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Biological and reproductive parameters of *Tribolium castaneum* in Brazil nut

Evaldo Martins Pires^{1,*}, Roberta Martins Nogueira¹, Leda Rita D'Antonino Faroni², Marcus Alvarenga Soares³, and Marco Antônio Oliveira⁴

Abstract

Brazil nut is used widely in the food industry because the nutritional content of this commodity contains a variety of lipids, proteins, and essential minerals, as well as vitamin A which is used in the cosmetic industry. Agricultural products such as grains, seeds, and nuts often are adversely affected by numerous organisms, including *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), that cause significant losses during storage. Therefore, the objective of the present study was to investigate the biological and reproductive parameters of *T. castaneum* on Brazil nut. Pre-oviposition, egg incubation, and oviposition lasted an average of 7.4, 4.2, and 28.4 d, respectively, with 79.4% egg viability. Oviposition began on the seventh d after mating and the last occurrence of laying was on the 46th d. An average of 1.2 eggs per d per female was produced during the first 20 d. The mean number of eggs per female produced during a lifetime was 28.9. The larval stage exhibited 8 instars during a period of about 86 d. The pupal stage averaged 6.5 d and the adult stage 40.8 d. Survival rate was 44.7% on d 40 and 22.7% on d 60 after adult emergence.

Key Words: red flour beetle; *Bertholletia excelsa*; food substrate; survival; stored product pests

Resumo

A castanha-do-brasil é amplamente utilizada nas indústrias alimentícia devido ao seu valor nutricional dessa “commodity” conter uma variedade de lipídeos, as proteínas e os minerais essenciais, tanto quanto a presença da vitamina A usada na indústria cosmética. Produtos agrícolas tais como, os grãos, as sementes e as castanhas são afetados por muitos organismos, incluindo *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) que causam perdas durante o armazenamento. Desta forma, o objetivo desse estudo foi investigar os parâmetros biológicos e reprodutivos de *T. castaneum* alimentados com castanha-do-brasil. A pré-oviposição, a incubação dos ovos e a última postura em média foi de 7,4; 4,2 e 28,4 d, respectivamente, com viabilidade de 79,4% dos ovos. A oviposição iniciou no sétimo d após o acasalamento e a última ocorrência de postura foi no 46º d. Uma média de 1,2 ovos por d por fêmea foi produzido durante dos 20 primeiros d. A média do número de ovos por fêmea foi 28,9. O estágio larval apresentou oito instares com duração de 86 d. O estágio pupal durou 6,5 d e a fase adulta 40,8 d. A taxa de sobrevivência foi de 44,7% no d 40 e 22,7% no d 60, após a emergência do adulto.

Palavras Chave: produto Amazônico; *Bertholletia excelsa*; substrato alimentar; sobrevivência; produtos armazenados

Brazil nuts are produced by the Brazil nut tree (*Bertholletia excelsa* HBK) (Lecythidaceae) and are native to the Amazon rainforest, which includes the countries Bolivia, Ecuador, French Guiana, English Guiana, Peru, and Brazil (Loureiro & Silva 1968; Loureiro et al. 1979; Souza et al. 2008). The nuts (seeds) are protected inside by a triangular-shaped woody bark encapsulated by a bulging carapace called “ourico” (the popular name for the fruit of Brazil nut tree). An outer covering is composed of a woody, thick, resistant material that protects the seeds from harsh climates, as well as physical and chemical damage (Scussel et al. 2014).

Brazil produced 37,664 tons of Brazil nuts in 2016 (Instituto Brasileiro de Geografia e Estatística 2017). Brazil nut production benefits the income of thousands of families that harvest the commodity. Postharvest processing of Brazil nuts is of considerable additional economic importance by providing employment along the production chain (Maciel & Reydon 2008).

The most significant efforts in Brazil nut research focus on the nutritional values of this product on human health (González & Salas-Salvado 2006; Berno et al. 2010; Colpo et al. 2013). In Brazil, there are few species of insect pests that are reported attacking Brazil nut, including coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) (Gumier-Costa 2009), and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *Tribolium castaneum* is popularly known as the “red flour beetle” and is considered a cosmopolitan insect found mainly in the tropics (Rees 1996; Faroni & Sousa 2006). It is classified as a secondary pest because adults and immature stages feed on grains previously cracked, broken, or damaged by primary pests. However, White (1982) reported the insect’s ability to survive in undamaged grains. Because this stored product pest has been reported previously to infest stored Brazil nuts (Pires et al. 2017) we investigated the biological and reproductive capacity of *T. castaneum* to develop in Brazil nuts.

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Material and Methods

Tribolium castaneum used in this study were obtained from a laboratory colony maintained on Brazil nuts for 2 yr in an acclimatized Biochemical Oxygen Demand chamber (Caltech EI-08F1-F, Caltech Ltda. Indústria e Comércio de Equipamentos de Laboratório, Recife, Pernambuco, Brazil) at 30 ± 2 °C and RH of $60 \pm 10\%$ situated in the Laboratory of Pests and Vectors of Amazon/Cerrado of the Universidade Federal de Mato Grosso - Campus de Sinop. Periodically, beetles were added to the colony from field collected material because this species has exhibited high mortality when reared on Brazil nuts.

Initially, 150 pupae were obtained from the colony and maintained until adult emergence in 6×1.5 cm Petri dishes lined with sheets of 75 g white paper at the bottom. One d after emergence, mating pairs were placed in similar Petri dishes for a total of 25 replications. Half Brazil nuts (cut with a surgical scalpel) were provided to each pair as a food substrate. Brazil nut meal was not provided to adult beetles because in preliminary tests individuals exhibited difficulty in moving about the dish. This was due to the high oil content of the nut meal that accumulated on the surface of the Petri dish from nut debris. This problem was similar to that for *T. castaneum* in maize meal (Li & Arbogast 1991). On the other hand, *T. castaneum* is classified as a primary pest for Brazil nuts, experiencing no difficulties when feeding on the intact product (Pires et al. 2017).

Duration of pre- and oviposition periods as well as daily and cumulative *T. castaneum* egg deposition per female were recorded. Eggs were collected daily with a soft bristle brush from nuts while those deposited on the sulphite papers were placed separately in another set of Petri dishes of the same dimensions as mentioned earlier. Egg hatch was monitored daily. Larvae were fed Brazil nuts. Duration and number of instars were determined by the presence of exuvium that characterizes an instar change. Daily and cumulative survivorship of *T. castaneum* adults fed Brazil nuts were determined using an additional aliquot of 150 pupae from the Laboratory of Pests and Vectors of Amazon/Cerrado colony and handled similarly as previously mentioned in Petri dishes. In preliminary tests, we found no difference between males and females daily or cumulative survivorship when fed Brazil nuts. Therefore, the sexes were not separated when recording those biological parameters. A regression analysis was performed on mean oviposition data (Ne) and percent adult survival (PS) over time fitted with a quadratic model, $P < 0.05$. All the statistical procedures were performed using Action Stat Pro software (Estatcamp & Digup 2017).

Results

Tribolium castaneum pre-oviposition, egg incubation, and oviposition periods were 7.4 ± 1.7 , 4.2 ± 0.4 , and 28.4 ± 7.2 d (\pm SD), respectively (Table 1), and egg viability was 79.4%. Oviposition began on the seventh d after males and females were placed together, and continued until 46 d after mating. Overall, females produced about 29 eggs per individual lifetime.

Generally, we found that egg production declined over time at a decreasing rate ($Ne = 0.0015t^2 - 0.1233t + 2.7824$; $R^2 = 0.8586$) (Fig. 1). The first four d of egg deposition averaged 1.9 per d while during d 5 to 12, the average was 1.3 eggs per d. After d 13, egg production declined to < 1 egg per d (Fig. 1). Peak oviposition was reached on d 4 with an average of 2.2 eggs deposited.

Overall duration of the larval stage averaged 85.9 ± 18.8 d, with 8 instars identified (Table 1). The pupal stage averaged 6.5 ± 0.5 d, whereas adult survivorship averaged 40.8 ± 28.5 d. The first occur-

Table 1. Biological parameters of *Tribolium castaneum* (Coleoptera: Tenebrionidae) in Brazil nuts.

Parameters	Duration in days (Mean \pm SD)
Pre-oviposition period	7.4 ± 1.7
Incubation period	4.2 ± 0.4
Oviposition period	28.4 ± 7.2
First instar	2.6 ± 0.6
Second instar	9.0 ± 1.3
Third instar	10.1 ± 1.4
Fourth instar	9.6 ± 1.2
Fifth instar	11.4 ± 1.9
Sixth instar	14.2 ± 3.1
Seventh instar	10.0 ± 3.2
Eighth instar	19.0 ± 6.0
Pupae period	6.5 ± 0.5
Adult period	40.8 ± 28.5

rence of death was recorded on the second d after emergence and the last was recorded at 129 d (Fig. 2). Generally, we found that adult *T. castaneum* survivorship declined over time ($PS = 0.0077t^2 - 1.7791t + 103.25$), with a coefficient of determination (R^2) = 0.9986 (Fig. 2). Approximately 45% of the population was alive at 40 d, then gradually declined to about 23% at 60 d.

Discussion

We found that the length of the pre-oviposition period in *T. castaneum* was in agreement with other studies, where *T. castaneum* females usually initiated oviposition about one wk after mating, and that the period from copulation to oviposition was, on average, four d (Good 1936). Also, the incubation period of *T. castaneum* eggs was similar to that found by Good (1936), Abdelsamad et al. (1988), and Devi and Devi (2015). However, the oviposition period of our beetles, and the number of eggs produced per female fed Brazil nuts, was considerably reduced compared with the same species reared on other diets (at the same temperature) where oviposition continued from 5 to 6 mo (Good 1936; Li & Arbogast 1991). In our study, mean egg viability was considerable lower (79%) than that reported for red flour beetles reared on flour, where egg hatch approached 90% (Good 1936). These results suggest that Brazil nut, when used as a food source for *T. castaneum*, may adversely affect the fertility and

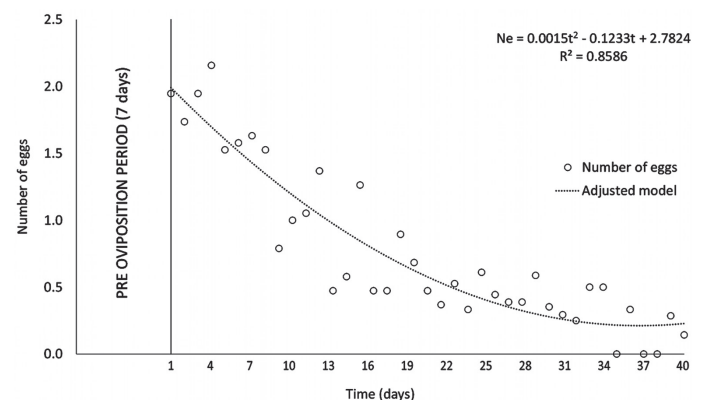


Fig. 1. Regression analysis of daily mean number of *Tribolium castaneum* eggs produced by individuals that fed on Brazil nuts.

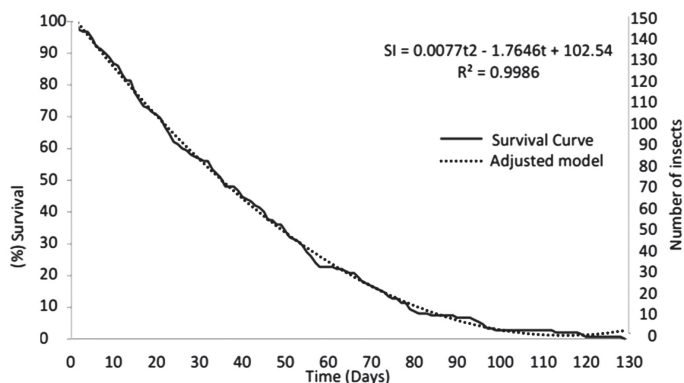


Fig. 2. Regression analysis of daily mean survival of adult *Tribolium castaneum* that fed on Brazil nuts.

biotic potential of this insect. This study was conducted under the optimum rearing conditions for *T. castaneum*, previously reported by others to be 30 °C (Good 1936; Howe 1956; Halliday & Blouin-Demers 2014; Halliday et al. 2015).

We also found that Brazil nut, as a food source, delayed larval developmental time in *T. castaneum*. Indeed, other workers have found similar results with whole wheat, corn flour, and bran and wheat flakes (Good 1936; Halliday & Blouin-Demers 2014; Devi & Devi 2015). We identified 8 instars in our rearing studies, which is within the range reported for this species (instar range 5–11 with 7–8 most common) (Good 1936; Devi & Devi 2015). Pupal stage duration was similar to previous reports for this species with other food sources at the same temperature range. Apparently, Brazil nuts provide the necessary nutritional requirements for successful pupation of *T. castaneum* (William 2000; Devi & Devi 2015).

However, survival of adult *T. castaneum* fed Brazil nut was lower than that observed in another study, in which males lived on average 547 d and females 226 d (Good 1936). The survival of adults on Brazil nuts, in our study, can be considered low given > 50% of adults died within 40 d after emergence and 75% within 60 d. Only 1 individual lived to 129 d. One might suggest that *T. castaneum* adults do not feed on Brazil nuts, but this hypothesis can be discarded because Good (1936) previously reported that this beetle can survive only 18 d without food at a temperature of 30 °C.

In summary, we found that the biological and reproductive parameters of *T. castaneum* were adversely affected when reared on Brazil nuts, reflected by production of fewer eggs, a considerable increase in larval stage, and short survivorship of adults compared with the results of other workers when reared on other food sources but under the same environmental conditions. This information suggests that even though *T. castaneum* may be considered a potentially important pest of stored Brazil nuts, it does not thrive on this commodity as a food substrate.

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