

Seasonal and daily patterns in the flight activity of *Trioza erytreae* (Del Guercio), vector of huanglongbing (HLB)

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Abstract

BACKGROUND: The psyllid *Trioza erytreae* (del Guercio, 1918) (Hemiptera: Triozidae) is one of the vectors of *Candidatus liberibacter spp.*, the causal agent of the huanglongbing (HLB) the main citrus disease worldwide. In this study, we investigated for the first time how the flight behavior of *T. erytreae* is influenced by factors such as sex or daily and seasonal pattern fluctuations by using a flight mill device under controlled laboratory conditions.

RESULTS: Our results showed that *T. erytreae* can fly an average distance of 16.20 m at a mean speed of 6 m per minute, but a few individuals were able to fly much longer, reaching a maximum distance of 395 m. Females displayed a higher flight capacity than males. Moreover, we found that *T. erytreae* is not confined to diurnal flight alone, as the psyllids demonstrated the ability to fly during the night, emphasizing the need for comprehensive vector control strategies that account for nocturnal activity.

CONCLUSION: Understanding the flight behavior of vector insects is essential for devising effective control measures aimed at mitigating the spread of vector-borne diseases. Further research in this area will contribute to a more nuanced understanding of vector movement and the development of more precise control measures.

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Supporting information may be found in the online version of this article.

Keywords: African citrus psyllid; citrus greening; flight mill; flight behavior; vector dispersion

1 INTRODUCTION

Huanglongbing (HLB), or Citrus Greening, induced by bacteria in the genus *Candidatus liberibacter*, is the most devastating disease in the citrus industry worldwide.¹ HLB is associated with three phloem α -proteobacteria, '*Candidatus liberibacter americanus*' (CLam), '*Ca. liberibacter asiaticus*' (CLas), and '*Ca. liberibacter africanus*' (CLaf). This disease is characterized by recognizable symptoms, including leaf yellowing, defoliation, reduced root growth, twig dieback, the formation of small and irregularly shaped bitter fruits, and an overall deterioration in health, eventually leading to plant death.¹ HLB is currently present in Asia, the Americas, Africa, and a few Indian Ocean islands (EPPO 2023, <https://gd.eppo.int/taxon/TRIZER/distribution>); but to date remains absent in Europe.² Once the pathogens causing HLB disease are introduced in a geographical location, they can be rapidly and widely spread across the landscape by their two known insect vectors, *Diaphorina citri* Kuwayama, 1908 and *Trioza erytreae* (Del Guercio 1918) (Hemiptera:Psyllidae).³⁻⁶ Since there is currently no known cure for HLB-infected trees, control strategies focus on controlling competent vectors, eradicating infected trees, and meticulously overseeing plant nurseries.¹

The African citrus psyllid, *T. erytreae* Del Guercio (Hemiptera: Triozidae), is one of the two known vector species of the pathogens *Candidatus Liberibacter asiaticus* (CLas) and *Candidatus*

Liberibacter africanus (CLaf).³⁻⁶ This psyllid feeds mainly on plants belonging to the Rutaceae family, displaying a preference for lemon trees over sweet or bitter orange trees.^{7,8} Due to its substantial fecundity, up to 2000 eggs per female,⁹ along with a lack of diapause and a multivoltine life cycle allowing for up to eight generations per year, the species exhibits a notable potential for invasion.¹⁰⁻¹²

The African citrus psyllid was first reported in Southern Africa in 1897.¹³ Since then, it has established a significant geographical range (EPPO 2023, <https://gd.eppo.int/taxon/TRIZER/>

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distribution), being reported in more than 25 countries across Africa, Europe, and the Middle East.^{14–16} Nonetheless, *T. erytrae* remains absent in the main citrus-growing regions in the Americas, Asia, and Australia (EPPO 2023, <https://gd.eppo.int/taxon/TRIZER/distribution>). In mainland Europe, this psyllid was initially identified in 2014 in the northwestern areas of the Iberian Peninsula.¹⁷ From that point onward, the insect has been limited to the Atlantic coastal zones of northern Spain and Portugal, with no reported expansion into other citrus-growing areas within the Mediterranean basin,¹⁶ mainly because of repetitive releases of the parasitoid *Tamarixia dryi*,¹⁸ other factors, such as climatic conditions¹⁹ and the availability of host plants,^{8,10} have also contributed to limiting the spread of *T. erytrae* populations beyond these regions.

Despite the widespread presence of *T. erytrae*, information regarding its flight patterns and dispersal abilities remains scarce and outdated. According to Catling (1973, 1978),^{20,21} the adults of *T. erytrae* have limited dispersal capabilities. Although they are able to jump and fly, their flying ability is not sustained, suggesting that *T. erytrae* mainly stays within an orchard setting and tends to move locally. Conversely, Van Den Berg and Deacon,²² reported that *T. erytrae*, in the absence of host plants, can travel distances of up to 1.52 km within 7 days with the assistance of prevailing winds, and females have a higher propensity than males for long-distance flights, probably due to the need to find plants with new shoots on which to oviposit. It is known that the *T. erytrae* can locate flush at distances of several hundred meters,^{23,24} suggested that *T. erytrae* may display highly dispersive behavior under stress conditions. Regarding diurnal parameters, the literature reports that most flight activity occurs shortly before sunset and ceases with the onset of darkness.²⁵ It is also known that psyllids can fly long distances transported by winds according to capture data from day and night aerial sampling carried out at a height of 200 m above ground at Cardington, Bedfordshire, UK, during summers between 1999 and 2007.²⁶

A better understanding of the flight behavior of *T. erytrae* is of paramount importance, as it wields a direct influence over the distribution of the pest itself and the potential for HLB transmission and spread. The ability of insects to disperse short and long distances plays a pivotal role in shaping the spatial dynamics of infestations and the emergence of disease outbreaks.²⁷ Thus, studying flight behavior is key to effective pest management and disease control.

Flight mill devices have been widely used to assess the flight behavior of various pest species including insect vectors of plant diseases under a controlled laboratory environment.^{28–35} Despite the diverse range of flight mill designs, most adhere to a common principle: an insect is attached to an arm connected to a supporting structure. This arrangement allows the insect to navigate a circular trajectory during flight, enabling data collection regarding various flight parameters, including flight incidence, distances flown, duration of flight sessions and even flight speed.^{36–38} Additionally, flight mill devices can assess how factors such as age, sex, host plants, season, time of the day, temperature, atmospheric pressure or population source can influence an insect's propensity to disperse.^{33,38}

In our study, we investigated for the first time how the flight propensity and dispersal ability of *T. erytrae* is influenced by factors such as sex, season, and time of day by using a flight mill device under controlled laboratory conditions. Such information is crucial to understand the dispersal ability of *T. erytrae* and its potential to move short or long distances, and thus understand

the risk of spread of HLB in case it is introduced in the Iberian Peninsula.

2 MATERIALS AND METHODS

2.1 Insects' origin and rearing

Trioza erytrae individuals were reared at the Estación Fitopatológica Areeiro (Pontevedra, Spain) in 1–2-year-old *Citrus limon* plants (cv. Lunario) in insect-proof cages in a glasshouse. The rearing conditions in the glasshouse varied between winter and summer, although the temperature was maintained between 10 and 30 °C and RH in the range of 60–80% throughout the year. No artificial light was provided in the glasshouse and light intensity and day length varied throughout the year.

The lemon plants, grown from seed, used for the rearing were regularly fertilized with a slow-release complex granulated blue fertilizer with an NPK (nitrogen, phosphorus, potassium) ratio of 12-8-16. Additionally, monthly fertigation was applied using Biomax solid (2%) (Agrinova Science, Spain). To promote the continuous availability of suitable sites for egg-laying and subsequent nymph development, the lemon plants were pruned regularly to stimulate new growth and the emergence of flushes.

2.2 Flight mill apparatus

The flight mill used was hand-made and based on the flight mill apparatus described in other studies.^{28,35} The flight mill was composed of a 10 cm optical fiber on the horizontal axis fixed to a sewing needle. At each extremity of the horizontal optical fiber, two smaller pieces of optical fiber (1 cm) were glued vertically, with a super glue (UHU, China), making a 90° angle with the horizontal fiber (Fig. 1).

The sewing needle was vertically positioned beneath a magnet, glued onto an acrylic platform. Another magnet was glued below onto a second platform to suspend the needle securely. The position of the second platform was adjusted using a micromanipulator (M3301R, World Precision Instruments, USA) to minimize friction and allow for smooth rotation of the sewing needle. The flight mill setup was then enclosed within a 32 × 32 × 24.5 cm acrylic chamber, and to provide consistent visual feedback to the insects, the acrylic chamber was covered with 2.5 cm wide vertical stripes alternating between black and white to provide visual cues that resemble illusion of movement,³⁹ during flight (Fig. 1).

2.3 Flight mill procedure

The effect of sex, season (Summer and Winter), and period of the day (morning, afternoon, and night) on the flight activity of *T. erytrae* was tested. For that, 5–10-day old adults of *T. erytrae* were submitted to flight mill assays divided into three different periods of the day: (i) 8:00–14:00, (ii) afternoon 14:00–20:00, and (iii) night 20:00–2:00 spanning across two specific seasons. The two seasons encompassed in the study were summer, occurring from June to August (2021 and 2022), and winter, spanning from November (2021 and 2022) to January (2022 and 2023).

Experiments were conducted under laboratory conditions, at a temperature of 23 ± 2 °C and relative humidity of 60 ± 10%. The flight mill tests conducted during the morning and afternoon were performed under two batteries of white linear LED lighting placed on the top of the acrylic chamber to provide constant overhead uniform light (artificial LED light (30 × 1.4 cm, 6000 K, 6 W 600 lm and 125 µm/Mol – 1)) to simulate natural light conditions. For night assays, the insects were kept in the dark during the

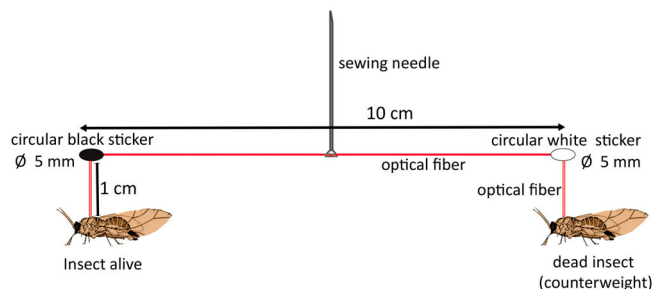
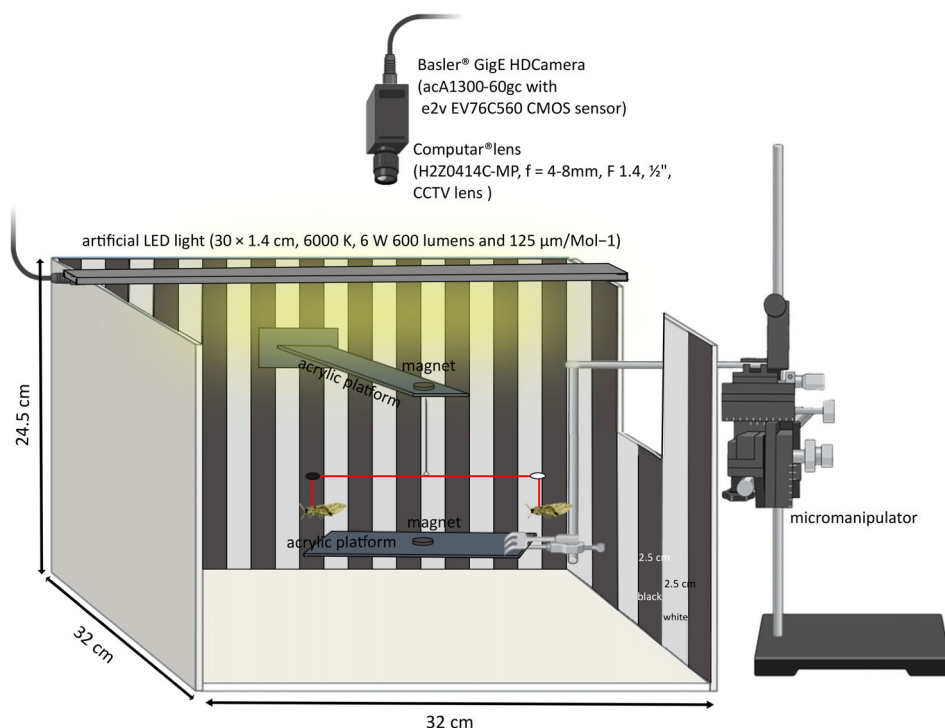


Figure 1. Schematic drawing of the flight mill apparatus built to conduct the assays (not drawn to scale).

whole process and an IR lamp (IR R125, 175 W, Havells Sylvania Europe) was used to record the flight activity.

Before starting the flight mill assays, adults of *T. erytrae* were sexed and placed in a refrigerator at 4 °C for 5 min to reduce their activity. Subsequently, the psyllids were immobilized by a vacuum device under a stereoscopic microscope. With the help of a micromanipulator, the 1 cm optical fiber attached to the end of the horizontal axis fiber of the flight mill was glued to the pronotum of the psyllids with a non-toxic washable glue (Elmer's products, Columbus, OH, USA). A dead *T. erytrae* was attached to the opposite end of the horizontal axis to counterbalance allowing insects to fly in a circular trajectory.

Additionally, to detect and track the flight activity, a circular black sticker was placed on one end of the horizontal axis fiber of the flight mill and a white sticker was placed on the opposite side to compensate for the weight. Then the flight mill with the *T. erytrae* glued on was placed gently between the magnets, and the behavioral assay was initiated. The flight activity of *T. erytrae* was filmed with a Computar® lens (H2Z0414C-MP, $f = 4-8$ mm, F 1.4, 1/2", CCTV lens) mounted on a Basler® GigE HD camera (acA1300-60gc with e2v EV76C560 CMOS sensor

(Fig. 1). The recording tool used was the Media Recorder 2.5 software.⁴⁰

Psyllids that did not fly within the first 10 min after attachment to the optical fiber were removed and discarded from the analysis. For the psyllids that engaged in flight activity, the assay was considered completed 5 min after the insect ceased flight. When insects performed several flights within the same recording, a new flight was considered each time that *T. erytrae* stopped the flight and engaged in a new flight again within a time lag of 20 s. Including non-flying insects in the analysis, which represent over 68% of the sample, could introduce significant issues, such as a large number of missing data (zeros). This can complicate the model's execution and interpretation, potentially distorting the results and affecting the analysis of flight parameters. However, non-flyers were included in the variable 'flight incidence' to explain the proportion of individuals that initiated flight, including those that did not fly among the different treatments.

2.4 Statistical analyses

The Noldus Observer XT 11.5 software,⁴¹ was used to analyze the flight incidence (i.e., the proportion of insects that flew or not), the

number of turns (n) during each flight, the number of single flights, and the duration of each single flight for each *T. erytrae* subject that underwent testing. With this information it was possible to calculate the flight duration (T) that corresponds to the time that one individual spent flying, the total distance travelled (D) by using the formula: $[D = d \times \pi \times n]$ (d - diameter = 10 cm), and mean speed of all the individual flights (V) by using the formula: $[V = D/T]$.

The flight performance of *T. erytrae* was evaluated through the assessment of several dependent variables, namely flight incidence, number of flights, distance travelled (m), flight duration (min), and mean speed (m/min). In total, 10 different models were constructed—two for each response variable—since both season and time of day were analyzed separately. For the seasonal pattern analysis, we examined the potential influence of sex (male and female), season (summer and winter), and their interaction on the aforementioned flight parameters. Conversely, in the daily pattern analysis, we focused on the impact of sex (male and female) and the time of day (morning, afternoon, and night) on the same flight parameters. The assessment of flight incidence (for the season and daily patterns) was undertaken using generalized linear models (GLMs), utilizing a binomial distribution of errors and a logit link function. These models were constructed using the 'glm' function in R. Concurrently, the other flight parameters (number of flights, distance travelled (m), flight duration (min), and mean speed (m/min)) were subjected to a linear regression model (LM) analysis. The model was created using the 'lm' function in R. Prior to analysis, the data underwent logarithmic transformation ($\log_{10}(x + 1)$) to achieve adherence to Gaussian distribution assumptions. All the 10 models were validated using the *simulateResiduals* function from the 'DHARMA' package.⁴² ANOVA tests with *post hoc* Tukey's HSD tests were performed to compare the mean values between the groups. The total number of individuals used for the studies was 448, being 208 females and 239 males. The number of individuals used in each model can be found in Table S1.

3 RESULTS

The flight mill device was conveniently modified testing different optical fiber materials and magnets until insects were able to complete a full spin soon after they started to fly. The flight mill prototype was considered functional when the horizontal optical fiber moved smoothly with almost no friction after flight initialization and a full spin was completed in less than 2 s. Then, after the flight mill was functional, a series of flight mill assays were conducted to study the flight performance of females and males of *T. erytrae* during two seasons at different periods of the day. Out of a total of 447 individuals tested, 141 of them successfully performed flights (31.54%). Overall, the results showed that the psyllids are capable of flying an average of 16.20 m at a mean speed of 6 m/min (Table 1). Females were shown to be capable of flying a maximum distance of 394.58 m at a mean speed of 6.45 m/min in a single flight on the flight mill device. In contrast, males could only fly a maximum of 11.94 m in a single flight. The rest of the descriptive statistics for the studied flight parameters, including overall data, females and males, are presented in Table 1.

The frequency distribution of the number of flights, distance travelled, flight duration, and mean speed for all insects that flew is shown in Fig. 2. Most insects were observed to perform only a single flight during the study. However, there were instances

Table 1. Descriptive statistics of all the flight parameters studied in the assay for a total of individuals ($n = 141$), females ($n = 98$), and males ($n = 43$)

	Flight parameters	Mean \pm SE	Median	Maximum
Total	Number of flights	1.45 \pm 0.07	1.00	4.00
	Distance travelled (m)	16.20 \pm 4.09	3.14	394.58
	Flight duration (min)	2.44 \pm 0.61	0.75	52.70
	Mean speed (m/min)	6.00 \pm 0.26	5.28	18.53
Females	Number of flights	1.55 \pm 0.09	1.00	4.00
	Distance travelled (m)	22.04 \pm 5.79	4.08	394.58
	Flight duration (min)	3.20 \pm 0.87	0.91	52.70
	Mean speed (m/min)	6.45 \pm 0.32	6.26	18.53
Males	Number of flights	1.21 \pm 0.09	1.00	4.00
	Distance travelled (m)	2.89 \pm 0.38	0.38	11.94
	Flight duration (min)	0.71 \pm 0.11	0.50	3.59
	Mean speed (m/min)	4.99 \pm 0.42	4.33	17.95

where a few insects exhibited an ability to perform successive flights for up to four times. In most cases, the insects covered relatively short distances, typically less than 10 m. However, there were exceptions—one female demonstrated exceptional flight capabilities, reaching maximum distances of almost 400 m. Regarding flight duration, the vast majority of insects exhibited relatively brief flights, lasting between 2 and 6 min. Nonetheless, three insects displayed prolonged flight time durations exceeding 40 min.

3.1 Seasonal pattern

One of the main objectives of this study was to investigate the influence of season and sex, as well as the interaction between both factors, on the flight activity of *T. erytrae*. The results indicate that the season significantly impacted the flight incidence and mean speed of males and females of *T. erytrae* (Table 2).

In the experiments conducted in summer, females showed a higher incidence of flight compared to males, but in winter there were no statistical differences between the sexes (Fig. 3(a)). Interestingly, males displayed a significantly greater incidence of flight during winter than those assessed during spring ($P < 0.001$). However, no statistically significant differences were observed between females in spring and winter ($P = 0.98$).

Concerning mean speed, during the summer, females showed significantly higher mean speeds than males ($P = 0.008$), while in the winter, no significant differences were observed between females and males ($P = 0.93$) (Fig. 3(b)). The results suggest that, in the summer, the females flew with significantly higher mean speeds than both females and males tested in the winter (P -values of 0.001 and < 0.001 , respectively).

As for the remaining parameters, such as flight duration, distance travelled, and the number of flights (Gaussian distribution) they were only affected by sex, as no significant differences were observed for the season (Table 2). Females were found to fly longer durations, cover greater distances, and performed more flights than males.

3.2 Daily pattern

All the flight parameters studied were significantly affected by sex and period of day. However, the interaction between these two factors did not significantly impact the flight parameters (Table 3).

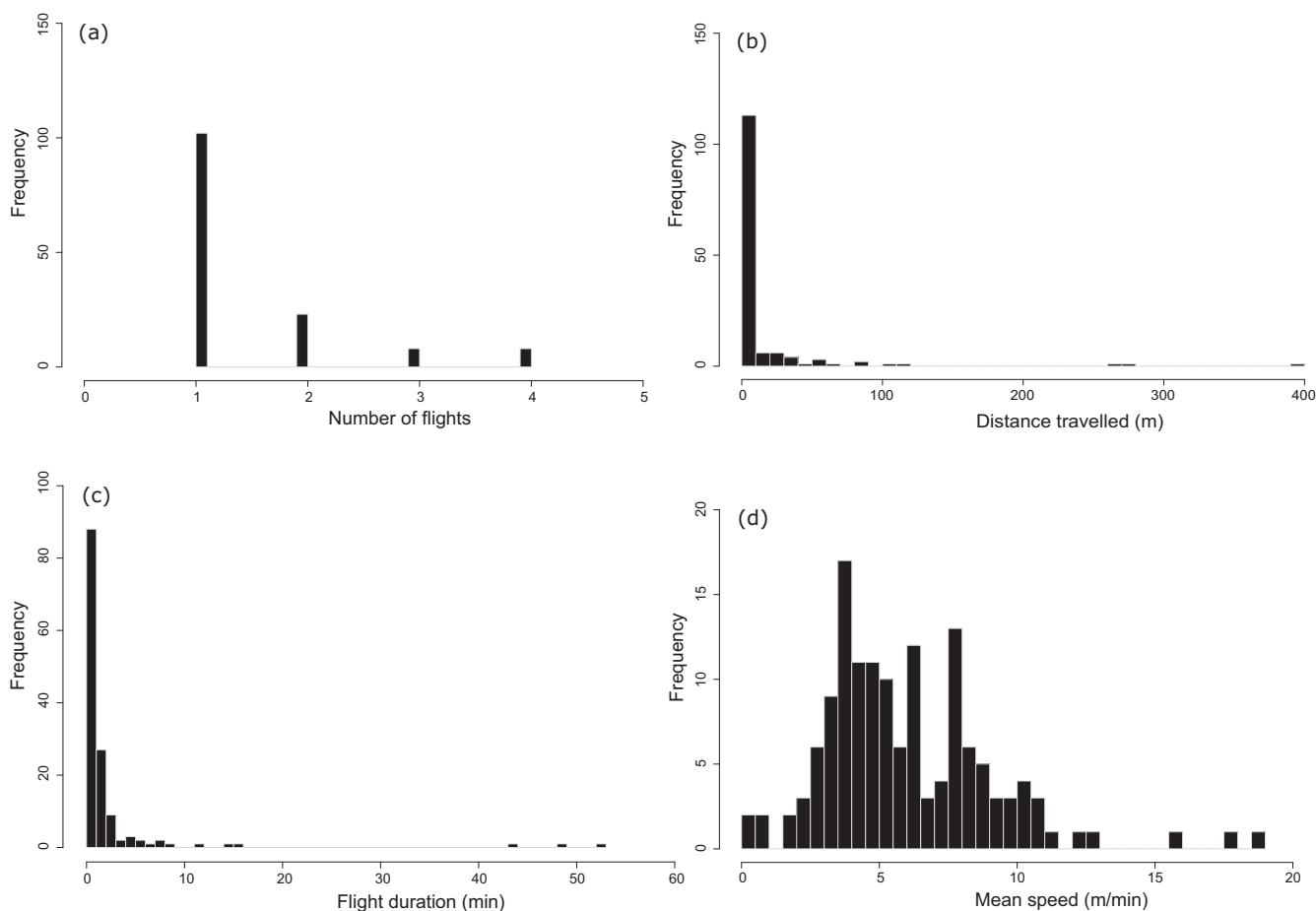


Figure 2. Frequency distribution of all the flight parameters studied in the flight activity of *Trioza erytrae*. (a) Number of flights performed; (b) Distance travelled (m); (c) Flight duration (min); (d) Mean speed (m/min).

Table 2. Results of the generalized linear model (GLM) developed for the effect sex, season as well as their interaction in the flight incidence and the results of the linear regression models (LMs) developed for the effect sex, season as well as their interaction on the flight duration, distance travelled, mean speed and number of flights

Independent variable	Response variable	df	F	P
Sex	Flight incidence	1	41.83	<0.01
Season		1	11.48	<0.01
Sex:Season		1	13.93	<0.01
Sex	Flight duration	1	0.61	0.01
Season		1	0.07	0.77
Sex:Season		1	0.05	0.52
Sex	Distance travelled	1	8.31	<0.01
Season		1	1.72	0.19
Sex:Season		1	3.20	0.07
Sex	Mean speed	1	3.27	0.07
Season		1	8.92	<0.01
Sex:Season		1	7.58	<0.01
Sex	Number of flights	1	5.55	0.02
Season		1	0.08	0.78
Sex:Season		1	1.32	0.25

Females displayed a considerably higher incidence of flight, covered greater distances, achieved higher speeds, had longer flight durations, and performed more flights compared to males, as illustrated in Fig. 4.

Regarding the time of day, flight incidence also showed variation, with a higher frequency in the morning (Fig. 5(a)). The distance travelled and the duration of flights were at their peak in the afternoon compared to the night time (Fig. 5(b),(d)). In terms of average speed, it was higher during the afternoon when compared to the morning (Fig. 5(c)); however, it did not differ from the speed recorded during the night (Fig. 5(c)). Thus, in summary flight activity of *T. erytrae* was higher in the afternoon than in the morning or night.

4 DISCUSSION

Predicting vector movement remains one of the foremost challenges in managing HLB. A fundamental aspect in assessing the risk of vector-borne pathogen dissemination is gaining insight into their dispersal capacity. This highlights the essential role of comprehending vector behavior in the effective management of the disease.

In the present study, for the first time, the flight capacity of *T. erytrae* was quantified by using a flight mill under controlled

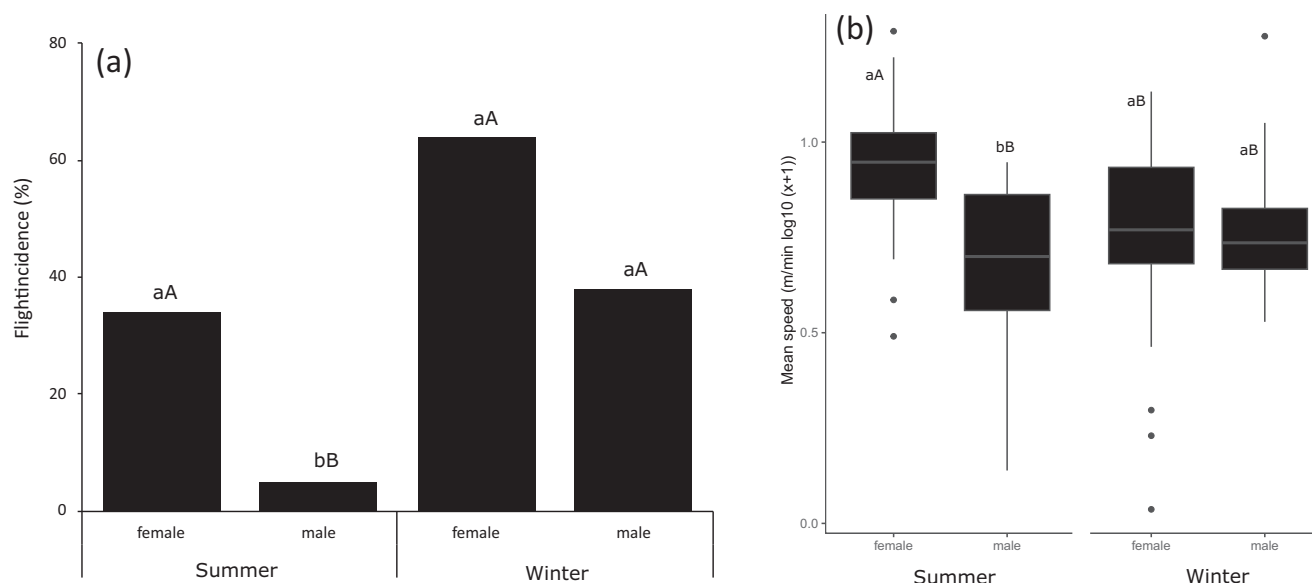


Figure 3. Season patterns in flight activity of *Trioza erytreae*: Comparisons between sexes (males and females) per season (summer and winter) (a) comparisons for flight incidence; (b) comparisons for mean speed (m/min); The capital letters refer to significant differences between the summer and winter, and the lowercase letters refer to significant differences between male as female. Groups accompanied by the same letter are not significantly different.

Table 3. Results of the generalized linear model (GLM) developed for the effect sex, period as well as their interaction in the flight incidence and the results of the linear regression models (LMs) developed for the effect sex, period as well as their interaction on the flight duration, distance travelled, mean speed and number of flights

Independent variable	Response variable	df	F	P
Sex	Flight incidence	1	43.66	<0.01
Period		2	37.40	<0.01
Sex:Period		2	1.13	0.5
Sex	Flight duration	1	2.71	<0.01
Period		2	1.53	0.05
Sex:Period		2	1.05	0.15
Sex	Distance travelled	1	2.71	<0.01
Period		2	1.53	0.05
Sex:Period		2	1.05	0.14
Sex	Mean speed	1	0.18	0.02
Period		2	0.38	<0.01
Sex:Period		2	0.04	0.56
Sex	Number of flights	2	0.09	0.03
Period		1	0.07	0.02
Sex:Period		2	0.06	0.11

laboratory conditions to determine how flight performance is affected by season, period of the day, and sex. Although flight mill devices do not fully replicate natural conditions and present limitations in terms of interpreting results and extrapolating them to real-world field scenarios, they can be used to make relevant comparisons. For example, flight mills can be valuable to make comparisons, between males and females, or between different temperatures,²⁸ and also indicate the flight potential under the most favorable conditions albeit in the absence of wind. They are also valuable tools to assess how plant pathogens may affect the dispersal ability of insect vectors. In fact, a similar flight mill

apparatus to the one we used in our study was tested by Martini *et al.*⁴³ to show the impact of CLAs on the flight ability of *D. citri*. They found that CLAs-infected psyllids were able to disperse more than non-infected psyllids. Additionally, the data obtained in studies using flight mills can be highly valuable in constructing dispersion models and offering insights that are not attainable through conventional field studies.^{37,44,45}

The results of our study indicate that, on average, *T. erytreae* can fly a distance of 16.20 m at a mean speed of 6 m/min. This suggests that this vector exhibits a lower dispersal capacity than the previously reported data for *Diaphorina citri*, the other confirmed vector of the HLB,^{28,35} or other psyllid species such as *Bactericera cockerelli* in flight mill experiments.²⁹ Martini *et al.* reported that, on average, *D. citri* can conduct long-distance flights, covering distances of up to 320 m, with a maximum observed distance of 2400 m.³⁵ Similarly, in another work conducted by Antolínez *et al.*²⁸ that tested the flight performance of *D. citri* under different temperatures, they observed that at a temperature of 26 °C, *D. citri* can fly a maximum distance of 2598 m. Furthermore, the flight ability of the potato psyllid (*Bactericera cockerelli*), which is the main vector of *Candidatus Liberibacter solanacearum* (CLSo), the causal agent of Zebra chip disease, was recently investigated by Antolínez *et al.*²⁹ They found that *B. cockerelli* can fly long-distances, flying an average distance of 185.33 m for CLSo-free insects and 122.99 m for insects infected with CLSo. The maximum flight capacity recorded in their study was 980, but the distance dispersed was not statistically different by the pathogen status of the vector. They also found that CLSo reduces the overall propensity to engage in long-distance flights.

Thus, it is clear that *T. erytreae* has less ability to fly long distances than other psyllid species reported, because the maximum distance we recorded in our study was only 395 m, a value well below than those reported for other psyllids.

Although our results suggest that *T. erytreae* has limited dispersal capacity compared to other psyllids, they also provide

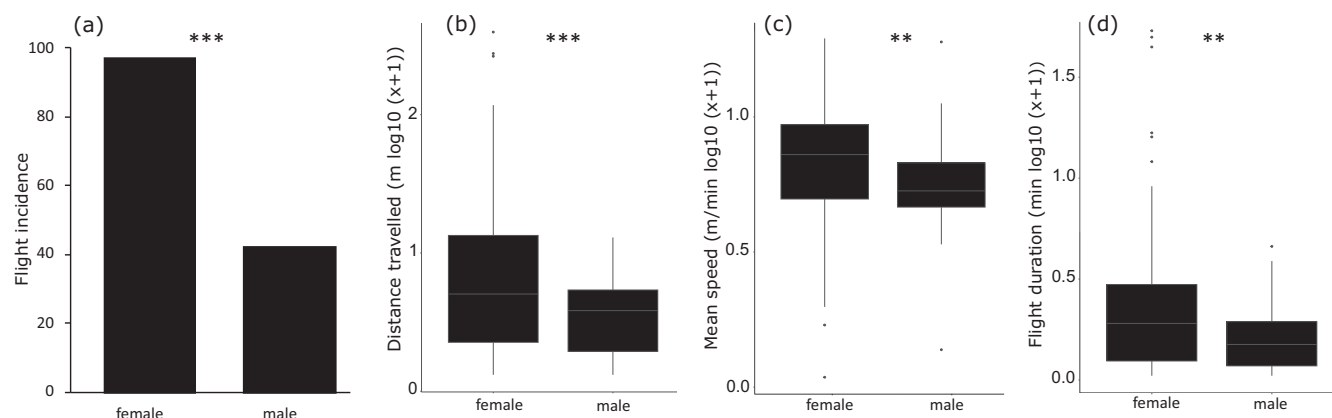


Figure 4. Comparisons between sexes (males and females) in the flight activity of *Trioza erytreae*: (a) flight incidence; (b) distance travelled (m); (c) mean speed (m/min); and (d) flight duration (min). The last three parameters were transformed by $(\log_{10}(x + 1))$, and the horizontal grey lines denote median values, boxes extend from the 25th to the 75th percentile of each group's distribution of values, and vertical extending lines denote the range of values. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

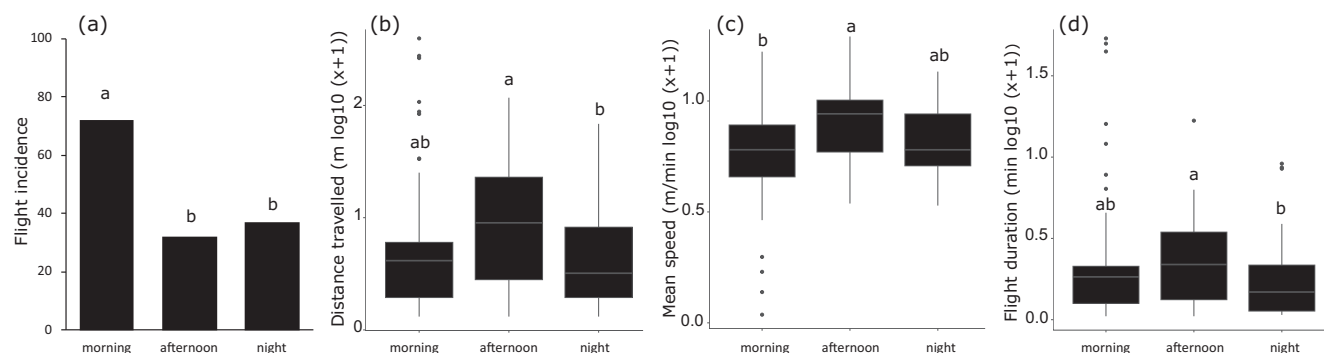


Figure 5. Daily patterns in flight activity of *Trioza erytreae*: Comparisons between different times of day (morning, afternoon and night) (a) Comparisons for Flight incidence; (b) Comparisons for distance travelled (m); (c) Comparisons for mean speed (m/min); and (d) Flight duration (min). The last three parameters were transformed by $(\log_{10}(x + 1))$, and the horizontal grey lines denote median values, boxes extend from the 25th to the 75th percentile of each group's distribution of values, and vertical extending lines denote the range of values.

evidence that some females of *T. erytreae*, can potentially disperse 395 m in a single flight without wind assistance. It is expected that in the presence of wind, the dispersal ability of this insect may be much greater. In fact, Greenslade *et al.*,²⁶ reported the presence of psyllids in the higher layers of the atmosphere, where they could be passively transported much farther by the wind, possibly even kilometers. Indeed, Benhadi-Marin *et al.*,⁴⁶ reported that more psyllids were flying from the northwest of Portugal, which coincides with the dominant wind direction in the study area; similar results were also reported by Van den Berg *et al.*²⁴ However, results of flight mill studies should be interpreted with caution because the flight performance of an insect tethered to a mill does not perfectly mirror its behavior when flying freely outdoors. Environmental factors, including variations in natural lighting conditions, the influence of sexual and feeding attractants, and other external stimuli, can significantly impact an insect's flight potential and behaviour.^{38,47} Additionally, it should be taken into consideration that flight mills can overestimate the distance covered by flying insects in natural conditions because it's unlikely for an insect to fly long distances in a straight line,⁴⁸ and due to inertia, it may possibly keep the insect in motion even if it actively stops for a rest.³⁶ Nonetheless, in epidemiological terms, if the

causal agent of HLB were to be introduced in Europe where the vectors are already present, the bacteria would quickly disseminate throughout citrus-producing regions. This is due to the fact that even a limited number of infective individuals flying more than 300 m can effectively transmit the pathogen from the infected source to distant locations.⁴⁹ The risk of HLB epidemics in Europe is high, not only because of the presence of *T. erytreae* in Portugal and Spain, but mainly because *D. citri* was detected in 2023 in Cyprus where there is more than 3000 ha of citrus.³

The season significantly impacted the flight incidence and mean speed of males and females of *T. erytreae*. During the summer, females exhibited considerably higher average speeds than males. In fact, the time of the year and weather conditions can strongly affect the behavior of several insect species due to changes in barometric pressure. These changes can affect the flight behavior of several insect species maintained under indoor conditions.⁵⁰ Furthermore, barometric pressure is known to affect the flight behavior of psyllids. Martini *et al.* found in an indoor flight mill experiment that the distance of flight of *D. citri* was reduced under decreasing barometric pressures.⁵¹ Thus, the results of our study suggest that the seasonal changes observed

in the flight behavior of *T. erytrae* are a consequence of the different weather conditions in winter and summer that were sensed by psyllids during the course of experiments. It is important to note here that in the roughly dozen studies that have compared flight speed from flight mills to free flight, most have shown lower velocities on flight mills.⁵² Thus, the flight speed of *T. erytrae* in our flight mill studies is probably lower than the real speed achieved under free flight. It is well-documented that temperature plays a crucial role in insects' survival, growth, and development, as well as the condition of flush shoots.^{53–57} Given that *T. erytrae* can complete its life cycle at elevated temperatures, such as 27 and 30 °C,⁵³ females may need to fly faster to locate host plants to lay their eggs in areas with temperatures more conducive to their development. Furthermore, females may be morphologically better adapted to sustain longer and faster flights than males as explained below. However, our experiments did not determine how temperature affects the flight performance of *T. erytrae*. Overall, the impact of temperature on the dispersal and flight performance of *T. erytrae* requires further investigation to understand the seasonal behavior better.

Regarding both seasonal and daily patterns of flight, females outperformed males in all the flight parameters assessed. These findings align with the research conducted by Van den Berg and Deacon,²⁵ where they reported that females possess a higher flight capacity, likely due to their need to locate plants with new shoots for oviposition. In contrast, males exert energy in finding females for mating. Furthermore, it's worth noting that females exhibit a larger body size and greater wing length than males. This physical advantage enables them to navigate the open field more effectively.⁵⁸ These findings point to the significant contribution of females in the population spatial spread and, consequently, the spread of the causal agent of HLB. Their enhanced flight capacity, possibly driven by the necessity to locate suitable plants for oviposition, could make them key vectors in disseminating this pathogen. Understanding the pivotal role of females in this context is essential for devising effective strategies to manage and control the spread of HLB.

Our results revealed that *T. erytrae* is able to fly during the night, which contradicts an earlier report by Van den Berg and Deacon in 1989.²⁵ This earlier study had noted a peak in flight activity just before sunset, suggesting the potential for night time flight, yet no actual night time flight activity was conclusively confirmed. Additionally, Sétamou *et al.* focusing on diurnal patterns and flight activity in the field of *D. citri* found that very few adults were captured on yellow sticky cards at night.⁵⁹ This observation implies that psyllids have a reduced nocturnal movement and raises the possibility of these insects struggling to locate host plants during nighttime hours as they are attracted to shortwave lengths for take-off and visible light for landing. In this context, the nighttime flight activity observed in our study seems to be prompted by the insects' stress under our experimental conditions, leading to flights during nighttime hours. Moreover, observing flight activity during sunset and sunrise suggests that this behavior may be part of a survival strategy.²⁵ Nevertheless, further research is imperative to comprehensively understand these insects' nocturnal behaviors in natural field conditions.

Trioza erytrae can cover considerably greater distances with longer flights during the afternoon compared to the nighttime, which aligns with prior research findings.⁶⁰ Understanding their flight patterns is crucial for developing effective pest management strategies, especially in managing diseases like HLB. The timing and flight performance play a pivotal role in transmitting

vector-borne pathogens, underscoring the need for thorough research to address this challenge.

In conclusion, this study sheds light on the flight capabilities of *T. erytrae*, a vector of significant concern in the management of HLB. It is important to highlight that the flight activity and ability to fly long distance of *T. erytrae* is lower than that of *D. citri*, the other psyllid vector species of HLB. Therefore, theoretically, *D. citri* would have a higher dispersal ability of HLB than *T. erytrae* under the same environmental conditions. Our research also demonstrates that these insects can have some nocturnal flights, which challenges previous assumptions and provides valuable insights into their behavior. Additionally, the findings emphasize the importance of females in population dispersion and the potential transmission of the HLB-causing agent. The impact of season and daily patterns on flight performance further underscores the complexity of vector behavior and its implications for disease management. While our study reveals some limitations of flight mill experiments, it highlights the need for further research to understand these insects' behavior under natural field conditions comprehensively. Understanding their flight patterns is crucial for developing effective pest management strategies, particularly in the context of HLB control.

AUTHOR CONTRIBUTIONS

JAP and AF conceived and designed research. IR and DFO conducted experiments and statistical analysis. RPO rearing the insects. IR wrote the first draft of the manuscript. All authors read and approved the manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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