



**Spatial distribution pattern and infestation rates of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in lemon orchards in the Northern Portugal**

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*“It is not possible to achieve true success without facing opposition and impediments. But it is possible to live the rest of your life without being defeated.”*

**David Schwartz**

*To my parents, Arlindo Costa and Maria Páscoa Costa and siblings, Kassi, Abdenayse, Adilson, Irigalete, Arlindo, Nazaré, Etefvina, who have always given me the strength to win the battles of life.*

*Offer*

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*Dedicate*

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## Abstract

The citrus leaf miner, *Phyllocnistis citrella*, poses a significant threat to citrus fruit cultivation worldwide, with its detrimental impact on citrus orchards well-documented. Direct damage caused by its larvae creating galleries in the leaves, along with increasing the plants' susceptibility to citrus canker disease, makes it essential to understand the infestation dynamics of this pest and develop effective management strategies. The main goals of this study were to evaluate the population fluctuations of adult males of *P. citrella* in a young lemon orchard in the parish of Ribela, Vila Nova de Famalicão, from May to December 2023, and to evaluate the infestation rate of *P. citrella* larvae in the same orchard from March to December 2023. The first aims to analyse the population dynamics of adults and develop a sampling methodology that, with minimal effort, provides accurate estimates of population density, and the second to evaluate the infestation rate. In the first study, Delta-type traps with sexual pheromones were utilized to capture males weekly, and thus infer the population levels of the pest. In the analysis of the results, the spatial distribution was determined using negative binomial tests, Iwao's method, Lloyd's indices and Taylor's power law, and the determination of the minimum number of observations necessary for a correct estimate of the population with a minimum sampling effort. In the second study, lemon tree shoots were randomly collected, which were examined in the laboratory by dividing leaves with a length of less than 3 cm and leaves with a longer length of 3 cm to identify the presence of eggs, larvae, pupae and galleries of *P. citrella*. The results indicate that the distribution of adults of the citrus leaf miner has an aggregate behavior obtained by the aggregation indices evaluated. In the determination of the minimum number of traps for an adequate estimate of the population, values between 2 and 5 traps per homogeneous area of the plot were obtained, with the lower values for high population levels and the higher values when the populations are lower. In the second study, the results show that there are different stages of insect development simultaneously in the field, as long as there is vegetation growth, and highlight the urgent need for a more detailed understanding of the dynamics of *P. citrella* infestation. The present work highlights the need for a more in-depth study on the dynamics of the pest in the region, and the importance of monitoring the populations to support decision-making and intervention at appropriate times, thus reducing the damage that *P. citrella* may cause.

**Keywords:** *Citrus Leafminer*, Population Dynamics, Integrated Pest Management, Dispersion Indices, Negative Binomial Distribution, Lloyd's Index, Taylor's Power Law, Minimum Sampling Effort.

## Resumo

A lagarta mineira dos citrinos, *Phyllocnistis citrella*, representa uma ameaça significativa para a cultura de citrinos em todo o mundo, sendo o seu impacto negativo em pomares de citrinos bem documentado. Os estragos diretos ocasionados pelas larvas deste lepidóptero, que criam galerias nas folhas, juntamente com o aumento da suscetibilidade das plantas ao cancro cítrico, fazem com que seja essencial compreender a dinâmica de infestação da praga e criar bases para fundamentar a decisão da necessidade de intervenção. Os principais objetivos deste estudo foram avaliar as flutuações populacionais dos machos adultos de *P. citrella* num pomar jovem de limoeiros na freguesia de Ribela, Vila Nova de Famalicão, de maio a dezembro de 2023, e avaliar a taxa de infestação de larvas de *P. citrella* no mesmo pomar de março a dezembro de 2023. A primeira pretende analisar a dinâmica populacional dos adultos e desenvolver uma metodologia de amostragem que com um esforço mínimo origine estimativas precisas da densidade populacional e na segunda avaliar a taxa de infestação. Na primeira parte utilizaram-se armadilhas tipo Delta com feromona sexual para captura de machos, inspecionadas semanalmente, e assim inferir dos níveis populacionais da praga. Na análise de resultados procedeu-se à determinação da distribuição espacial utilizando testes binomiais negativos, método de Iwao, índices de Lloyd e a Lei da Potencia de Taylor, e a determinação do número mínimo de observações necessárias para uma estimativa correta da população com um esforço mínimo de amostragem. No segundo estudo, foram colhidos aleatoriamente rebentos de limoeiro, que foram examinados em laboratório dividindo as folhas com comprimento menor que 3 cm e folhas com comprimento maior igual a 3 cm para identificar a presença de ovos, larvas, pupas e galerias de *P. citrella*. Os resultados indicam que a distribuição dos adultos da mineira dos citrinos tem um comportamento agregado obtido pelos índices de agregação avaliados. Na determinação do número mínimo de armadilhas para uma estimativa adequada da população obtiveram-se valores entre 2 a 5 armadilhas por área homogenia da parcela, com os valores inferiores para níveis populacionais elevados e os superiores quando as populações são mais baixas. No segundo estudo, os resultados mostram haver diferentes estados de desenvolvimento do inseto simultaneamente no campo, desde que haja crescimento de vegetação, e destacam a necessidade urgente de uma compreensão mais detalhada da dinâmica de infestação da *P. citrella*. O presente trabalho destaca a necessidade de um estudo mais aprofundado sobre a dinâmica da praga na região, e a importância do acompanhamento das

populações para a fundamentação da tomada de decisão e intervenção em momentos adequados, reduzindo desta forma os prejuízos que a *P. citrella* possa ocasionar.

**Palavras-chave:** Lagarta mineira dos citrinos, Dinâmica populacional, Proteção Integrada, Índices de dispersão, Distribuição binomial negativa, Índice de Lloyd, Lei de potência de Taylor, Esforço mínimo de amostragem.

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# CHAPTER 1

## General Introduction and Objectives

# 1. General Introduction and Objectives

The citrus industry is a keystone of global agriculture, with citrus fruits serving as economically and nutritionally essential supplies worldwide (Barbieri et al., 2023). However, there are many problems that affect the health and productivity of citrus orchards. Diseases and pests are some of the biggest challenges facing citrus farmers (Barbieri et al., 2023). One such pest is *Phyllocnistis citrella*, also known as the citrus leaf miner, which is especially concerning because it can damage citrus trees and reduce the quality of their fruits (Tipu et al., 2021).

Originally from Asia, *P. citrella* has spread quickly to citrus-growing areas around the world (Ali et al., 2023). It is very adaptable and can survive in different environments, making it a serious threat to citrus farming. The larvae of this small moth tunnel through the leaves of citrus trees, leaving behind distinctive trails that affect the leaves' ability to photosynthesize and function properly (Nath & Deka, 2019; Ali et al., 2023). As a result, citrus trees become weaker, produce fewer fruits, and the quality of the fruits decreases, which is bad news for citrus farmers and the industry (Iqbal et al., 2020).

Even though we know that *P. citrella* is a serious problem, finding effective ways to manage it is difficult. There are challenges like the pest becoming resistant to insecticides and concerns about the environmental impact of using pesticides (Ali et al., 2023; Bourtzis et al., 2012). That's why it's important to learn more about *P. citrella* – how it lives, behaves, and interacts with citrus trees – so we can develop sustainable and effective ways to control it. This study addresses these knowledge gaps by scrutinizing the intricate dynamics of *P. citrella* within citrus ecosystems. Specifically, our main goal is to understand the population dynamics of male adults of *P. citrella* in lemon orchards in northern Portugal and provide insights for the development of effective monitoring and control strategies. The main goals will be achieved through the fulfillment of the following specific objectives:

(a) Investigate population fluctuations of male adults of *P. citrella* in lemon orchards in northern Portugal.

(b) Evaluate the spatial distribution of male adults of *P. citrella* in the studied orchard.

(c) Estimate the minimum sampling effort required to obtain a good estimate of male adults of *P. citrella* population density.

(d) Evaluate the infestation rate in lemon orchards throughout the year.



# CHAPTER 2

## Theoretical Framework

## 2. Theoretical Framework

### 2.1. Citrus production in the world

Citrus belongs to the Rutaceae family, encompasses a genus of flowering trees and shrubs primarily found in southeastern Asia, New Guinea, Australia, and the East Indian Archipelago (Wu et al., 2018) (Figure 2.1). Citrus species have thrived in these regions with their wild counterparts, celebrated both for their fruit production and ornamental appeal. Their significance extends globally, as they constitute a vital component of fruit agroecosystems, contributing substantially to human health and dietary preferences across various cultures. Rich in minerals, vitamins, dietary fiber, flavonoids, and carotenoids, Citrus fruits are esteemed for their nutritional value and diverse applications (Sun et al., 2022).



**Figure 2.1.** The World's Top Citrus Producing Countries. Source: [paises productores de citricos 2019 Archivos - Citricas.Com](#).

According to Norton (2024), Brazil, China, the United States, México, India, and Spain have emerged as the top producers of Citrus fruits worldwide. Brazil leads the pack with a staggering 20,682,309 tonnes, followed closely by China with 19,617,100 tonnes, renowned for emphasizing orange production. The United States ranks third with 10,017,000 tonnes, most citrus fruits produced in the U.S. go into juice processing, especially orange juice, consumed locally end most of it is exported in frozen form.

Citrus holds significant economic and social importance in the Mediterranean region. Despite being not among the top producers globally, Portugal maintains a notable position, emphasizing the Algarve region (FAO, 2016). Covering 20,357 hectares, Portugal's citrus production, totalling 354,294 tonnes, contributes substantially to both local and international markets. The Mediterranean climate, characterized by mild winters and hot, dry summers, provides an ideal environment for citrus cultivation, with the Algarve orange standing out for its exceptional quality (Cavaco et al. 2021). Beyond the Algarve, regions such as the Vale of Tejo and Alentejo also contribute to Portugal's citrus production, benefiting from favorable climatic conditions and a longstanding tradition of cultivation (Cavaco et al. 2021).

## **2.2. Worldwide threats to citrus production**

Citrus plants are constantly threatened by a diverse array of diseases and pests, posing significant challenges to the citrus industry worldwide.

### **2.2.1. Diseases**

The Citrus Variegated Chlorosis (CVC), Citrus leprosis, and Huanglongbing (HLB), are among the most damaging diseases, each causing substantial losses in citrus production. CVC, caused by the gram-negative bacterium *Xylella fastidiosa*, manifests with symptoms appearing on branches, leaves, and fruits. Infected trees typically experience a drastic reduction in production rates and fruit quality, characterized by small interveinal chlorotic yellow spots on adult leaves, resembling symptoms of zinc deficiency (Rapicavoli et al., 2018).

In contrast, the leprosis caused by *Citrus leprosis virus* of the type cytoplasmic (CiLV-C) exhibit necrotic injuries, often accompanied by a yellow crown on leaves and fruits. Early-stage fruit injuries lack color and gradually deteriorate, developing a yellow crown at the center. Stem injuries display flattened concentric chlorotic rings that expand over time, leading to shell shedding and, in severe cases, premature fruit fall, leaf abscission, and branch dieback (Chung & Brlansky, 2006).

However, perhaps the most alarming threat to the global citrus industry is Huanglongbing (HLB), also known as citrus greening disease whose etiologic agent is *Candidatus Liberibacter* spp. This devastating disease has spread rapidly, with major citrus-producing regions worldwide affected, except for the Mediterranean basin and Australia and New Zealand. HLB weakens citrus trees progressively, ultimately resulting in minimal fruit production and poor fruit quality, rendering commercial production unfeasible due to

drastically reduced yields. Despite efforts, there is currently no cure for HLB, posing a severe challenge to citrus growers globally (Bové, 2014; Costa et al., 2021).

In recent years, HLB has wreaked havoc on orange juice production in Florida (Hodges & Spreen, 2012; Costa et al., 2021) and has had a significant impact in Brazil, affecting not only oranges but also grapefruits and mandarins in regions such as Florida and Texas. The disease is associated with three species of Gram-negative alpha proteobacters: *Candidatus Liberibacter asiaticus* (Clas), *Candidatus Liberibacter americanus* (Clam), and *Candidatus Liberibacter africanus* (Claf). Despite ongoing research efforts, the management and containment of HLB remain a formidable challenge for the citrus industry worldwide.

## **2.2.2. Pests**

### **2.2.2.1. *Phyllocnistis citrella***

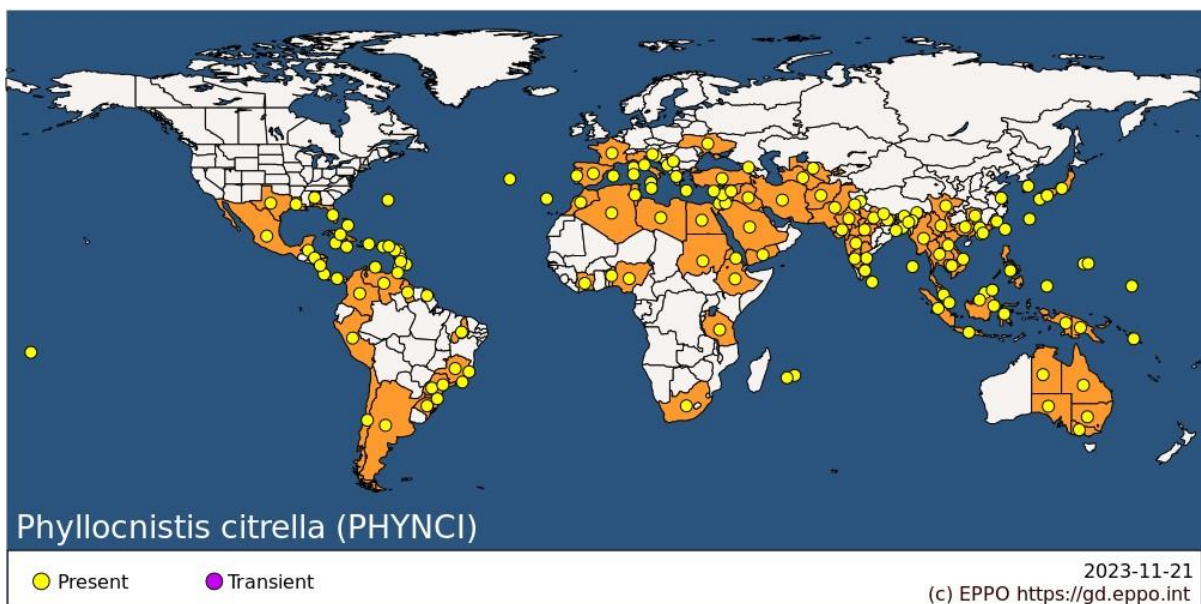
*Phyllocnistis citrella*, Stainton, 1856 (Lepidoptera: Gracillariidae), commonly known as the Citrus Leafminer, stands out as a significant threat to citrus farming across various regions (Santos et al., 2009). This species of Lepidoptera inflicts considerable damage on citrus trees, affecting both fruit production and quality (Iqbal et al., 2020). The insect primarily targets new shoots of all citrus varieties, with its larvae laying eggs on the fresh leaves, subsequently emerging to feed on the foliage and create distinctive galleries (Carvalho et al., 2017). This feeding behavior results in leaf atrophy, characterized by a silvery effect, ultimately reducing the photosynthetic area of the affected foliage (Santos et al., 2009). Although the juvenile primary target is the leaves, it can also be found infesting branches of young vegetation and occasionally even on the fruits, particularly in cases of high population density (Carvalho et al., 2017). In the final larval stage, the leafminer relocates to the leaf edges, where it constructs a protective shell during the pupal phase until adulthood (Carvalho et al., 2017).

Furthermore, *P. citrella* in citrus orchards intensifies the risk of bacterial contamination, particularly by the pathogen responsible for citrus canker, *Xanthomonas axonopodis* pv. Citri (Carvalho & Macedo, 2015). This association with citrus canker underscores the broader impact of this pest, with nurseries and newly established orchards particularly susceptible due to the vulnerability of new leaves and shoots to infestation. Therefore, effective management strategies for *P. citrella* are crucial for mitigating direct damage to citrus trees and minimizing the risk of associated diseases, ultimately safeguarding citrus production and orchard health.

### ***Geographical distribution***

*Phyllocnistis citrella* known for its proficient flying abilities and remarkable capacity for expansion (Brown et al., 2019). This ability to fly, coupled with its high fertility rate, enables the species to generate multiple generations annually in regions conducive to its development, thus facilitating its widespread distribution (Jones & Smith, 2017). Originating from South Asia, this pest made its first appearance in Spain during the summer of 1993, proliferating rapidly along the Mediterranean coast by 1995 (Lee et al., 2015) (Figure 2.2).

Portugal encountered the pest in 1994, with initial sightings in Conceição de Faro, subsequently extending its presence to Tavira, Faro, and Silves. By 1995, citrus orchards in Northern Portugal reported infestations (Alves, 2020). The invasion continued, with Madeira Island detecting the pest in April 1995, followed by its appearance in the Azores in 1996 (Figueiredo & Lopes, 2005). This relentless spread underscore the adaptability and resilience of the citrus leafminer, posing significant challenges to citrus cultivation and requiring robust pest management strategies to mitigate its impact.



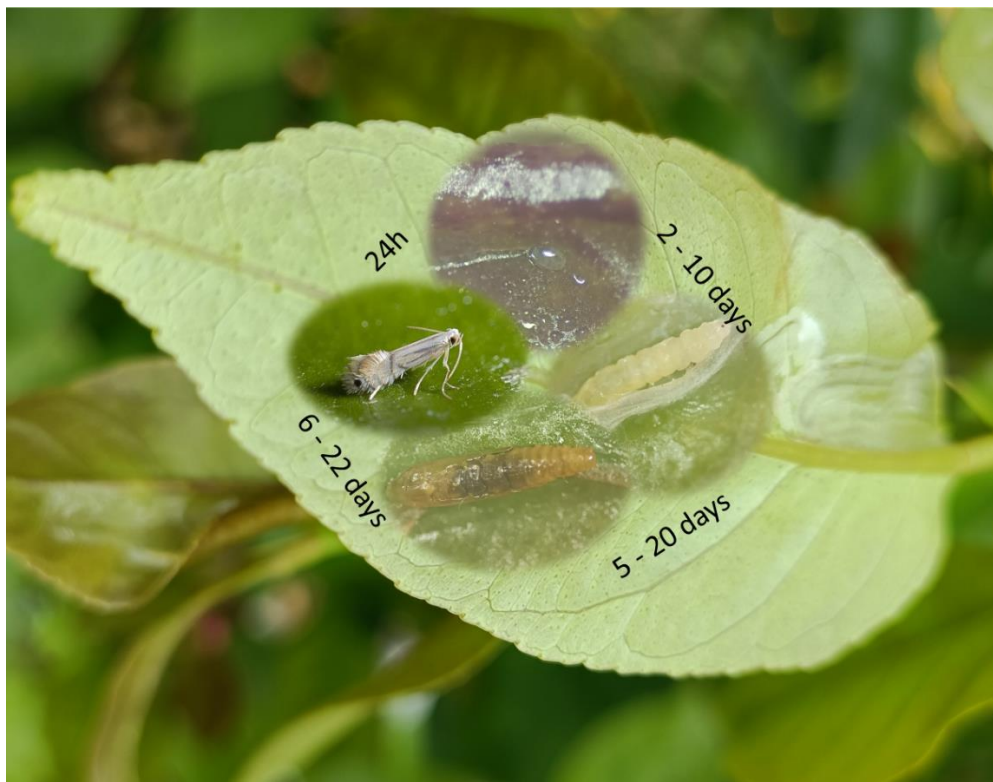
**Figure 2.2.** Distribution of *Phyllocnistis citrella* worldwide. Source: EPPO Global Database, 21-11-2023)

### ***Bioecology and life cycle***

The bioecology of *P. citrella* presents a detailed account of its life stages and habits (Figure 2.3). Initially, the eggs, measuring approximately 0.2 to 0.3 mm in length, are laid by

females in the shoots, particularly near the main vein of leaves, typically on the lower surface. These translucent eggs become opaque and yellow, with females depositing around 7 to 108 eggs. Upon hatching, larvae emerge, penetrating the leaf mesophyll to begin constructing characteristic serpentine mines, predominantly on the lower leaf surface (Parra et al., 2004). Over the course of four larval instars, they reach about 3mm in length before ceasing feeding and forming pupal chambers, typically bending the margin of the leaf. The resulting chrysalises are yellowish-brown and measure approximately 2.3 mm in length (Parra et al., 2004). Adults have only 2mm of length (CABI, 2021), with Wingspan 4.0 in male e 4.7 mm in female (Kim, et al., 2015).

This intricate life cycle of the caterpillar, *P. citrella*, spans from 11.5 to 32.7 days, influenced by temperatures ranging from 18° C to 32° C, with variations noted among citrus varieties. Notably, for example in Brazil, the sprouting period from September to April overlaps with the occurrence of the pest, potentially leading to up to 14 generations in regions with warmer climates (Parra et al., 2004). Environmental conditions significantly impact the biological cycle of this insect (Dantas, 2002).



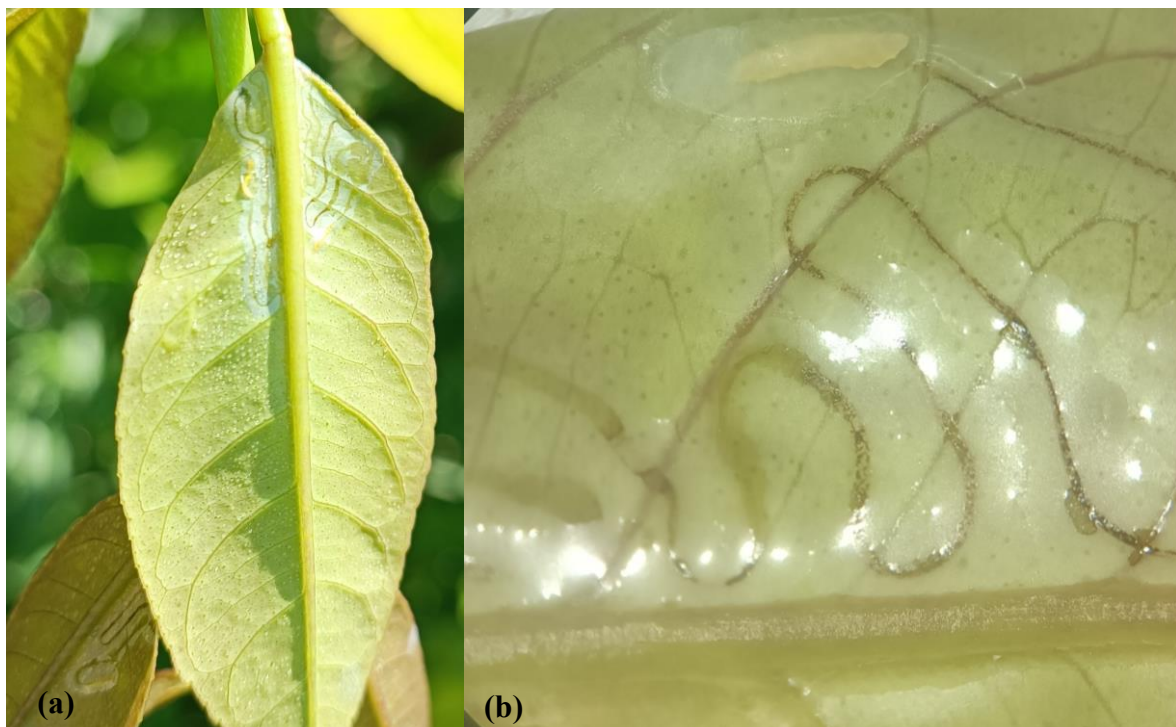
**Figure 2.3.** Life cycle of *Phyllocnistis citrella* (egg-larvae end pupae, Photo Author) and (Adult, Source: [Minadora da folhas \(\*Phyllocnistis citrella\*\) \(agrolink.com.br\)](http://Minadora da folhas (Phyllocnistis citrella) (agrolink.com.br))).

### ***Symptomatology and damage***

The damage caused by *P. citrella* larvae is recognizable, as it is evident from both the observation of galleries and the affected plant organs (Pereira, 2008). Particularly in nurseries and young orchards, the larvae stage of the citrus leafminer is notorious for its destructive behavior, as it creates serpentine-shaped galleries within the leaves (Figure 2. 4) (Carvalho & Macedo, 2015). These galleries, formed on both the adaxial and abaxial surfaces of young leaves, cause curling, chlorosis, necrosis, and stunted growth (Dantas, 2002).

The presence of this pest significantly compromises the growth and development of shoots, ultimately impacting plant productivity. Moreover, it serves as a gateway for diseases such as citrus canker, caused by the bacterium *Xanthomonas axonopodis* pv. *citri*, which exploits the lesions inflicted by the mining moth (Carvalho & Macedo, 2015). Beyond direct damage, the infestation of *P. citrella* can lead to economic losses due to reduced crop yield and quality.

Furthermore, the spread of this pest can be facilitated by environmental conditions favorable to its development. Temperature, humidity, and host plant availability all play significant roles in the population dynamics of *P. citrella* (Parra et al., 2004).



**Figure 2.4.** (a) and (b) - Leaf attacked by the caterpillar and formation of galleries in the leaf in the shape of a serpentine (Photo Author).

### ***Control methods***

Effective control of *P. citrella* begins with rapid action upon detecting the first larvae. Continuous monitoring of pest development is crucial, assessing whether the level of damage surpasses the economic threshold for intervention. The economic threshold denotes the population density at which combating the pest becomes necessary to prevent its numbers from reaching harmful levels. This critical level is the lowest density, which leads to noticeable damage (Alves, 2020). The recommended economic level of attack for citrus sprout miner on trees in production is as follows: Young or regrafted trees – 10 to 15 % of shoots with L1 and/or L2 larvae (fresh galleries, on the leaves at the ends); Mature trees (in the most important budding periods) – 20 to 55% of shoots with L1 and/or L2 larvae (AGROTEC, 2018).

Biological control aims to reduce the pest population to an economically acceptable level avoiding harmful effects to the ecosystem such as ecotoxicological effects (Oliveira et al., 2022), is the best option to control this pest (Elekciyolu, 2017). The classic effort in this practice aims at the introduction of host-specific insects within the native distribution area of the citrus leaf miner (Garcia-Marí et al., 2004). The best biological control agents in the areas of origin of *P. citrella* are parasitoids. The species considered most abundant and efficient are the encyrtide *Ageniaspis citricola* Logvinovskaya and the eulophyd *Citrostichus phyllocnistoides* Narayanan (Garcia-Marí et al., 2004; Elekciyolu, 2017).

Cultural practices play a fundamental role in *P. citrella* management. Techniques such as light pruning at the end of winter, coupled with nitrogen-rich leaf fertilization and the application of growth-regulating hormonal substances like gibberellic acid, facilitate uniform spring sprouting. Additionally, reducing watering during summer months helps deter excessive sprouting, while early fruit harvesting, particularly for late varieties, aids in pest control (Figueiredo & Lopes, 2005).

Chemical control methods rely on insecticide applications tailored to the severity of infestation and the timing of outbreaks, safeguarding emerging leaves from *P. citrella* damage. Among the insecticides recommended for combating these pests are abamectina, acetamiprida, azadiractina, metoxifenozyda tebufenozyda (DGAV, 2020).

The use of chemical products to control the population level of an insect pest, in principle, seems advantageous, because it reduces the damage in a short period of time, but in the same way that they modify the populations of the pests, they also modify the populations

of the natural control agents, in addition to the fact that the solution obtained with the use of insecticides is temporary. However, studies have shown that *P. citrella* has developed a high degree of resistance to a range of insecticides (Santos, 2009).

#### 2.2.2.2. *Trioza erytreae*

*Trioza erytreae* (Del Guercio, 1918), commonly referred to as the African citrus psylla, is a quarantine organism belonging to the order Hemiptera, family Triozidae and superfamily Psylloidea (Urbaneja-Bernat et al., 2023) (Figure 2.5).

The African citrus psylla is a sucking-biting insect that has as its exclusive host plants of the Rutaceae family, with a special preference for the genus *Citrus*. It has a particular preference for lemon trees (*Citrus lemon*) (Benhadi-Marín et al., 2021) and limes (*Citrus aurantiifolia*), and can also feed on orange, tangerine, grapefruit and cunquates (DGAV, 2017).



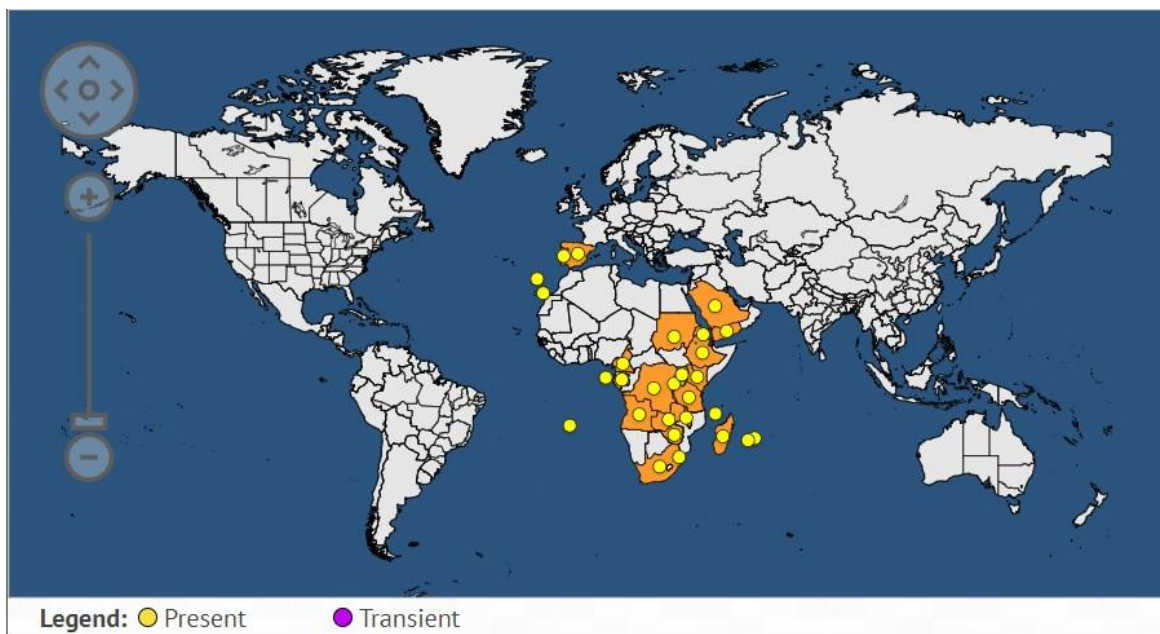
**Figure 2.5.** Adult of *Trioza erytreae* (Photo Author).

*Trioza erytreae* is of great importance in citrus because it transmits the phloem-restricted bacterium *Candidatus liberibacter* spp., the huanglongbing intermediate (HLB), the disease that causes the most devastating damage to citrus. Both the nymphs of the African citrus psylla and the adults are vectors of the bacterium, however, only the adults can spread the pathogen it causes serious damage to plants because of saliva toxicity (Urbaneja-Bernat et al., 2023).

### ***Geographical distribution***

*Trioza erytreae* occurs widely in sub-Saharan Africa and in Madagascar, Mauritius, Réunion, Saint Helena, Saudi Arabia, and Yemen (OEPP/EPPO). In recent decades, African psylla has been introduced in Europe, in Madeira (1994) and later in the Canary Islands (2002), and in 2014, it was detected in the mainland of the Iberian Peninsula ((Benhadi-Marín et al., 2021).

In 2015, it was detected in mainland Portugal in the greater Porto area (EPPO, 2015). A record of the presence of the psyllid has been observed in the following countries (Figure 2.6).



**Figure 2.6.** *Trioza erytreae* distribution map. Source: EPPO Global Database *Trioza erytreae* Distribution Map 2022-11-08 in [Trioza erytreae \(TRIZER\)\[World distribution\]](#) | [EPPO Global Database](#)

At present, the plague is currently dispersed in Asia, the Americas, Africa, and some islands in the Indian Ocean. The other common symptoms associated with HLB include yellowing of the leaves, defoliation, decrease in the abundance of roots, death of branches, production of small, irregularly shaped and bitter fruits, with a deficient sanitary condition, leading to the death of the plant. In this sense, the disease causes enormous economic costs added to considerable reductions in production, as well as in the use of chemicals (pesticides)

in sanitary treatment and in the implementation of expensive control action plans (Godefroid, 2023).

### ***Bioecology and life cycle***

The life cycle of *T. erytreae* is intricately linked with its ecological adaptations. This psyllid exhibits remarkable adaptability to various ecological conditions, ranging from equatorial to temperate climates. *T. erytreae* can thrive in diverse environments, including regions with different temperatures and rainfall patterns (Cocuzza et al., 2017).

The life cycle of *T. erytreae* consists of distinct stages: egg, nymph, and adult (Figure 2.7). Eggs are laid on young citrus leaves and hatch within 1-2 weeks (Godefroid, 2023). The nymphs undergo five instars, with development ranging from 2 to 8 weeks, depending on environmental factors such as temperature and humidity (Godefroid, 2023). During this period, they feed on phloem sap, injecting toxins into the plant tissues and inducing gall formation (Saraiva, 2021)

Adult of *T. erytreae*, characterized by their green coloration, emerge from nymphs after a developmental period of 40 to 100 days (DGAV, 2017). Females can lay up to 2000 eggs in their 30-day lifespan, contributing to the propagation of the species (Saraiva, 2021). The eggs are laid on the margins of new leaf growth and are attached by short stems (Patil, 2023). The dispersal of *T. erytreae* is limited naturally, with a maximum range of 1.5 km from citrus fruits (DGAV, 2017).

The ecological dynamics of *T. erytreae* are further elucidated by its preference for new citrus shoots, where nymphs settle and induce characteristic blistering (Magalhães et al., 2022). This dependence on citrus shoots for reproduction underscores the intricate relationship between the psyllid and its host plant.

Moreover, the life span and reproductive success of *T. erytreae* are influenced by air temperature and the nutritional status of the host plant (Aidoo, 2023). The adaptability of this species to varying environmental conditions underscores its resilience and potential for rapid population growth, with up to 8 generations per year (Alves, 2020).



**Figure 2.7.** Life cycle of *T. erytrae* -Adult, Eggs, and Nymphs, (Photo Author).

#### ***Symptomatology and damage***

The plants of the Rutaceous family are the hosts of *T. erytrae*, preferably of the genera Citrus, in particular the Lemon and Lime. However, there are other species such as Fortunella, Poncirus, and also of the genus Casimiroa, Clausena, Vepris, Zanthoxylum and Choisya (DGAV, 2016).

The characteristics of the symptoms associated with *T. erytrae*, the nymphs are largely sedentary that form conspicuous colonies, establish themselves on the underside of the young leaves where, they feed on the sap and produce open galls in the form of holes, (Figure 2.8) (OEPP/EPPO, 2005). According to the same author, *T. erytrae* can cause distorted leaves, curl, dwarfism, abrasions and chlorosis in plants. It transmits huanglongbing disease (HLB) or citrus greening, the disease of which may indicate the presence of this insect. The disease causes irregular yellow spots on the leaves, the fruits are underdeveloped, crooked, non-uniform in color, early ripening, and may have a bitter taste.

*T. erytrae* is a pest with little relevance, but when populations are excessive, there can be an imbalance in the vegetative vigor of the plant, and thus cause loss of productivity. Nymphs, like adults, produce a large amount of honeydew that can in one way lead to the appearance of fungi on the leaves (Saraiva, 2021).



**Figure 2.8.** A - Typical symptoms of leaves attacked by *T. erytreae*, B - Appearance of leaves infested by *T. erytreae* nymphs. (Photo Author)

### ***Control methods***

According to Poudel et al. (2022), among a series of available strategies and/or methods for citrus pest control, such as quarantine measures (the objective is to prevent the introduction or dissemination of psyllid to vector-free areas). Chemical control is one of the most common alternatives to keep the population at low levels of abundance. However, the indiscriminate use of pesticides can pose serious risks to human health and the environment, as well as create resistance in pests, making them less effective over time (Poudel et al., 2022). Therefore, it is important for citrus growers to use pesticides responsibly and following regulatory standards.

Integrated pest management is used control tactics, alone or associated with a strategy based on cost/benefit analyses that take into account the interest and/or impacts on producers, satiety and the environment. In this sense, with the demand of consumers for healthy foods, it requires a rationalization in the use of pesticides (Schrader et al., 2019).

Possible preventive measures should be taken to prevent the entry and spread of this pest. Prevent the entry of infected plant material, particularly those from areas where its presence has been declared. In implementing new plantations, plant material must be purchased from authorized nurseries of reliable origin where the pest is not present (DGAGPA, 2015).

In any case, the entry of the Trioza vector bacterium (*candidatus liberibacter* sp.) should be avoided, since the conjunction of both agents is a very serious problem for citrus farming.

The General Directorate of Agriculture and Veterinary (DGAV), in order to prevent the rapid spread of citrus fruit (*T. erytraea*) throughout the country, has issued some measures for its control and immediate prevention, which have been adopted and implemented by citrus producing regions (Alves, 2020).

According to the same author, for the control and monitoring of the pest, an area called the Demarcated Zone was created, which consists of the infested zone and the buffer zone, a surrounding area with a radius of three kilometers, with or without the presence of the pest or even its symptoms.

All branches with symptoms of the pest should be cut off, and the plant material infected by the pest should be immediately buried or burned. In areas where the presence of these insects is confirmed, a phytosanitary treatment should be carried out with approved neonicotinoid products (insecticides), based on acetamiprid (EPIK SG), imidacloprid (CONFIDOR O-TEQ and NUPRIDE 200 SL) and thiamethoxam (ACTARA 25 WG), for the control of *T. erytraea* (IVDP, 2015).

According to the same author, the application of these products should coincide with the burst periods, especially late winter – spring and autumn, only in the affected areas and in neighboring areas.

Always keep records of the treatments, the respective products, the dose and the dates of their application. In regions belonging to buffer zones. It is mandatory for owners to monitor the orchards and report the presence of some symptoms, and above all, to avoid the displacement of any plant or part of a plant from the crop (branches, leaves, peduncles – except fruits and seeds). For the monitoring of these insects, the use of yellow chronotropic traps is advised (Alves, 2020).

Another pest control alternative is biological control, which consists of the use of natural predators that can be native or introduced, parasitoids or entomopathogens to control or limit pest populations (Pérez-Rodríguez et al., 2021). For the same authors, this technique has proven to be an efficient and more sustainable option than the use of chemical pesticides, as it does not leave toxic residues in the environment, does not harm biodiversity and helps preserve plant health.

### **2.2.2.3. *Toxoptera citricida***

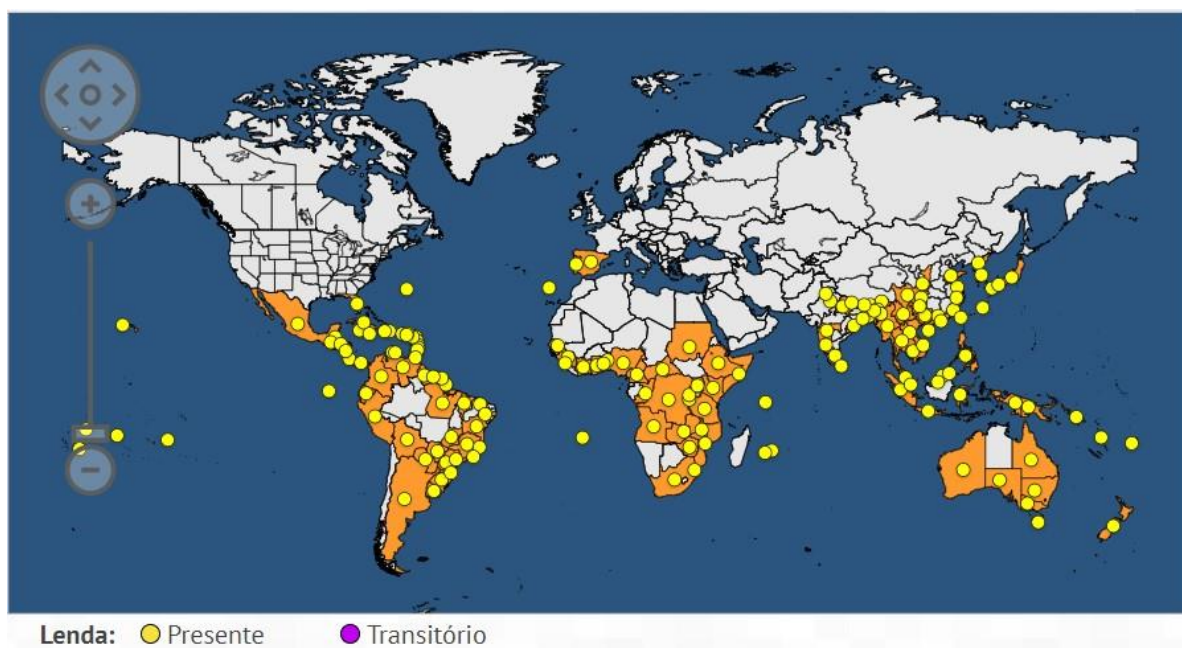
*Toxoptera citricida* (Kirkaldy, 1907), commonly known as aphids or aphids, of the order Hemiptera, constitute the superfamily Aphidoidea, of which the family Aphididae is the largest and most common (Toledo, 2006). Are important pests in citrus as vectors of the phytopathogenic virus *Citrus tristeza virus* (CTV) Persad & Hoy (2004), with the winged forms being the most efficient (Rocha et al., 2008). It is one of the main pests of citrus trees such as: lemon (Sun et al., 2022).

The citrus black aphid measures about 2 mm in length and can usually form large colonies mainly on young plants and plant shoots (Teodoro et al., 2014). The same author reports that these same colonies are formed by adults and nymphs, and the adults can become rough and/or winged in which they are responsible for dispersal. Females reproduce without the participation of males, by asexual reproduction, giving rise to nymphs that will give rise to other females.

#### ***Geographical distribution***

*Toxoptera citricida* Kirkaldy, probably originating in Southeast Asia, is now widely found in the humid tropical areas of Asia, Australia, Africa, South America, and North America (Figure 2.9). This species feeds mainly on citrus fruits but occasionally attacks other Rutaceae (Mou et al., 2017).

It was first detected in Portugal in 1994 on the island of Madeira and on the mainland in 2003. It was also detected and identified in several parishes located in the intervention area of the Regional Directorate of Agriculture and Fisheries of Lisbon and Vale of Tejo (DRAPLVT) (SNAA, 2022).



**Figure 2.9.** Distribution of *Toxoptera citricida kirkaldy* (citrus aphid) worldwide. Source: EPPO 2022-10-13 *Aphis citricidus* (TOXOCI)[World distribution]| EPPO Global Database.

### ***Bioecology and life cycle***

Aphids, in their almost totality, go through four nymphal stages, becoming Adults After the 4th Instar (Toledo, 2006).

According to Jeger et al. (2018) and Schrader et al. (2019), this insect's life cycle is intimately tied to the phenology of citrus plants and the environmental conditions of its habitat (Leyva & Flores, 2013). *T. citricida* exhibits a reproductive cycle involving parthenogenetic females, males, and sexual females, with parthenogenetic females giving birth to nymphs that perpetuate the cycle (Santos, 2011; Teodoro et al., 2014).

In terms of ecology, *T. citricida's* feeding habits significantly impact citrus trees. Adults and nymphs feed on tender shoots, leaves, and flower buds, causing direct damage such as twisted shoots and wrinkled leaves. The production of honeydew by the insects attracts ants and promotes fungal growth, further affecting tree health and fruit quality (Jeger et al., 2018).

The dynamic interplay between the insect's life cycle and its ecological interactions underscores the importance of understanding both aspects for effective management strategies. By comprehending how environmental factors influence the insect's behavior and reproductive patterns, as well as its impact on citrus plants, researchers and growers can develop targeted approaches to mitigate damage and preserve crop yields.

### ***Symptomatology and damage***

The black aphid, due to its attack, causes dwarfism, curling of leaves and shoots, retardation of growth, of shoots and especially in young plants and reduces flowering (Figure 2.10). In the feeding process, the insects excrete honeydew, a sweet substance in plants that develops fungi, this honeydew attracts ants to the young shoots (Teodoro et al., 2014; SNAA, 2022). Aphids cause reduced photosynthesis and are vectors of other pathogens such as the Citrus tristeza virus (CTV) (SNAA, 2022).



**Figure 2.10.** Leaf attacked by *Toxoptera citricida* kirkaldy (Photo Author).

### ***Control methods***

*Toxoptera citricida*, is a quarantine body in the European Union (EU), in this sense, its presence requires the application of necessary phytosanitary measures in control and eradication in order to prevent its dispersion (Jeger et al., 2018).

The control of *T. citricida*, the natural factor such as rain which is important in the mortality of these pests and the other is the natural enemies that help in biological control (the auxiliaries) (SNAA, 2022). There are predators that can be native or induced, parasitoids or entomopathogens that can be used to control or limit the population of this pest (Pérez-Rodríguez et al., 2021). For the same authors, this technique has proven to be an efficient and more sustainable option than the use of chemical pesticides, as it does not leave toxic residues in the environment, does not harm biodiversity and helps preserve plant health. One of the ways to control the appearance of this pest in citrus crops is the study of the natural enemies of *T. citricida*. Some of these studies have focused on predatory insects, mainly on beetles (Coccinellidae) and dipterans (Diptera: Syrphidae) as the insects that cause the highest mortality in black aphid populations (Toledo, 2006).

As a cultural method, we have to cut the branches affected by the insect and destroy them by burning, crushing or burying them in the same place as the plant debris and not moving them (branches, leaves, stalks) to other places because they may be part of host plants. And the chemical one, to carry out phytosanitary treatments on plants infected with authorized plant protection products (SNAA, 2022). Chemical control is carried out with pesticides registered in Mapa (AGROFIT, 2014) and the level of control is suggested when 10% of orchard plants are in formation or more than 10% of adult plants present the pest, (Teodoro et al., 2014).

# CHAPTER 3

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



# 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.

### 3.1. 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, Perdikis, & Lykouressis, 2011). Its rapid global dissemination reached Portugal in the 1990s, first reported in the country's south and subsequently in 1995 in citrus orchards in the Northern part of the country (Alves, 2020).

Adults of *P. citrella* are small moths, measuring approximately 2mm in length and 4mm in wingspan and displaying a white to silvery hue with distinctive wing characteristics (Parra et al., 2004). Females oviposit single eggs on the young, tender citrus leaves about 24 hours 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 & Braham, 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 undergoes a transition into the prepupal stage, characterised by a cessation of feeding behaviours (Heppner & Fasulo, 2010). 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 cycle spans 11.5 to 32.7 days, with up to 14 annual generations in tropical conditions (Parra et al., 2004; Dantas, 2002).

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 (Carvalho et al., 2017; 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 (Elekçioğlu & Uygün, 2006; Hall et al., 2010; Atiq et al., 2013; Elekçioğlu & Uygün, 2013).

The spatial pattern of an arthropod species, it is an inherent and constant characteristic of the species (Taylor, 1984). The knowledge of such patterns, especially species considered as

pests, holds paramount significance, especially within the domains of agriculture and pest control. Since the understanding of spatial distribution patterns of a pest is crucial for the formulation and application of effective sampling, monitoring, and control strategies (Liu et al., 2008; Benhadi-Marín et al., 2021; He et al., 2022; Padala et al., 2023).

The development of a 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 (Pedigo, 1994; Boeve & Weiss, 1998; Southwood & Henderson, 2000; Arnaldo & Torres, 2005). This critical sampling framework finds applications in various branches, including ecological investigations the study of population dynamics (Jarosik 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. These plans have demonstrated significant efficiency gains compared to traditional sampling approaches, providing a practical and resource-efficient approach to monitor and control citrus leafminer. Therefore, the objectives of this work were as follows: (a) Study the population fluctuations of males' adults of *P. citrella* in a 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.

## 3.2. Material and methods

### 3.2.1. 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 four 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).

For the surveillance of adult citrus leafminer populations, a Delta-type trap equipped with a sexual pheromone (BIOSANI, Portugal) was strategically placed at a central point of each plot. A total of five Delta-type traps were deployed across the study area. Weekly observations were conducted from May 2023 to December 2023, during which the number of captures in each trap was recorded. The pheromone was regularly replaced in accordance with the manufacturer's guidelines.

During the surveillance period, a phytosanitary treatment was administered on June 16, 2023. Align (SIPCAM, Portugal), a plant-based insecticide containing azadirachtin as its active substance, and SEQURA TOP (SIPCAM, Portugal), a biological insecticide consisting of crystals of endotoxins and viable spores of *Bacillus thuringiensis*, subspecies *kurstaki*, serotype 3a, 3b, strain HD-1, were employed.

### 3.2.2. Data analyse

#### 3.2.2.1. 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 underwent testing against the negative binomial distribution ( $\alpha = 0.01$ ), as outlined by Waters (1959), utilising the `fitdist` and `gofstat` functions from the `fitdistrplus` package (Delignette-Muller & Dutang, 2015).

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. As k approaches zero, it signifies an aggregated distribution, while k approaching infinity suggests a more random distribution, as discussed by (Waters, 1959).

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:  $m^* = \alpha + \beta \bar{X}$ ,  $\alpha$  and  $\beta$  are the Iwao's parameters and  $m^*$  is the Lloyd index, Lloyd (1967), calculated as:  $m^* = \bar{X} + (S^2 / \bar{X} - 1)$ . Then of the parameter  $\beta$  is set to 1, it denotes a random distribution. A value of  $\beta$  greater than 1 indicates an aggregated distribution, while  $0 < \beta < 1$  suggests a uniform distribution.

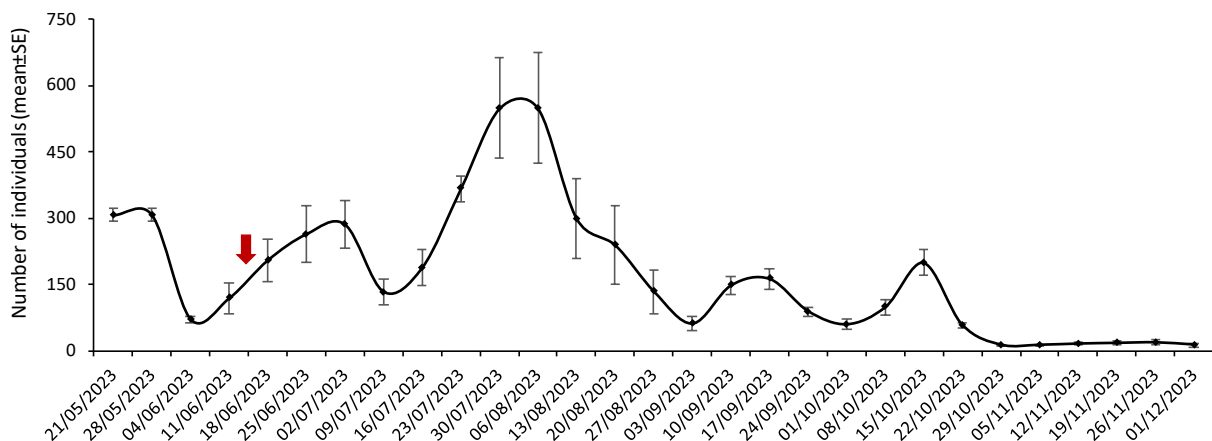
Regarding the Taylor's law, as outlined by Taylor & Woiwod (1980), characterises the relationship between the logarithm of the variance ( $\text{Log}(S^2)$ ) and the logarithm of the mean ( $\text{Log}(\bar{X})$ ) through the equation:  $\text{Log}(S^2) = \text{Log} \alpha + \beta \text{Log}(\bar{X})$ , in this equation,  $\alpha$  represents the intercept,  $\beta$  denotes the slope of the regression line. When  $\beta < 1$ ,  $\beta = 1$ , and  $\beta > 1$ , it signifies a uniform, random, and aggregated distribution, respectively. The extent at which  $\beta$  deviates from 1 was assessed using a t test.

### 3.2.2.2. 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 (Green, 1970):  $N = (\alpha + \bar{X}^{\beta-2}) / D^2$ . N is the number of required samples,  $\alpha$  and  $\beta$  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.

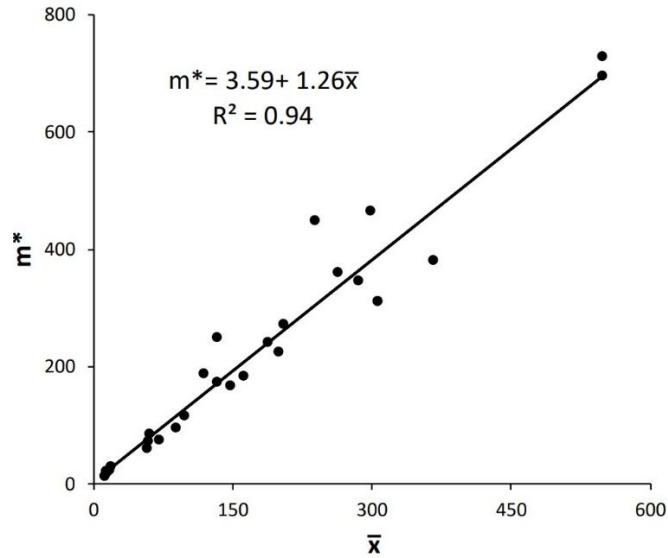
### 3.3. Results

A total of 24528 male adults of *P. citrella* were captured throughout the sampling period. The population dynamics of *P. citrella* exhibited five density peaks (Figure 3.1). One in late May, another in early July, followed by one in late July to early August, one in mid-September, and another in mid-October. The highest population peak was recorded between July 30 and August 6 ( $548.75 \pm 113.62$  and  $548.93 \pm 125.81$ , respectively). The population started to drop gradually in 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 June 16, did not demonstrate any discernible effect on the population of *P. citrella*.

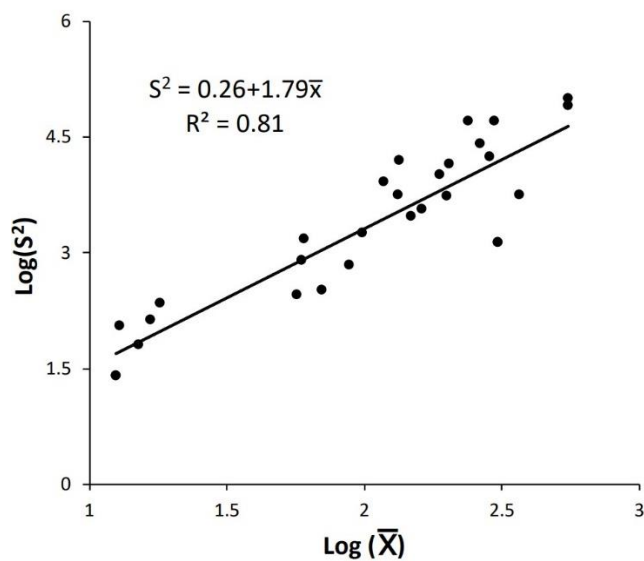


**Figure 3.1.** Mean number ( $\pm$ SE) of male adults of *Phyllocnistis citrella* captured using delta traps. Red arrow means day of treatment.

In general, the data fitted a negative binomial distribution according to the goodness of fit test and Iwao's patchiness regression method (Table 1). The various aggregation indices employed to evaluate the spatial pattern revealed a consistent extent of distribution. The parameters of the Iwao regression were  $\alpha = 3.59$  and  $\beta = 1.26$  ( $R^2 = 0.94$ ;  $t = 6.33$ ;  $p < .001$ ) (Figure 3.2), and since the variances and means were significantly related according to Taylor's power law ( $\alpha = 0.26$  and  $\beta = 1.79$  ( $R^2 = 0.81$ ;  $t = 25.34$ ;  $p < .001$ )) (Figure 3.3), suggesting that in general *P. citrella* it has aggregated distribution.



**Figure 3.2.** Relationship between the mean crowding index of Lloyd ( $m^*$ ) and the mean ( $\bar{X}$ ) of male adults of *Phyllocnistis citrella* captured using delta traps.



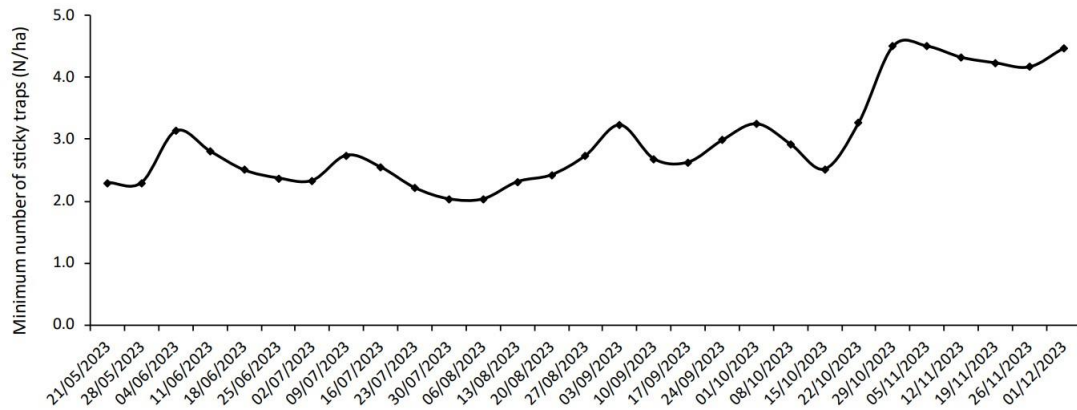
**Figure 3.3.** Regression between the log variance ( $S^2$ ) and the log mean ( $\bar{X}$ ) of male adults of *Phyllocnistis citrella* captured using delta traps according to Taylor's law.

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.

**Table 1:** Mean ( $\bar{x}$ ) and variance ( $S^2$ ) of the captured male adults of *Phyllocnistis citrella*. goodness of fit test for the negative binomial distribution ( $\chi^2$ ; P), k parameter of the negative binomial distribution (k), Lloyd's index of patchiness (Lloyd), mean crowding index ( $m^*$ ).

Date	$\bar{x}$	S2	$\chi^2$	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

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 between 2 to 3 traps per hectare, except for the month of November, where 4 to 5 traps are needed (Figure 3.4).



**Figure 3.4.** Minimum number of sticky traps (N/ha)

### 3.4. Discussion

The findings of this study provide a comprehensive understanding of the population dynamics and spatial distribution of *P. citrella* 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 Algeria (Mitidja) *P. citrella* has been reported to have four generation (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 to nine to 13 (Lal 1950), 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; Dantas, 2002). 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 suggest a critical period of increased activity, which aligning with existing literature (Dahmane & Chakali; 2022, Patel et al. 1994; Mansour et al., 2021; Garcia-Mari et al., 2002). 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 (Mafi & Ohbayashi, 2006; Whalon et al., 2008; Faskha et al., 2022). Additionally, because of its multiple generations per year, frequent applications of insecticides are required to enhance 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 (Mafi & Ohbayashi, 2006; Faskha et al., 2022).

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* also have 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 considered a strategic response to avoid unfavourable conditions (Allee, 1926). Furthermore, it is worth noting that aggregation behaviour is typical among various species of Lepidoptera (Sétamou et al., 2000; Cocco et al., 2014; Sujithra & Chander, 2016; He et al. (2022); Karimzadeh et al., 2023), 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 suggest 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 as like others citrus species show

different flushing patterns throughout the year; thus, interspecific differences in 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 to 3 Delta-traps with pheromone per hectare during periods of high population density and increase 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 (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 incorporating of specific pheromones transcend the limitations of direct visual observation. They provide the capacity to systematically cover a larger spatial expanse efficiently, and are more objective and quantifiable *modus operandi*, enhancing the sensitivity of detection even during the incipient phases of infestation (Vanaclocha et al., 2016).

### **3.5. Conclusion**

Our 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.



# CHAPTER 4

*Phyllocnistis Citrella* (Lepidoptera: Gracillariidae)  
Infestation Rate in Lemon Orchards in Northern  
Portugal

# ***Phyllocnistis citrella* (Lepidoptera: Gracillariidae) Infestation Rate and plant distribution pattern in Lemon Orchards in Northern Portugal**

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## Abstract

The citrus leaf miner, *Phyllocnistis citrella*, poses a significant threat to citrus fruit cultivation worldwide, with its detrimental impact on citrus orchards well-documented. The direct damage caused by its larvae creating galleries in the leaves, along with increasing the plants' susceptibility to citrus canker disease, makes it essential to understand the infestation dynamics of this pest and develop effective management strategies. Given the pervasive nature of the citrus leaf miner, understanding its infestation dynamics becomes imperative for devising efficient pest management strategies. Therefore, the present study aimed to investigate the infestation dynamics of the citrus leaf miner in lemon orchards in the northern region of Portugal. To achieve the research objective, two lemon orchards located in the Ribela, Vila Nova de Famalicão, Braga, Portugal, were sampled. New shoots with leaves smaller than 3 cm and greater equals the 3 cm were collected and subsequently examined in the laboratory to identify the presence of eggs, larvae, pupae and galleries of *P. citrella*. Intriguing patterns of *P. citrella* infestation emerged, with a noticeable trend of increasing infestation of shoots and leaves observed over time. Particularly, higher infestation rates were observed in smaller leaves, raising concerns about the pest's impact on citrus productivity. Surprisingly, despite applying insecticides, we found no significant reduction in infestation levels, indicating potential inefficiencies in the current control strategies employed. The findings of this study underscore the pressing need for a more nuanced understanding of the infestation dynamics of the citrus leaf miner in lemon orchards. Infestation Rate Identification and factors influencing insecticide efficacy provides valuable insights for developing more targeted and effective integrated pest management strategies. Moreover, the study highlights the importance of continuous monitoring and adaptation of control measures to effectively mitigate the impact of *P. citrella* infestation on citrus fruit production.

**Keywords:** *Citrus Leafminer*, Population Dynamics, Integrated Pest Management,

## 4.1. Introduction

The citrus leaf miner, *Phyllocnistis citrella* Stainton, 1856, stands as a formidable menace to citrus fruit cultivation, as documented by various studies (Awalekar et al., 2020; Ullah et al., 2023). Its initial sighting in Portugal in 1994, notably in the Conceição de Faro area, marked the beginning of a swift spread to citrus orchards in Northern Portugal within a year (Lopes et al., 2008; Alves, 2020). This invasive pest causes destruction through direct and indirect damage to citrus crops, impacting leaves, fruits, and branches alike (Ribeiro, 2002). Furthermore, its presence exacerbates citrus plants' susceptibility to infestation by the citrus canker bacterium, *Xantomonas axonopodis* pv. Citri (Ribeiro, 2002; Carvalho & Macedo 2015).

The intricate life cycle of *P. citrella*, spanning from translucent eggs to the adult stage composed of bright microlepidoptera, underscores its remarkable adaptability and widespread presence throughout various parts of the plant. Their activities, ranging from egg-laying on shoots to forming galleries mined by larvae, profoundly compromise plant growth, directly impacting citrus productivity (Pereira, 2008; Carvalho & Macedo, 2015).

Observing subepidermal leaf galleries facilitates the recognition of symptoms caused by citrus miners. As they feed and progress on the epidermal surface, larvae leave behind a distinctive serpentine pattern with traces of excrement. Upon concluding the feeding period, they migrate towards the leaf margin through folds, a conducive site for transitioning from the larval to the pupal stage (Lopes et al., 2008).

Plant species' morphological characteristics play a pivotal role in insect pest colonization. *Phyllocnistis citrella* exhibits a close relationship with the phenological status of its citrus host, with population densities often correlating with new bursts of hosts. Leaves colonized by *P. citrella* manifest deformations, escalating severity in younger leaves and intensifying with higher infestation rates (Lopes et al., 2008). The detrimental impact extends beyond visible symptoms, encompassing a reduction in the plant's photosynthetic capacity, consequently retarding the growth of young trees and diminishing fruit production (Awalekar et al., 2020; Loureiro et al., 2020). Given its remarkable flight capacity and high reproduction rate, this pest poses a substantial threat to citrus plantations, with multiple annual generations flourishing in conducive environments (Dantas, 2002).

This study aims to investigate the infestation dynamics of the citrus leaf miner in lemon orchards in the northern region of Portugal. Specifically, it seeks to identify infestation patterns, assess factors influencing insecticide efficacy, and explore opportunities to enhance integrated pest management strategies for mitigating the impact of *P. citrella* infestation on citrus fruit production.

## **4.2. Material and methods**

### **4.2.1. Study area**

The study was carried out in a lemon orchard located in Ribela, Vila Nova de Famalicão, district of Braga, Portugal in two plots, one with the coordinates (41°26'34.3"N 8°30'16.7"W and the other with coordinates 41°26'39.7"N 8°30'15.7"W). Both plots contained lemon trees of the "Lunario" variety, which were approximately four years old since planting and had an average height of two meters.

The selection of the orchards took into account the application of good agricultural practices and the production method used with a preference for orchards in Organic Production Mode (MPB), without the application of chemical synthesis products and Integrated Protection (IPM) modes, where the application of phytosanitary products is carried out in a sustainable way, and the vegetation cover is maintained for a large part of the year.

A phytosanitary treatment was administered during the surveillance period on 16 June 2023. Align (SIPCAM, Portugal), a plant insecticide containing azadirachtin as an active substance, and SEQURA TOP (SIPCAM, Portugal), a biological insecticide composed of endotoxin crystals and viable spores of *Bacillus thuringiensis*, subspecies *kurstaki*, serotype train HD-1.

### **4.2.2. Sampling of Lemon Trees**

In each plot, ten lemon trees were randomly selected for sampling. In every ten trees, the branches distributed in the respective cardinal points were observed for the absence and presence of *P. citrella*. In addition, in another 10 trees, five in each plot, five random shoots were collected from the trees and carefully transported to the laboratory for examination under a stereo microscope. In the laboratory, these branches were divided into leaves with a length of less than 3 cm and leaves with a length greater than 3 cm. These leaves were observed under a

microscope to observe the presence of eggs, larvae, pupae and galleries of *P. citrella*. Sampling was conducted weekly from March to October 2023. In addition, in each aerial part collected, the number of leaves was counted to allow the calculation of the leaf infestation rate per aerial part (Mustafa et al., 2014).

#### 4.2.3. Data Analysis

The data were tested for normality using the Shapiro-Wilk test and normalized using square root transformation when necessary. The data were analyzed by unidirectional analysis of variance (ANOVA) to verify the difference between leaf size and cardinal group for *P. citrella* infestation. Means were compared using the paired Tukey HSD comparison test.

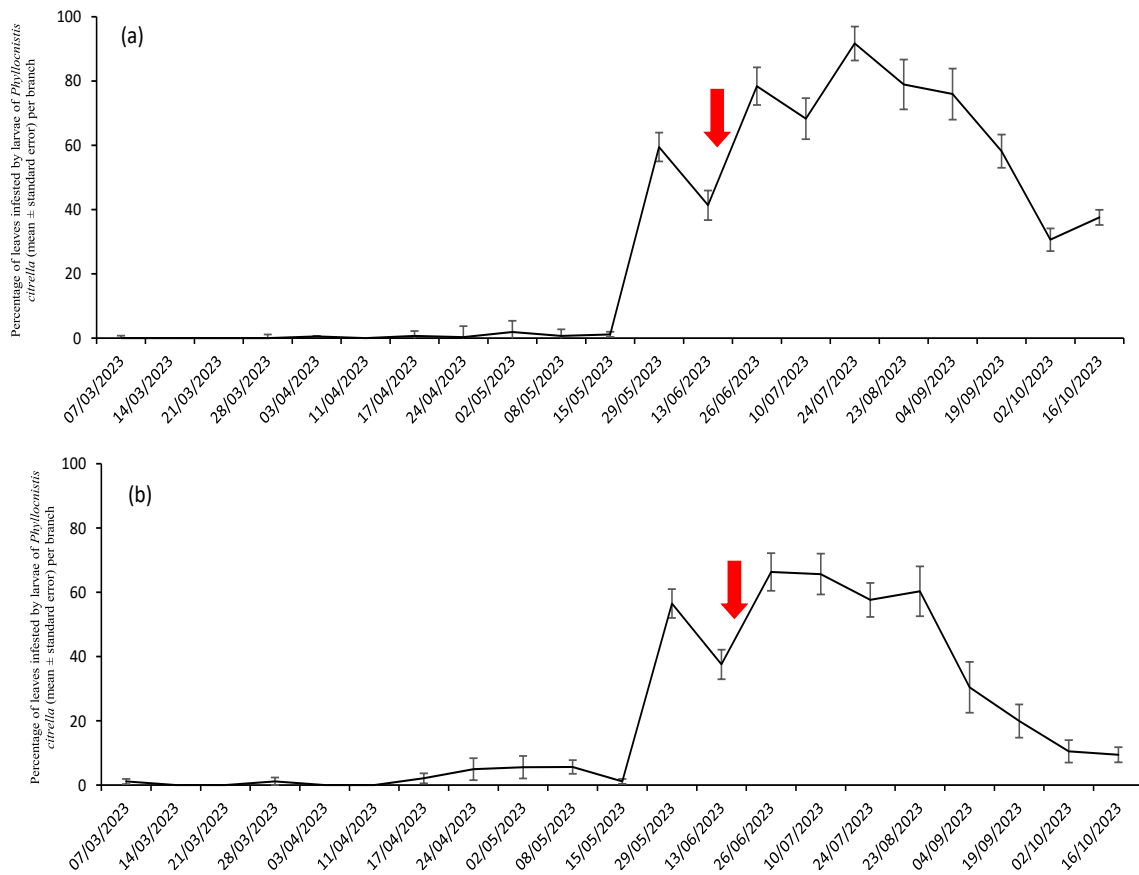
### 4.3. Results

In this study, we unveiled valuable insights into the infestation of *P. citrella* in the two lemon orchards situated in Ribela, Vila Nova de Famalicão, Braga, Portugal. Systematic sampling of trees and shoots revealed intriguing patterns regarding this pest's presence.

On average, the sampled shoots exhibited  $5.114 \pm 0.07$  leaves measuring less than 3 cm and  $3.99 \pm 0.07$  leaves measuring 3 cm or more. During the sampling period, we didn't observe eggs on the leaves, probably due to their translucent appearance, which makes them difficult to identify (Parra et al., 2004).

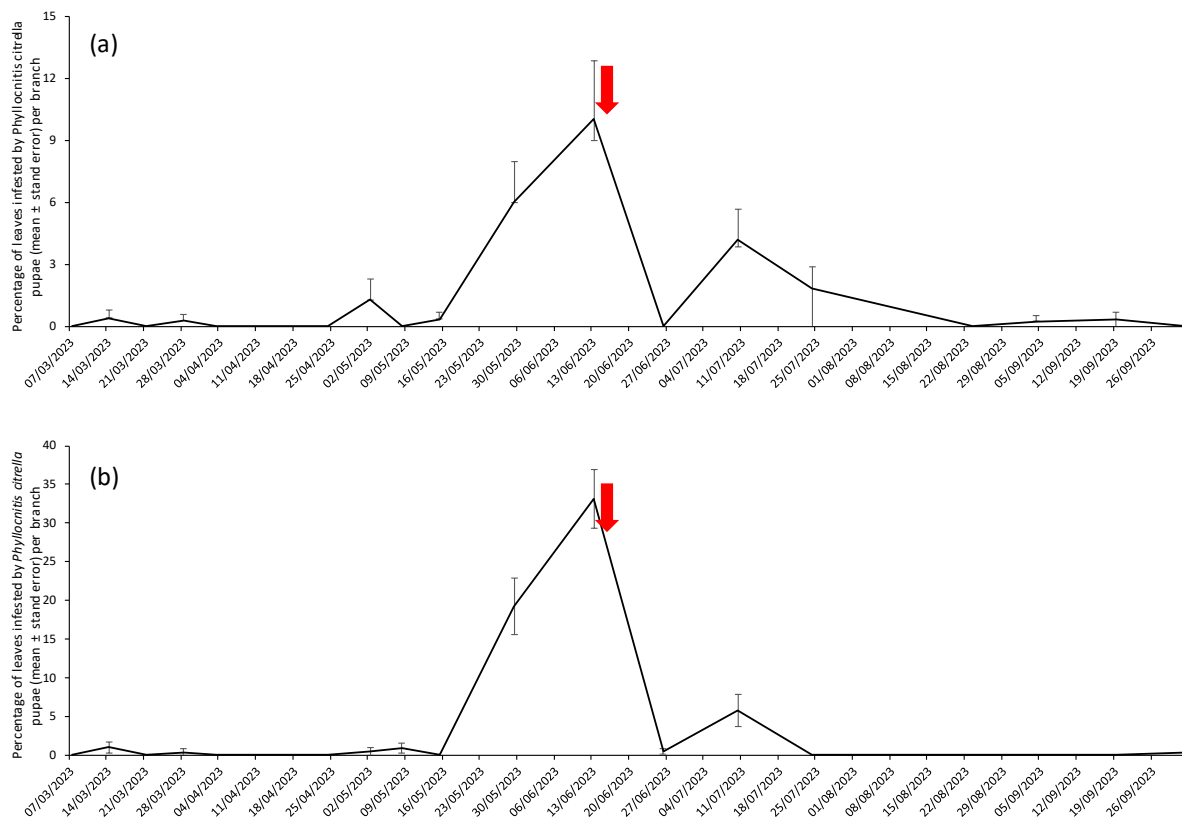
The lemon tree branches exhibited leaves infested with larvae of *P. citrella* right at the beginning of sampling. However, the infestation rate was very low, with only 1% of the branch leaves being infested with larvae (Figure 4.1). Both shorter and longer leaves, measured at less than 3 cm and 3 cm or more, respectively, began to show infestation rates surpassing 50% as of May 15, 2023. Subsequent sampling dates indicated a gradual increase in infestation rates. However, a gradual decline in the infestation rate was observed starting from September 4, 2023 (Figure 4.1).

Overall, leaves measuring less than 3 cm in length exhibited significantly higher larval infestation rates of *P. citrella* than those measuring 3 cm or more ( $F=47.82$ ;  $df=1$ ;  $P<0.001$ ). The application of the two insecticides on June 16, did not demonstrate any discernible effect on the infestation rate of *P. citrella* (Figure 4.1).



**Figure 4.1.** Percentage of leaves with length (a) less than 3 cm and (b) equal to or higher than 3 cm infested by larvae of *Phyllocnistis citrella* (mean ± standard error) per sampled branch. The red arrow means a day of treatment.

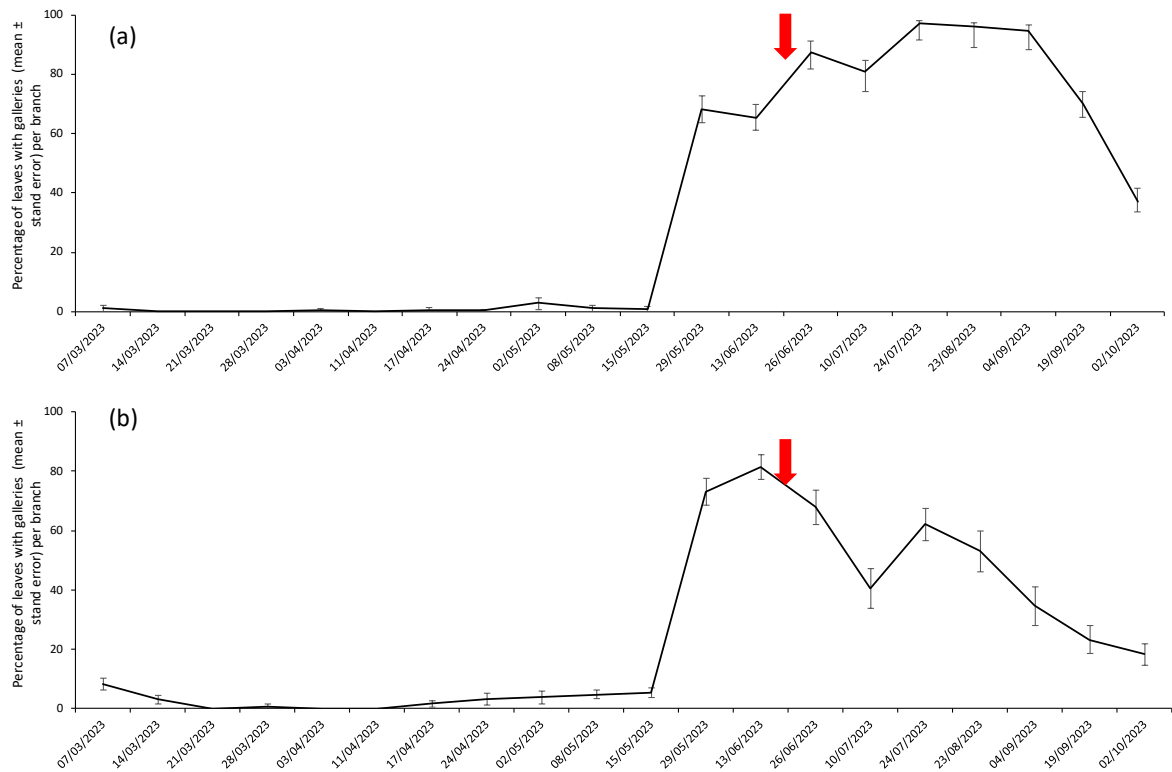
Concerning pupae, a consistent pattern emerged across leaves measuring less than 3 cm and those measuring 3 cm or more. The peak infestation occurred especially on June 13th. Notably, a higher abundance of pupae was observed on leaves measuring 3 cm or more (Figure 4.2).



**Figure 4.2.** Percentage of leaves with length (a) less than 3 cm and (b) equal to or higher than 3 cm infested by pupae of *Phyllocnistis citrella* (mean  $\pm$  standard error) per sampled branch. The red arrow means a day of treatment.

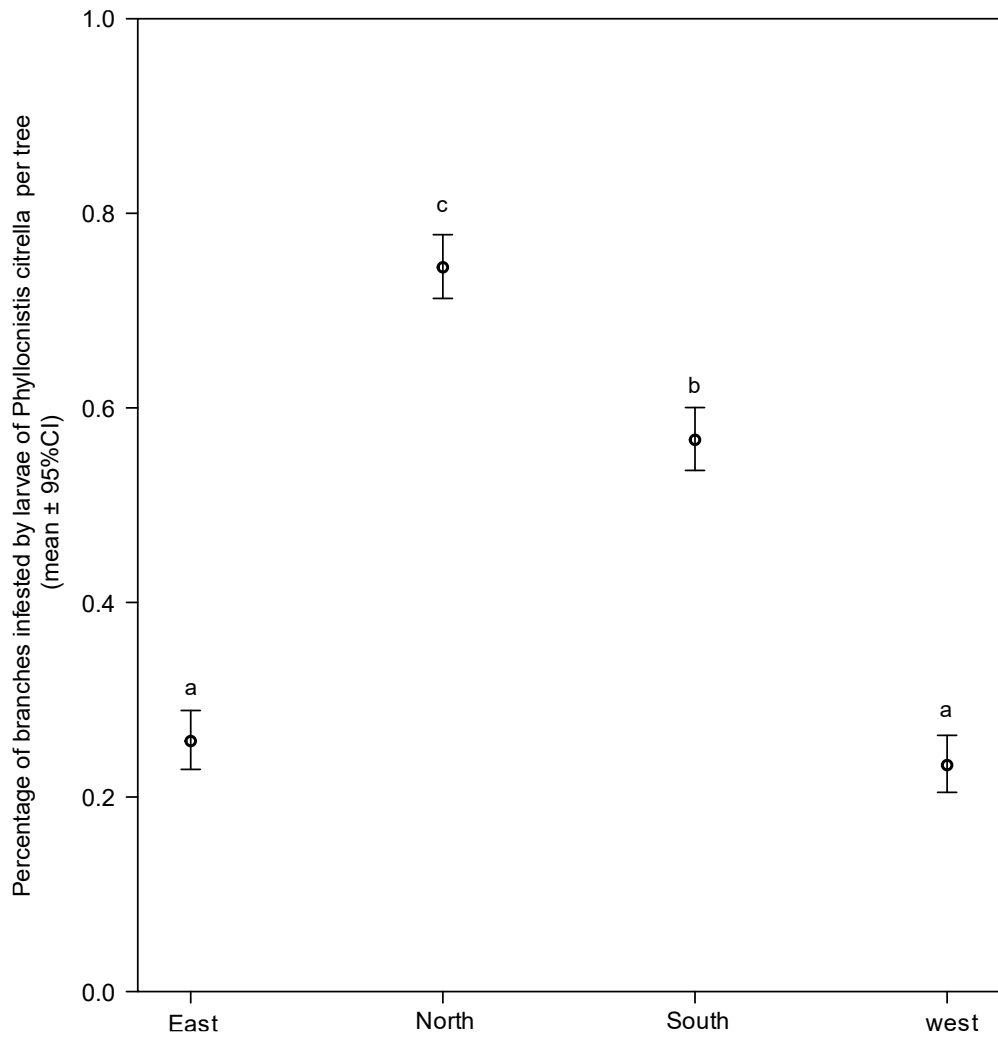
From the second week of May onward, there was an exponential rise in the percentage of leaves exhibiting galleries, a trend observed consistently across leaves measuring both less than and equal to or greater than 3 cm (Figure 4.3).

Leaves measuring less than 3 cm reached a peak infestation of 96% (on 24/07/2023), whereas those equal to or greater than 3 cm showed a maximum of 78% (on 13/06/2023) of affected leaves.



**Figure 4.3.** Percentage of leaves with length (a) less than 3 cm and (b) equal to or higher than 3 cm with galleries (mean  $\pm$  standard error) per sampled branch. The red arrow means a day of treatment.

The infestation rate of branches varied significantly among cardinal points ( $F=30$ ;  $df=3$ ;  $P<0.001$ ). A higher infestation rate was observed in the north, followed by the south. East and West exhibited the lowest infestation rates among the cardinal points (Figure 4.4).



**Figure 4.4.** Percentage of branches infested by *Phyllocnistis citrella* (mean  $\pm$  standard error) in the different cardinal points.

#### 4.4. Discussion

Our results provide valuable insights into the infestation dynamics of *P. citrella* in lemon orchards in northern Portugal. The observed patterns of *P. citrella* infestation on lemon tree shoots align with findings reported in the literature (Garcia-Marí et al. 2002; Kheder et al. 2002, Caleca et al. 1998). The results indicating higher infestation rates from mid-May 2023 onwards on shorter leaves data that is consistent with studies by Garcia-Marí et al. (2002) and Kheder et al. (2002), which suggest that leaf flushes during the summer-autumn seasons are more susceptible to *P. citrella* damage compared to those appearing in the spring. Similarly, Caleca et al. (1998) noted a delay in infestation onset until the second half of June for spring

flushes in Sicily, Italy. Likewise, Lo Pinto and Fucarino (2000) noted that this lepidoptera is active during the summer and fall months. This supports the notion that seasonal variations influence *P. citrella* infestation dynamics, with spring flushes being relatively spared.

Our study revealed a peak pupae infestation, notably on June 13th, with a higher abundance observed on larger leaves (3 cm or more). This insight could be essential for optimizing pest management strategies, such as targeted pesticide applications or biological control measures. Furthermore, the exponential increase in the percentage of leaves exhibiting galleries from the second week of May indicates a rapid infestation spread within the orchard. Investigating the factors driving this rapid spread, such as pest behavior and environmental conditions, could inform the development of more effective management strategies.

The results presented indicate a concerning trend of infestation by the *P. citrella* over the observation period. The infestation of leaves with galleries, especially in young plants and nurseries, is a serious issue due to the direct damage caused by the larvae creating serpentine galleries in the leaves (Pereira, 2008). In addition to direct plant damage, infestation by the citrus leafminer can further compromise plant productivity as it creates an entry point for diseases such as citrus canker (Carvalho & Macedo, 2015). It is important to address this infestation in an integrated manner, combining physical, cultural, and biological control methods and judicious use of insecticides when necessary. Furthermore, monitoring environmental conditions favoring the insect's development is crucial to implement appropriate preventive measures. Effective control of the citrus leafminer is essential to prevent significant economic losses due to reduced fruit production and quality.

The ineffectiveness of the insecticide application on June 16 in reducing the infestation rate of *P. citrella* raises concerns about the control method's efficacy or the timing of its implementation. Several factors could contribute to this: (i) firstly, resistance development in *P. citrella* populations could render the insecticides ineffective (Mafi & Ohbayashi, 2006; Faskha et al., 2022). Using the same insecticides without rotation or incorporating alternative methods can accelerate resistance; (ii) secondly, the insecticides' formulation may not have been suitable for effectively targeting *P. citrella*. Different insecticides have varying modes of action, and selecting the appropriate formulation is crucial for successful control (Mafi & Ohbayashi, 2006; Faskha et al., 2022). (iii) thirdly, the timing of insecticide application may not have aligned with the peak activity of *P. citrella* larvae or pupae, reducing its effectiveness (Yumruktepe et al., 1996). Precision in timing is essential to target vulnerable stages of the

pest's life cycle. Moreover, environmental factors such as temperature, humidity, and rainfall can influence insecticide efficacy (Mao et al., 2019).

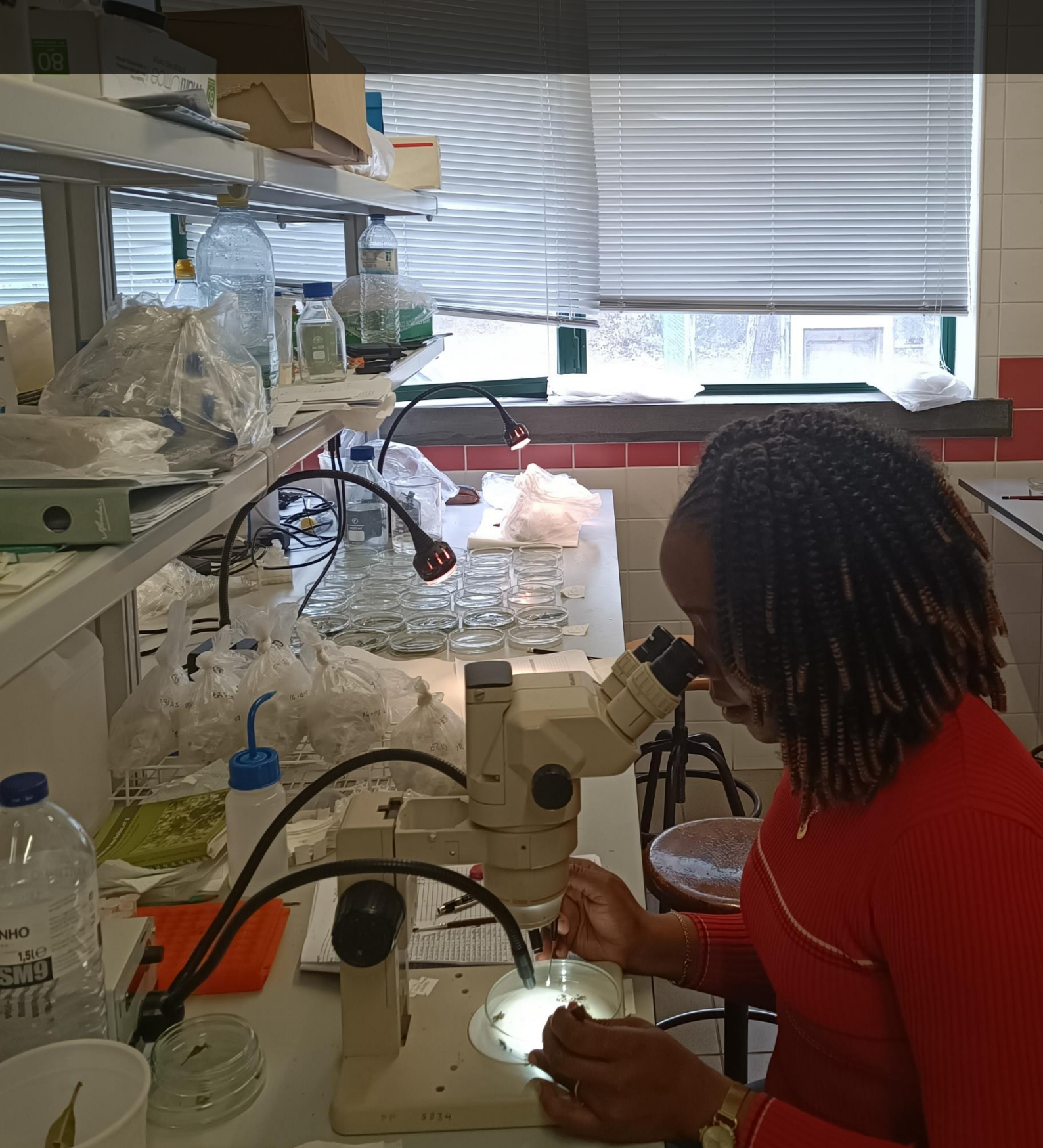
## **4.5. Conclusion**

In conclusion, this study provides valuable insights into the infestation dynamics of *P. citrella* in lemon orchards in northern Portugal. The observed infestation patterns are aligned with previous research, highlighting seasonality as a significant factor in lemon tree susceptibility to infestation. The identification of a peak pupal infestation, especially on June 13th, and its correlation with leaf size suggest opportunities to enhance pest management strategies, such as targeted pesticide applications or biological control measures.

Furthermore, the ineffectiveness of the insecticide application on June 16 raises concerns about the efficacy or timing of control methods. Resistance development in *P. citrella* populations, insecticide formulation suitability, and precision in application timing emerge as critical areas to address in future management strategies.

# CHAPTER 5

## General Conclusions and Future Perspectives



## 5. General Conclusions and Future Perspectives

The study of *P. citrella* population dynamics and spatial distribution in lemon orchards in Northern Portugal sheds light on critical factors influencing infestation patterns and control measures. From this study, several overarching conclusions and future directions come into view:

-The observed density peaks underscore the complex interplay between environmental factors and insect development cycles. Understanding these dynamics is essential for effective pest management.

-The aggregation pattern of *P. citrella* suggests the importance of localized management strategies. Future research could delve deeper into the factors driving temporal variations in spatial arrangement.

-Green's model provides valuable guidance for optimizing trap placement, but ongoing monitoring efforts are necessary to adapt to changing population densities.

-Integrated pest management approaches should be prioritized, incorporating cultural, biological, and chemical control methods to mitigate resistance development and environmental impact.

Further investigation into the mechanisms driving resistance development in *P. citrella* populations is crucial for developing sustainable pest management strategies. Understanding the impact of climate change on population dynamics and distribution patterns is essential for predicting future infestation trends and devising adaptive management approaches. Exploring innovative technologies, such as remote sensing and predictive modeling, could enhance monitoring efforts and facilitate early detection of infestations.

In conclusion, this study advances our understanding of *P. citrella* infestation dynamics. By addressing existing challenges and embracing emerging opportunities, future research can contribute to more effective and sustainable pest management practices in citrus orchards.

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