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Locomotor Activity of Adult Olive Fruit Flies Recorded under Conditions of Food or Water Deprivation

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Abstract: The olive fruit fly, known as *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is causing substantial economic losses in olive crops worldwide. Studying the activity patterns of the insect may expand our knowledge to eventually adopt more sustainable and effective pest control approaches. In the present study, we investigated the impact of food and water deprivation on the mobility of olive fruit flies using a modified version of the LAM25 system (locomotor activity monitor)—Trikinetics, an automated locomotor activity electronic device. Both male and female flies at four different age groups, reared on olives in the laboratory, were individually placed in glass tubes. Their locomotor activity was recorded every minute by three monitors within the digital device over a three-day period. Our observations revealed that adults exhibited significantly reduced movement during nighttime compared to daytime. The greatest mobility was observed during the period of 15:00 to 20:59. Additionally, younger flies demonstrated higher levels of mobility compared to older ones. Flies subjected to both food and water deprivation exhibited higher mobility compared to the control group. These insights offer valuable insights for enhancing pest management strategies aimed at controlling olive fruit flies adopting a more sustainable approach.

Keywords: pest control; olive groves; Tephritidae; activity patterns; *Bactrocera oleae*

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1. Introduction

Bactrocera oleae (Rossi) (Diptera: Tephritidae), commonly known as the olive fruit fly, is considered a major pest in olive crops, causing significant economic losses worldwide, with some cases experiencing up to 70% loss in olive production due to *B. oleae* [1]. This monophagous species is native to the Mediterranean region but has spread to other regions with olive cultivation, including California, Mexico, and South and Central Africa [2]. The larvae of *B. oleae* feed on olive fruits after females' oviposition [3], rendering the fruit unsuitable for human consumption and reducing the yield and quality of olive oil [4]. Studying the factors affecting *B. oleae*'s mobility is crucial for developing effective pest control management strategies. Recent studies were conducted on *B. oleae* regarding the lifecycle [5] and how landscape affects insect movement [6]. Additionally, the difference in mobility between wild olive fruit flies and artificially reared insects was studied using a LAM25 system (locomotor activity monitor)—Trikinetics [7]. It would be very useful to expand such studies to the adults of *B. oleae* to investigate the effects of abiotic stress conditions such as food and water deprivation.

Previous studies on *B. oleae* [8] and *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) [9] examined activity patterns during the day and the sleep behavior of *B. oleae*. This research served as our initial motivation to investigate the movement response of *B. oleae* under conditions of food or water deprivation. Circadian rhythms and sleep have already been studied in many species and model organisms [8,10–13]. Sleep is ubiquitous throughout the animal kingdom, and recent research has convincingly demonstrated its presence in arthropods and nematodes [14]. Numerous studies have investigated factors influencing sleep in *D. melanogaster* in which sleep is influenced by age, sex, and pharmacological agents [11,15]. Additionally, according to J.Yano, *Drosophila mojavensis* (Patterson) (Diptera: Drosophilidae), a fly that has adapted to extreme desert environments, exhibits different sleep requirements compared to *D. melanogaster* [16].

In the past, visual observations of monitoring the activity patterns of flies were used, but this approach had a serious drawback: it was time-consuming and prone to observer bias. Fortunately, recent technological advances have introduced automated monitoring systems, such as the DAM and LAM systems to meet the need for the constant monitoring of individual insects and tracking their movements under inconstant laboratory conditions [17]. These systems provide effective records of the activity of flies by utilizing infrared (IR) beams for the precise detection and quantification of their movement patterns over extended periods. In a typical experimental setup, flies are housed within transparent tubes equipped with a food and water source. As they traverse the enclosure, their locomotor behavior is recorded [18]. LAM systems have already been employed to explore the correlation between insects' activity and an adult diet rich in protein and nutrients. Researchers achieved this via daily recordings of locomotory activity in *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) adults [19,20]. Researchers also compared the mobility of irradiated and unirradiated *B. tryoni* males [21]. Furthermore, a more recent study successfully used the LAM system to reveal differences in daily activity patterns among *B. tryoni* adults with or without access to a diet rich in endosymbiotic bacteria [22]. The same system has been utilized to assess the effect of radiation-induced sterilization on the activity of adult *B. tryoni* and to identify differences in the daily behavior of fertile and sterile adults using a video system [23].

In this study, we examined whether food and water deprivation can affect the circadian clock of adult male and female *B. oleae* of varying ages at different periods in daytime and nighttime using a Trikinetics device (locomotory activity monitor—LAM25 system). The main objectives of this study are to enhance sustainable pest control strategies against *B. oleae* by investigating the locomotory activity patterns of the insects during different time periods throughout the day under constant laboratory conditions and to assess their response to stress conditions that they may encounter in the wild, such as food and water deprivation. This study represents the first attempt to investigate the activity response of *B. oleae* adults to stress conditions in the laboratory during the 24 h of the day for three consecutive days.

2. Materials and Methods

This study was conducted in the Laboratory of Applied Zoology and Parasitology, School of Agriculture, Aristotle University of Thessaloniki, Greece, during the period between October 2022 to January 2023. The insects used in the experiments derived from infested olive fruits collected from olive groves in Chalkidiki, North Greece. The experiments took place under constant and continuously controlled laboratory conditions, a temperature (T) of 25 ± 2 °C, a relative humidity (RH) of 45 ± 5%, and a photoperiod of L14:D10 (with photophase starting at 07:00 and ending at 20:59). The light intensity of the illumination (fluorescent tubes) ranged from 1500 to 2000 lux.

The experiment consisted of the following stages (see Figure 1): (1) gathering non-infested and infested olives; (2) preparing adults and ensuring constant, stable laboratory conditions; (3) conducting the experiment: recording insect activity at one-minute intervals

for three days using a Trikinetics device (TriKinetics Inc., PO Box 325 Princeton, MA, USA); and (4) performing data processing and statistical analysis.

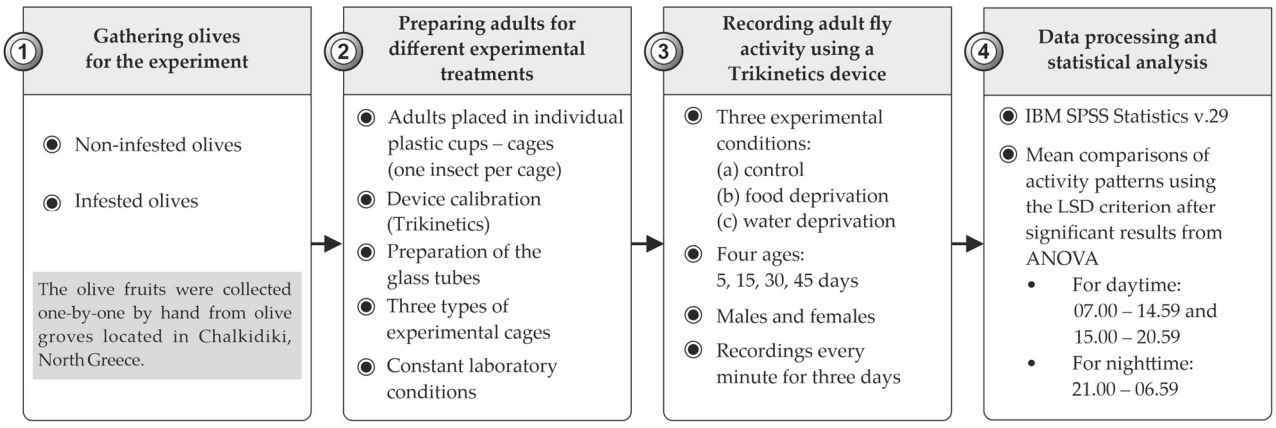


Figure 1. Stages of conducting the experiment.

2.1. Gathering Non-Infested and Infested Olives

Non-infested and infested olive fruits were collected by hand one by one from olive groves located in Chalkidiki, North Greece. For non-infested olives, special care was given to visually ensure that they were not infested from pests and diseases. After gathering, the non-infested olive fruits were placed into glass jars and then stored in a laboratory refrigerator (NIKIInox) at a temperature of 6 ± 1 °C (Figure 2).

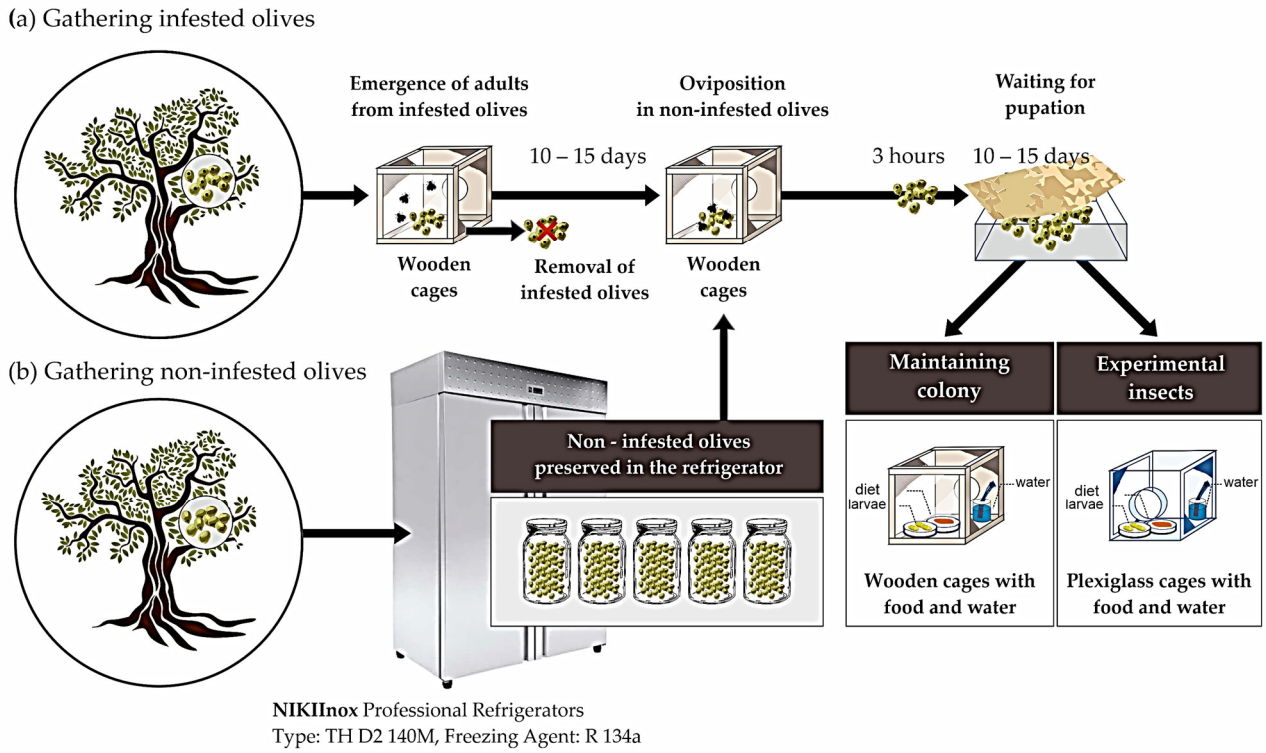


Figure 2. Collection of non-infested and infested olives from the wild to maintain the colony and to obtain the number of adults needed for the experiments.

To ensure the genetic diversity of the insects, the adults used in the experiments were collected from infested olives every week for a period of three months (from October to December). Gathering new infested olives was necessary not only to obtain the required number of flies for the experiments but also to maintain the colony.

2.2. Preparing Adults for Different Experimental Treatments

The types of cages used in the experiments were as follows:

(a) BugDorm-type cages (30 × 30 × 30 cm^3) for rearing insects to maintain the colony [24].
(b) Plexiglass transparent cages (20 × 20 × 20 cm^3) for obtaining experimental insects from pupae in Petri dishes (9.0-cm Ø).
(c) Individual cages (modified plastic cups) (6.5 × 8 × 9.5 cm^3) for maintaining the insects (with access to food and water) until they were placed in the Trikinetics device.

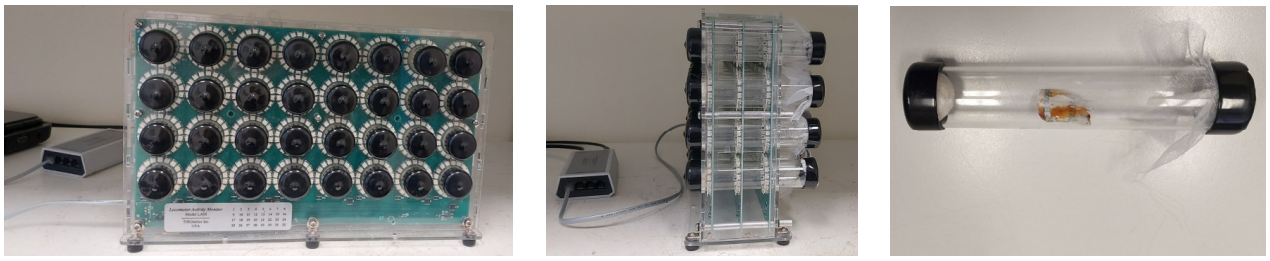
The steps followed for preparing the adults needed for the experiments were as follows:

(a) Obtaining the adults: pupae derived from the infested olives were placed in Petri dishes within Plexiglass cages.
(b) Upon emergence, males and females were placed in individual plastic cups—cages (one insect per cage) with water and diet until the day (5, 15, 30, and 45 days) they entered the Trikinetics device to avoid mating and adverse effects of crowding. The selection of the four age classes (5, 15, 30, and 45 days) aimed to explore the activity patterns of adults due to food or water deprivation as they get older.

A total of 480 adults (20 adults in each treatment × 24 treatments) were used in the experiments. Flies that died during the preparation stage of the experiment were immediately replaced to maintain a constant number of 20 adults per treatment.

2.3. Recording Adult Fly Activity Using a Trikinetic Device

The experiment was based on recordings of insect activity inside glass tubes using an electronic device that utilizes beams to identify potential insect movements (Trikinetics). Locomotory activity patterns were recorded under constant laboratory conditions using a LAM25 system (locomotory activity monitor—LAM25)—Trikinetics (Figure 3)—away from noise or other disturbances (i.e., vibrations) that would possibly affect their normal (expected) activity. Recordings of insect activity started at 7:00 am for all treatments. A few moments before the recordings began, the insects were placed in the glass tubes of Trikinetics with access only to water for the food-deprived treatments and with access only to food for the water-deprived treatments. Control groups inside the glass tubes had full access to food and water throughout the experiment.



(**a**) (**b**) (**c**)

Figure 3. Front (**a**) and side view (**b**) of the Trikinetics (LAM25system) set in laboratory. The tube setup: vinyl plastic stoppers (black color) were placed on one end (**a**,**b**) and cups with organdy cloth placed on the other end for the ventilation of the tubes (**c**) (photos taken by E. Balampekou).

Before conducting the experiment, adults were individually placed in plastic cups containing a food mixture of hydrolyzed yeast in a ratio of 5:4:1 (water, sugar, and yeast hydrolysate as protein), along with access to water. The food and water levels inside each plastic cup were continuously monitored to ensure the flies had access to fresh, appealing food and an adequate water supply.

Within the device, activity in each tube was monitored using three infrared light beams (referred to as activity monitors). These beams were positioned at three planes: two near the ends of each tube and one in the middle (as shown in Figure 4). At the ages of 5, 15, 30, and 45 days, flies were placed individually in 32 glass tubes (each with a diameter of

25 mm and a length of 125 mm), where they remained for 72 h. Inside the tubes, the control adults had access to both food and water. In contrast, food-deprived adults had access only to water, while water-deprived adults had access only to food (as depicted in Figure 5). The activity was recorded every minute, with the system counting the number of times an adult insect passed through one of the three infrared beams. The results included the total number of recorded movements from the three monitors (for each tube and per insect).

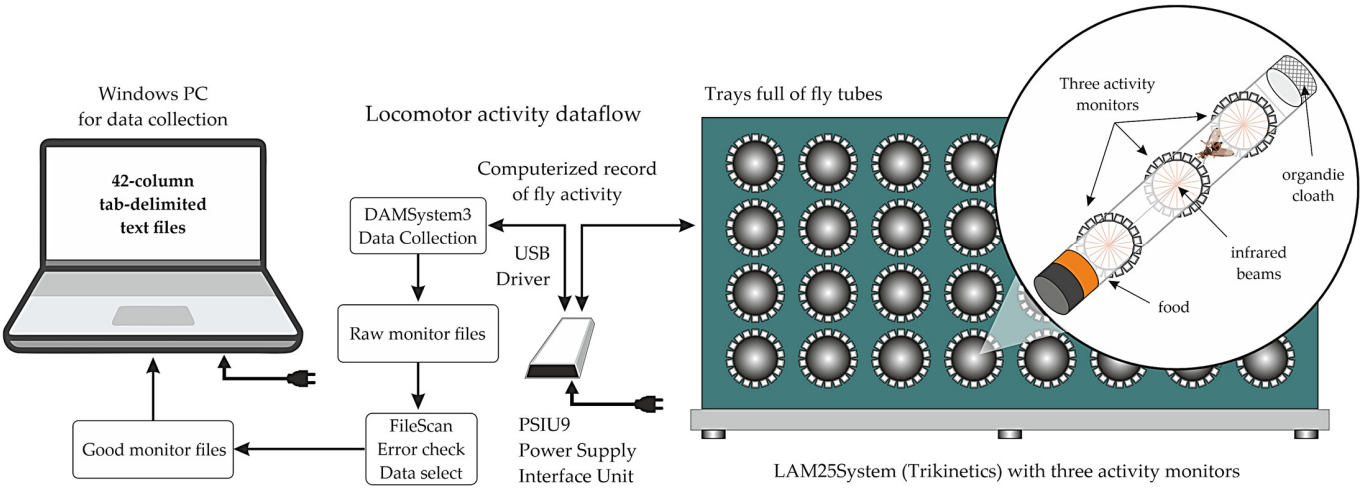


Figure 4. Locomotor activity dataflow using LAM25 System (Trikinetics).

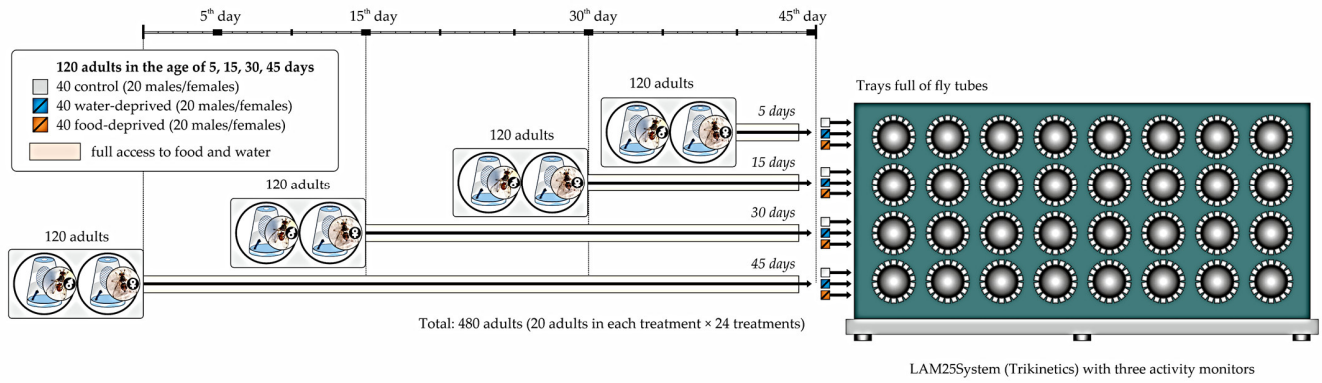


Figure 5. Experimental process.

We conducted a total of 24 experiments (treatments): (a) control adults with access to both food and water; (b) food-deprived adults with access only to water; and (c) water-deprived adults with access only to food. These experiments were conducted across four age groups, 5, 15, 30, and 45 days, and included both sexes (males and females).

2.4. Data Processing and Statistical Analysis

The mean locomotory activity of the adults placed in the tubes within the Trikinetics system was recorded every minute on a daily (24 h) basis for three days. The LAM25 system of Trikinetics was configured to record the number of insect movements every minute in each of the three monitors. Subsequently, we pooled the recordings from the three monitors to obtain the total activity, which represented the cumulative movements across all three monitors (a total of 1440 recordings: 840 during the daytime and 600 during the nighttime). Next, we calculated the mean value for each individual adult every two hours, following the approach described by Terzidou et al. [7]. Based on these results, we constructed twelve radar diagrams per age group and type of stress.

Data regarding the number of movements (insect activity) during daytime (07:00–20:59) were analyzed using general linear models with the ANOVA method. The model considered the main effects and interactions (2-way, 3-way, and 4-way) of four factors:

1. Sex (with 2 levels: males, females);
2. Ages (with 4 levels: 5, 15, 30, and 45 days);
3. Types of stress (with 3 levels: control, food-deprived, and water-deprived);
4. Daytime periods (with 2 levels: 07:00–14:59 and 15:00–20:59).

The factors sex, ages, and types of stress were included in the model as between-subjects factors, while the factor referring to daytime periods was considered a within-subjects factor. Each treatment had 20 replicates (insects).

The analysis of this model is equivalent to a factorial $2 \times 4 \times 3 \times 2$ (48 treatments) design with a split-plot arrangement [25,26]. In this approach, the combinations of the levels of the three between-subjects factors were treated as the main plots, and the levels of the within-subjects factor were considered the subplots.

A preliminary analysis of the raw data revealed violations of the model's assumptions related to the residuals' normality and homoscedasticity. Consequently, we tested a series of data transformations to ensure that the residuals conformed to these assumptions. Ultimately, a square root transformation of the raw data was selected as the most appropriate. The ANOVA and all subsequent analyses were performed on the transformed data (see Supplementary Table S1). Mean values of the transformed data, along with those of the observed data, are reported in the tables (Supplementary Tables S5–S12).

Data regarding the number of movements (insect activity) during nighttime (21:00–06:59) were analyzed using general linear models with the ANOVA method. The model considered the main effects and interactions (2-way and 3-way) of three factors:

1. Sex (with 2 levels: males and females);
2. Ages (with 4 levels: 5, 15, 30, and 45 days);
3. Types of stress (with 3 levels: control, food-deprived, water-deprived).

Thus, the model fits a $2 \times 4 \times 3$ (24 treatments) factorial design. The three factors were entered in the model as between-subjects factors. Each treatment had 20 replications (insects). A preliminary analysis of the raw data revealed violations of the model's assumptions related to the residuals' normality and homoscedasticity. Consequently, we tested a series of data transformations to ensure that the residuals conformed to these assumptions. Ultimately, the $\log_{10}(x + 1)$ transformation of the raw data was selected as the most appropriate. The ANOVA and all subsequent analyses were performed on the transformed data (see Supplementary Table S4). Mean values of the transformed data, along with those of the observed data, are reported in the tables (Supplementary Tables S13–S15).

In all cases, the normality assumption of residuals was evaluated through a visual inspection of the corresponding histogram and boxplot, a comparison of the residuals' median values with zero, an assessment of skewness and kurtosis indices, and the results of the Kolmogorov–Smirnov test for normality ($p > 0.05$). The homoscedasticity assumption was examined by visually inspecting the residuals' scatter plot against the model's predicted values and assessing the magnitude of Spearman's rho rank correlation coefficients between the residuals' absolute values and the predicted values estimated from the model.

In both analyses (during daytime and during nighttime), to further understand the impact of the independent variables on the insects' activity, the partial eta squared ($n^2_{partial}$) index was calculated. The partial eta squared, η^2, indicates the effect size (ES) of an effect (main or interaction) after all other effects have been removed. It is calculated by the formula SSA/(SSA + SSE), where SSA is the sum of squares of the effect of interest (let uss say A), and SSE is the sum of squares of the error [27]. The quantities SSA and SSE are provided from the correct ANOVA table. It must be noted that the ANOVA method was mainly employed to estimate the correct standard errors of the differences between the mean values of the factor levels' combinations. Statistically significant differences among mean values were highlighted using the "protected" least significant difference criterion.

Preliminary statistical analyses showed substantial differences between the movements occurring during daytime (light) and nighttime (dark) periods (see Table 1). The effect of these differences was masking all other effects in the tested models. Additionally, in previous studies that analyzed circadian rhythms in the same or related species for 24 h, activity levels were estimated separately for daytime and nighttime [7,8]. Thus, we decided to perform two separate analyses: one for the data collected during the daytime and another for the data collected during the nighttime.

Table 1. Mean and transformed mean (squared root transformation) of IR sensor interruptions (insect movements) with standard errors for the two periods of the day.

Periods of Day	Mean (with Std. Error)	Transformed Mean (with Std. Error)
Daytime	308.34 ± 9.3	15.64 ± 0.8
Nighttime	21.38 ± 1.9	0.91 ± 0.1

The statistical analyses were conducted using IBM SPSS Statistics ver. 29.0 Software (IBM Corp., Armonk, NY, USA) [28], and the significance level in all statistical hypothesis testing procedures was preset at $\alpha = 0.05$ ($p \leq 0.05$).

3. Results

We conducted two separate statistical analyses: one for the data collected during the daytime and another for the data collected during the nighttime. Detailed ANOVA results are available for both the daytime and nighttime periods in Supplementary Tables S1 and S4, respectively. We applied the least significant difference (LSD) criterion to assess statistically significant differences among the treatments (Supplementary Tables S5–S15).

In the daytime period, there are significant main effects of the four factors such as type of stress, age, sex and daytime period, as indicated by ANOVA ($F(2, 47) = 30.964$, $p < 0.001$, $n^2_{partial} = 0.120$; ($F(3, 47) = 12.160$, $p < 0.001$, $n^2_{partial} = 0.074$; ($F(1, 47) = 7.981$, $p = 0.005$, $n^2_{partial} = 0.017$; and ($F(1, 47) = 267.662$, $p < 0.001$, $n^2_{partial} = 0.370$, respectively (Supplementary Table S1). In the nighttime period, there are significant main effects of two out of the three factors, namely the type of stress and age, as indicated by ANOVA ($F(2, 23) = 11.508$, $p < 0.001$, $n^2_{partial} = 0.048$ and ($F(3, 23) = 12.160$, $p = 0.006$, $n^2_{partial} = 0.027$, respectively (Supplementary Table S4). In the daytime period, there are statistically significant differences with LSD = 2.34 (for the transformed data) and in the nighttime period with LSD = 0.36 (for the transformed data). Based on the values of the corresponding partial eta squared indices, the factor "Daytime Periods" and the factor "Types of Stress" had the largest effects on the insects' activity (see also Supplementary Tables S3 and S4).

3.1. Comparisons between Type of Stress

The results based on the comparison between the movements of adults in different types of stress indicate that food and water deprivation lead to increased mobility in insects, both during the daytime and the nighttime. For males, it is apparent that lower mobility is observed in the control insects, while higher mobility is observed in insects deprived of food both during the day and at night (Figure 6). In females, higher mobility is observed in individuals deprived of food. During the daytime period, in both male and female individuals 5 days old, the mobility of the control insects is lower and differs from the mobility of those deprived of food or water. In individuals deprived of food, mobility is higher and differs from that of individuals deprived of water (Figure 6). Detailed comparisons between the types of stress across the same age and in the same daytime period in males and females are described in Supplementary Tables S7 and S8 and in Supplementary Table S13 for nighttime.

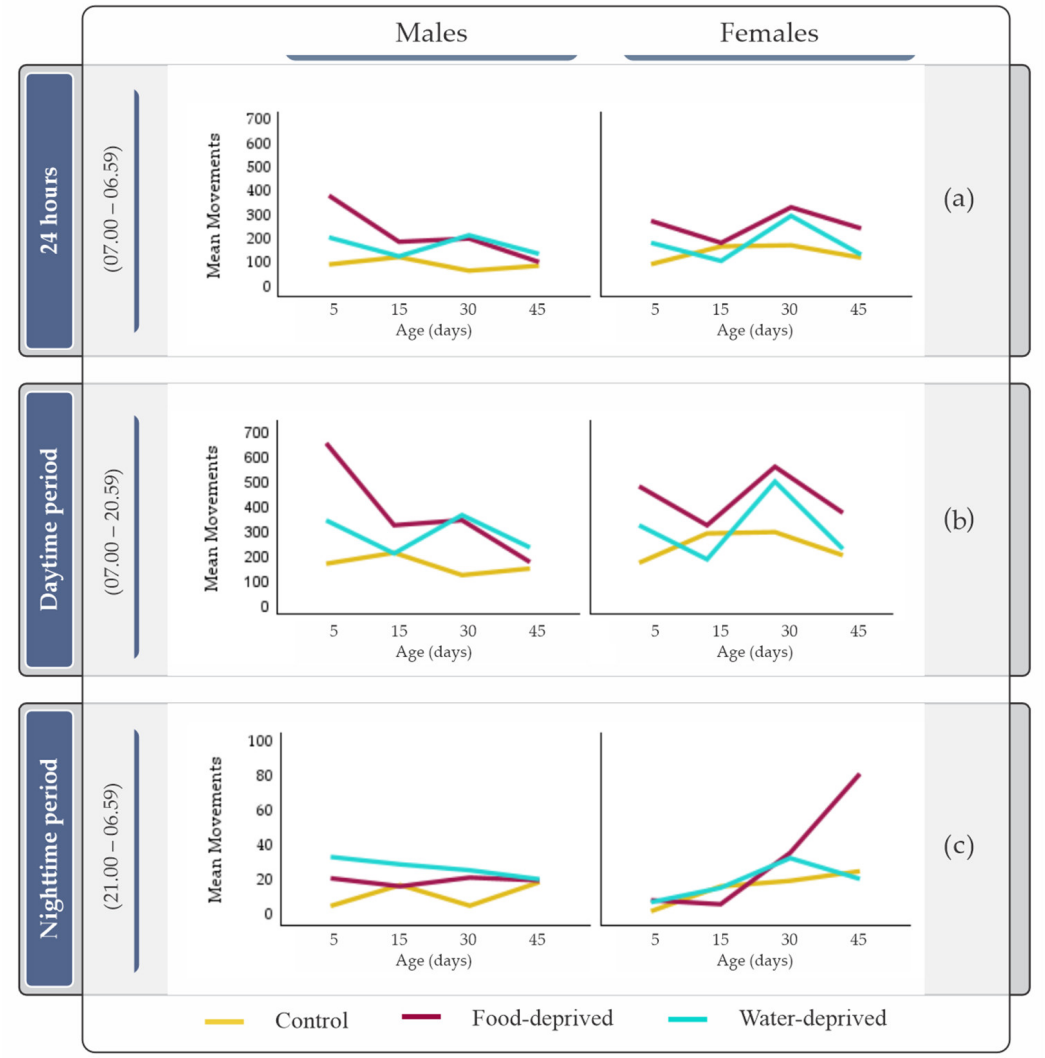


Figure 6. The total mean movements (untransformed data) over the three-day recording period (72 h) were analyzed for both males and females under each type of stress condition, including (**a**) both daytime and nighttime periods; (**b**) daytime only; (**c**) nighttime only.

3.2. Comparisons between Males and Females

Both males and females exhibit higher mobility during the last six hours of the daytime period, while mobility sharply declines during the night. Specifically, 5-day-old males deprived of food as well as those deprived of water at 15:00–20:59 are more mobile compared to their female counterparts. During the daytime period, 30-day-old females in all types of stress are more mobile compared to their male counterparts. The same trend is observed in 45-day-old females deprived of food (Figure 7). According to ANOVA (Supplementary Table S4), no differences were observed during the nighttime between the sexes. Detailed comparisons between the sexes across the same age and the same type of stress (control, food-deprived, and water-deprived), in the daytime periods 07:00–14:59 and 15:00–20:59 are given in Supplementary Tables S11 and S12 and in Supplementary Table S15 for the nighttime.

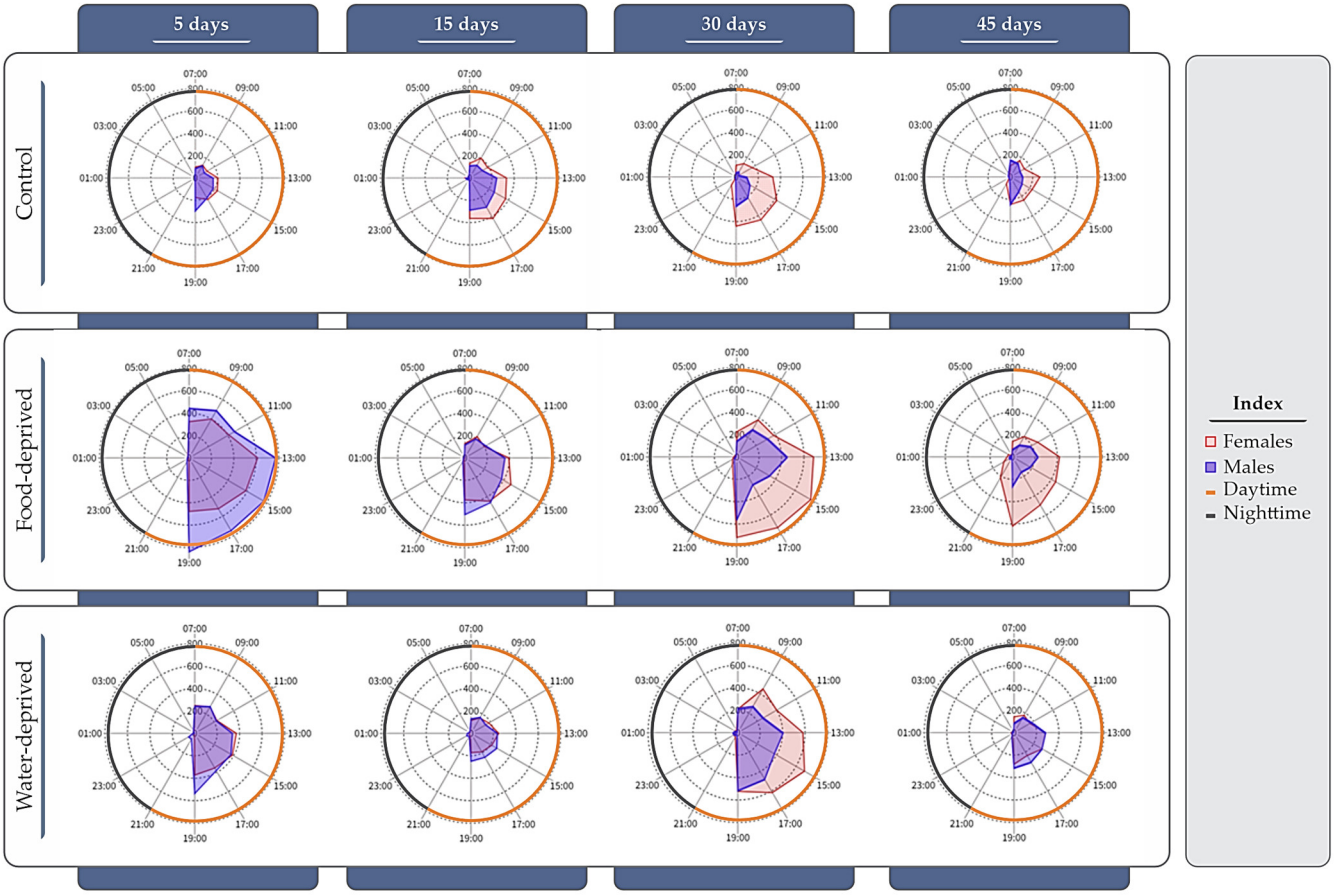


Figure 7. Radar diagrams are employed to visualize the differences in mean movement patterns (untransformed data) between males and females across various types of stress and age groups. Movement recording starts at 07:00 in the morning, with averaged movement values plotted for every two-hour interval throughout the three-day experiment.

3.3. Comparisons between Age Groups and Daytime–Nighttime Periods

The 5-day-old males exhibit higher mobility in almost all types of stress compared to their counterparts 45 days old in both daytime period groups. Similarly, 5-day-old females deprived of water show greater mobility compared to their counterparts 45 days old in both daytime period groups, as well as those deprived of food in the early daytime period 07:00–14:59 (Figure 8).

During the nighttime period, the mean highest value in the recorded mobility was 82.01 ± 21.7 (movements) observed in 45-day-old females deprived of food, while the mean lowest value in the recorded mobility was 2.30 ± 0.6 (movements), observed in control females aged 5 days (Figure 8). Detailed comparisons between ages across the same type of stress (control, food-deprived, and water-deprived) and in the same daytime period in males and females are given in Supplementary Tables S9 and S10 and in Supplementary Table S14 for the nighttime period.

Increased mobility is observed in almost all treatments during the daytime period 15:00–20:59. Specifically, males exhibit greater mobility during the 15:00–20:59 period in all treatments except for 45-day-old individuals in the control and food-deprived groups. Females exhibit greater mobility during the 15:00–20:59 period in all treatments except for 5-day-old individuals in the control and food-deprived groups and 5- and 15-day-old individuals in the water-deprived groups (Figure 8). Detailed comparisons between the daytime periods (07:00–14:59 and 15:00–20:59) across the same age and the same type of stress in males and females are given in Supplementary Tables S5 and S6.

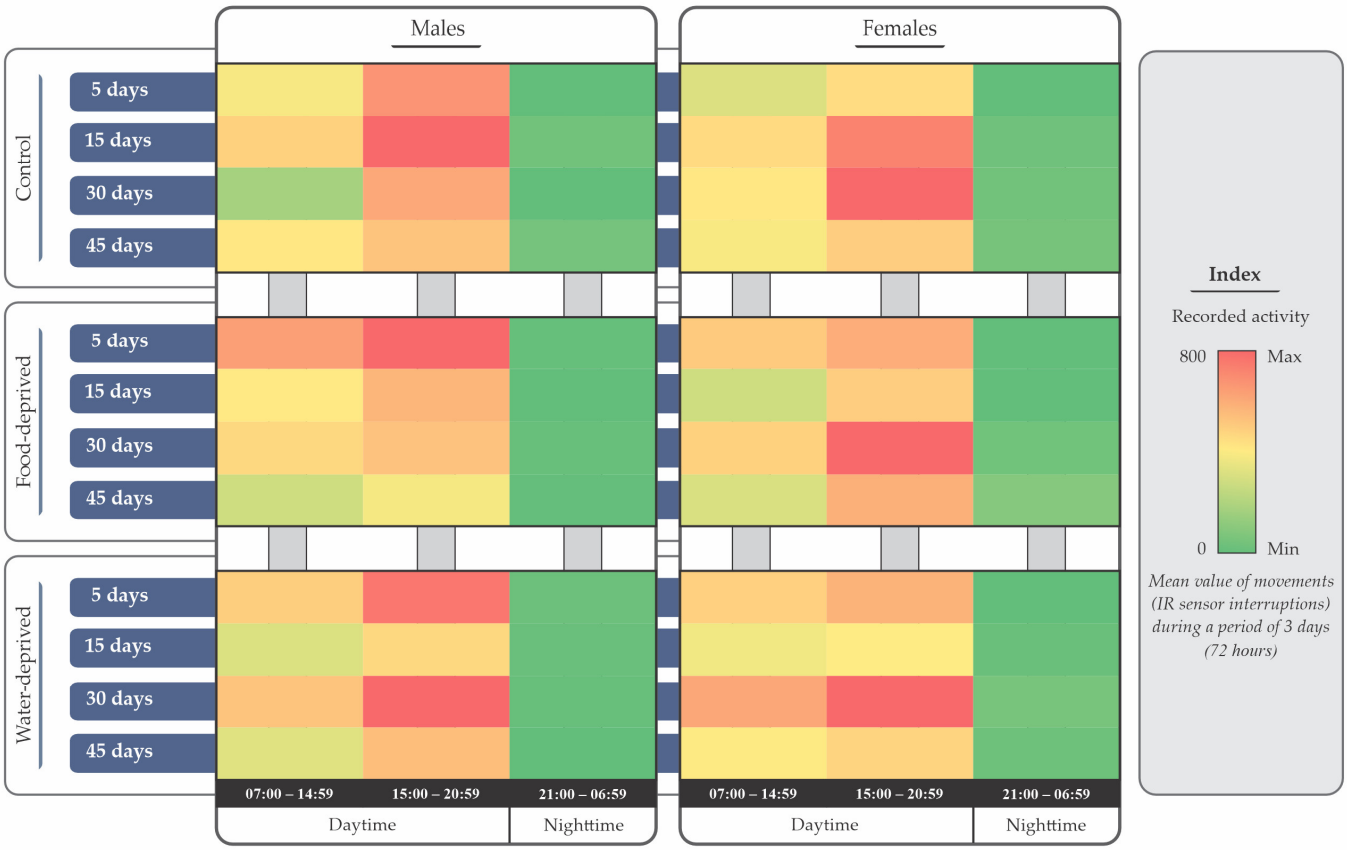


Figure 8. Heatmap with mean value of movements—IR sensor interruptions (untransformed data) for males and females in three types of stress (control, food-deprived, and water-deprived) and for four age groups (5, 15, 30, and 45 days).

In both sexes, low mobility was recorded during the nighttime across all stress and age treatments. In the daytime period, moderate to high mobility was recorded for both sexes and specifically flies were more active between 15:00 and 20:59 than 7:00 and 14:59 ($p < 0.001$, $n^2_{partial} = 0.370$). Detailed information on the statistically significant differences (SSDs) between the daytime periods (07:00–14:59 and 15:00–20:59) across the same age and the same type of stress is given in Supplementary Tables S5 (males) and S6 (females). Generally, younger flies were more active than older flies across daytime and nighttime periods, but some differentiation between different age and stress groups was observed (Figure 8).

4. Discussion

In this study, we investigated the locomotory activity response of *B. oleae* adults to stress conditions in a laboratory using an automated, modern, and technically sophisticated approach of using the Trikinetics device. These investigations aimed to uncover the factors influencing the mobility of adult flies and assess the periods of circadian adult activity.

The highest level of activity (measured by the average number of movements) was observed in both males and females during the daytime period from 15:00 to 20:59 compared to the daytime period from 07:00 to 14:59. According to previous studies, *B. oleae* engages in mate searching and courtship in the late afternoon [7,29–32]. The device records movement that may be due either to wing movement or insect walking. However, increased activity after 15:00 may certainly be related to courtship, as it is observed for both sexes in the control and in food and water-deprived flies.

The activity patterns of males and females are quite similar in all treatments with only some exceptions. For example, in the daytime period 15:00–20:59, 30-day-old females

exhibited higher activity compared to males in the control and both types of stress (food- and water-deprived). In these last six hours of the daytime period (15:00–20:59), the 5-day-old males deprived of food and 15-day-aged males deprived of water were more active than the corresponding females. The recording of this high activity may be attributed to the movement of male wings. Males begin to perform wing vibration after the first 5 days of their adult life, which corresponds with females becoming receptive to mating. Females of *B. oleae* have immature oocytes in their ovaries in the first 3 to 5 days of their adult life, and during this period, they do not mate. Mating occurs after this period and reaches a maximum at 10 days of age [31,33], while wing vibration and mating occur only in the last 6 h of the photophase [34]. Females produce sex pheromones from the third to the fifth day after emergence, and this is repeated after 10 days when males repeat the courtship call with wing vibration [31,33,35]. The courtship call of males is extremely energy-consuming and increases their need for food [36]. In addition, the increase in the mobility of virgin males during the daytime period 15:00–20:59 at ages 5, 15, and 30 days may be interpreted as their search for females at these ages, which represent the reproductive period of males [37].

During the daytime, both 5- and 30-day-old male and female individuals, as well as 45-day-old females, in the control group exhibited the lowest activity levels compared to their counterparts deprived of food or water. This notable observation concerning the consistent increase in mobility observed in response to both food and water deprivation, regardless of sex or time of day, suggests that insects exhibit a behavioral response to resource scarcity, potentially driven by the need to search for alternative sources of sustenance or navigate to more favorable environments. Our results are in line with another study conducted with *Bactrocera minax* (Enderlein) (Diptera: Tephritidae) in which the removal of food and water resulted in increased activity [38]. Knoppien et al. found that food deprivation makes *D. melanogaster* more active [39]. A similar study on *Locusta migratoria* (Meyen) (Orthoptera: Acrididae) showed that food or water deprivation affected locomotor patterns [40]. This adaptive response highlights the resilience and adaptability of insects in the face of changing environmental conditions.

In our study, younger individuals generally exhibit higher activity levels compared to older individuals, consistent with the findings of Rodovitis et al. in *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) [8]. Specifically, differences are observed in food-deprived and water-deprived males between younger (5-day-old) and older (45-day-old) individuals. In females, differences are observed during the daytime between younger and older food-deprived and water-deprived individuals, while during the daytime period 07:00–14:59, differences exist only in food-deprived females. This may be attributed to the fact that younger individuals actively seek food and water as they mature prior to mating, while older individuals have reduced energy needs for reproduction [41]. Moreover, this age-related decline in mobility could be attributed to factors such as changes in physiological fitness or metabolic rates as individuals mature. Additionally, the observed differences between age groups underscore the dynamic nature of locomotor behavior and highlight the importance of considering developmental stages when analyzing mobility data.

Furthermore, the recorded data illustrate distinct responses to light–dark cycles in mobility patterns. Increased mobility is consistently observed during the daytime period, particularly during the 15:00–20:59 period, across almost all experimental treatments, which is consistent with similar responses observed in many organisms, where activity peaks during periods of light. During the nighttime period, mobility levels generally decrease compared to the daytime period, reflecting the natural tendency of organisms to be less active during darkness. The results of our study are in line with studies conducted on *D. melanogaster* regarding the factors that can influence sleep [11,15]. Interestingly, the recorded data highlight a notable difference in mobility between age groups and types of stress during the nighttime period, with 45-day-old females deprived of food exhibiting the highest mean mobility value recorded. This unexpected increase in mobility in older females under food deprivation conditions warrants further investigation and may indicate age-specific responses to environmental stress conditions. It is worth noting that based on

our experience and the existing baseline data in our laboratory, virgin females of *B. oleae* typically live for approximately 70 days under constant laboratory conditions.

The comparison of the mean highest and lowest mobility values across different types of stress provides additional insights into the variability of locomotor behavior. Notably, the highest mean mobility values are consistently observed during the 15:00–20:59 period, particularly in 5-day-old males deprived of food. Conversely, the lowest mean mobility values are recorded during the nighttime period, with 5-day-old females in the control group exhibiting the lowest mobility levels.

To the best of our knowledge, this study represents the first attempt to evaluate and compare variations in mobility among different age groups of *B. oleae* adults across various time periods throughout the day and night, under conditions of food or water deprivation. The findings of our work indicate that insects exhibit an increase in their mobility under food or water deprivation. In the wild, during a period of food or water scarcity (i.e, during a drought), a potential increase in insect mobility may lead to depletion of their energy reserves [42,43] and therefore a reduction in their population. Thus, this period may be preferred for the application of sterile insect techniques or other genetic control strategies. These findings offer valuable insights into potential vulnerabilities that can be targeted for pest control strategies and can be used to answer questions regarding (a) the biological response of male and female adult flies under food and water deprivation, (b) the role of the aging process in their mobility, and (c) the activity patterns during daytime and nighttime periods. Further research is needed to explore the genetic mechanisms underlying the impact of food and water deprivation on insect mobility, as well as the role of age and other environmental factors in influencing activity patterns. In addition, experiments in the wild are needed to confirm our findings in the laboratory.

Given the fact that in our research, the 5-day-old food-deprived males exhibited the highest activity, we expect that these findings may open new ways in sterile insect techniques, if, theoretically, young males were preferred to be released instead of pupae. For future research, we suggest repeating the same experiment with 5-day-old adult males that emerge from irradiated pupae (sterilized by radiation) and to verify if the mobility of the food-deprived groups is greater than that of the control. Additionally, similar experiments can be conducted on sterilized males resulting from other genetic control strategies to control various harmful species.

5. Conclusions

The current study highlights the use of an automated electronic device (Trikinetics) to record the individual locomotory activity patterns of adult olive fruit flies (*B. oleae*) under stress conditions. Our study reveals increased locomotory activity when insects are subjected to food or water deprivation, while decreased mobility is observed with aging. It should also be noted that insect activity during nighttime was also studied, revealing very low levels of activity. The findings of our study revealed distinct activity patterns between males and females depending on the type of stress and the period of daytime.

The main conclusions of the study are as follows:

- Marked differences were observed in insects' activity between daytime and nighttime.
- Food-deprived adult flies of both sexes exhibited the highest locomotory activity followed by water-deprived and then by control adult flies.
- During the daytime period 15:00 to 20:59, adults showed higher activity compared to the daytime period 07:00 to 14:59.
- Higher activity levels were recorded in 5-day-old males and females compared to those that were 45 days old.
- The 30-day-old female flies exhibited higher levels of locomotory activity than males of the same age during the daytime period.

These insights could be exploited in enhancing existing or new pest control management dealing with stress insect physiology, the aging process, and functional senescence and could be used in quality control tests performed in SIT. Our study forms the basis for

future research on locomotory activity of *B. oleae* under stress conditions to formulate more effective and sustainable pest control strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14051051/s1, Table S1: Tests of between-subjects effects (ANOVA) for testing the effects (main and interactions) of types of stress (control, food-deprived, water-deprived), ages (5 days, 15 days, 30 days, 45 days), sexes (males, females), and daytime periods (07:00–14:59, 15:00–20:59) on movements during the daytime (square-root-transformed data); Table S2: Mean and transformed mean (squared root transformation) with standard errors for the two daytime periods ($n^2_{partial} = 0.370$); Table S3: Mean and transformed mean (squared root transformation) with standard errors for the three types of stress ($n^2_{partial} = 0.120$); Table S4: Tests of between-subjects effects (ANOVA) for testing the effects (main and interactions) of types of stress (control, food-deprived, water-deprived), ages (5 days, 15 days, 30 days, 45 days), sexes (males, females) on movements during nighttime period [$\log_{10}(x + 1)$ transformed data]; Table S5: Statistically significant differences (SSDs) between the daytime periods (07:00–14:59 and 15:00–20:59) across the same age and the same type of stress in males with different lower-case letters that correspond to statistically significant differences among mean values of transformed data (squared root transformation) at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 2.34); Table S6: Statistically significant differences (SSDs) between the daytime periods (07:00–14:59 and 15:00–20:59) across the same age and the same type of stress in females with different lower-case letters that correspond to statistically significant differences among mean values of transformed data (squared root transformation) at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 2.34); Table S7: Statistically significant differences (SSDs) between types of stress (control, food-deprived, and water-deprived) across the same age and in the same daytime period in males with different lower-case letters that correspond to statistically significant differences among mean values of transformed data (squared root transformation) at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 2.34); Table S8: Statistically significant differences (SSDs) between types of stress (control, food-deprived, and water-deprived) across the same age and in the same daytime period in females with different lower-case letters that correspond to statistically significant differences among mean values of transformed data (squared root transformation) at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 2.34); Table S9: Statistically significant differences (SSDs) between ages across the same type of stress (control, food-deprived, and water-deprived) and in the same daytime period in males with different lower-case letters that correspond to statistically significant differences among mean values of transformed data (squared root transformation) at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 2.34); Table S10: Statistically significant differences (SSDs) between ages across the same type of stress (control, food-deprived, and water-deprived) and in the same daytime period in females with different lower-case letters that correspond to statistically significant differences among mean values of transformed data (squared root transformation) at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 2.34); Table S11: Statistically significant differences (SSDs) between sexes across the same age and the same type of stress (control, food-deprived, and water-deprived) in the 07:00–14:59 daytime period with different lower-case letters that correspond to statistically significant differences among mean values of transformed data (squared root transformation) at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 2.34); Table S12: Statistically significant differences (SSD) between sexes across the same age and the same type of stress (control, food-deprived, and water-deprived) in the 15:00–20:59 daytime period with different lower-case letters that correspond to statistically significant differences among mean values of transformed data (squared root transformation) at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 2.34); Table S13: Statistically significant differences (SSD) between the types of stress (control, food-deprived, and water-deprived) across the same age and the same sex in nighttime period with different lower-case letters that correspond to statistically significant differences among mean values of transformed data [$\log_{10}(x + 1)$ transformation] at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 0.36); Table S14: Statistically significant differences (SSDs)

between ages across the same type of stress (control, food-deprived, and water-deprived), and the same sex in nighttime period with different lower-case letters that correspond to statistically significant differences among mean values of transformed data [$\log_{10}(x + 1)$ transformation] at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 0.36); Table S15: Statistically significant differences (SSDs) between sexes across the same age and the same type of stress (control, food-deprived, and water-deprived) in nighttime period with different lower-case letters that correspond to statistically significant differences among mean values of transformed data [$\log_{10}(x + 1)$ transformation] at a significance level of $a = 0.05$, according to the results of LSD criterion (for transformed data common LSD value = 0.36).

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