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# Foraging Behavior of *Latheticus oryzae* (Coleoptera: Tenebrionidae) Is Affected by the Type of Food Source

**Maria C. Boukouvala *, Demeter Lorentha S. Gidari, Constantin S. Filintas, Anna Skourti, Vasiliki Panagiota C. Kyrpislidi, Irene N. Kypriotaki, Angelos M. Kontopoulos and Nickolas G. Kavallieratos ***

Laboratory of Agricultural Zoology and Entomology, Faculty of Crop Science, Agricultural University of Athens, 75 Iera Odos Str., 11855 Athens, Greece; dlgidari@aua.gr (D.L.S.G.); kfilintas@aua.gr (C.S.F.); annaskourti@aua.gr (A.S.); kyrpvp@aua.gr (V.P.C.K.); stud122079@aua.gr (I.N.K.); stud123051@aua.gr (A.M.K.)
* Correspondence: mbouk@aua.gr (M.C.B.); nick_kaval@aua.gr (N.G.K.)

## Abstract

*Latheticus oryzae* has emerged as a notorious invasive insect species affecting stored products globally. In the present study, the effect of different types of flour (i.e., whole barley, chickpea, date, potato, white rice, and white soft wheat) on the time *L. oryzae* required to reach the food source, the time it spent at the food source, the time it spent away from the food source, and the number of visits to the food source were evaluated. The Preference Index (PI, %) was calculated as the percentage of the total observation time (900 s) spent by each insect at the food source. The type of flour had a decisive effect on *L. oryzae* response. Specifically, potato flour exhibited the highest approach success rate (27 out of 30 insects) and the shortest estimated mean approach time (258.7 s) for *L. oryzae*. However, the mean PI (%) for potato flour was 10.9%, ranking it third after white soft wheat flour (33.9%) and white rice flour (23.4%). In contrast, significant approach delays, fewer visits, and longer times away from the food source were recorded for whole barley flour and white rice flour. The findings indicate that *L. oryzae* exhibited differential behavioral responses to the six tested types of flour, with differences in approach success, approach time, time spent at the flour source, and visitation frequency. These results provide evidence of substrate-specific differences in foraging behavior.

**Keywords:** long-headed flour beetle; type of flour; Preference Index; behavioral traits

## 1. Introduction

Foraging behavior determines the fitness of organisms by leading to their access to resources and energy. Two components play a vital role in this process: food preference, which is characterized as the acceptance of a given resource, and mobility related to the food source, reflecting the overall search to locate and exploit it [1]. The study of foraging behavior is particularly relevant for insect pests of stored products, where feeding and movement directly influence infestation levels [2]. Mobility is a functionally important trait of insect behavior within storage environments, since resources such as food, refuge, and mates are unevenly distributed throughout storage facilities [3]. Insects locate food primarily through olfactory cues and then taste cues, and their movement changes once a suitable source has been found [4]. However, not all food sources are equally exploited since chemical and nutritional differences among them shape whether they will be accepted or avoided [5].

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The foraging behavior and the food consumption from the stored-product pests result in postharvest losses in stored cereals ranging from 30 to 40% on a global scale [6]. *Latheticus oryzae* Waterhouse (Coleoptera), the long-headed flour beetle, is a small tenebrionid measuring approximately 3 mm in length with a yellowish-brown coloration, a flattened body, an elongated head and the ability to fly [7]. This species has emerged as an invasive secondary pest of stored food products worldwide [8]. Both adult and larval stages of *L. oryzae* feed directly on a plethora of stored products, including cracked grain kernels, wheat and barley bran, and various flours, tea, rice, sorghum, and maize products [9,10]. It has also recently emerged as a major pest of fermented grain products, including Daqu starter cultures used in East Asian food and beverage production [11]. Similarly to other stored-product tenebrionids, such as *Tribolium* spp., *L. oryzae* causes qualitative damage to the infested flour commodities, including larval and adult excreta, discoloration, and unpleasant pungent odors due to the secretion of defensive compounds [12–14].

Existing research on the biology of *L. oryzae* is limited, including few studies regarding the development under varying temperature and relative humidity conditions [15,16]. According to these studies, *L. oryzae* exhibits optimal development (shortest developmental rate and lowest recorded mortality) under elevated temperature and relative humidity (R.H.) settings (35 °C and 85% R.H.). *Latheticus oryzae* requires a minimum temperature of 25 °C to complete its life cycle. Recent molecular analysis revealed the complete mitochondrial genome of *L. oryzae*, providing new data for future integrated pest control strategies [11]. The overall knowledge about the management of *L. oryzae* is poor, including the efficacy of different old active ingredients (a.i.s) [17] or new a.i.s [18] against different life stages, and lethal doses of far infrared radiation as a physical control method [19].

Apart from the restricted knowledge regarding the biology and control of *L. oryzae*, its behavior has also attracted insufficient attention. In a most recent study, Boukouvala et al. [20] investigated the courtship and mating behavior of *L. oryzae*. To our knowledge, there is no behavioral work addressing the foraging and substrate-specific behavior of this species. Therefore, the aim of the current study was to investigate different traits relating to the foraging behavior of *L. oryzae* in the presence of six food sources. Specifically, this study examined the walking behavior of adult individuals by quantifying the time required to reach the food source, the time spent on versus away from the source, and the number of visits made to the source over a fixed observation period.

## 2. Materials and Methods

### *2.1. Rearing of Latheticus oryzae*

The cultures of *L. oryzae* individuals were maintained at 65% R.H. and 30 °C, under continuous darkness in the Agricultural University of Athens, Greece, Laboratory of Agricultural Zoology and Entomology. The rearing medium was white soft wheat flour with 5% brewer's yeast. Adult individuals of <2 weeks old were selected for the tests [18].

### *2.2. Tested Substrates*

Six different flour varieties, free from insecticidal treatments and infestations, were used as test substrates: (1) whole barley flour (*Hordeum vulgare*, BioAgros, Krya Vrysi, Greece), (2) chickpea flour (*Cicer arietinum*, Ola Bio, Greece), (3) date flour (*Phoenix dactylifera*, Scientific and Technical Research Center on Arid Regions, Biskra, Algeria), (4) potato flour (*Solanum tuberosum*, Ola Bio, Greece), (5) white rice flour (*Oryza sativa*, BioAgros, Krya Vrysi, Greece), and (6) white soft wheat flour (*Triticum aestivum*, St. George Mills, Sourpi, Greece), which was considered as the reference flour, since it was the main constituent of the rearing medium. Before starting the tests, all six types of flour were uninfested and environmentally equilibrated (i.e., maintained at 65% R.H. and 30 °C for one week) [21].

*2.3. Bioassays*

The experimental arenas were constructed using Petri dishes with a surface area of 50.27 $cm^2$ (∅ 8 cm × 1.5 cm height), having their bottoms coated with a 0.5 cm thick layer of cement (GIGAS Low Carbon Portland Composite Cement material, Heidelberg Materials Hellas, Aspropyrgos, Greece). The arenas were allowed to dry for 24 h (h) and were then ready for use [18]. For the tests, 0.3 g of whole barley flour, chickpea flour, date flour, potato flour, white rice flour, or white soft wheat flour were separately placed in the center of the arena [22] (Figure 1).

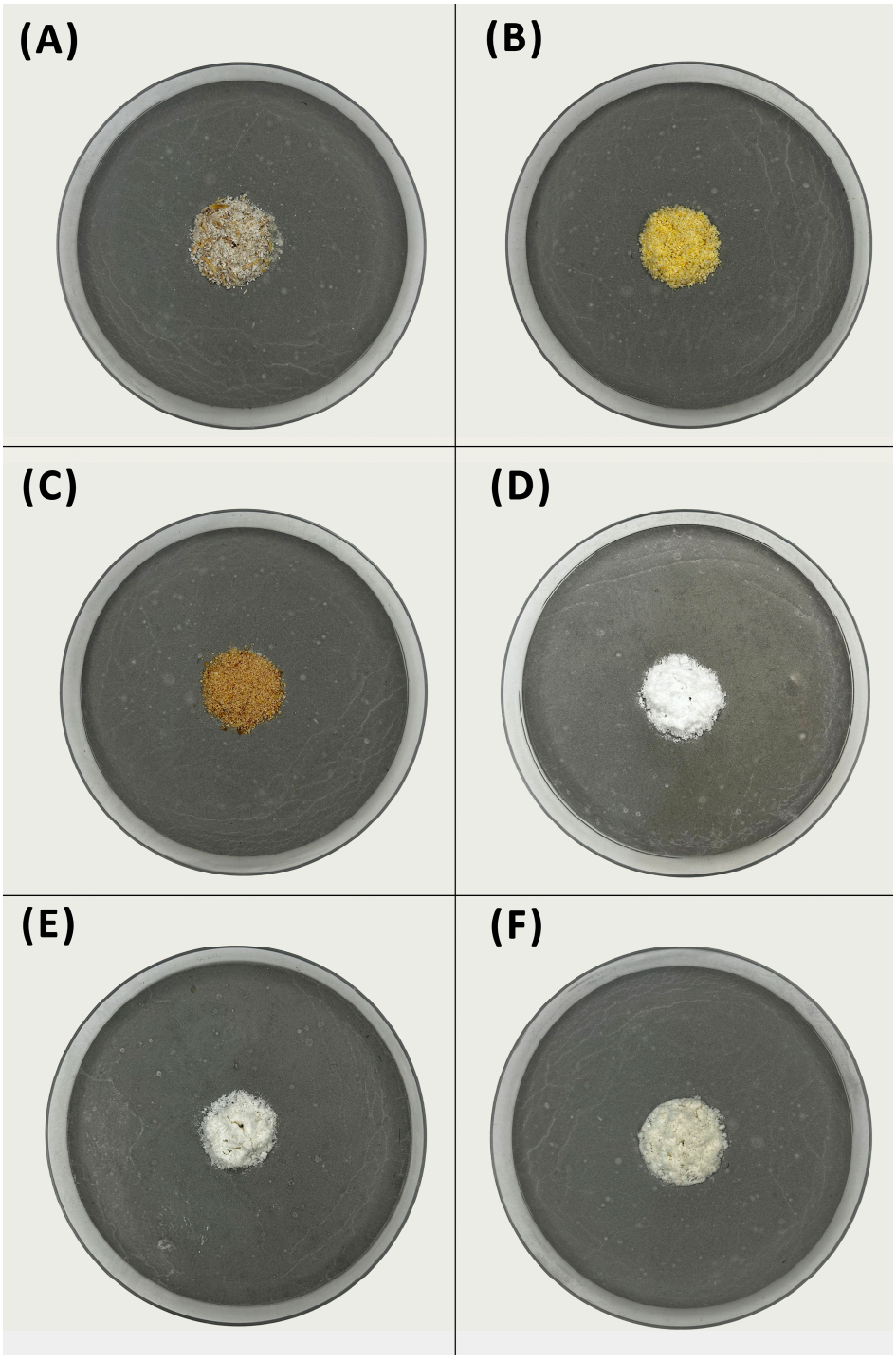



**Figure 1.** Quantity (0.3 g) of (**A**) whole barley flour, (**B**) chickpea flour, (**C**) date flour, (**D**) potato flour, (**E**) white rice flour, and (**F**) white soft wheat flour in the center of the experimental arenas.

Insects were deprived of food for a whole day prior to the observations [22]. Subsequently, an unsexed adult individual of *L. oryzae* was released at the periphery of the arena (the internal walls of the dish). Then, the required time to reach the food source, the time spent at the food source, the time spent away from the food source, and the number of visits to the food source were recorded visually for 900 s (15 min) at 30 °C [22] (Figure 2).

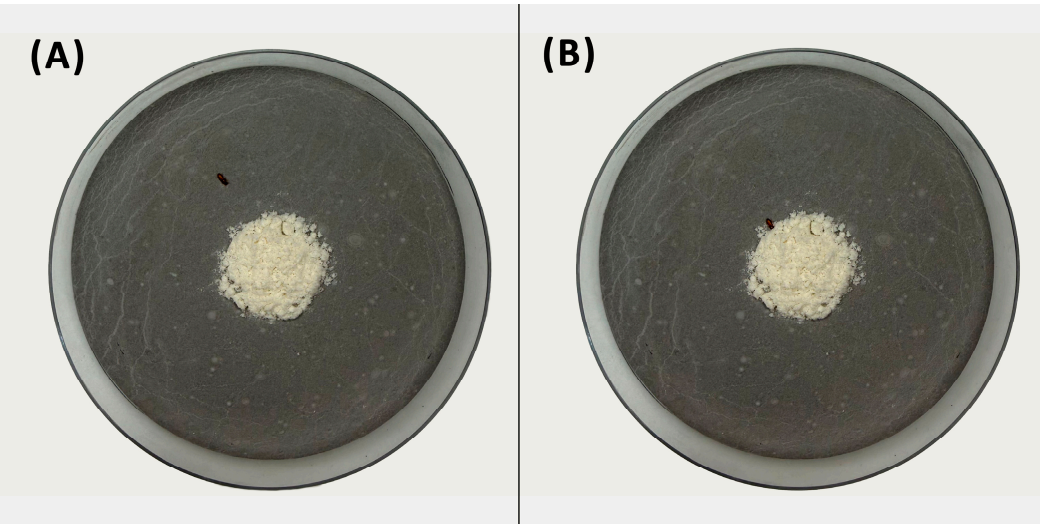



**Figure 2.** *Latheticus oryzae* individual (**A**) walking to the food source and (**B**) reaching the food source.

In addition, the Preference Index (PI, %) was calculated as the percentage of the total observation time that each insect spent at the food source, according to the following equation:

$$\text{PI} = \frac{\text{Time spent at the food source}}{\text{Total time of observation}} \times 100$$

For insects that did not visit the food during the observation period (i.e., 900 s), the time spent at the food was recorded as zero (PI = 0), and these individuals were retained in the dataset. In the context of the present study, PI was used as a measure of behavioral association or retention at the tested flour substrate and should not be interpreted as a direct measure of feeding preference or food acceptance. There were 30 repetitions for each food source (i.e., 30 *L. oryzae* adult individuals per flour type) [22,23]. For each repetition, a new arena, *L. oryzae* adult, and food source were used. In total, 180 individuals were tested (30 repetitions × 6 substrates). The experiments were carried out at 65% R.H., 30 °C and natural light between 8 a.m. and 8 p.m. [22].

### *2.4. Statistical Analysis*

The time required for insects to reach food was analyzed as time-to-event data using the Life Distribution procedure in JMP 16.2 [24]. An event was defined as the insect reaching the food source, while *L. oryzae* individuals that did not reach the food within the predefined time limit of 15 min were treated as right-censored observations. A lognormal distribution was applied for parametric modeling of the time-to-event data [25,26]. Differences among the six flour types were assessed using the global log-rank, Peto–Peto, and Wilcoxon tests [27]. To evaluate the overall effect of flour type on the probability of success/failure of *L. oryzae* individuals to reach the food source, a Generalized Linear Model (GLM) with binomial distribution and logit link was applied, using white soft wheat flour as the reference group. The statistical significance level was set at $p \leq 0.05$. The Shapiro–Wilk test was used to test the normality of *L. oryzae* data for the PI, time spent away from the food source, and number of visits to the food source [28]. Since the data did not follow a

normal distribution in most groups (except for the trait "time spent away from the food source" in the potato flour; $p = 0.1208$), nonparametric tests were applied. Specifically, the Kruskal–Wallis test was used for group comparisons [29] and the Steel–Dwass test for multiple pairwise comparisons [30,31]. The graphical presentation of the distribution and variability of the observed values per trait for each type of flour was achieved using box plots, displaying the median, quartiles (Q1 and Q3), and data range. The significance level was set at $p \leq 0.05$. The statistical package JMP 16.2 was used for the analysis [24].

## 3. Results

### *3.1. Effect of Flour Type on Latheticus oryzae Individuals to Reach the Food*

The analysis of time to reach the food showed that the type of flour significantly affected the time insects required to reach the food source (Figure 3). Specifically, the distribution curves differed significantly among the six flours (log-rank test, $\chi^2 = 18.31$, df = 5, $p = 0.0026$). Accordingly, significant differences also emerged from the Peto–Peto ($\chi^2 = 15.15$, df = 5, $p = 0.0097$) and Wilcoxon ($\chi^2 = 15.15$, df = 5, $p = 0.0097$) tests. Insects approached potato flour faster than the other flours, presenting the shortest estimated mean time to reach the food source (258.7 s), followed by date flour (299.5 s), white soft wheat flour (304.8 s), and chickpea flour (351.7 s). Longer estimated mean times were recorded for white rice flour (434.0 s) and especially for whole barley flour (570.8 s), which showed the longest estimated mean times of reaching the food source (Figure 3).

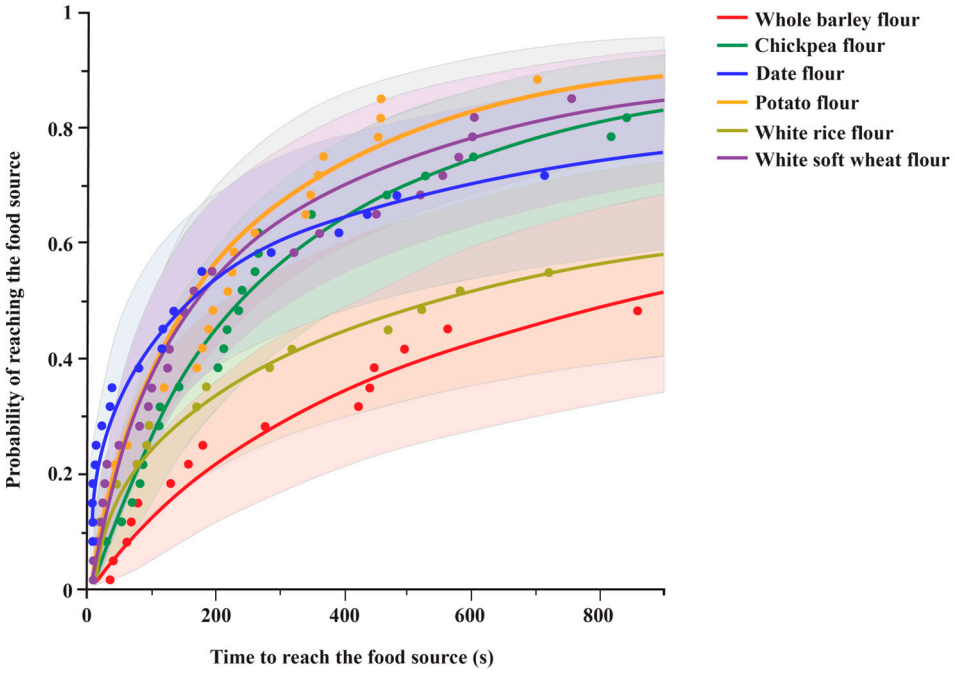



**Figure 3.** Probability of reaching the food source as a function of time (s) for *Latheticus oryzae* populations on six different flour types (whole barley, chickpea, date, potato, white rice, and white soft wheat). The solid lines represent the estimated curves (lognormal distribution) with the corresponding 95% confidence intervals (shaded areas) and the colored dots represent the experimentally observed data.

The type of flour had a statistically significant effect on the probability of insects reaching the food source ($\chi^2 = 21.23$, df = 5, $p = 0.0007$). Potato flour exhibited the highest success (27 out of 30 insects reached the food), followed by the white soft wheat flour (26 individuals), chickpea flour (25 individuals), and date flour (22 individuals), with statistically significant differences. In contrast, the lowest success was recorded in white

rice flour and whole barley, where 17 out of 30 and 15 out of 30 individuals managed to reach the food source, respectively, without significant differences (Figure 4).

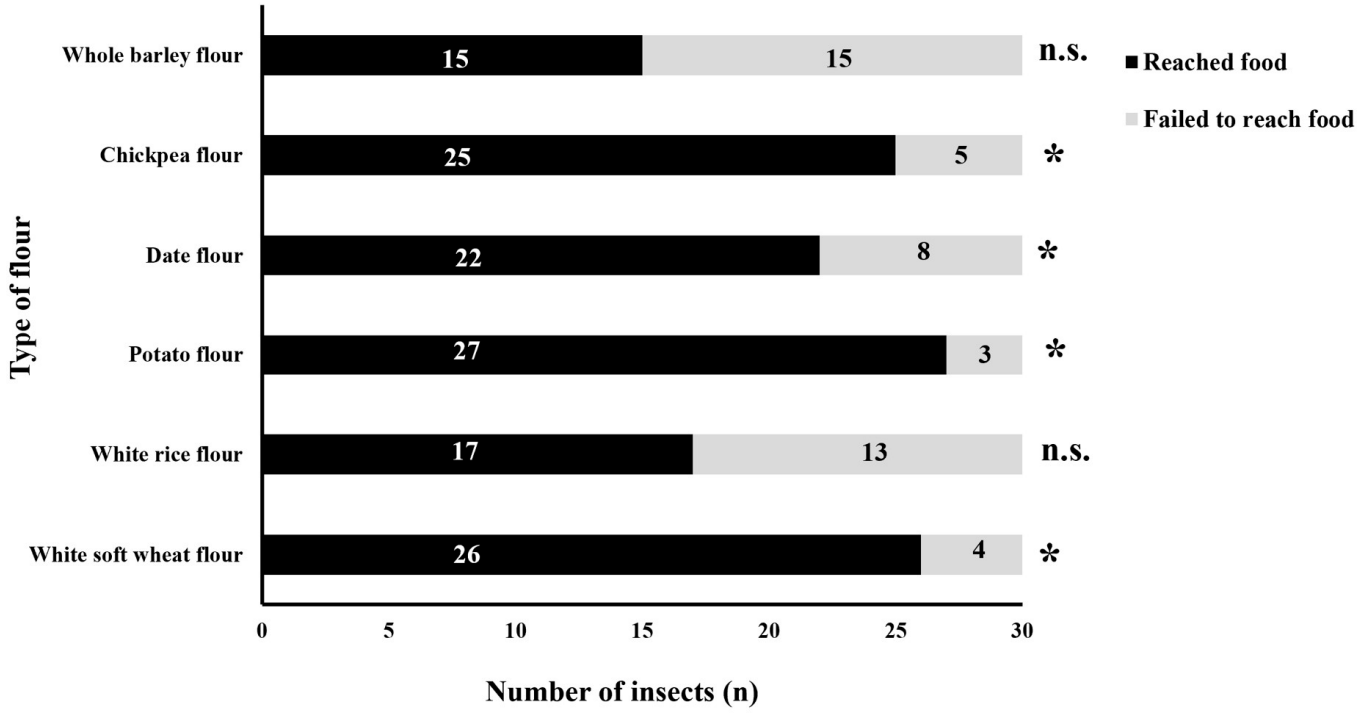



**Figure 4.** Number of *Latheticus oryzae* individuals that reached or did not reach the food source (i.e., whole barley flour, chickpea flour, date flour, potato flour, white rice flour, and white soft wheat flour). Asterisks indicate statistically significant differences (GLM, binomial distribution, $p \leq 0.05$), while n.s. indicates no statistically significant difference.

According to the GLM of the probability of *L. oryzae* individuals reaching the food source, whole barley flour and white rice flour showed negative and statistically significant estimate values (−1.1597 and −0.8915, respectively), indicating lower log-odds of insects reaching the food source compared to white soft wheat flour (Table 1). In contrast, potato flour showed a positive and statistically significant estimate value (1.0375), indicating higher log-odds of insects reaching the food source successfully compared to the reference group (white soft wheat flour) (Table 1). Results for chickpea flour and date flour did not differ statistically from those on white soft wheat flour, indicating that insects behaved similarly to the reference group.

**Table 1.** Parameter estimates and results of the GLM for the probability of reaching a food source by flour type (with binomial and logit link distributions). White soft wheat flour serves as the reference category represented by the Intercept. Asterisks indicate statistically significant differences ($p \leq 0.05$). White soft wheat flour is the reference category.

| Term | Estimate | Standard Error | $\chi^2$ | *p* | Lower CL | Upper CL |
|---|---|---|---|---|---|---|
| Intercept | 1.1597 | 0.1928 | 45.04 | <0.0001 * | 0.7974 | 1.5586 |
| Whole barley flour | −1.1597 | 0.3551 | 10.53 | 0.0012 * | −1.8655 | −0.4644 |
| Chickpea flour | 0.4497 | 0.4440 | 1.13 | 0.2927 | −0.3664 | 1.4088 |
| Date flour | −0.1481 | 0.3883 | 0.14 | 0.7051 | −0.8882 | 0.6520 |
| Potato flour | 1.0375 | 0.5330 | 4.86 | 0.0275 * | 0.1044 | 2.2658 |
| White rice flour | −0.8915 | 0.3573 | 6.03 | 0.0140 * | −1.5947 | −0.1839 |

Lower CL and Upper CL correspond to the lower and upper 95% confidence limits (CLs), respectively.

### 3.2. Behavioral Response of *L. oryzae* to Different Types of Flour

The mean PI of *L. oryzae* differed significantly among the six flour types ($\chi^2 = 19.3$, $p = 0.002$) (Table 2). The highest mean PI was recorded for white soft wheat flour (33.9%), with significant differences among flours (except white rice flour, 23.4%), while the lowest values were observed for chickpea flour (8.5%) and date flour (8.4%). Results for white soft wheat flour and white rice flour showed a wider data dispersion with several higher outliers, while the remaining flours were tightly concentrated near the lower end of the index (Figure 5a).

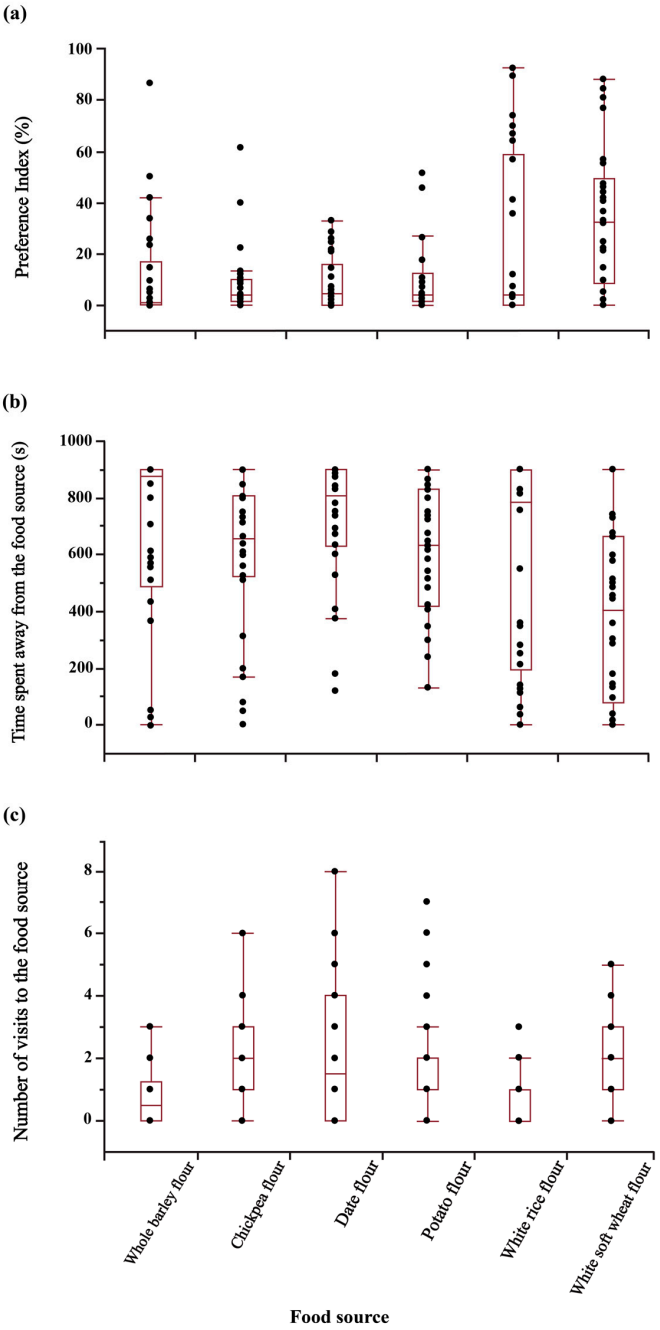



**Figure 5.** Box plots showing the distribution: (**a**) Preference Index (PI, %), (**b**) time spent away from the food source (s), and (**c**) number of visits to the food source of *Latheticus oryzae* on six different types of flour (i.e., whole barley, chickpea, date, potato, white rice, and white soft wheat). The horizontal line inside each box corresponds to the median, the box limits correspond to the first (Q1) and third quartiles (Q3), the lines (whiskers) extend to the extreme non-outliers, and the dots represent individual observations (i.e., each insect).

**Table 2.** Mean values (±Standard Deviation (SD)) of the Preference Index (PI, %), time spent away from the food source (s), and number of visits (n) of *Latheticus oryzae* on six types of flour (i.e., whole barley flour, chickpea flour, date flour, potato flour, white rice flour, and white soft wheat flour). Different letters in each trait indicate statistically significant differences (Steel–Dwass, $p \leq 0.05$).

| Type of Flour | PI (%) | Time Spent Away from Food (s) | Visits to Food (n) |
|---|---|---|---|
| Whole barley flour | 11.5 ± 19.8 b | 654.5 ± 327.5 a | 0.8 ± 0.9 b |
| Chickpea flour | 8.5 ± 12.9 b | 612.5 ± 272.4 ab | 1.9 ± 1.4 a |
| Date flour | 8.4 ± 10.3 b | 715.3 ± 222.2 a | 2.1 ± 2.1 ab |
| Potato flour | 10.9 ± 14.9 b | 613.5 ± 217.5 ab | 2.0 ± 1.6 a |
| White rice flour | 23.4 ± 32.3 ab | 567.9 ± 357.4 ab | 0.9 ± 1.0 b |
| White soft wheat flour | 33.9 ± 28.1 a | 391.0 ± 315.4 b | 2.1 ± 1.4 a |
| $\chi^2$ | 19.3 | 18.3 | 26.7 |
| $p$ | 0.002 | 0.003 | <0.01 |
| df | 5 | 5 | 5 |

The mean time spent away from the food source differed significantly among the six flour types ($\chi^2 = 18.3$, $p = 0.003$) (Table 2). The shortest mean time that *L. oryzae* remained away from the food source was recorded for white soft wheat flour (391.0 s), which differed significantly from date flour (715.3 s) and whole barley flour (654.5 s). The values for white soft wheat flour shifted noticeably towards the lower durations, while the remaining flours generally exhibited higher durations with a wider dispersion of data (Figure 5b).

The mean number of visits to the food source differed significantly among the six flour types ($\chi^2 = 26.7$, $p < 0.01$) (Table 2). The highest mean number of visits was observed for date flour (2.1) and white soft wheat flour (2.1), followed by potato flour (2.0) and chickpea flour (1.9). The lowest mean number of visits was observed for whole barley flour (0.8) (Table 2). The data distributions for whole barley flour and white rice flour are tightly clustered at the lower end (near 0 and 1 visit), while the box plots for white soft wheat flour, date flour, and potato flour extend upward, exhibiting a higher frequency of *L. oryzae* visits (Figure 5c).

## 4. Discussion

The present study provides evidence that food type strongly modulates the foraging behavior of *L. oryzae* adults. Significant differences among the six flour types were detected across multiple behavioral parameters, including time to reach the food source, probability of reaching the food, PI, time spent away from the food source, and visitation frequency. Collectively, these findings indicate that the response of *L. oryzae* to a food substrate cannot be adequately characterized by a single behavioral measure. In contrast, these parameters represent distinct components of the foraging process. This distinction is particularly relevant for stored-product pests whose survival and infestation dynamics depend strongly on their ability to locate and exploit spatially heterogeneous food sources [5].

The significant differences in time to reach the food source provide evidence that flour type influenced the initial foraging response of *L. oryzae*. Potato flour resulted in the fastest approach, whereas whole barley flour resulted in the longest. Additionally, the relatively strong response to potato flour is noteworthy. Most of the tested individuals reached potato flour, representing the highest approach success among the tested substrates, and the probability of successful food detection was significantly higher compared to the one observed for the reference substrate (white soft wheat). A possible interpretation is that potato flour releases cues that facilitate food source detection. In stored-product insects, movement toward food can be influenced by chemical components associated with the food source (attractants), since faster approach indicates stronger chemoattraction or preference [32–35].

For instance, Trematerra et al. [35] compared the bioactivity of volatiles produced by cereal- and legume-based pasta on *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) adults in multi-choice behavioral bioassays. Analyzing the response indices in the two-choice behavioral biological tests, the authors suggested that the low attractiveness of legume pasta was mainly due to the lack of attractants rather than the emission of repellent compounds. Thus, potato flour might emit attractive cues. In contrast, the lack-of-attractant interpretation may provide a possible explanation for the comparatively weak responses observed in the present study for whole barley flour and white rice flour. Both substrates resulted in lower approach success and fewer insect visits to the food source, while whole barley flour also showed the longest estimated mean approach time. Chickpea flour showed an intermediate behavioral response, with most individuals successfully locating the food source while spending limited time on it. Such responses may therefore reflect a lack of attractive cues associated with these substrates rather than active avoidance due to repellent compounds. The results obtained for date flour provide further evidence that individual behavioral metrics could capture different aspects of the food source evaluation. Date flour produced the second-shortest estimated mean approach time, indicating relatively rapid detection, while 22 out of 30 insects successfully reached this food source. Yet, the PI of the date flour was the lowest value observed. At the same time, insects made an average number of visits to date flour, equal to the highest visitation frequency recorded (white soft wheat flour). However, since the current study did not identify volatile compounds, the involvement of chemical cues remains hypothetical. The faster response to potato flour should therefore be interpreted primarily as evidence of enhanced behavioral orientation under the tested experimental setup rather than as direct evidence of chemoattraction. Interestingly, the high approach success of potato flour was not combined with the highest PI. Potato flour had a mean PI of 10.9%, significantly lower compared to that recorded for white soft wheat flour. This discrepancy represents one of the most informative findings of the study, since it demonstrates that the food source detection and insect retention are not necessarily equivalent behavioral processes. Further experiments combining chemical characterization, controlled olfactory assays, and direct feeding or consumption measurements will be necessary to determine whether particular volatile or non-volatile constituents explain the responses observed in *L. oryzae*.

The response to the white soft wheat flour provides a contrasting pattern. White soft wheat flour produced the highest PI, the shortest mean time spent away from the food source, and a high mean number of visits among the tested substrates. In addition, 26 out of 30 individuals successfully reached the white soft wheat flour. These results indicate a strong tendency of *L. oryzae* to remain associated with this substrate once it detects it. The strong response to white soft wheat flour may also have been influenced by the rearing history of the tested insects. Previous exposure to a particular food influences subsequent behavioral responses through experience. For instance, Yilmaz & Bayram [36] examined species-specific behavioral preferences of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae), and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) on wheat, bulgur, maize, and chickpea. *Callosobruchus maculatus* and *S. oryzae* resulted in a preference for chickpea and wheat, respectively, which were their rearing media. From a foraging perspective, the observed behavioral heterogeneity is consistent with the concept that resource selection involves sequential decisions rather than a single response [37]. Such findings provide a possible comparative framework, but they do not establish that the same mechanism occurred in *L. oryzae*. Experiments using insects reared on different substrates would be required to separate effects of prior experience from intrinsic responses to the tested flours.

## 5. Conclusions

These findings could have practical implications for stored-product pest management. Substrate-specific differences in movement influence the probability that *L. oryzae* detects and then exploits certain commodities. In addition, substrates that elicit rapid approach or repeated visitation warrant investigation as potential components in trapping monitoring systems. By quantifying several complementary behavioral traits, the present study expands the available behavioral framework for this species and provides a basis for future investigation into how food source properties influence movement and food exploitation. Future research could combine behavioral assays with chemical characterization of the substrates to determine whether specific volatile or non-volatile components are associated with rapid detection of the food source or prolonged retention in the food source. Additionally, experiments involving *L. oryzae* individuals reared on different substrates would also be valuable for separating intrinsic substrate-specific behavior from effects associated with previous feeding experience. Future studies could also compare individual and grouped-insect assays, or directly examine responses to conspecific-derived cues, to determine whether social or semiochemical factors modify the responses to different food sources. Furthermore, it would be interesting to analyze food preference and time spent on food depending on sex, age of the tested adults (in days post-eclosion), and whether they have mated.

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