Received: 24 November 2020 | Revised: 15 January 2021 | Accepted: 25 January 2021

DOI: 10.1111/jen.12870

ORIGINAL CONTRIBUTION

JOURNAL OF APPLIED ENTOMOLOGY WILEY

Sampling and distribution pattern of *Trioza erytreae* Del Guercio, 1918 (Hemiptera: Triozidae) in citrus orchard

Jacinto Benhadi-Marín[1] | **Luís F. Pereira**[1] | **Isabel Rodrigues**[1] | **Alberto Fereres**[2] | **José Alberto Pereira**[1]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal

[2]Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, ICA-CSIC, Madrid, Spain

Correspondence
José Alberto Pereira, Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.
Email: jpereira@ipb.pt

Funding information
Fundação para a Ciência e a Tecnologia, Grant/Award Number: UIDB/00690/2020; project PRE-HLB-Preventing HLB epidemics for ensuring citrus survival in Europe, Grant/Award Number: H2020-SFS-2018-2 Topic SFS-05-2018-2019-2020, proj

Abstract

Developing efficient sampling protocols is essential to monitor crop pests. One vector of the citrus disease HLB, the African citrus psyllid *Trioza erytreae* Del Guercio, 1918 (Hemiptera: Triozidae), currently threatens the lemon industry throughout the Mediterranean region. In this work, a pool of sampling methods devoted to monitoring the population of *T. erytreae* was compared, its spatial distribution in the orchard was assessed, and the minimum sampling effort for the best sampling method was estimated. Three lemon orchards in North-western Portugal were sampled for one year using two types of yellow sticky traps (standard yellow and fluorescent Saturn yellow), B-vac sampling and sweep net sampling. The method that best performed, in terms of cost-efficiency, was the yellow sticky traps. The two colours of the sticky traps tested did not yield a significantly different number of catches. The spatial distribution throughout the orchards was found to be aggregated towards the borders. A minimum of three sticky traps per hectare was found to be enough to estimate the population at 90% accuracy for the mean during the outbreak. These results should help to monitor and anticipate outbreaks that may even colonize neighbour orchards. Studies on the local dispersion patterns of *T. erytreae* throughout the orchard are mandatory to further refine and optimize efficient monitoring protocols.

KEYWORDS

African citrus psyllid, aggregation, HLB, minimum sampling effort, sticky trap, yellow traps

1 | INTRODUCTION

The African citrus psyllid *Trioza erytreae* Del Guercio, 1918 (Hemiptera: Triozidae) is an important citrus pest since it is a vector of the phloem-limited bacteria *Candidatus* Liberibacter spp., the causal agents of huanglongbing (HLB) or 'Citrus greening disease', the most devastating disease of citrus plants (de Graça, 1991).

Most likely native from south-eastern Africa (Cocuzza et al., 2016), *T. erytreae* was found for the first time in Europe in the islands of Madeira (Portugal) in 1994 (Carvalho & Aguiar, 1997) and the Canary Islands (Spain) in 2002 (González-Hernández, 2003). More than a decade later, it was reported at mainland (north-western) Spain and mainland (north-western) Portugal in 2014 (Pérez-Otero et al., 2015). Since then, *T. erytreae* has been continuously spreading though the north coast regions of Spain and central and southern areas in Portugal (DGAV, 2016–2020) representing a serious threat for the citrus industry throughout the Iberian Peninsula. At the present time, it has reached the Basque Country, very few kilometres away from the French border (EPPO, 2020).

Due to the alarm raised among citrus growers in Europe, the development of monitoring schemes and the definition of fast and efficient sampling protocols for *T. erytreae* is mandatory. Chromotropic sticky traps have been suggested for a long time to detect reservoirs of adults of *T. erytreae*, because of their broad usefulness

(Aubert, 1987; Samways, 1987a). Advantages of chromotropic sticky traps include minimal colour deterioration, weatherproof properties, a relative cleanliness if replaced weekly, and ease of use (Samways, 1987a). Moreover, since *T. erytreae* is positively phototaxic responding optimally to a wavelength of 500–550 nanometres (i.e., yellow-green), specific traps, such as the so-called fluorescent Saturn sticky yellow traps, have been commonly used to sample adults (Urban, 1977) In Florida and Texas (EEUU) yellow sticky card traps yielded significantly higher captures of *Diaphorina citri* Kuwayama, 1908, the Asian citrus psyllid, than blue sticky card traps over a 4-week period in 'Temple' orange trees (Hall et al., 2007). *D. citri* is the main vector of *Ca. Liberibacter asiaticus* in America and Asia (Bové, 2006).

Suction methods have been widely used to sample arthropods (Sunderland et al., 1995). Among them, one of the most popular is the Dietrick vacuum insect net (D-vac), consisting of a backpack motor fan for suction sampling of insect populations (Dietrick, 1961). D-vac sampling has been used to compare the actual number of winged adults collected on canopies by aspiration to the sequential catches of *D. citri* on yellow sticky traps (Aubert & Quilici, 1988). However, D-vac devices are relatively heavy and hard to handle, thus cheaper and easier to handle modified 'blower-vacs' based on inverted leaf blowers raised as alternative for suction sampling (Zou et al., 2016).

Sweep net sampling has been also used to develop fixed-precision sequential sampling plans for density estimation (e.g., O'Rourke et al., 1998) and to monitor the seasonal abundance of psyllids in different agroecosystems such as carrots, potato, or celery (e.g., Antolínez et al., 2019), as well as *D. citri* in Florida's citrus groves (e.g., Arevalo et al., 2011). Moreover, for the latter species, presence–absence sampling plans based on in situ counts of adults, nymphs and eggs have been developed to provide an estimation of its population density in citrus orchards (Sétamou et al., 2008).

Since eradication of *T. erytreae* is very difficult once introduced and spread, the scenarios where *T. erytreae* and HLB are simultaneously present or not is crucial for planning control strategies (Cocuzza et al., 2016). Moreover, despite the amount of suitable methods used to monitor psyllids, a simple monitoring scheme simultaneously based on the comparison of several methods, the minimum sampling effort and the study of the spatial distribution of *T. erytreae* within a citrus grove is still lacking. Thus, the objectives of this work were as follows: (a) to test and compare different sampling methods for monitoring the population of adults of *T. erytreae* in North-western Portugal on citrus orchards, (b) to assess the spatial distribution of the population in the studied orchards and (c) to estimate the minimum sampling effort to have a good estimate of population density.

2 | MATERIAL AND METHODS

2.1 | Study area

The study area encompassed three *Citrus limon* (L.) (variety 'Lunario') farms in Vairão, Porto (North-western Portugal). The first experimental area was part of an experimental farm located at the Agrarian Campus of Vairão (University of Porto) (41°19'38.5"N 8°40'32.4"W) of 0.14 ha. The experimental farm houses other species such as tangerine, bitter orange and other fruit plants. The two other experimental areas were established in two adjacent groves located near Caracoi (Vairão) hereafter referred as Caracoi 1 and Caracoi 2, respectively. Caracoi 1 (41°18'46.4"N 8°38'09.7"W) is a commercial grove of 1.35 ha. surrounded by a dense fringe of eucalyptus trees (*Eucalyptus globulus* Labill., 1,800), Caracoi 2 (41°18'45.6"N 8°38'17.3"W) is an adjacent commercial grove of 0.98 ha mostly surrounded by roads and some dispersed eucalyptus trees. The three orchards present a flat topography and an average altitude of 80, 160 and 170 m.a.s.l, respectively. Age of lemon trees is more than 20 years and the average distance of trees are 6 m (between rows) and 2 m (between trees). During the sampling period, the soil was not ploughed, not irrigated, no phytosanitary treatments were applied except a single foliar application of copper sulphate.

2.2 | Sampling design

The population of *T. erytreae* adults was monitored at each orchard using different sampling methods for comparison: (1) Standard chromotropic yellow sticky traps, (2) fluorescent chromotropic Saturn yellow sticky traps, (3) a modified leaf blower-vac (hereafter B-vac) and (4) sweep net. Methods (1) and (2) were used in the three sampling areas, whereas methods (3) and (4) were used in Caracoi 1 and Caracoi 2. Both types of yellow sticky traps were manufactured by Sanidad Agrícola Econex, S.L. (Siscar, Murcia, Spain). Sampling was conducted from March 2019 to April 2020 on a weekly basis for the case of the sticky traps and on a fortnightly basis on the case of B-vac and sweep net sampling.

A total of 10 sticky traps (5 standard and 5 fluorescent Saturn) each one with a sticky area of 25 × 20 cm were installed in each experimental farm. The traps were spaced 36 m away each from another and placed at 1.75 m above ground (Figure S1). The number of *T. erytreae* adults captured was counted on both sides of the traps. Traps were aligned with the plantation row so that each side faced two consecutive inter-rows. Side 'A' was north-west oriented, whereas side 'B' was south-east oriented. Weekly, the traps were transported to the laboratory, the individuals were counted, and the traps were cleaned, re-glued and replaced in the field.

In addition to the sticky traps, B-vac and sweep net sampling was conducted in Caracoi 1 and Caracoi 2 experimental farms. Each sampling was conducted on a fortnightly basis at 10 randomly selected trees per grove. Each B-vac suction sample lasted 30 s, and two samples were collected in each tree canopy by aspiration of tree shoots at the opposite side of each tree canopy (sides A and B). The B-vac followed the design of Zou et al. (2016) with a nozzle of 3.6 cm in diameter and an air intake rate of 27 m/s.

The sweeping sampling was conducted using an entomological sweep net (45 cm in diameter) at the canopy level in 10 randomly selected trees per grove. Each sample consisted of four sweeps around the tree. The individuals collected using the B-vac and sweep net were transported to laboratory and counted.

2.3 | Data analysis

2.3.1 | Efficiency of sampling methods

Data analysis was conducted in R (R Core Team, 2018). The effect of the type of yellow trap (Standard and fluorescent Saturn), the trap side (A and B) and the trap position throughout the grove on the number of captured adults of *T. erytreae* was analysed by developing a generalized linear mixed model (GLMM) using the abundance data as response, whereas type of trap, side and position was used as drivers following Zuur et al. (2009). Farm was considered as a random term and since the response corresponded to count data, a Poisson distribution was used. Statistical significance among levels of factors was assessed using a post hoc Tukey test ($\alpha = 0.05$).

2.3.2 | Spatial distribution of T. erytreae and minimum sampling effort

The mean ($\overline{X}$) and variance (S^2) of captured individuals using the yellow sticky traps were calculated for 26 sampling dates from 02/06/2019 to 02/12/2019 using the data of the three experimental farms. The adjustment of observed and expected frequencies of captured individuals was tested for the negative binomial distribution ($\alpha = 0.01$) (Waters, 1959) using the *fitdist* and *gofstat* functions of the *fitdistrplus* package (Delignette-Muller & Dutang, 2015). Then, the spatial distribution of *T. erytreae* was assessed using a pool of aggregation indexes. The k parameter of the negative binomial distribution was calculated as:

$$k = \overline{X}^2 / S^2 - \overline{X} \quad (1)$$

where $\overline{X}$ is the sample mean, and S^2 is the sample variance. The k parameter of the negative binomial distribution is an indicator of aggregation so that k tends to zero for an aggregated distribution whereas k tends to infinity in the case of random distribution (Waters, 1959). Iwao´s patchiness regression method (Iwao, 1968) quantifies the relationship between the mean crowding index of Lloyd (m^*) and the mean ($\overline{X}$) as:

$$m^* = \alpha + \beta \overline{X} \quad (2)$$

where α and β are Iwao´s parameters, and m^* is the Lloyd index (Lloyd, 1967) calculated as:

$$m^* = \overline{X} + (S^2/\overline{X} - 1) \quad (3)$$

where $\overline{X}$ is the mean, and S^2 is the variance. In Equation 2, a β parameter equals to 1 indicates a random distribution, whereas $\beta > 1$ and indicates an aggregated distribution, and $0 < \beta < 1$ a uniform distribution. Lloyd's index of patchiness was calculated using the function *agg_index* of *epiphy* package (Gigot, 2018), respectively.

Taylor´s law (Taylor & Woiwod, 1980) describes the regression between logarithm of the variance and the logarithm of the mean as:

$$\mathrm{Log}\left(S^2\right) = \mathrm{Log}\,\alpha + \beta\,\mathrm{Log}\,(\overline{X}) \quad (4)$$

where α is the intercept, β is the slope of the regression line, S^2 is the variance, and $\overline{X}$ is the mean. $\beta < 1$, $\beta = 1$, and $\beta > 1$ indicate uniform, random, and aggregated distribution respectively.

The extent at which β deviates from 1 was assessed using a *t* test.

Finally, the minimum number of samples necessary for estimating the mean at a given precision was calculated by solving Green's model (Green, 1970) as:

$$N = (\alpha + \overline{X}^{\beta - 2}) / D^2 \quad (5)$$

where N is the number of required samples, α and β are the parameters of Taylor´s model, and D is the precision level. D = 0.1 (90%) was used as a standard for the fixed level of precision (Waters et al., 2014). Since each side of the sticky traps was considered a sample, the estimated number of traps was divided by two. Since all the traps were used to calculate the required number of samples, the estimate was divided by the total area of the farms to provide the minimum number of traps per hectare.

3 | RESULTS

The population dynamics of *T. erytreae* showed one main peak density at the beginning of August in the 3 orchards surveyed according to the yellow trap catches. The population started to drop gradually in September reaching very low densities in October and remained close to zero until the beginning of April (Figure 1). The peak density according to the B-vac sampling (Figure 2A) and sweep net sampling (Figure 2B) was mid-July; however, the latter methods provided in average a lower mean number of captures compared to the yellow sticky traps (Figures 1 and 2). In addition, the number of captures was quite similar in Caracoi 1 and Caracoi 2, using the sticky traps method (Figure 1B,C). Nevertheless, the population in Caracoi 2 was much lower especially during the population outbreak in July, compared with Caracoi 1, according to B-vac and sweep net captures (Figure 2A,B)..

Side 'A' (the one facing north-west) of the yellow sticky traps (either standard or fluorescent Saturn) captured a statistically significantly higher number of adult individuals than the opposite side 'B' ($\chi^2 = 548.47$; $df = 1$; $p < .001$) (Figure 3A). The trap position within the grove (one to five representing the trap location throughout the orchard from one edge to the opposite) had a significant effect on the number of captured individuals ($\chi^2 = 8{,}422.33$; $df = 4$; $p < .001$), being the border traps across the orchard (i.e., position one and five) the most successful (Figure 3B). On the contrary, the trap colour

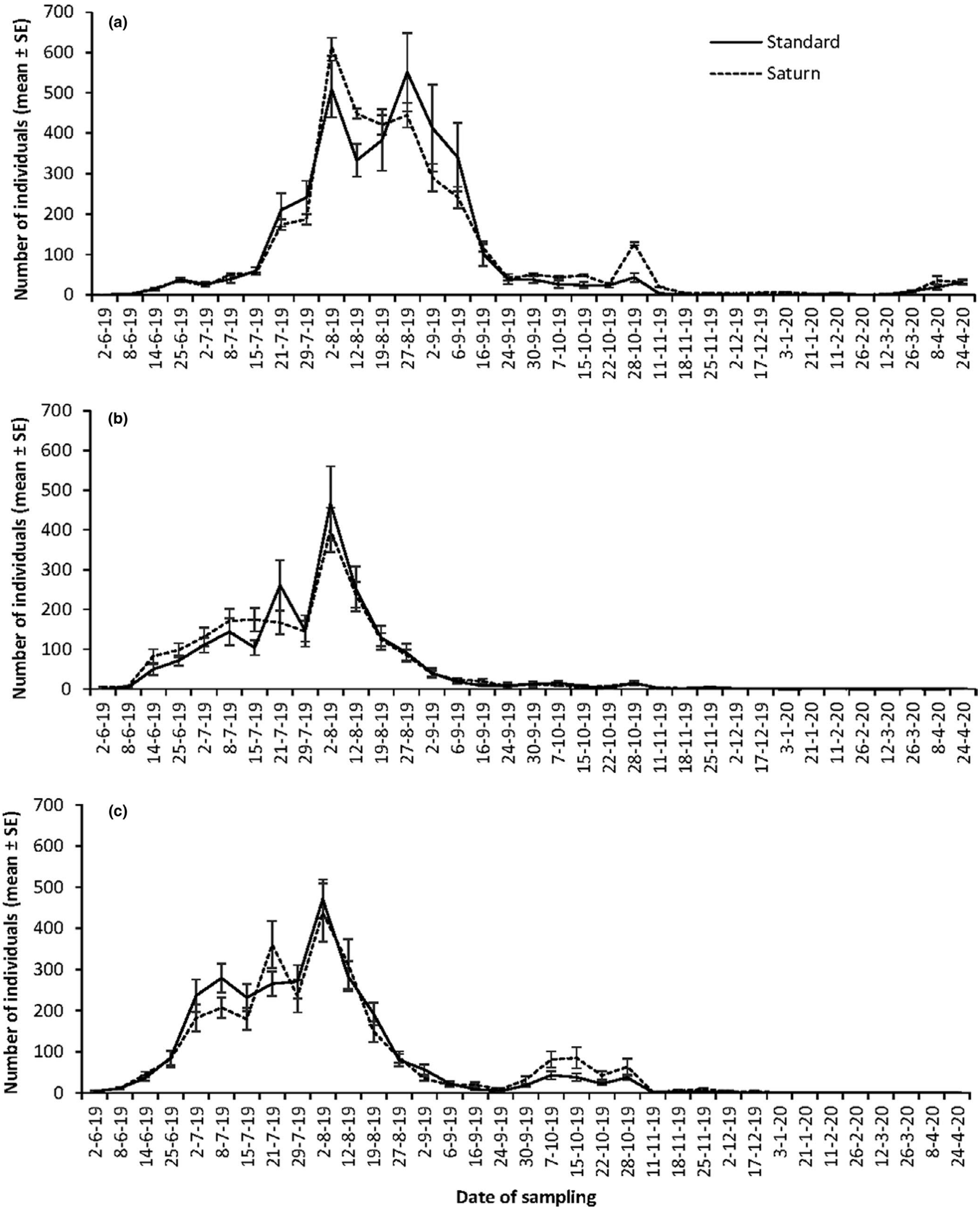

FIGURE 1 Mean number (±SE) of adults of *Trioza erytreae* captured using standard yellow sticky traps and Saturn yellow sticky traps in Vairão (A), Caracoi 1 (B) and Caracoi 2 (C)

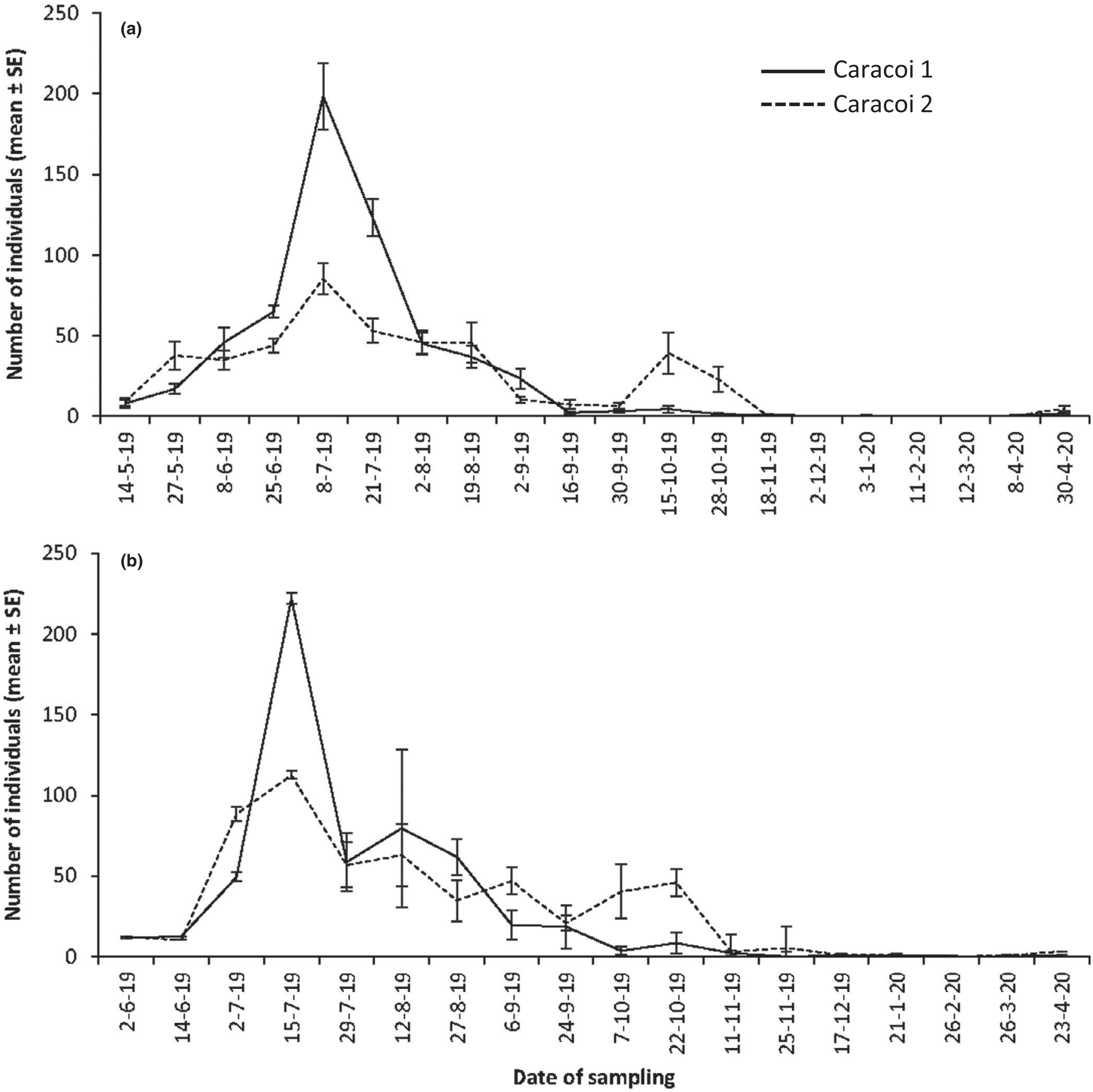

FIGURE 2 Mean number (±SE) of adults of *Trioza erytreae* captured by B-vac (A) and sweeping (B) in Caracoi 1 and Caracoi 2

(standard or fluorescent Saturn) did not significantly affect the number of captured individuals ($\chi^2 = 0.98$; $df = 1$; $p = .32$) (Figure 3C).

The data fitted a negative binomial distribution according to the goodness of fit test and Iwao´s patchiness regression method (Table 1) (Figure 4). The different methods used to assess the spatial pattern highlighted a similar extent of distribution; the parameters of the Iwao regression were $\alpha = 43.51$ and $\beta = 1.37$ ($R^2 = 0.72$; $t = 10.36$; $p < .001$), indicating an aggregated distribution; the low values of the k parameter of the negative binomial distribution indicated an aggregated distribution (Table 1), and Lloyd's index of patchiness was higher than 1 for each sampling date. The estimated parameters of Taylor´s law were $\alpha = 0.42$ and $\beta = 1.75$ ($R^2 = 0.96$; $t = 25.34$; $p < .001$) (Figure 5). Despite the trap type, the average minimum sampling effort during the outbreak (8/07/2019 to 24/09/2019) estimated for a precision level (D) of 0.1 according to Green's model was three sticky traps per hectare (i.e., the six sides of each trap) corresponding with the maximum of abundance of *T. erytreae* adults (Table 1).

4 | DISCUSSION

In this work, different sampling methods were used to monitor the population of adult individuals of *T. erytreae* in three citrus orchards

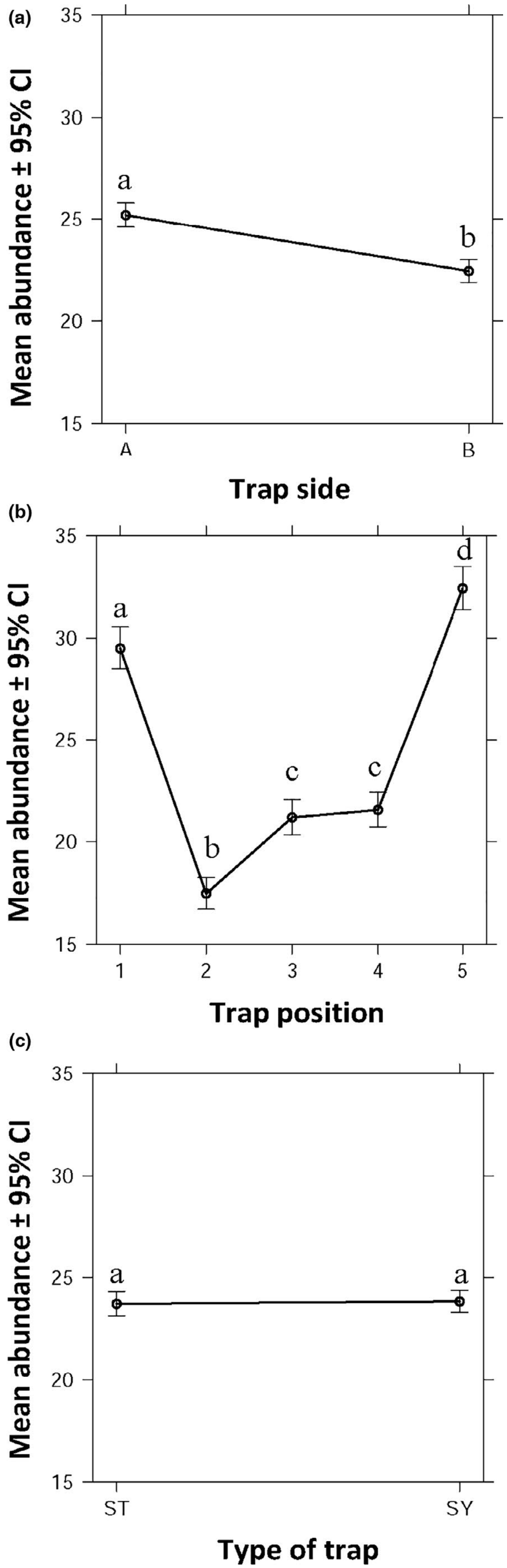

FIGURE 3 Effect of trap side (A), trap position (B) and type of trap (C) on the number of captured adults of *Trioza erytreae*. Different letters above bars indicate significant differences. ST: Standard yellow sticky trap; SY: Fluorescent Saturn yellow sticky trap

in North-western Portugal. We found clear population peaks in the first week of August using yellow sticky traps, whereas the B-vac and sweep net methods registered the maximum captures in mid-July. This difference may be due to flight activity-related factors, such as age and sex (see Van den Berg & Deacon, 1989). The sweep net and B-vac sampling could be collecting insects settled and feeding on the trees, while the sticky traps were detecting insect movement. This could reflect that there was movement of insects flying around the trees in early August, whereas in July insects may be settled on the trees and do not need to move around searching for new young sprouts (flushing). This could explain the lag between the abundance peaks when using different sampling methods; however, a detailed study is needed focusing on this purpose.

Different methods have been also used for monitoring *D. citri* such as chromotropic sticky traps with different colours (yellow, different shades of green, red, white and blue), sweep net sampling, suction devices, visual inspection and stem tap sampling (Miranda et al., 2018; Sétamou et al., 2014). In general, our results agree with a series of works in which those different methods were compared for *D. citri*. For example, Miranda et al. (2018) found that although all the sampling methods detected *D. citri* in untreated orange orchards in Brazil during short-term experiments, only the yellow sticky traps were able to do so in orchards where insecticide was applied.

Although the fluorescent Saturn yellow traps have been suggested as optimal to attract a range of homopterans such as *T. erytreae* (Aubert, 1987; Aubert & Quilici, 1988), their usefulness in this work for creating a continuous record of density of winged individuals did not significantly differ from standard and cheaper yellow sticky traps. Our results agree with those of Samways (1987a), in which the fluorescent Saturn yellow attracting efficiency was significantly higher than other models of trap such as green paper and yellow flexi pipe traps, but it did not significantly differ from other yellow traps. Therefore, our results suggest that standard yellow sticky traps should be recommended to sample *T. erytreae* populations because they are cheaper and easier to manufacture than the Saturn yellow sticky traps.

The yellow sticky traps clearly revealed an accumulation of individuals at opposite sides of the orchards. Wind direction may play a role in clumping high numbers of *T. erytreae* (Van den Berg et al., 1991). However, the presence of a wall along the plantation row in Vairão, and the surrounding large eucalyptus trees and a wall at Caracoi 1 and Caracoi 2 acting as windbreaks, suggests that wind direction may not be a factor to be considered in our analysis. On the other hand, local abiotic factors such as light, temperature and movement patterns and mainly because of differences in flushing within the orchard may explain the significantly higher number of

TABLE 1 Mean ($\bar{X}$) and variance (S^2) of the captured adults of *Trioza erytreae* during the period considered for the study of the spatial distribution, goodness of fit test for the negative binomial distribution (χ^2; P), k parameter of the negative binomial distribution (k), Lloyd's index of patchiness (Lloyd), mean crowding index (m*) and estimated minimum number of sticky traps per hectare for a 90% level of accuracy (D = 0.1). ^{op} indicates the outbreak period

Date	$\bar{X}$	S^2	χ^2	P	k	Lloyd	m*	*n* min (D = 0.1)
02/06/2019	2.48	9.91	-	-	0.83	2.21	5.48	7.18
08/06/2019	6.08	28.69	-	-	1.64	1.61	9.80	5.74
14/06/2019	40.25	1,500.90	0.87	1.00	1.11	1.90	76.54	3.58
25/06/2019	68.15	2,408.37	0.10	1.00	1.98	1.50	102.49	3.14
02/07/2019	118.08	11,447.09	9.72	0.21	1.23	1.81	214.02	2.73
08/07/2019	148.33	13,415.31	13.41	0.06	1.66	1.60	237.77	2.58^{op}
15/07/2019	133.70	9,148.04	3.44	0.84	1.98	1.50	201.12	2.65^{op}
21/07/2019	239.60	22,913.50	10.21	0.18	2.53	1.39	334.23	2.29^{op}
29/07/2019	204.02	17,948.25	5.91	0.55	2.35	1.43	290.99	2.38^{op}
02/08/2019	483.42	60,984.11	6.29	0.51	3.86	1.26	608.57	1.92^{op}
12/08/2019	311.05	24,435.57	8.38	0.30	4.01	1.25	388.61	2.15^{op}
19/08/2019	232.92	33,881.06	10.94	0.14	1.61	1.62	377.38	2.31^{op}
27/08/2019	223.10	65,071.38	15.42	0.03	0.77	2.30	513.77	2.33^{op}
02/09/2019	145.77	46,885.98	11.11	0.13	0.45	3.20	466.42	2.59^{op}
06/09/2019	110.72	30,554.85	16.27	0.02	0.40	3.48	385.69	2.78^{op}
16/09/2019	45.88	4,719.19	3.25	0.86	0.45	3.22	147.74	3.46^{op}
24/09/2019	17.83	637.73	0.40	1.00	0.51	2.95	52.59	4.39^{op}
30/09/2019	27.00	850.47	0.12	1.00	0.89	2.13	57.50	3.95
07/10/2019	36.12	1861.46	1.65	0.98	0.71	2.40	86.66	3.68
15/10/2019	34.45	2,295.68	1.17	0.99	0.52	2.91	100.09	3.72
22/10/2019	20.80	498.20	-	-	0.91	2.10	43.75	4.22
28/10/2019	49.90	2,603.55	0.35	1.00	0.98	2.03	101.08	3.39
11/11/2019	4.75	71.95	-	-	0.34	3.98	18.90	6.11
18/11/2019	2.72	12.44	-	-	0.76	2.32	6.30	7.02
25/11/2019	4.15	24.84	-	-	0.83	2.20	9.14	6.31
02/12/2019	2.00	6.44	-	-	0.90	2.11	4.22	7.58

individuals captured on the north-west oriented side of the traps (Samways, 1987b; Samways & Manicom, 1983; Van den Berg, Deacon, et al., 1991).

The efficiency of vacuum devices have been tested for detection and census purposes of the Asian citrus psyllid, *D. citri*, resulting in different levels of efficiency according to the model (e.g., electronic or gas powered device) (Thomas, 2012). In this work, the low efficiency of the gasoline powered B-vac used to sample adults of *T. erytreae* compared to sticky traps was probably due to the behavioural response of the insects to disturbance. According to Catling (1978), adults of *T. erytreae* show a strong jump and fly behaviour when disturbed. A fast escape response could make difficult for the B-vac to collect individuals since shoots are disturbed with the nozzle during sampling. This fact together with the small diameter of the nozzle coupled to the suction device could explain the low efficiency of this method for *T. erytreae* in the canopy despite the intake air velocity.

Sweep net sampling is an inexpensive method useful when presence/absence data are required and can provide a good estimate of total abundance (Kent et al., 2019). In our work, sweeping seemed to perform worse than sticky traps. However, direct comparisons are difficult to make since sticky traps captured insects per area unit within a week (based on passive trapping due to colour-based attraction of the insects) whereas the sweep net and B-Vac reflect the number of insects per sample a single catch estimate per week. Despite this, differences could be related to carrying out this method on a non-optimal target stratum since sweeping tend to be used away of large branches (Kent et al., 2019) such as those sampled in the citrus trees. In fact, thorns of citrus trees can break the nets when sweeping and adults can jump and fly when branches are disturbed. Interestingly, Cooper et al. (2012) argued that sweep netting usually takes a long sampling time in the field and sample processing time thus being less efficient than other methods, whereas Morente at el. (2018) found that sweep netting was the most effective and least time-consuming

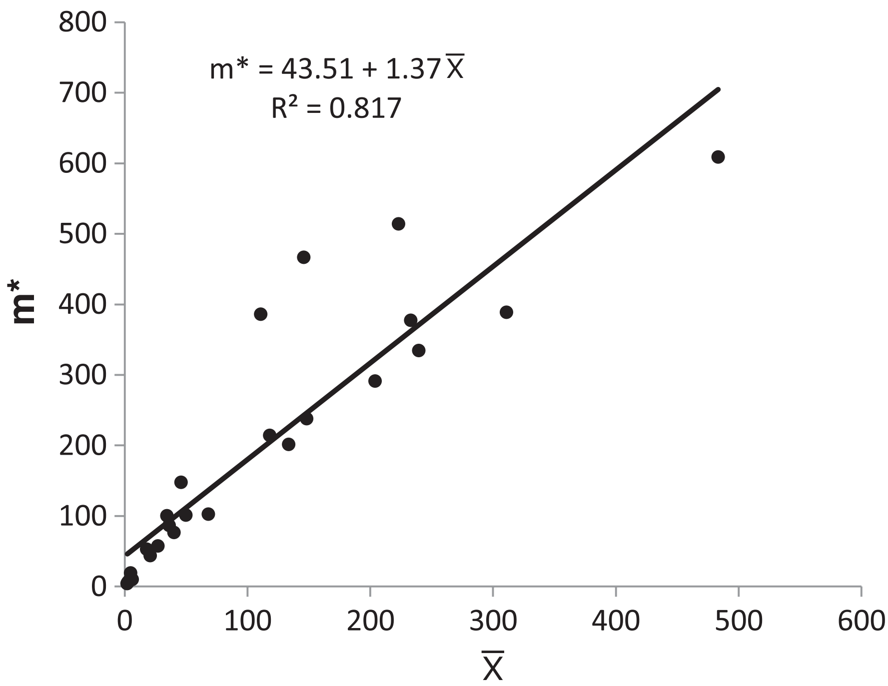

FIGURE 4 Relationship between the mean crowding index of Lloyd (m*) and the mean (X̄) of adults captured using sticky traps following Iwao´s patchiness regression method for the abundance data of *Trioza erytreae*

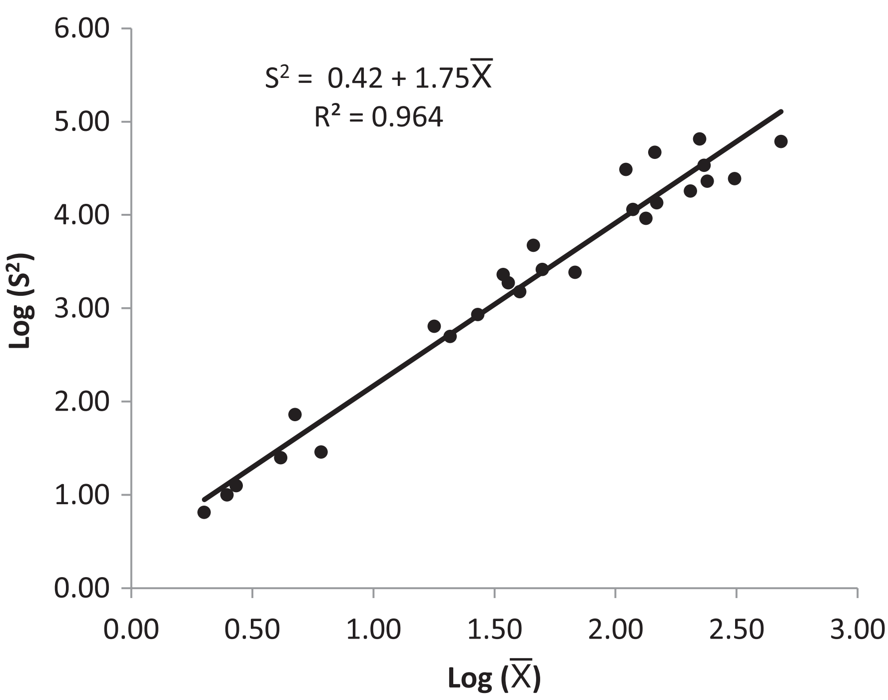

FIGURE 5 Regression between the variance (S^2) and the mean (X̄) of adults of *Trioza erytreae* captured using sticky traps according to Taylor´s law

sampling method among those tested for spittlebugs both in the ground cover and olive tree canopies in Portugal and Spain.

It is worth to mention that beyond the sampling methods used in this study, other possibilities such as Malaise traps could be used as an alternative or supplement to the sticky traps in descriptive studies (see Thanou et al., 2020; Tsagkarakis et al., 2018) such as absence/presence of psyllids or in migration studies. Besides, this type of trap collects the individuals in containers with ethanol preserving the material for DNA studies.

The significant effect of sticky trap position within the orchard on the number of captured individuals could reveal a border effect. Moreover, the larger number of captures on side A than side B suggests that trap orientation is very important. Our results suggest that there were more psyllids flying from the north-west than from the south-east. This could be explained by the dominant wind direction in the study area that coincides with this flying pattern (personal observation). Moreover, the k parameter of the negative binomial distribution, Lloyd's index of patchiness, the parameters of Iwao´s method and Taylor´s law indicated an aggregated spatial distribution of individuals. The relationship of the mean crowding and the mean (i.e., Iwao´s patchiness regression) found in this work suggests compact colonies and a process of contagion (Southwood & Henderson, 2000). The parameter β of Taylor´s law is specific of the species and independent of local environment variables (Taylor & Woiwod, 1980). The value estimated through the regression of the variance on the mean (β = 1.75), although slightly higher, agreed to the β reported by Samways and Manicom (1983) for *T. erytreae* (β = 1.69) which suggest an increase in contagion with time.

The pattern of aggregation has been already reported for *T. erytreae* (Samways & Manicom, 1983; Van den Berg, Deacon, et al., 1991) and even for other psyllids such as the apple psylla, *Cacopsylla mali* (Schmidberger, 1836) (Tret'yakov, 1984). Samways and Manicom (1983) found an exponential increase in clumping of adults of *T. erytreae* in the border row of plantation due to adjacent infested orchards. In our study, taking Caracoi 1 as example, the orchard is adjacent to Caracoi 2 which may act as source of flying adults and could explain the pattern observed. Moreover, on the opposite side of the orchard (no more citrus orchards nearby), the accumulation of individuals could be due to a physical barrier (Samways & Manicom, 1983), such as the dense border of large eucalyptus trees that surrounds Caracoi 1. However, in order to further investigate the border effect, larger farms with higher number of traps in the interior part of them would be necessary, in order to conclude if the number of catches is significantly higher in the border than in central areas.

In conclusion, according to our results, the standard yellow sticky traps were the most efficient, low-effort and cheapest sampling method for monitoring *T. erytreae*, in lemon orchards. Only three traps per hectare during the outbreak, two of them located at the opposite borders of the orchard before an outbreak of *T. erytreae* would achieve an accuracy of 90% in terms of mean number of adults captured. The strong pattern of aggregation of *T. erytreae* is crucial since the resulting border effect is directly related to more or less efficiently HLB managed adjacent orchards (Bové, 2014). Advising stakeholders on the best position of the minimum number of traps to accurately monitor and fight the pest is also crucial to anticipate strong outbreaks of *T. erytreae* that may even colonize neighbour orchards. Further research regarding the local dispersion patterns of *T. erytreae* throughout the orchard is mandatory to refine sampling and monitoring protocols towards the development of successful control strategies of a vector of HLB.

ACKNOWLEDGEMENTS

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal), for financial support through national funds FCT/MCTES to CIMO (UIDB/00690/2020) and to the project PRE-HLB-Preventing HLB epidemics for ensuring citrus survival in Europe (H2020-SFS-2018-2 Topic SFS-05-2018-2019-2020, proj. No. 817526).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

J.A.P., A.F. and J.B.-M. conceived the idea, J.B.-M. and J.A.P analysed the data and prepared the figures, all authors contributed to developing fieldwork, writing and reviewing the paper. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

Data supporting the information shown in the results have been uploaded to figshare (https://figshare.com/account/home). Benhadi-Marín, J. (2020): Trioza erytreae sampling data set. Data set figshare. xlsx. https://doi.org/10.6084/m9.figshare.13182566.

ORCID

Jacinto Benhadi-Marín https://orcid.org/0000-0002-9804-4145
Luís F. Pereira https://orcid.org/0000-0002-3423-8450
Isabel Rodrigues https://orcid.org/0000-0002-4827-2115
Alberto Fereres https://orcid.org/0000-0001-6012-3270
José Alberto Pereira https://orcid.org/0000-0002-2260-0600

REFERENCES

Antolínez, C. A., Moreno, A., Ontiveros, I., Pla, S., Plaza, M., Sanjuan, S., Palomo, J. L., Sjölund, M. J., Sumner-Kalkun, J. C., Arnsdorf, Y. M., Jeffries, C. J., Ouvrard, D., & Fereres, A. (2019). Seasonal abundance of psyllid species on carrots and potato crops in Spain. *Insects*, *10*(9), 287. https://doi.org/10.3390/insects10090287

Arevalo, H. A., Qureshi, J. A., & Stansly, P. A. (2011). Sampling for Asian citrus psyllid (ACP) in Florida citrus groves. Entomology and Nematology Department, Florida Cooperative Extension Service, Institute o Food and Agricultural Sciences, University of Florida. Document, ENY-857 (IN867), 7 pp.

Aubert, B. (1987). *Trioza erytreae* Del Guercio and *Diaphorina citri* Kuwayama (Homoptera: Psylloidea), the two vectors of citrus greening disease: Biological aspects andpossible control strategies. *Fruits*, *42*, 149–162.

Aubert, B., & Quilici, S. (1988). Monitoring adult psyllas on yellow traps in Reunion Island. In: L. W. Timmer, S. M. Garnsey, & L. Navarro (Eds.). *Proceedings of the 10th conference of the International Organization of Citrus Virologists – IOCV* (pp. 249–254). University of Florida-IFAS-CREC. Riverside: IOCV.

Bové, J. M. (2006). Huanglongbing : A destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology*, *88*, 7–37. www.jstor.org/stable/41998278

Bové, J. M. (2014). Huanglongbing or yellow shoot, a disease of Gondwanan origin: Will it destroy citrus worldwide? *Phytoparasitica*, *42*, 579–583. https://doi.org/10.1007/s12600-014-0415-4

Carvalho, J. P., Aguiar, A. M. F. (1997). Espécies da entomofauna nociva. In Secretaria Regional de Agricultura Florestas e Pescas, & Instituto Nacional de Investigaçao Agrária (Eds.), *Pragas dos citrinos na Ilha da Madeira. Direçao Regional de Agricultura da Regiao Autónoma da Madeira*, (pp. 83–91).

Catling, H. D. (1978). Citrus psylla *Trioza erytreae* (Del Guercio). In: E. C. G. Bedford (Ed.). *Citrus pests in the Republic of South Africa*. Science bulletin (South Africa. Dept. of Agricultural Technical Services), *391*, (pp. 71–75).

Cocuzza, G. E. M., Alberto, U., Hernández-Suárez, E., Siverio, F., Di Silvestro, S., Tena, A., & Carmelo, R. (2016). A review on *Trioza erytreae* (African citrus psyllid), now in mainland Europe, and its potential risk as vector of huanglongbing (HLB) in citrus. *Journal of Pest Science*, *90*, 1–17. https://doi.org/10.1007/s10340-016-0804-1

Cooper, N. W., Thomas, M. A., Garfinkel, M. B., Schneider, K. L., & Marra, P. P. (2012). Comparing the precision, accuracy, and efficiency of branch clipping and sweep netting for sampling arthropods in two Jamaican forest types. *Journal of Field Ornithology*, *83*, 381–390. https://doi.org/10.1111/j.1557-9263.2012.00388.x de Graça, J. V. (1991). Citrus greening disease. *Annual Review of Phytopathology*, *29*, 109–136. https://doi.org/10.1146/annurev.py.29.090191.000545

Delignette-Muller, M. L., & Dutang, C. (2015). fitdistrplus: An R package for fitting distributions. *Journal of Statistical Software*, *64*, 1–34. https://doi.org/10.18637/jss.v064.i04

DGAV. (2016–2020). Direção Geral de Alimentação e Veterinária. Retrieved from http://www.dgv.min-agricultura.pt/portal/page/portal/DGV/genericos?generico=221911&cboui=221911

Dietrick, E. J. (1961). An improved backpack motor fan for suction sampling of insect populations. *Journal of Economic Entomology*, *54*, 394–395. https://doi.org/10.1093/jee/54.2.394

EPPO. (2020). Update of the situation of *Trioza erytreae* in Spain. EPPO Reporting Service no. 08 - 2020 Num. article: 2020/164. Retrieved from https://gd.eppo.int/reporting/article-6842

Gigot, C. (2018). epiphy: Analysis of plant disease epidemics. *R Package Version*, (3), 4. Retrieved from https://CRAN.R-project.org

González-Hernández, A. (2003). *Trioza erytreae* (Del Guercio 1918): Nueva plaga de los cítricos en Canarias. *Phytoma España*, *153*, 112–117.

Green, R. H. (1970). On fixed precision level of sequential sampling. *Researches on Population Ecology*, *12*, 249–251. https://doi.org/10.1007/BF02511568

Hall, D. G., Hentz, M. G., & Ciomperlik, M. A. (2007). A comparison of traps and stem tap sampling for monitoring adult Asian citrus psyllid (Hemiptera: Psyllidae) in citrus. *Florida Entomologist*, *90*, 327e334.

Iwao, S. (1968). A new regression method for analyzing the aggregation pattern of animal populations. *Researches on Population Ecology*, *10*, 1–20. https://doi.org/10.1007/BF02514729

Kent, C. M., Peele, A. M., & Sherry, T. W. (2019). Comparing four simple, inexpensive methods for sampling forest arthropod communities. *Journal of Field Ornithology*, *90*, 57–69. https://doi.org/10.1111/jofo.12278

Lloyd, M. (1967). Mean crowding. *Journal of Animal Ecology*, *36*, 1–30. https://doi.org/10.2307/3012

Miranda, M. P., Dos Santos, F. L., Bassanezi, R. B., Montesino, L. H., Barbosa, J. C., & Sétamou, M. (2018). Monitoring methods for *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) on citrus groves with different insecticide application programmes. *Journal of Applied Entomology*, *142*, 89–96. https://doi.org/10.1111/jen.12412

Morente, M., Cornara, D., Plaza, M., Durán, J., Capiscol, C., Trillo, R., Ruiz, M., Ruz, C., Sanjuan, S., Pereira, J., Moreno, A., & Fereres, A. (2018). Distribution and relative abundance of insect vectors of *Xylella fastidiosa* in olive groves of the Iberian peninsula. *Insects*, *9*(4), 175. https://doi.org/10.3390/insects9040175

O'Rourke, P. K., Burkness, E. C., & Hutchison, W. D. (1998). Development and validation of a fixed-precision sequential sampling plan for Aster leafhopper (Homoptera: Cicadellidae) in carrot. *Environmental Entomology*, *27*, 1463–1468. https://doi.org/10.1093/ee/27.6.1463

Pérez-Otero, R., Mansilla, J. P., & del Estal, P. (2015). Detección de la psila africana de los cítricos, *Trioza erytreae* (Del Guercio, 1918) (Hemiptera: Psylloidea: Triozidae), en la Península Ibérica. *Arquivos Entomolóxicos*, *13*, 119–122.

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Retrieved from https://CRAN.R-project.org

Samways, M. J. (1987). Phototactic response of *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae) to yellow-coloured surfaces, and an attempt at commercial suppression using yellow barriers and trap trees. *Bulletin of Entomological Research*, *77*, 91–98. https://doi.org/10.1017/S0007485300011573

Samways, M. J. (1987b). Weather and monitoring the abundance of the adult citrus psylla, *Trioza erytreae* (Del Guercio) (Hom., Triozidae). *Journal of Applied Entomology*, *103*, 502–508. https://doi.org/10.1111/j.1439-0418.1987.tb01015.x

Samways, M. J., & Manicom, B. Q. (1983). Immigration, frequency distributions and dispersion patterns of the psyllid *Trioza erytreae* (Del Guercio) in a citrus orchard. *Journal of Applied Ecology*, *20*, 463–472. https://doi.org/10.2307/2403520

Sétamou, M., Flores, D., French, J. V., & Hall, D. G. (2008). Dispersion patterns and sampling plans for *Diaphorina citri* (Hemiptera: Psyllidae) in citrus. *Journal of Economic Entomology*, *101*, 1478–1487. https://doi.org/10.1093/jee/101.4.1478

Sétamou, M., Sanchez, A., Saldaña, R. R. R., Patt, J. M. M., & Summy, R. (2014). Visual responses of adult Asian citrus psyllid (Hemiptera: Liviidae) to colored sticky traps on citrus trees. *Journal of Insect Behavior*, *27*, 540–553. https://doi.org/10.1007/s10905-014-9448-2

Southwood, T. R. E., & Henderson, P. A. (2000). *Ecological methods*, (3rd ed.) (p. 575). Blackwell Science.

Sunderland, K. D., de Snoo, G. R., Dinter, A., Hance, T., Helenius, J., Jepson, P. C., Kromp, B., Lys, J.-A., Samu, F., Sotherton, N. W., Toft, S., & Ulber, B. (1995). Density estimation for invertebrate predators in agroecosystems. *Acta Jutlandica*, *70*, 133–162.

Taylor, L. R., & Woiwod, I. P. (1980). Temporal stability as a density-dependent species characteristic. *Journal of Animal Ecology*, *49*, 209–224. https://doi.org/10.2307/4285

Thanou, Z. N., Kontogiannis, E. G., & Tsagkarakis, A. E. (2020). Impact of weeds on Auchenorrhyncha incidence and species richness in citrus orchards. *Phytoparasitica*, https://doi.org/10.1007/s12600-020-00857-w

Thomas, D. B. (2012). Comparison of Insect vacuums for sampling Asian citrus psyllid (Homoptera: Psyllidae) on citrus trees. *Southwestern Entomologist*, *37*(1), 55–60. https://doi.org/10.3958/059.037.0107

Tret'yakov, N. N. (1984). The distribution of the apple weevil and the apple sucker in orchards. *Zashch Rast*, *9*, 42–43.

Tsagkarakis, A. E., Afentoulis, D. G., Matared, M., Thanou, Z. N., Stamatakou, G. D., Kalaitzaki, A. P., Tzobanoglou, D. K., Goumas, D., Trantas, E., Zarboutis, I., & Perdikis, D. C. (2018). Identification and seasonal abundance of auchenorrhyncha with a focus on potential insect vectors of Xylella fastidiosa in olive orchards in three regions of Greece. *Journal of Economic Entomology*, *111*(6), 2536–2545. https://doi.org/10.1093/jee/toy239

Urban, A. J. (1977). Colour vision of the citrus psylla *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae) in relation to alightment colour preferences. Ph.D. thesis, Rhodes Univ., Grahamstown, South Africa. (pp 95).

Van den Berg, M. A., Anderson, S. H., & Deacon, V. E. (1991). Population studies of the citrus psylla, *Trioza erytreae*: Factors influencing dispersal. *Phytoparasitica*, *19*, 283–289. https://doi.org/10.1007/BF02980962

Van den Berg, M. A., & Deacon, V. E. (1989). Flight activities of the citrus psylla, *Trioza erytreae* (Hemiptera:Triozidae). *Phytophylactica*, *21*, 391–395. https://hdl.handle.net/10520/AJA03701263_1173

Van den Berg, M. A., Deacon, V. E., & Steenekamp, P. J. (1991). Dispersal within and between citrus orchards and native hosts, and nymphal mortality of citrus psylla, *Trioza erytreae* (Hemiptera: Triozidae). *Agriculture Ecosystems & Environment*, *35*, 297–309. https://doi.org/10.1016/0167-8809(91)90080-H

Waters, E. K., Furlong, M. J., Benke, K. K., Grove, J. R., & Hamilton, A. J. (2014). Iwao's patchiness regression through the origin: Biological importance and efficiency of sampling applications. *Population Ecology*, *56*, 393–399. https://doi.org/10.1007/s10144-013-0417-y

Waters, W. E. (1959). A quantitative measure of aggregation in insects. *Journal of Economic Entomology*, *52*, 1180–1184. https://doi.org/10.1093/jee/52.6.1180

Zou, Y., van Telgen, M. D., Chen, J., Xiao, H., de Kraker, J., & Bianchi, F. J. & van der Werf, W. (2016). Modification and application of a leaf blowervac for field sampling of arthropods. *Journal of Visualized Experiments*, *114*. 5. https://doi.org/10.3791/54655

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed Effects Models and Extensions in Ecology with R* (p. 574). Springer. https://doi.org/10.1007/978-0-387-87458-6

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Benhadi-Marín J, Pereira LF, Rodrigues I, Fereres A, Alberto Pereira J. Sampling and distribution pattern of *Trioza erytreae* Del Guercio, 1918 (Hemiptera: Triozidae) in citrus orchard. *J Appl Entomol*. 2021;145:601–611. https://doi.org/10.1111/jen.12870