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# Feeding preference of *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) adults on Brazil nuts combined with rice, corn or soybeans

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**Abstract.** *Tribolium castaneum* (Herbst, 1797) is a major pest of stored products, and understanding its feeding preferences among different food sources is essential for developing effective monitoring and control strategies. The objective was to investigate the behavior of *T. castaneum* adults regarding food preference in tests with a chance of choice using Brazil nuts combined with rice, corn, or soybeans. In condition I, the insects preferred Brazil nuts (51.16%), followed by rice (41.72%) and, lastly, not feeding (7.16%); in condition II, the most attractive was corn (80.88%), followed by Brazil nuts (17.04%) and not feeding (2.08%). In condition III, the preference was for Brazil nuts (72.52%) followed by soybeans (21.80%) and not feeding (5.68%). The insect chose to change its food throughout the days evaluated for condition I, which did not occur in conditions II and III. Brazil nuts may be used to capture and thereby reduce the population of *T. castaneum* present in stored rice and soybeans, and this could be a proposal for developing a tool for the integrated management of *Tribolium* in storage units of these products.
**Keywords:** Behavioral method,*Bertholletia excelsa*,feed alternation,stored products.

## Introduction

Research into Brazil nuts (*Bertholletia excelsa* H.B.K) is recent, and efforts are mainly concentrated in areas related to post-harvest (Nogueira et al., 2014; Costa et al., 2016; 2017; Monteiro et al., 2016; Carneiro et al., 2017; 2018; Pires et al., 2018; 2017; Pires & Nogueira, 2018) and in the food industry (Carvalho et al., 2022; Kluczkovski et al., 2021; 2015; Yang 2009; Chunhieng et al. 2008), pharmaceutical (Pena Muniz et al., 2015; Fiori et al., 2017; Gustmann et al., 2017) and cosmetics (Carvalho et al., 2022; Gomes et al., 2020).

Due to its importance for the subsistence of many families living in the Amazon Biome region, and also the high added value of this product, mainly for export, significant efforts have been made by groups of researchers from the states of Acre, Amazonas, Mato Grosso and Pará in Brazil to guarantee the quality of this product.

Through the research results, it has been possible to raise awareness among those involved in the Brazil nut chain to promote better conditions for this product, from the forest to the consumer. Storage units are being built to promote the rapid removal of this product from the forest and its proper storage until processing.

Among the problems surrounding the storage of agricultural products, insect pests of stored grains are responsible for major losses in rice, corn, soybeans, sorghum etc. (Martins et al., 1985; Santos et al., 2002; Caneppele et al., 2003; Silva et al., 2003; Alves et al., 2008; Faroni & Silva 2008; Elias et al., 2009; Alencar et al., 2011) and, recently, some of these such as *Hypothenemushampei*(Coleoptera: Scolytidae), *Plodia interpunctella*(Lepidoptera: Piralidae), *Tribolium castaneum*(Coleoptera: Tenebrionidae), *Rhyzoperthadominica*(Coleoptera: Bostrichidae), *Ephestiakuehniella*(Lepidoptera: Pyralidae) and *Sitotrogacerealella*(Lepidoptera: Gelechiidae) have been reported as potential pests of Brazil nuts in storage conditions (Gumier-Costa, 2009; Gomes et al., 2015; Pires et al., 2019; 2018; 2017; Pires & Nogueira, 2018).

The genus *Tribolium* has species considered pests commonly found in grain storage units, causing considerable losses in products such as cereals, bran, feed, flour and cornmeal (Trematerra& Sciarretta, 2004; Daglish, 2006).

The *T. castaneum*species is classified as a secondary pest of stored grains because the adults and immatures feed on grains that have been previously cracked, broken or damaged by primary pests, with feeding behavior that is characterized by a preference for food in the form of small particles such as flour, even when compared to the same substrate in coarser particles (Jang et al., 1982). However, reports show that these insects can survive on undamaged grains (White, 1982). Recently, this insect was reported as a potential pest of Brazil nuts capable of damaging intact kernels (without lesions), which may classify it as a primary pest for this Amazonian product (Pires et al., 2017).

Food preference can be understood as a behavior that determines the choice of the most appropriate food for the organism's needs (Jang et al., 1982; Zavala-Camin, 1996) or even in a deterrent situation in which the insect is forced by the plant's defense mechanisms (secondary metabolites, proteins with toxic, repellent and/or anti-nutritional effects) (Albiero et al., 2020; Rani & Jyothsna, 2010; War et al., 2012) to seek another food source. However, food preference is only one parameter capable of evaluating the behavior of choice and suitability for a type of food (Jang et al., 1982), which can be of fundamental knowledge in determining, from a quantitative point of view, the probability of this insect choosing a specific food product over another (Jang et al., 1982), since this knowledge can be used as a pest control strategy in IPM programs (Pires et al., 2016; 2015).

In studies of insect feeding behavior, it is common for individuals to exhibit a preference for food sources different from those on which they were reared (Jang et al., 1982). Research focusing on insect preference for particular food types remains limited, as most studies on feeding behavior primarily aim to understand strategies involved in host selection and the influence of plant defense mechanisms (Brochu et al., 2020; Kaplan et al., 2014; Lankau, 2007).

The aim was to investigate the behavior of *T. castaneum*adults in terms of food preference in choice tests using Brazil nuts with rice, corn or soybeans.

## Material and Methods

The experiments were carried out at the “Laboratório de Pragas e Vetores da Amazônia/Cerrado” (LPVAC) of the “Universidade Federal de Mato Grosso”, Campus of Sinop, where the Brazil nuts (*Bertholletia excelsa*) were offered to *T. castaneum* adults.

Plastic pots with lids and a capacity of 250 mL (9.5 cm in diameter and 7.5 cm high) were used and 10 adult individuals of *T. castaneum*, unsexed, obtained from mass rearing at the LAPVAC, were placed inside each pot, along with the substrates for evaluation.

The *T. castaneum* food preference study was evaluated in choice tests carried out in plastic trays (arenas) measuring 21 x 14 x 8 cm (Figure 1) for 10 days. Brazil nut kernels and crushed rice, corn or soy were offered in quantities equivalent to the weight of the nut offered. Brazil nut kernels were not offered whole, as they are rarely removed intact and typically present lesions. Moreover, *T. castaneum* is a secondary pest that preferentially feeds on damaged material. Therefore, all substrates were deliberately fragmented to avoid biasing their attractiveness and to simulate natural conditions in stored products better.

**(A)** **(B)**

**Figure 1.** (A) Experiment on the food preference of insect pests of stored grains. (B) Detail of the arena where the insects were introduced in the food preference study.

The data was subjected to the Anderson-Darling normality test, followed by two-way ANOVA to see if there was an effect of food source (Food), time (days) and the Food*Time interaction for the insects subjected to the following conditions: I - rice or Brazil nuts or not feeding; II - corn or Brazil nuts or not feeding and III - soy or Brazil nuts or not feeding. The food source data was submitted to Tukey's test at a 5% probability level to check which food condition presented the greatest preference for the adults of *T. castaneum*. All statistical procedures were carried out using the software Action Stat Pro (Equipe Estatcamp 2014) and a significant level of 0.05 was adopted for the type I error.

## Results and discussion

The tests to verify the feeding preference of *T. castaneum*adults submitted to conditions I - Rice/Brazil nuts/not feeding; II - Maize/Brazil nuts/not feeding and III - Soybeans/Brazil nuts/not feeding, showed that in all conditions there was an effect of Food, Time and the Food*Time interaction on choice (Table 1).

Adults *T. castaneum*showed greater acceptance of Brazil nuts when compared to rice and the condition of not feeding (Figure 2 A). However, when analyzing the feeding behavior of this insect over the days under the Food*Time interaction, it can be seen that there were changes in the types of food on which the insect was reported and that the choice of the most acceptable substrate began to be evident from the sixth day onwards (Figure 3 A).

Compared to Brazil nuts, corn or no contact with the substrate, corn was preferred by *T. castaneum*(Figure 2 B). The feeding behavior of this

insect over the days under the Food*Time interaction showed changes, especially between corn and Brazil nuts, with emphasis on the observation made from the first to the second day and, after the third day, the occurrences of food changes were lower, which may characterize stability in the choice of food type (Figure 3 B).

**Table 1.** Effect of Food, Time and the interaction Food*Time on the feeding behavior of *Tribolium castaneum*in tests with chance of choice considering $p<0.05$

| Condition | Food | Time | Food*Time |
|---|---|---|---|
| I | (F= 441.33; $p< 0.001$) | (F= $4.38x10^{-4}$; $p= 1$) | (F= 29.38; $p< 0.001$) |
| II | (F= 1627.15; $p< 0.001$) | (F= $1.42x10^{-31}$; $p= 1$) | (F= 2.68; $p< 0.001$) |
| III | (F= 1141,76; $p< 0.001$) | (F= $1.44x10^{-31}$; $p= 1$) | (F= 3.82; $p< 0.001$) |

Conditions: I - Rice/Brazil nuts/not feeding; II - Corn/Brazil nuts/not feeding and III - Soybeans/Brazil nuts/not feeding.

Compared to Brazil nuts, soybeans or those without contact with the substrate, Brazil nuts were preferred by *T. castaneum*(Figure 2 C). There was also a change in the feeding behavior of *T. castaneum*over the days, mainly between soy, Brazil nuts and not feeding. For this test, a slight increase in the percentage of insects on the Brazil nut could be observed, with a consequent reduction in this value for those observed on the soybean. For those observed outside the food substrate, it was found that from the third to the ninth day, this occurrence was less than 10%, with only the record obtained on the 10th day being greater than 10% (Figure 3 C).

For corn, there was a preference for this cereal over the other options (Figure 2 B). For soy, Brazil nuts and not feeding, the insect preferred Brazil nuts over the other conditions (Figure 2 C).
In the Rice/Brazil nut condition, the preference for Brazil nuts can be seen when compared to rice and the condition of not feeding (Figure 2 A). The feeding behavior of this insect in this condition varied over the days evaluated, showing that after the 6th day, the insects began to choose Brazil nuts (Figure 3 A).

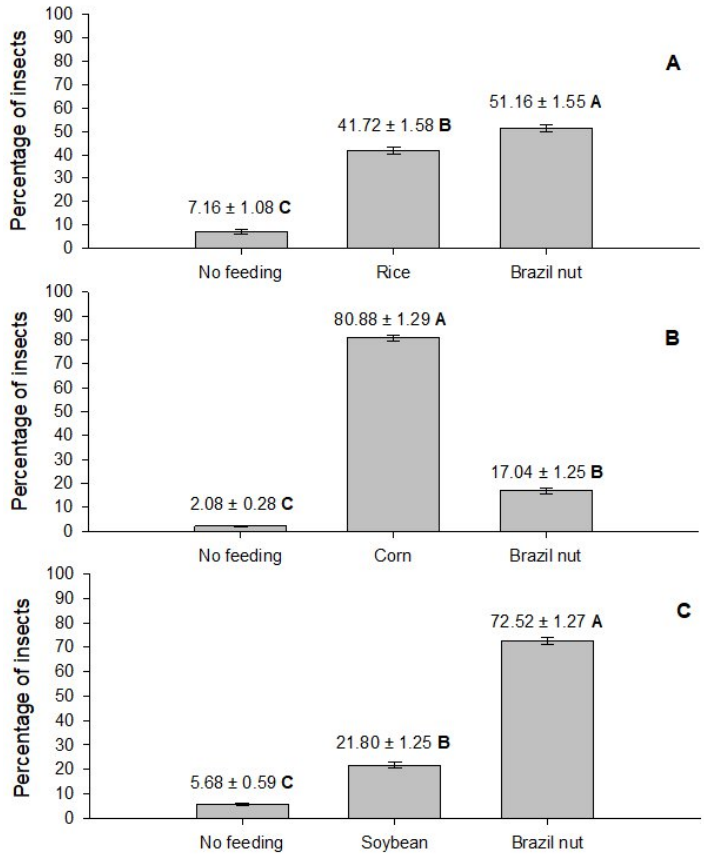


**Figure 2.** Adults of *Tribolium castaneum*(Coleoptera: Tenebrionidae) submitted to the food choice test. Mean followed by the same do not differ by Tukey's test at the 5% probability level.

In the condition in which the insects were tested to choose between Brazil nuts, rice and not feeding, there was an effect only between treatments (food sources) (F= 441.30; $p< 0.001$). This beetle showed greater acceptance of Brazil nuts (Figure 3 A). There was no change in food preference during the evaluation time (F= 0.0004; $p= 1$).

Also, for other associations made in this same study using Brazil nuts and corn or Brazil nuts and soybeans, there were effects only between food sources (F= 1563.09; $p< 0.05$) and (F= 1068.22; $p< 0.05$), respectively. As in the first case, days did not influence this coleoptera's food preference. For those whose preference was tested between Brazil nuts, corn and those who were not fed, there was greater acceptance of corn (Figure 3 B). For the evaluation between Brazil nuts, soybeans and not feeding, there was greater acceptance of Brazil nuts (Figure 3 C).

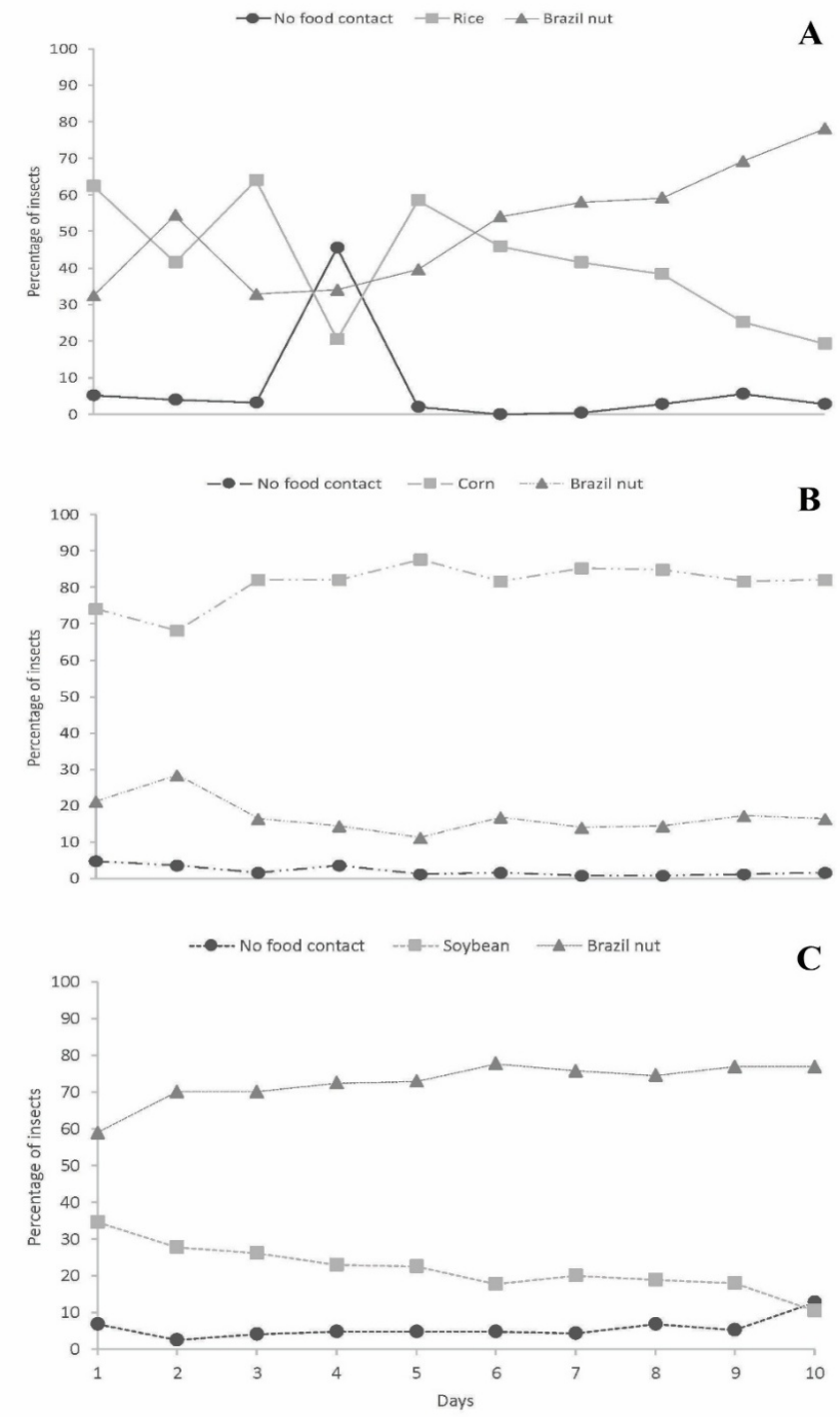


**Figure 3.**Daily feeding dynamics of adults of *Tribolium castaneum*(Coleoptera: Tenebrionidae) on rice (A), corn (B) and soybeans (C) subjected to the choice-of-food test. Mean followed by the same do not differ by Tukey's test at the 5% probability level.

The study of food preference is an important parameter for assessing the suitability of an insect for food (Bellec et al., 2022; Jang et al., 1982). For this reason, it can be valuable to know, from a quantitative point of view, the probability of this insect choosing a specific food product over another (Bellec et al., 2022; Jang et al., 1982).

The effect of time (days) is important in studies of food preference, as this variable can be used to verify conditions such as staying on just one food item, changing food substrates for good; the change and returning to the first food substrate and even the option for the insect not to feed on any of these offered and, in some cases, the behavior of changing the variety of food items may be a strategy to dilute the ingestion of toxins (Freeland and Janzen, 1974) and to counterbalance nutritional deficiencies with complementary food sources (Pulliam, 1975; Westoby, 1978).

It can be common for insects to show a preference for a different product to the one they were reared on (Jang et al., 1982) and conditions such as food particle size and nutritional values can motivate them to look for new sources that can meet their nutritional needs (Bellec et al., 2022; Jang et al., 1982).

It is known that *T. castaneum*is an insect that shows a preference for more refined substances (flours) compared to coarser particles (Shweil& Al-Jubouri, 2024; Jang et al., 1982); however, in this work, as it is known that *T. castaneum*can be considered a primary pest for Brazil nuts (Pires et al., 2017) the aim is to quantify the food preference of this Coleoptera when compared to the original product in order to use chestnut traps in storage units.

The ability of adults and immatures of *T. castaneum* to feed on the intact Brazil nut may be because this kernel provides less resistance to penetration by the chewing mouth apparatus on its surface when compared to other grains or seeds such as rice, beans, corn, soybeans, etc. In this way, *T. castaneum* can be classified as a primary pest for Brazil nuts.

The lesions caused by this Coleoptera on Brazil nuts in the adult and immature stages, group this pest as forming galleries that evolve into more extensive lesions and, because of this feeding behaviour, the types of injury caused by *T. castaneum* can be characterized in terms of quantitative and quantitative parameters (Pires et al., 2017). For the former, we can mention the apparent loss of matter that will culminate in the weight that the nut presents as a result of the attack by this coleopteran and; for the latter, the capacity that the lesion formed can represent in the loss of the germinative power of the seeds, cause the depreciation of the grain and loss of nutritional value that may be a consequence of the attack by pests of seeds and stored grains that produce this damage.

*Tribolium castaneum* adults showed greater preference for Brazil nuts (51.16%) when compared to rice (41.72%) and in the non-feeding condition (7.16%); when this test refers to Brazil nuts or corn or not feeding, the corn was the most attractive to these insects, with 80.88% of the preference, followed by Brazil nuts and not feeding with values of 17.04 and 2.08%, respectively. In the condition in which soybeans were offered, this insect preferred to feed on Brazil nuts (72.52%), followed by soybeans (21.80%) and not feeding (5.68%). Throughout the days evaluated, the change in food source was verified when rice was an option, with occurrences of alternation in the food source, which did not occur in the conditions in which corn and soybeans were used.

Therefore, Brazil nuts can be considered as a potential product capable of capturing and, therefore, reducing the population of *T. castaneum* in rice and soybean warehouses, and based on the results observed, open a line of studies aiming to seek its use as an IPM tool through the behavioral method of controlling *Tribolium* populations in storage units.

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