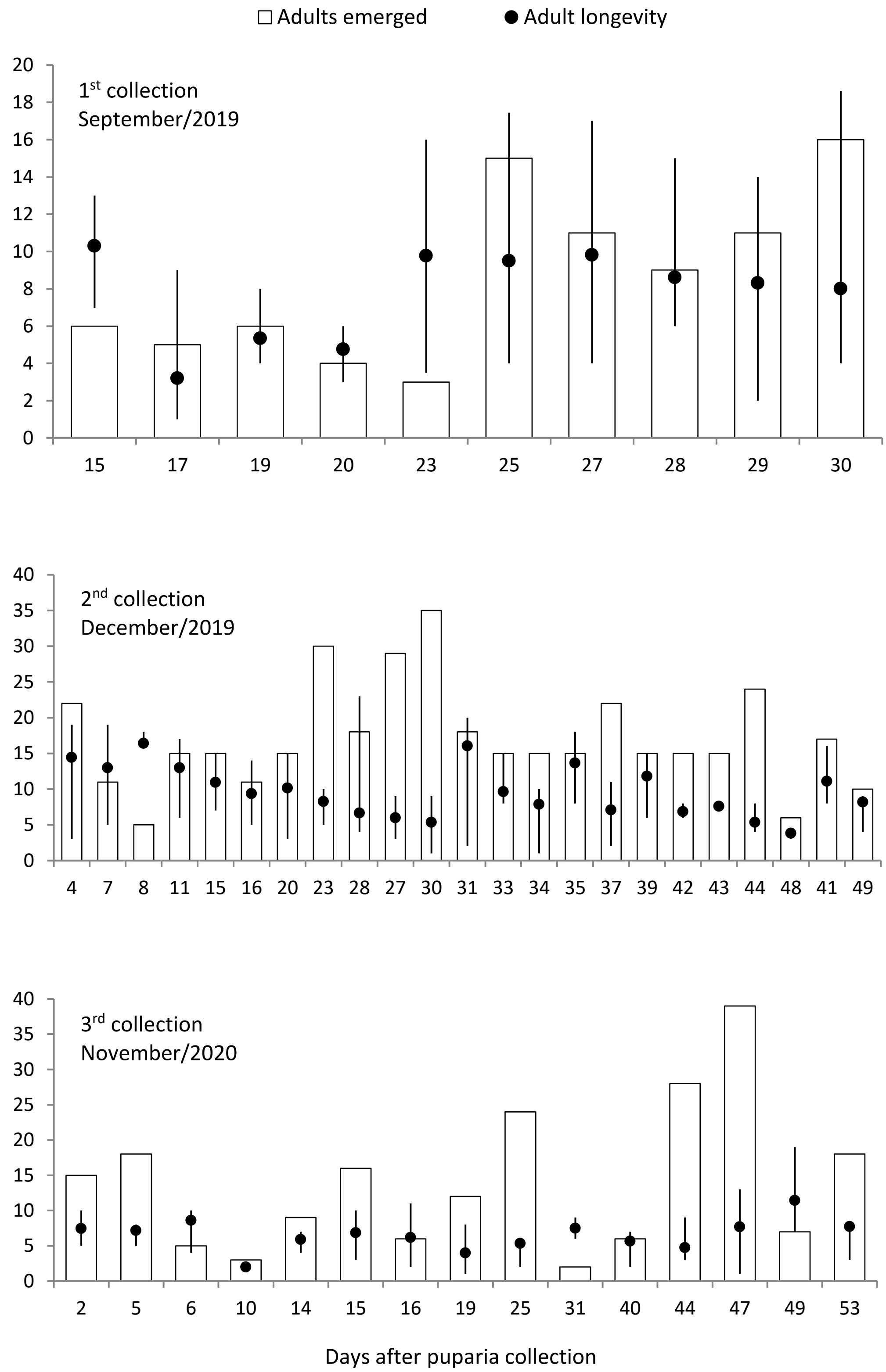

Figure 2. Longevity (mean ± confidence interval), in days, and number of adults of Billaea rhynchophorae *emerged in the laboratory from* Rhynchophorus palmarum *cocoons collected from naturally infested oil palms at Jaguaripe and Taperoá, Bahia, Brazil.*

Aya et al. (2019) [40] obtained a longevity of 11.2 days for *B. claripalpis* adults in *Diatraea* spp., with no significant effect of host species. Female longevity is an important factor since the maturation of planidia can be lengthy. *Billaea claripalpis* reaches sexual maturity at 10-12 days [44]. The longevity of *B. rhynchophorae* individuals may be related to the type of diet and environmental conditions to which they are subjected [45, 46], making it necessary to improve the management of females with better food and suitable habitat for their maintenance.

The planidia obtained from females were characterized by the large mass of individuals with whitish color and small size. The planidia were immobile due to the non-occurrence of copulation between males and females in the laboratory. According to Gaviria and Löhr (2020) [44], the planidia of *B. claripalpis* are so active that it is necessary to place them in a vaseline circle for manipulation, thus reducing the speed of movement and avoiding escaping.

Given the high rates of parasitism obtained in the field, it is recommended to continue biological studies with *B. rhynchophorae* to improve the management of puparia and flies, enabling their rearing and multiplication in the laboratory and aiming at the development of strategies that could be used in the biological control of *R. palmarum*.

3.3 Food resources on the parasitoid longevity

In the first trial, it was found that the longevity of *B. rhynchophorae* was greater in the treatment with sugarcane (13.62 days), statistically differing from the treatments with *A. merrillii* flowers (10.35 days) and chamomile flowers (8.53 days), which, in turn, did not differ from each other ($F_{2, 132} = 4.33$; $p < 0.01$). In the second trial, bee pollen and 20% honey provided greater longevity (16.33 and 15.67 days, respectively), not differing from each other, but differing from 33% sucrose (7.63 days) ($F_{2, 87} = 4.86$; $p < 0.01$) (Table 4).

Table 4. Longevity (mean ± EP) of Billaea rhynchophorae *adults submitted to different food sources in the laboratory.*

Trial	Treatment	Longevity (days)
1	*Saccharum officinarum* stalks	13.62 ± 0.77 a
	Adonidia merrillii flowers	10.35 ± 0.75 b
	Matricaria chamomilla flowers	8.53 ± 0.53 b
	C.V. (%)	42.88
2	Bee pollen	16.33 ± 1.56 a
	20% honey	15.67 ± 1.35 a
	33% sucrose	7.63 ± 0.59 b
	C.V. (%)	51.26

Means followed by the same letter within the same trial are not different by the Tukey's test ($p < 0.01$).

The food resources that provided greater longevity for *B. rhynchophorae* adults were pollen and 20% honey in the second trial and sugarcane stalks in the first. Dindo et al. (2019) [20] observed that females of *Exorista larvarum* L., 1758 (Diptera: Tachinidae) have greater longevity when fed with pollen and sucrose (29.2 and 25.9 days, respectively) than when fed with 20% honey (19.9 days). According to the authors, the number of eggs laid per female in the host larvae was not significantly affected by the food sources offered to the adults, however, the difference in female longevity influenced the number of eggs laid throughout their lives. Zhang et al. (2010) [47] emphasize that there is no record of pollen being offered as food to Tachinidae in laboratory rearings. However, according to the authors, pollen has been used as a food source for Hymenoptera parasitoids, alone or in combination with other carbohydrates.

Controversially, in the present study, sugarcane, which is very rich in sucrose, provided good longevity to *B. rhynchophorae* adults, but 33% sucrose did not provide good longevity. Any substrate rich in sugar and protein can contribute to insect survival. However, it has been observed that different concentrations supplied to a parasitoid can affect its development, altering the physiological functions of adults and their reproductive performance [48].

Billaea rhynchophorae presents indirect oviposition as its reproductive strategy, which consists of laying larvae called planidia. These, in turn, have the ability to locate host larvae by waiting for their passage or actively searching for them [49]. Indirect oviposition probably evolved as an adaptive response by which tachinid females can reach the host within plant tissues, often protected by plugs [50], as is the case with *B. rhynchophorae* and *R. palmarum*. Gaviria and Löhr (2020) [44] report that flies of the genus *Billaea* reach sexual maturity at 10-12 days. In the present study, flies aged 8 to 14 days had developed planidia, however, they were not viable, regardless of the food resource used, most likely because the females were not mated.

Thus, knowing that flowers produce nectar and pollen, which contribute to the survival and reproduction of parasitoid insects in agroecosystems [25, 51], the diversification of these systems could contribute to the permanence of *B. rhynchophorae* and the increase in its parasitism rates on *R. palmarum* in the field, or even in laboratory rearings. However, the biology of *B. rhynchophorae* in the laboratory and the role of local flora in biology and parasitism still need to be studied further.

3.4 Food resources on parasitism in the laboratory

The dissection of *R. palmarum* larvae revealed no parasitism by *B. rhynchophorae* with any food resources evaluated. Likewise, when adult females of *B. rhynchophorae* were dissected, no viable planidia were found in flies of any age or food resource.

4. CONCLUSION

Extraordinarily high field parasitation rates make *B. rhynchophorae* a promising candidate for introduction biocontrol of native and invasive palm weevils. However, the very long life cycle, the low rate of adult emergence from field-collected specimens, the short adult longevity, and the failure to reproduce in captivity require further studies to convert this species into a viable biocontrol agent.

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