Online ISSN: 2299-9884
Polish Journal of Entomology 89(1): 1–6 (2020) DOI: 10.5604/01.3001.0014.0230

Distribution pattern of developmental stages of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) on the surface of citrus leaves

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Abstract: The leafminer *Phyllocnistis citrella* (Stainton, 1856) (Lepidoptera: Gracillariidae), is considered a potential serious pest of citrus in Algeria. The aim of this work was to investigate the relationship between the ecophases of the citrus leafminer and the leaf surface of the Washington navel citrus variety. Practically all the examined leaves of the young shoots were occupied by at least a developmental stage of this insect. More than 80% of the leafminer ecophases were distributed on the lower surfaces of the leaves. A relationship is highlighted between the leaf surfaces and the stages evolution of citrus leafminer. The choice of area laying by females is decisive for the survival and evolution of stages of development of the insect in relationship with leaf age. The analysis of the eggs distribution revealed that young leaves, with leaf surface smaller than 2 cm², were significantly preferred by females for oviposition. However, the larvae were abundant on leaf surfaces smaller than 6 cm² which provide a favorable environment for the development of larvae in order to realize their respective gallery. The pupal stage was observed almost uniformly on all the analyzed area classes. These results could contribute to the guidelines for pest risk assessment highlighting the intervention for the protection of citrus orchards against heavy infestations of *Phyllocnistis citrella*.

Key words: citrus, *Phyllocnistis citrella*, distribution, ecophases, surface, Washington navel

Introduction

The species *Phyllocnistis citrella* (Stainton, 1856) (Lepidoptera: Gracillariidae) is considered a stenophagous pest of citrus, originating from East and South Asia (Urbaneja *et al.* 2000). It was first introduced in India and spread very rapidly in most citrus-growing areas of the world (Bermudez *et al*. 2004). In Algeria, this species was first reported in citrus orchards in the summer of 1994 (Berkani 1995), and consequent attacks by this species are found on different varieties of citrus fruits. Colonization strongly damages the leaves of young shoots, tender stems and sometimes the fruits. Larvae of this species prefers the lower surface of the leafs, destroying the epidermis of the leaves, which curls up and becomes sclerotic and necrotic (Uygun *et al*. 2000), and it is mainly found on lemon trees, while orange was found the less susceptible, followed by mandarin trees (Batra & Sandhu 1981, Singh *et al*. 1988, Thompson 1988, Verma 1989, Batra *et al*. 1992). Economic losses reflect the increased pest's population, with direct impact on the leaves of young tree shoots (Garcia-Marí *et al*. 2002, Kheder *et al*. 2002). During the infestation phase, *P. citrella* can affect seriously the photosynthetic activity of infested leaves, thus stopping the growth of young citrus trees in orchards and nurseries (Schaffer *et al*. 1997, Elekcioglu & Uygun 2013). Due to the high capacity of migration of the populations outside orchards and their high fertility, control alternatives are becoming very complex. Damage caused by

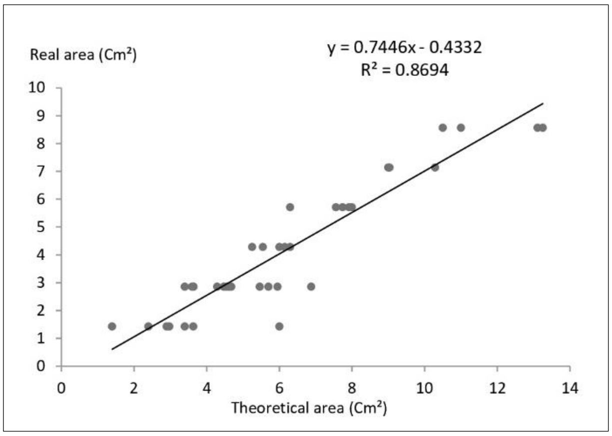

Fig. 1. Model equation for calculating the surfaces of the leaves of the Washington navel variety.

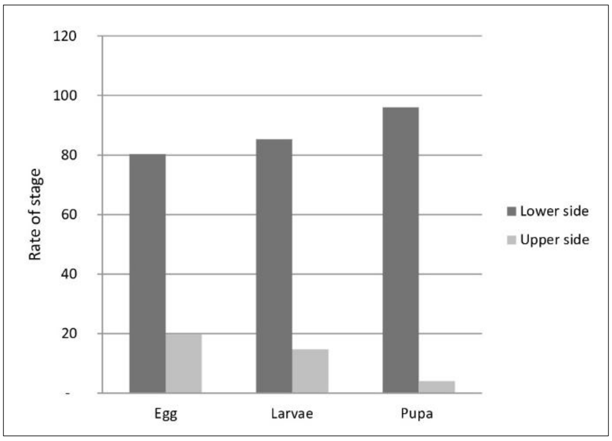

Fig. 2. Distribution of ovo-larval stages of *Phyllocnistis citrella* on citrus leaves.

this leafminer is visually estimated by the percentage of leaf area damaged (Peña & Duncan 1993). The main objective of this study was to assess the state of infestations and establish the distribution strategy of the *P. citrella* ecophases on the surface classes of young shoots of Washington navel citrus fruits.

Material and Methods

Study area and data collection

This investigation was conducted in an orchard of 2 ha, including 150 plants of Washington navel, 30 years old, spaced 3 m between plants and 5 m between rows. The study area site is located in Algeria, in the region of Mitidja (latitude 35°55' and longitude 2°55'), at a sub-humid Mediterranean bioclimatic stage at an altitude of 50 m asl. No treatment was applied throughout the experimental period. Samplings were carried out during all the year 2017. In each sample five plants were randomly selected. At each selected plant, five shoots were collected at a height of 2 m, for a total of 25 samples per 10 days. The collected shoots were individually packed in an organized manner and were examined the same day of sampling, at the laboratory, using stereomicroscope (Motic Digital Microscope). The numbers of ecophases (egg, larvae and pupa) were counted and registered from the both sides of leaves. The maximum length and width of each leaf according to their position on the shoot were measured to determine the leaf area. Based on representative data samples, a model equation was developed for calculating the real surface area of all the samples collected during the experimentation (Fig. 1).

Data analysis

The study was concerned with two variables, the leaf area and the frequency of the leaves infested with each stage. The objective of this work was to deduce model equations in order to describe the relationship between the two variables as well as to derive their possible correlations. Regression analysis was focused on the attack densities as a function of leaf area. The frequency analysis determined the whole populations and presented the data without losing most of the information in the values of the variable to set; this analysis concerned the distribution of citrus leafminer stages on the surface classes. The data were classified and regrouped in order to delimit five surfaces classes expressed in cm²: $x<2$, $2\leq x<4$, $4\leq x<6$, $6\leq x<8$ and $8\leq x<10$.

The means and variances of numbers of eggs and larvae and their dispersion indices were calculated using the ratio of variance/mean (s^2/x) (Myers 1978), which correspond to random dispersion when it equals 1, uniform dispersion when it is below 1, and aggregate dispersion when it is above 1.

Table 1. Descriptive analysis of data on ovo-larval stages. The means differ very significantly (ANOVA; LSD; $P < 0.001$); Wilks lambda = 0.90706; F(8.2518) = 15.731; p = 0.0001***.

Leaf area (cm²)	Observed frequency	Eggs		Larvae	
		Mean ± SD	(s^2/x)	Mean ± SD	(s^2/x)
<2	700	0.81±0.74	0.37	1.84±0.92	0.39
2.1 - 4	283	0.14±0.46	0.33	1.22±1.05	1.01
4.1 - 6	139	0.09±0.39	0.26	1.18±1.15	1.5
6.1 - 8	63	0.07±0.24	0.06	0.97±1.03	1.11
8.1 - 10	40	0.02±0.15	0.04	0.67±0.97	1.32

ANOVA analyses were conducted using Excel stat package and Statistica software (Statsoft Inc. 2001; version 6.0; Tulsa, Oklahoma, USA).

Results

The data of the distribution of ovo-larval stages in relation to surface classes are shown in Table 1. The developmental stages of leaf miner are mainly distributed over areas with less than 10 cm², and the ecophases were found to be significantly correlated to the phenological growth of the host plant (p=0.0001***). More than 80% of the leafminer's ecophases were observed on the underside of the leaves (Fig. 2). The decrease in ecophase presence rates on the upper side of the leaf indicates the non-preference of the evolution of the insect at the upper side of the leaf. In the event of overcrowding, the upper surface also becomes a site of choice for the miner to ensure its survival.

Ovo-larval stage

An average of one egg per leaf was evidenced in this study. The occupancy percentages of the ovo-larval stages in relation to the surface classes are expressed on the curves in Figure 3.

The distribution of ovo-larval stages is related to the leaf surface and attack density. Across the leaf areas, there is a gradual decrease in the number of eggs. Oviposition was significantly more frequently observed on the leaves with surfaces smaller than 2 cm². Fewer eggs are observed when the surface increases. The analysis of the eggs distribution revealed that young leaves, less than 2 cm², were significantly preferred by females for oviposition, with 83% of eggs counted in this surface class. The analysis of larvae distribution showed a high correlation (R^2=0.99) between leaf size and the number of larvae per leaf. The first three larval stages were more abundant in leafs smaller than 6 cm²; beyond this threshold, there are fewer larval attacks.

Pupal stage

The larvae complete their development according to their environmental site, consuming less food on smaller leaves and on large surfaces, it has the opportunity to freely consume its food until it pupates. The pupal stage was more frequent in the surface

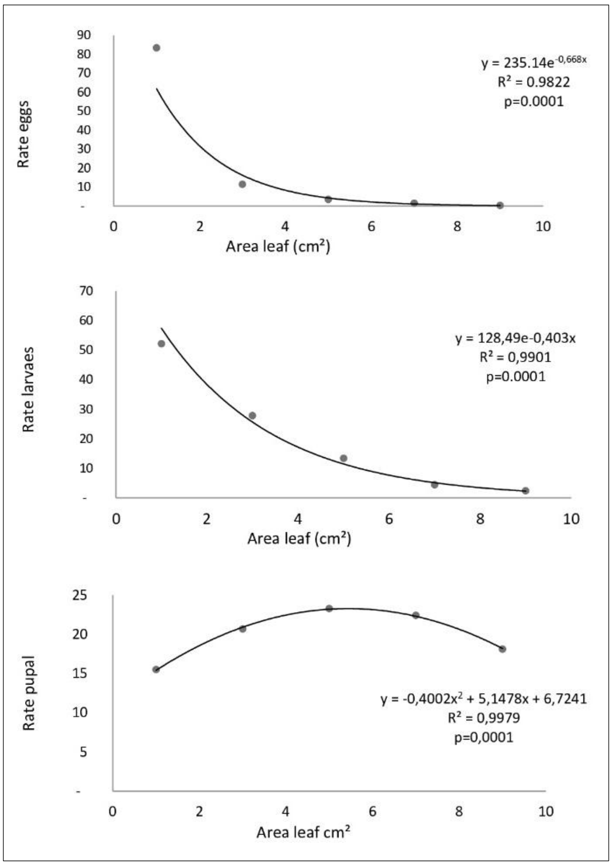

Fig. 3. Distribution of *Phyllocnistis citrella* stages on the surface of Washington navel leaves.

classes ranging from 2 to 10 cm², with a maximum presence on leaves of about 6 cm², accounting for 65% of the population's pupation, therefore revealing a perfect synchronization between the evolution of the pupa and the growth of the leaf. This stage is weakly achieved on 2 cm² leaves, as on large 10 cm² leaves (Fig. 3).

Discussion

About 20 years ago, citrus orchards in Mitidja were heavily infested by the citrus leafminer. Berkani (1995) reported that *Phyllocnistis citrella* caused spectacular damage to citrus orchards in north-western Algeria in 1994.

Thanks to its life strategies, the citrus leafminer is extending and expanding in citrus-growing areas, therefore practically all the leaves of the examined young shoots were occupied by at least one developmental stage of the insect. The oviposition and the first hatching occurrs mainly on small leaves, when they are much fresher and heavily targeted for feeding by young instar larvae, whereas low number of eggs was noted on larger leaves. This suggests that the female of the citrus leafminer can distinguish the leaf age according to its lignification. The larger the leaf, the less it is sought by the female in search of an oviposition site. It was also noted that most of the developmental stages of *P. citrella* are found on the lower surface of the leaves examined. Like most species of Lepidopteran leafminers, this species is specialized on the underside of the leaves (Díaz *et al*. 2006), but can exploit both sides, depending on the characteristics of the leaf (Reavey & Gaston 1991) and the occurrence of population outbreaks. In fact, the females tend to avoid laying eggs on areas of the leaf that already contain eggs, which can explain their presence on larger leaves or on both sides during high population densities. These results confirmed of those of Jarraya *et al*. (1997), who mentioned that females tend to choose young leaves from the apical part of the twigs. Vercher *et al*. (2008) noted that the pest attacks young leaves that are 10 to 20 mm long. Knapp *et al*. (1995) reported that oviposition is largely localized on young leaves less than 0.63 cm long. The insect prefers tender young leaves because they are conducive to the release of their eggs and the development of young larvae at hatching (Sohi & Sandhu 1968, Knapp *et al*. 1994). Ikemoto (1972) confirmed that egg distribution on the leaves is uniform at low densities but becomes contagious at high density in the *Citrus unshui* in Japan. Many studies have shown that the preference for egg laying sites could be explained by the performance of the offspring in relation to leaf nutrients and defense characteristics, which could lead to a concentration of mines on limited resources at different spatial scales (Ayabe & Shibata 2008). Thus, the preference and choice of oviposition site by an insect is determined by the quantity and quality of food available (Ohgushi 1992). Older foliage with more lignified parenchyma is practically avoided, as Kalaitzaki *et al*. (2011) reported that leafminer attacks are less severe in mature plants. Females aim to optimize the survival chances of their progeny on young leaves that are better for insect feeding (Dixon 1985). The rate of larval presence was related to the leaf surface, with occupancy gradually decreasing on leaf surfaces ranging from 2 to 10 cm², despite the availability of leaf area for mining. Bell (1991) reported that there was a high correlation between female host selection and larval performance. The larval population is concentrated on areas less than 6 cm², which provide a favorable environment for the development of larvae in order to hollow their respective gallery. This preference may impose constraints on the availability of resources. For their development, larvae preferred young leaves because the surface has a role in the survival of individuals, the smaller leaf, the more the overpopulation phenomenon was encountered and therefore a sharp drop in feeding was observed, leading directly to wilting of the plant support and, hence, the mortality of larvae. The distribution of developmental stages of the leaf miner is correlated to the phenological stage and leaf composition. The phenology of the host plant and the availability of resources has a decisive role in fluctuations density of leaf miner species, with preference for a particular leaf age class (Auerbach *et al*. 1995).

This work allows us to conclude that the citrus miner has developed an activity strategy based on the growth of young shoots. The choice of the oviposition area by the females is decisive for the survival and evolution of stages of development of the insect in relation to the leaf area. The stages of the citrus leafminer are distributed according to precise surfaces. For the majority of examined samples, the stages of leafminer development are mostly distributed over the lower surface of the leaves. Through this strategic behaviour, the citrus leafminer female ensures a better evolution for progeny. These results could contribute to the guidelines for pest risk assessment highlighting the intervention for the protection of citrus orchards against heavy infestations of *Phyllocnistis citrella.*

Acknowledgements

This study was carried out with a support of Luís Bonifácio, Senior Researcher in Forest Entomology and Acarology at Instituto Nacional de Investigação Agraria e Veterinaria, I.P. Portugal and Sara Hezil, National School of Agronomy, Algeria. Their useful suggestions were important for the realization of this work.

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