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Spatial distribution pattern of male adults of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in lemon orchards in Northern Portugal

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Abstract

Phyllocnistis citrella Stainton, commonly known as the citrus leafminer, threatens global citrus production. This study focuses on elucidating the population dynamics and spatial distribution of *P. citrella* in lemon orchards located in Northern Portugal. From May to December, using delta traps with sexual pheromones, the levels of the adult population were monitored, and aggregation indices were calculated. Five distinct density peaks are observed, with the highest recorded in late July to early August. Spatial distribution consistently indicated an aggregated pattern. However, temporal variation in distribution was observed on specific dates. According to our results, it is suggested deploying two or three traps per hectare during peak density periods. This study significantly advances our understanding of *P. citrella* dynamics, emphasizing the need to consider spatial and temporal patterns for effective pest management. The outcomes underscore the importance of further exploration into factors influencing distribution patterns to refine control strategies. These insights are crucial for devising targeted and efficient measures to mitigate the impact of *P. citrella* on citrus orchards globally.

KEYWORDS
citrus leafminer, dispersion indices, negative binomial distribution, population dynamics, Taylor's power law

1 | INTRODUCTION

Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae), commonly known as the citrus leafminer, is native to Southeast Asia, displaying a wide-ranging and globally expanding geographic distribution (Mansour et al., 2021). Initially documented in 1856 in Calcutta (India), its impact has become pronounced in citrus-producing regions since the 1990s (Tsagkarakis et al., 2011), with rapid global dissemination reaching Portugal during that decade. First reported in the country's south, it was subsequently observed in citrus orchards in the northern part of Portugal in 1995 (Alves, 2020).

Adults of *P. citrella* are small moths, measuring approximately 4 mm and displaying a white to silvery hue with distinctive wing characteristics (Parra et al., 2004). Females oviposit single eggs on the young and tender citrus leaves about 24 h after mating (Amalin et al., 2002). Newly hatched larvae swiftly initiate the creation of a mine by penetrating the delicate leaf beneath the epidermal layer (Mansour et al., 2021). The life cycle of *P. citrella* unfolds through four distinct larval instars. In the first three instars, the larvae predominantly feed on sap and epidermal cells, while the fourth instar transitions into the prepupal stage, characterized by a cessation of feeding behaviours (Beattie & Hardy, 2004). During the prepupal stage, the larvae meticulously construct a protective silken cocoon, forming a pupal chamber often shielded by the folded leaf margin (Chermiti et al., 2001). Influenced by environmental conditions, the leafminer's life spans from 11.5 to 32.7 days, with up to 14 annual generations in tropical conditions (Parra et al., 2004).

The economic significance of *P. citrella* is underscored by its detrimental effects on citrus leaves, leading to adverse consequences for fruit production and quality (Arshad et al., 2020; Dahmane & Chakali, 2022). Larval infestation targets new shoots, creating galleries within the leaves, resulting in atrophy and a reduction in the photosynthetic area (Santos et al., 2009). Although *P. citrella* is not considered a key pest in mature Mediterranean citrus orchards, it is an important citrus threat in nurseries as well as on young plants and top-grafted trees (Garcia-Marí et al., 2018). Furthermore, the presence of this pest in orchards heightens the risk of contamination by *Xanthomonas axonopodis* pv. citri, the bacterium responsible for citrus canker (Atiq et al., 2013; Elekçioğlu & Uygun, 2006, 2013; Hall et al., 2010).

The spatial pattern of an arthropod is an inherent and constant characteristic of the species (Taylor, 1984). The knowledge of such patterns, especially species considered pests, holds paramount significance, especially within the domains of agriculture and pest control. The understanding of spatial distribution patterns of a pest is crucial for the formulation and application of effective sampling, monitoring and control strategies (Bakry et al., 2023; Benhadi-Marín et al., 2021; He et al., 2022; Liu et al., 2008; Padala et al., 2023).

The development of reliable sampling protocols is imperative, encompassing considerations such as the identification of optimal sampling times, selection of appropriate sampling units, determination of sampling patterns (randomness) and establishment of sample sizes (Arnaldo & Torres, 2005; Boeve & Weiss, 1988). This critical sampling framework finds applications in various branches, including ecological investigations, the study of population dynamics (Jarošík et al., 2003), the identification of pest levels warranting control measures (Arnaldo & Torres, 2005) and the assessment of crop losses (Liu et al., 2008).

The most common methods employed to characterize the dispersion patterns of arthropod populations rely on the dispersion coefficient, k, of the negative binomial distribution and indices of aggregation, including methods such as Taylor's power law (Taylor, 1961) and Iwao's regression (Iwao, 1968). These methods fundamentally involve regressions reflecting changes observed in the aggregation among population density. Sampling strategies utilizing these indices not only enhance the efficiency of sampling efforts but also improve precision (Kuno, 1991). Sequential sampling plans are deployed to identify mean pest populations at or exceeding the economic threshold more effectively. Remarkably, these plans have demonstrated a reduction in the sampling effort by up to 50%, as compared to conventional sampling approaches (Pedigo & Zeiss, 1996; Subramanyam et al., 1997).

In the context of *P. citrella* management, implementing sequential sampling plans utilizing dispersion indices offers a promising avenue for reducing sampling efforts while maintaining accuracy (Bakry et al., 2023; Benhadi-Marín et al., 2021; He et al., 2022; Liu et al., 2008; Padala et al., 2023). These plans have demonstrated significant efficiency gains compared to traditional sampling approaches (Bakry et al., 2023; Benhadi-Marín et al., 2021; Padala et al., 2023), providing a practical and resource-efficient approach to monitor and control citrus leafminer. Therefore, the objectives of this work were as follows: (a) to study the population fluctuations of male adults of *P. citrella* in lemon orchards in Northern Portugal, (b) to assess the spatial distribution of the population in the studied orchard and (c) to estimate the minimum sampling effort to have a good estimate of population density.

2 | MATERIALS AND METHODS

2.1 | Study area and sampling design

The study was conducted in five plots within one extensive lemon orchard in Ribela, Vila Nova de Famalicão, Portugal (41° 26′ 39.700" N, 8° 30′ 15.700" W), covering a total of 3.4 ha. The lemon trees (*Citrus limon* (L.), variety 'Lunario') are 4 years old and are planted with a spacing of 2 m between trees and 6 m between rows. The cultivation is under an integrated pest management (IPM) approach. The prevailing climate in Ribela's region is classified as Csb, characterized by a wet winter and a dry and slightly hot summer (Pimentel-Rodrigues & Silva-Afonso, 2023). The dominant wind direction is from west to east (Oliveira et al., 2022).

To monitor adult citrus leafminer populations, we strategically positioned delta-type traps in each plot, each equipped with a sexual pheromone (BIOSANI, Portugal), at a central location. A total of five delta-type traps were deployed across the study area. Observations were conducted weekly between May and December 2023, documenting the number of captures in each trap. The pheromone was regularly replaced in accordance with the manufacturer's guidelines.

During the surveillance period, a phytosanitary treatment was administered on 14 May 2023. Align (SIPCAM, Portugal), a plant-based insecticide containing azadirachtin, and SEQURA TOP (SIPCAM, Portugal), a biological insecticide based on *Bacillus thuringiensis* kurstaki, serotype 3a, 3b, strain HD-1, were employed.

2.2 | Data analysis

2.2.1 | Spatial distribution of *Phyllocnistis citrella*

The mean ($\bar{x}$) and variance (S^2) of individuals captured by delta-type traps across the 29 sampling dates were calculated. The adjustment of observed and expected frequencies of captured individuals fitted a negative binomial distribution ($\alpha = 0.01$), as outlined by Waters (1959), utilizing the fitdist and gofstat functions from the fitdistrplus package (Delignette-Muller & Dutang, 2015) in R (R Core Team, 2020).

The spatial distribution of male adults of *P. citrella* was evaluated by applying various aggregation indexes such *k* parameter for the negative binomial, Lloyd's patchiness index and Taylor's power law.

The calculation of the *k* parameter for the negative binomial distribution was conducted as follows: $k = \bar{x}^2 / S^2 - \bar{x}$ where $\bar{x}$ represents the sample mean and S^2 denotes the sample variance. In the context of the negative binomial distribution, the *k* parameter serves as an indicator of aggregation.

The Iwao's patchiness regression method, Iwao (1968), quantifies the relationship between the mean crowding index of Lloyd (m^*) and the population mean ($\bar{x}$) as follows: $m^* = \alpha + \beta\,\bar{x}$, α and β are the Iwao's parameters and m^* is the Lloyd index, Lloyd (1967), calculated as: $m^* = \bar{x} + (S^2 / \bar{x} - 1)$.

Regarding the Taylor's law, as outlined by Taylor and Woiwod (1980), it characterizes the relationship between the logarithm of the variance ($\mathrm{Log}(S^2)$) and the logarithm of the mean ($\mathrm{Log}(\bar{x})$) through the equation: $\mathrm{Log}\,(S^2) = \mathrm{Log}\,\alpha + \beta\,\mathrm{Log}\,(\bar{x})$, in this equation, α represents the intercept and β denotes the slope of the regression line.

2.2.2 | Minimum sampling effort

The determination of the minimum number of delta-type traps required to estimate the mean with a specified precision was computed by applying the Green's model: $N = (\alpha + \bar{x}^{\beta-2}) / D^2$, *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). The estimate required number of samples was divided by the total area of the cultivation to provide the minimum number of traps per hectare.

TABLE 1 Mean ($\bar{x}$) and variance (S^2) of the captured male adults of *Phyllocnistis citrella*.

Date	$\bar{x}$	S^2	χ^2	*P*	*k*	Lloyd	m^*
21/05/2023	307	1360.6	–	–	89.2	1.0	310
28/05/2023	307	1360.6	–	–	89.2	1.0	310
04/06/2023	70	319.5	–	–	19.8	1.1	74
11/06/2023	118	8180.7	1.0	1.0	1.7	1.6	186
18/06/2023	204	13866.8	3.3	0.9	3.1	1.3	271
25/06/2023	264	25625.9	10.0	0.2	2.7	1.4	360
02/07/2023	285	17501.2	3.7	0.8	4.7	1.2	346
09/07/2023	133	5614.9	0.3	1.0	3.2	1.3	174
16/07/2023	188	9976.7	2.9	0.9	3.6	1.3	240
23/07/2023	367	5493.7	0.3	1.0	26.2	1.0	381
30/07/2023	549	80687.8	9.2	0.2	3.8	1.3	695
06/08/2023	549	98924.6	14.4	0.0	3.1	1.3	728
13/08/2023	299	50155.7	8.0	0.3	1.8	1.6	466
20/08/2023	238	50069.2	7.5	0.4	1.1	1.9	447
27/08/2023	134	15419.3	4.7	0.7	1.2	1.9	248
03/09/2023	61	1508.5	0.2	1.0	2.5	1.4	85
10/09/2023	147	2935.9	2.0	1.0	7.8	1.1	166
17/09/2023	162	3569.1	3.4	0.8	7.7	1.1	183
24/09/2023	88	684.0	–	–	13.1	1.1	95
01/10/2023	59	795.8	–	–	4.7	1.2	72
08/10/2023	98	1760.3	0.9	1.0	5.8	1.2	115
15/10/2023	199	5429.1	1.6	1.0	7.6	1.1	225
22/10/2023	57	284.3	–	–	14.3	1.1	61
29/10/2023	12	25.5	–	–	11.9	1.1	14
05/11/2023	12	25.5	–	–	11.9	1.1	14
12/11/2023	15	63.5	–	–	4.8	1.2	18
19/11/2023	17	131.6	–	–	2.4	1.4	24
26/11/2023	18	220.3	–	–	1.6	1.6	29
01/12/2023	13	112.4	–	–	1.7	1.6	21

Note: 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) and mean crowding index (m^*).

3 | RESULTS

A total of 24,528 male adults of *P. citrella* were captured throughout the sampling period. The population dynamics of *P. citrella* exhibited

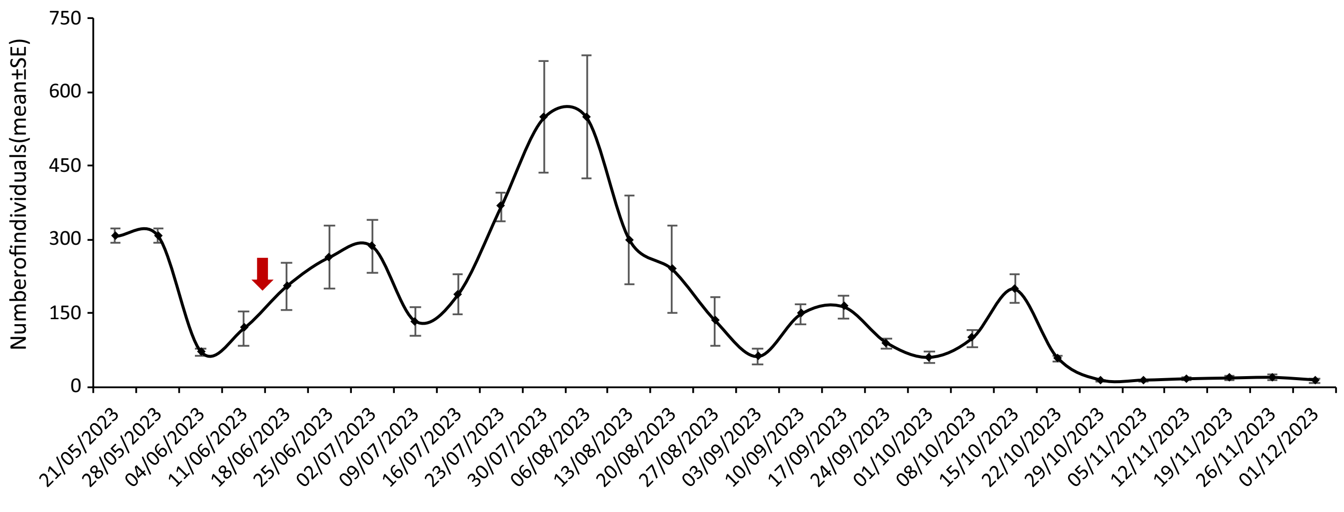

FIGURE 1 Mean number (±SE) of male adults of *Phyllocnistis citrella* captured using delta traps. Red arrow means day of treatment.

five density peaks (Figure 1), suggesting the existence of five generations in the study area: one in late May and another in early July, followed by one in late July to early August, one in mid-September and another in mid-October. The peak population was observed during the period from 30/07/2023 to 06/08/2023, with values of 548.75 ± 113.62 individuals per trap and 548.93 ± 125.81 individuals per trap, respectively. The population started to drop gradually at the end of October, ultimately reaching low densities in November, with no more than 20 captures per trap documented during this period. The application of the two insecticides on 14/05/2023 did not demonstrate any evident effect on the population of *P. citrella*.

The data fitted a negative binomial distribution according to the goodness-of-fit test and Iwao's patchiness regression method (Table 1) (Figure 2). The various aggregation indices employed to evaluate the spatial pattern revealed a consistent extent of distribution. The Iwao regression yielded parameters $\alpha = 3.59$ and $\beta = 1.26$ ($R^2 = 0.94$; $t = 6.33$; $p < 0.001$) (Figure 2). Additionally, Taylor's power law showed significant relationships between variances and means with $\alpha = 0.26$ and $\beta = 1.79$ ($R^2 = 0.81$; $t = 25.34$; $p < 0.001$) (Figure 3), indicating a generally aggregated distribution for *P. citrella*.

On the dates 21/05/2023, 28/05/2023 and 23/07/2023 (Table 1), a Lloyd's index of patchiness equal to 1 was observed. Additionally, high values of the *k* parameter in the negative binomial distribution indicated that on these specific dates, *P. citrella* exhibited a random distribution.

Regarding the minimum number of traps required, according to Green's mode (with a precision level (*D*) of 0.1), it is necessary to have

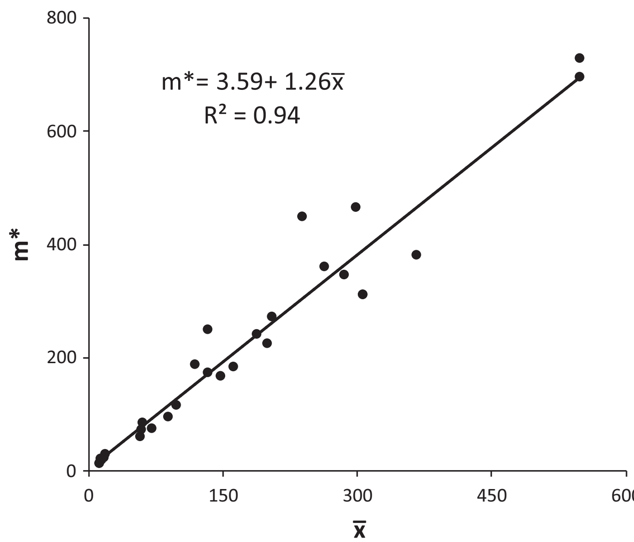

FIGURE 2 Relationship between the mean crowding index of Lloyd (m^*) and the mean ($\bar{x}$) of male adults of *Phyllocnistis citrella* captured using delta traps.

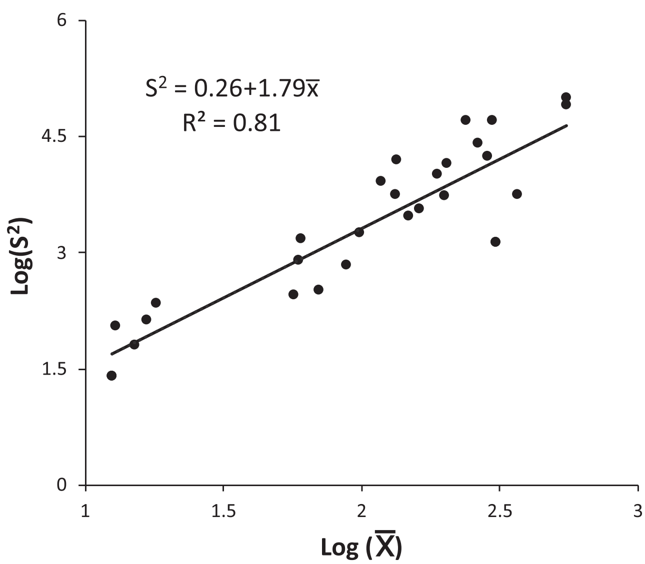

FIGURE 3 Iwao's patchiness and regression between the variance (S^2) and the mean ($\bar{x}$) of male adults of *Phyllocnistis citrella* captured using delta traps according to Taylor's law.

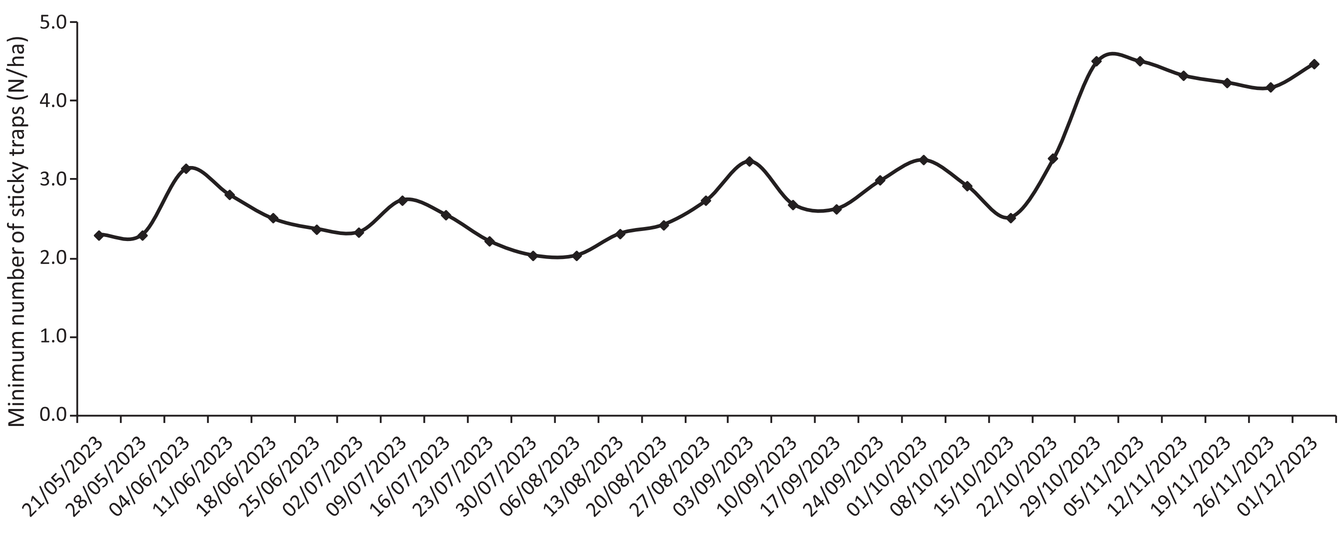

FIGURE 4 Minimum number of sticky traps (N/ha).

between 2 and 3 traps per ha, except for the month of November, where 4–5 traps are needed (Figure 4).

4 | DISCUSSION

The findings of this study provide a comprehensive understanding of the population dynamics and spatial distribution of citrus leafminer in lemon orchards in Northern Portugal, offering valuable insights for pest management strategies.

The observed density peaks at different time points throughout the sampling period suggest that from May to December, *P. citrella* may exhibit up to five generations within lemon orchards in Northern Portugal.

Notably, diverse studies underscore the variability in the number of generations per year for this lepidopteran species. In Mitidja (Algeria), *P. citrella* has been reported to have four generations (two in summer and another two in autumn) (Dahmane & Chakali, 2022), whereas in Tuscany (Italy), *P. citrella* has been reported to complete seven to eight annual generations, spanning from May to December (Garcia-Marí et al., 2004). Conversely, in southern Japan, the species undergoes six generations Clausen, 1931, while in north-central India, the range expands from nine to 13, and in southern India, the number of generations reaches ten (Pandey & Pandey, 1964). Furthermore, under tropical conditions, the species may manifest up to 14 annual generations (Parra et al., 2004). The duration of the life cycle and the annual number of generations of this insect appear to be intricately influenced by a group of factors, including temperature, average relative humidity, photoperiod and foliage flushing cycles (Nawaz et al., 2021). This intricate interplay of environmental variables underscores the adaptability and responsiveness of *P. citrella* to climatic conditions, shaping its population dynamics across different regions.

The highest peak of abundance in late July to early August suggests a critical period of increased activity, aligning with existing literature (Dahmane & Chakali, 2022, Garcia-MarÍ et al., 2002; Mansour et al., 2021; Patel et al., 1994). The observed increase in population coincides with the availability of new flushes and temperatures conducive to the optimal development of the insect. Indeed, Patel et al. (1994) identified that the high population peaks of the citrus leafminer during the month of August were associated with elevated temperature and relative humidity, accompanied by a reduction in the number of days. Subsequently, Nawaz et al. (2021) validated and confirmed this relationship.

The lack of impact from insecticide application may suggest resistance or ineffectiveness against *P. citrella*. Achieving effective chemical control is challenging due to the protective barrier provided by the leaf cuticle, which shields the larvae from the impact of insecticides (Faskha, 2022; Mafi & Ohbayashi, 2006; Whalon et al., 2008). Additionally, because of its multiple generations per year, frequent applications of insecticides are required to increase control (Yumruktepe et al., 1996). However, *P. citrella* has the capacity to develop resistance to insecticides, thereby complicating the challenge of attaining sufficiently effective control (Faskha, 2022; Mafi & Ohbayashi, 2006).

The aggregation indices have contributed to a comprehensive understanding of the spatial pattern in the adults of *P. citrella*. According to our results, this Lepidoptera demonstrates an aggregation pattern. Previously, the larvae of *P. citrella* have also been described as typically displaying an aggregated pattern (Jahnke et al., 2008; Jesus & Redaelli, 2008; Liu et al., 2008; Tsagkarakis et al., 2011). This aggregation behaviour is a strategic response to avoid unfavourable conditions (Allee, 1926). Furthermore, it is worth noting that aggregation behaviour is typical among various species of Lepidoptera (Cocco et al., 2015; He et al., 2022; Karimzadeh et al., 2023; Sétamou et al., 2000; Sujithra & Chander, 2016), highlighting its general behaviour as a survival strategy in response to environmental challenges.

The observation of a random distribution on specific dates (21/05/2023, 28/05/2023 and 23/07/2023) based on the *k* parameter and Lloyd's index of patchiness suggests that *P. citrella's* spatial arrangement may vary temporally. This temporal variation could be attributed to factors such as host plant availability or other ecological dynamics that influence the dispersal and aggregation patterns of *P. citrella*. Lemon trees, like other citrus species, show different flushing patterns throughout the year; thus, interspecific differences in the temporal availability of young leaves could greatly affect host use by *P. citrella* (Goane et al., 2008).

According to Green's model, it is recommended to place 2–3 delta traps with pheromone per hectare during periods of high population density and increasing the number of traps during periods when the population is typically low. The estimation of optimum sample size is fundamental for reducing the time required for monitoring pests (Bakry & Shakal, 2020; Ward et al., 1985). Typically, the monitoring of *P. citrella* population involves the observation of larvae on young branches (Jahnke et al., 2008; Jesus & Redaelli, 2008; Liu et al., 2008; Tsagkarakis et al., 2011). However, the use of delta traps introduces a paradigm shift in this approach. These traps, designed with precision and with the incorporation of specific pheromones, transcend the limitations of direct visual observation. This type of trap provides the capacity to systematically cover a larger spatial expanse efficiently and a more objective and quantifiable modus operandi, enhancing the sensitivity of detection even during the incipient phases of infestation (Vanaclocha et al., 2016).

The results of this research can be applied and shared to formulate a sampling strategy to measure population size accurately. Moreover, the sampling methodology outlined will serve as a valuable resource to inform decisions in the field of integrated pest management (Bakry & Arbab, 2020).

In summary, the study provides valuable information on the population dynamics and spatial distribution of *P. citrella*. Additionally, understanding the spatial and temporal patterns of distribution is crucial for developing targeted and effective control measures. Further research could explore the factors influencing aggregation and distribution patterns, contributing to the development of more precise pest management strategies.

AUTHOR CONTRIBUTIONS

Isabel Rodrigues: Conceptualization; writing – original draft; writing – review and editing; data curation; supervision. **Jaciley Costa:** Writing – original draft; methodology. **Marta Madureira:** Writing – original draft; methodology. **José Alberto Pereira:** Conceptualization; funding acquisition; writing – original draft; writing – review and editing; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data supporting the information shown in the results have been uploaded to Zenodo (https://zenodo.org/; Data set reference Costa et al., 2024).

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REFERENCES

Allee, W. C. (1926). Studies in animal aggregations: Causes and effects of bunching in land isopods. *Journal of Experimental Zoology*, *45*(1), 255–277. https://doi.org/10.1002/jez.1400450108

Alves, H. D. C. (2020). *Estudo da Fenologia do Limoeiro (Citrus lemon) e Outros Citrinos Associado à Atividade da Psila Africana (Trioza erytreae) e Outras Pragas*. Master's Thesis in agricultural engineering. Faculty of Sciences of the University of Porto.

Amalin, D. M., Peña, J. E., Duncan, R. E., Browning, H. W., & McSorley, R. (2002). Natural mortality factors acting on citrus leafminer, *Phyllocnistis citrella*, in lime orchards in South Florida. *BioControl*, *47*(3), 327–347. https://doi.org/10.1023/A:1014815000826

Arnaldo, P. S., & Torres, L. M. (2005). Spatial distribution and sampling of *Thaumetopoea pityocampa* (Den. & Schiff.) (Lep. Thaumetopoeidea) populations on Pinus pinaster Ait. in Montesinho, N. Portugal. *Forest Ecology and Management*, *210*(1–3), 1–7. https://doi.org/10.1016/j.foreco.2005.02.041

Arshad, M., Ullah, M. I., Guz, N., Afzal, M., & Qureshi, J. A. (2020). Cultivar-specific infestation by *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), leaf morphology and trace elements in citrus. *Crop Protection*, *129*, 105025. https://doi.org/10.1016/j.cropro.2019.105025

Atiq, M., Khan, M. A., Sahi, S. T., & Ahmad, R. (2013). Genetic response of citrus germplasm against citrus leaf miner. *Journal of Animal Plant Sciences*, *23*, 240–243.

Bakry, M. M., & Shakal, S. Y. (2020). Population size and spatial distribution pattern of *Schizaphis graminum* (Hemiptera: Aphididae) on some wheat cultivars and lines. *Acta Entomology and Zoology*, *1*, 1–9. https://doi.org/10.33545/27080013

Bakry, M. M. S., & Arbab, A. (2020). Monitoring of the scale insect *Icerya seychellarum* (Westwood) infesting guava. *Indian Journal Of Entomology*, *82*(1), 1. https://doi.org/10.5958/0974-8172.2020.00001.2

Bakry, M. M. S., Badawy, A. M. M., & Mohamed, L. (2023). Spatial distribution and abundance of the mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) infesting okra plants. *SVU-International Journal of Agricultural Sciences*, *5*(3), 1–17. https://doi.org/10.21608/svuijas.2023.223578.1297

Beattie, A., & Hardy, S. (2004). Citrus leafminer. Agfact H2.AE.4, 4th edition. NSW department of primary industries. https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0006/137634/citrus-leaf-miner.pdf

Benhadi-Marín, J., Pereira, L. F., Rodrigues, I., Fereres, A., & Alberto Pereira, J. (2021). Sampling and distribution pattern of *Trioza erytreae* Del Guercio, 1918 (Hemiptera: Triozidae) in citrus orchard. *Journal of Applied Entomology*, *145*(6), 601–611. https://doi.org/10.1111/jen.12870

Boeve, P. J., & Weiss, M. (1988). Spatial distribution and sampling plans with fixed levels of precision for cereal aphids (Homoptera: Aphididae) infesting spring wheat. *The Canadian Entomologist*, *130*(1), 67–77. https://doi.org/10.4039/Ent13067-1

Chermiti, B., Braham, M., Znaidi, M., Gahbiche, H., Messelmani, B., Dali, M., & Messelmani, H. (2001). First results on releasing experiments of *Ageniaspis citricola* Logvinovskaya (Hym., Encyrtidae), a parasitoid of *Phyllocinistis citrella* Stainton (Lep., Gracillariidae) in Tunisia. *Journal of Applied Entomology*, *125*(1–2), 45–52. https://doi.org/10.1046/j.1439-0418.2001.00493.x

Clausen, S. P. (1931). *Two citrus leaf miners of the Far East*. USDA, Washington, D.C. *TEC Bulletin*, *252*, 1–13.

Cocco, A., Serra, G., Lentini, A., Deliperi, S., & Delrio, G. (2015). Spatial distribution and sequential sampling plans for Tuta absoluta (Lepidoptera: Gelechiidae) in greenhouse tomato crops. *Pest Management Science*, *71*(9), 1311–1323. https://doi.org/10.1002/ps.3931

Costa, J., Madureira, M., Pereira, J. A., & Rodrigues, I. (2024). Adults of *Phyllocnistis citrella* [Data set]. Zenodo https://doi.org/10.5281/zenodo.10519658

Dahmane, M., & Chakali, G. (2022). Population dynamic monitoring of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) using immature stages sampling and male moth pheromone-baited traps. *International Journal of Tropical Insect Science*, *42*(4), 3107–3113. https://doi.org/10.1007/s42690-022-00853-z

Delignette-Muller, M. L., & Dutang, C. (2015). Fitdistrplus: An R package for fitting distributions. *Journal of Statistical Software*, *64*(4), 1–34. https://doi.org/10.18637/jss.v064.i04

dos Santos, J. P., Redaelli, L. R., Soglio, F. K. D., Foelkel, E., & Costa, V. A. (2009). Variação sazonal de lepidópteros minadores e seus parasitoides em plantas de crescimento espontâneo em pomar orgânico de citros em Montenegro, RS, Brasil. *Arquivos do Instituto Biológico*, *76*(3), 381–391. https://doi.org/10.1590/1808-1657v76p3812009

Elekçioğlu, N. Z., & Uygun, N. (2006). The parasitoid complex of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in the East Mediterranean region of Turkey and their role in biological control. *Turkish Journal of Zoology*, *30*, 155–160.

Elekçioğlu, N. Z., & Uygun, N. (2013). Population fluctuation of citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and its parasitoids in the eastern Mediterranean region of Turkey. *Pakistan Journal of Zoology*, *45*, 1393–1403.

Faskha, S. (2022). Efficiency of some insecticides in controlling citrus Leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae).

Entomologia Hellenica, *31*(1), 19–29. https://doi.org/10.12681/eh.28188

Garcia-Marí, F., Faiza, A., Forti, J., Aroun, M., Rachida, B., Benfekih, L., Farid, B., Chermiti, B., Conti, F., Fernandes, J., Franco, J. C., Giannakou, I., Kapaxidi, E., Kormpi, M., Markellou, E., Ramdani, B., Razi, S., Satar, S., Siscaro, G., & Varikou, K. (2018). Questionnaire on the current situation of citrus pests and diseases in the Mediterranean basin. *IOBC/WPRS Bull*, *132*, 2–5.

Garcia-MarÍ, F., Granda, C., Zaragoza, S., & AgustÍ, M. (2002). Impact of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) on leaf area development and yield of mature citrus trees in the Mediterranean area. *Journal of Economic Entomology*, *95*(5), 966–974. https://doi.org/10.1093/jee/95.5.966

Garcia-Marí, F., Vercher, R., Costa-Comelles, J., Marzal, C., & Villalba, M. (2004). Establishment of *Citrostichus phyllocnistoides* (Hymenoptera: Eulophidae) as a biological control agent for the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Spain. *Biological Control*, *29*(2), 215–226. https://doi.org/10.1016/S1049-9644(03)00155-5

Goane, L., Valladares, G., & Willink, E. (2008). Preference and performance of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) on three citrus hosts: Laboratory and field assessment. *Environmental Entomology*, *37*(4), 1025–1034. https://doi.org/10.1093/ee/37.4.1025

Hall, D. G., Gottwald, T. R., & Bock, C. H. (2010). Exacerbation of citrus canker by citrus Leafminer *Phyllocnistis citrella* in Florida. *Florida Entomologist*, *93*(4), 558–566. https://doi.org/10.1653/024.093.0413

He, Y., Wang, K., Du, G., Zhang, Q., Li, B., Zhao, L., He, P., & Chen, B. (2022). Temporal and spatial distribution patterns of *Spodoptera frugiperda* in mountain maize fields in China. *Insects*, *13*(10), 938. https://doi.org/10.3390/insects13100938

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

Jahnke, S. M., Redaelli, L. R., Diefenbach, L. M. G., & Efrom, C. F. (2008). Spatial distribution of parasitism on *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae) in citrus orchards. *Brazilian Journal of Biology*, *68*(4), 813–817. https://doi.org/10.1590/S1519-69842008000400016

Jarošík, V., Honěk, A., & Dixon, A. F. G. (2003). Natural enemy ravine revisited: The importance of sample size for determining population growth. *Ecological Entomology*, *28*(1), 85–91. https://doi.org/10.1046/j.1365-2311.2003.00485.x

Jesus, C. R., & Redaelli, L. R. (2008). Spatial distribution of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) larvae in three scales. *Brazilian Journal of Biology*, *68*(1), 205–210. https://doi.org/10.1590/S1519-69842008000100030

Karimzadeh, R., Malekzadeh, M., Iranipour, S., & Trematerra, P. (2023). Spatio-temporal distribution and mass-trapping of leopard moth, *Zeuzera pyrina* in walnut orchards. *International Journal of Pest Management*, *95*(5), 1–10. https://doi.org/10.1080/09670874.2023.2179126

Kuno, E. (1991). Sampling and analysis of insect populations. *Annual Review of Entomology*, *36*(1), 285–304. https://doi.org/10.1146/annurev.en.36.010191.001441

Liu, Z. M., Meats, A., & Beattie, G. A. C. (2008). Seasonal dynamics, dispersion, sequential sampling plans and treatment thresholds for the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), in a mature lemon block in coastal New South Wales, Australia. *Australian Journal of Entomology*, *47*(3), 243–250. https://doi.org/10.1111/j.1440-6055.2008.00651.x

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

Mafi, S. A., & Ohbayashi, N. (2006). Toxicity of insecticides to the citrus leafminer, *Phyllocnistis citrella*, and its parasitoids, *Chrysocharis pentheus* and *Sympiesis striatipes* (Hymenoptera: Eulophidae). *Applied Entomology and Zoology*, *41*(1), 33–39. https://doi.org/10.1303/aez.2006.33

Mansour, D., Pérez-Hedo, M., Catalán, J., Karamaouna, F., Braham, M., Jaques, J. A., & Urbaneja, A. (2021). Biological control of the citrus leafminer 25 years after its introduction in the Valencia citrus growing area (Spain): A new player in the game. *Biological Control*, *155*, 104529. https://doi.org/10.1016/j.biocontrol.2020.104529

Nawaz, R., Abbasi, N. A., Hafiz, I. A., Khan, M. F., & Khalid, A. (2021). Environmental variables influence the developmental stages of the citrus leafminer, infestation level and mined leaves physiological response of Kinnow mandarin. *Scientific Reports*, *11*(1), 7720. https://doi.org/10.1038/s41598-021-87160-8

Oliveira, D. F., Benhadi-Marín, J., Neto, J., Sanz, L., Garzo, E., Aguiar, A., Fereres, A., & Pereira, J. A. (2022). Kaolin particle films disrupt landing, settling behavior and feeding of *Trioza erytrae* on lemon plants. *Pest Management Science*, *78*(11), 4753–4763. https://doi.org/10.1002/ps.7095

Padala, V. K., Ramya, N., Kumar, M., Rajna, S., Rajashekhar, M., Venkatesh, Y., Raut, S., Chander, S., Singh, I., & Monobrullah, M. (2023). Spatial distribution and optimum sample size for monitoring of water lily aphid *Rhopalosiphum nymphaeae* (L.) in *makhana Euryale ferox* Salisb. *International Journal of Tropical Insect Science*, *43*(6), 2167–2177. https://doi.org/10.1007/s42690-023-01119-y

Pandey, N. D., & Pandey, Y. D. (1964). Bionomics of *Phyllocnistis citrella Stt*. (Lepidoptera: Gracillariidae). *Indian Journal Of Entomology*, *26*, 417–423.

Parra, J. R. P., Bento, J. M. S., Chagas, M. C. M., & Yamamoto, P. T. (2004). O controle biológico da larva-minadora-dos-citros. *Visão Agrícola*, *1*(2), 64–67.

Patel, N. C., Valand, V. M., Shekh, A. M., & Patel, J. R. (1994). Effect of weather factors on activity of citrus leaf-miner (*Phyllocnistis citrella*) infesting lime (*Citrus aurantifolia*). *Indian Journal of Agricultural Sciences*, *64*, 132–134.

Pedigo, L. P., & Zeiss, M. R. (1996). *Analyses in insect ecology and management* (p. 168). University Press, Ames, Iowa.

Pimentel-Rodrigues, C., & Silva-Afonso, A. (2023). The feasibility of rainwater harvesting systems in buildings with green roofs: A case study based on the Köppen climate classification. *Sustainability*, *15*(24), 16859. https://doi.org/10.3390/su152416859

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Sétamou, M., Schulthess, F., Poehling, H.-M., & Borgemeister, C. (2000). Spatial distribution of and sampling plans for *Mussidia nigrivenella* (Lepidoptera: Pyralidae) on cultivated and wild host plants in Benin. *Environmental Entomology*, *29*(6), 1216–1225. https://doi.org/10.1603/0046-225X-29.6.1216

Subramanyam, B., Hagstrum, D. W., Meagher, R. L., Burkness, E. C., Hutchison, W. D., & Naranjo, S. E. (1997). Development and evaluation of sequential sampling plans for *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) infesting farm-stored wheat. *Journal of Stored Products Research*, *33*(4), 321–329. https://doi.org/10.1016/S0022-474X(97)00014-3

Sujithra, M., & Chander, S. (2016). Distribution pattern and sequential sampling plan for spotted pod borer, *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae) on pigeon pea, Cajanus cajan L. *International Journal of Pest Management*, *62*(1), 64–68. https://doi.org/10.1080/09670874.2015.1102360

Taylor, L. R. (1961). Aggregation, variance and the mean. *Nature*, *189*(4766), 732–735. https://doi.org/10.1038/189732a0

Taylor, L. R. (1984). Assessing and interpreting the spatial distributions of insect populations. *Annual Review of Entomology*, *29*(1), 321–357. https://doi.org/10.1146/annurev.en.29.010184.001541

Taylor, L. R., & Woiwod, I. P. (1980). Temporal stability as a density-dependent species characteristic. *The Journal of Animal Ecology*, *49*(1), 209. https://doi.org/10.2307/4285

Tsagkarakis, A. E., Perdikis, D. C. H., & Lykouressis, D. P. (2011). Seasonal abundance and within tree spatial distribution of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). *Entomologia Generalis*, *33*(3), 165–174. https://doi.org/10.1127/entom.gen/33/2011/165
Vanaclocha, P., Jones, M. M., Monzó, C., & Stansly, P. A. (2016). Placement density and longevity of pheromone traps for monitoring of the citrus Leafminer (Lepidoptera: Gracillariidae). *Florida Entomologist*, *99*(2), 196–202. https://doi.org/10.1653/024.099.0207
Ward, S. A., Rabbinge, R., & Mantel, W. P. (1985). The use of incidence counts for estimation of aphid populations. 1. Minimum sample size for required accuracy. *Netherlands Journal of Plant Pathology*, *91*(2), 93–99. https://doi.org/10.1007/BF01974304
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*(2), 393–399. https://doi.org/10.1007/s10144-013-0417-y
Waters, W. E. (1959). A quantitative measure of aggregation in Insects1. *Journal of Economic Entomology*, *52*(6), 1180–1184. https://doi.org/10.1093/jee/52.6.1180
Whalon, M. E., Mota-Sanchez, D., & Hollingworth, R. M. (2008). Analysis of global pesticide resistance in arthropods. In *Global pesticide resistance in arthropods* (pp. 5–31). CABI. https://doi.org/10.1079/9781845933531.0005
Yumruktepe, R., Aytas, M., Erkiliç, L., Yigit, A., Canhilal, R., Uygun, N., Karaca, I., Elekçioglu, N. Z., & Kersting, U. (1996). Chemical control of the citrus leafminer and side effects of effective pesticides on natural enemies in Turkey. In M. A. Hoy (Ed.), *Proceedings, international meeting: Managing the citrus Leafminer, 22–25 April 1996, Orlando, Florida* (p. 103). University of Florida.

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