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Population Dynamics of the Citrus Leaf Miner, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its Parasitoids on Marsh Seedless Grapefruit in Algeria

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ABSTRACT

This study is the first report carried out on the plantation of grapefruit in the region of Tizi Ouzou, intented to determine the fluctuations of the population of *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae) and its parasitoids in Algeria. The citrus leaf miner develops three generations, mainly on summer and autumn flushes. We noted a severity of the attack during autumn. The first eggs were observed on the foliage at the beginning of July 2005, the vegetative growth stage of the plant. The centre of the tree is the preferred direction with 25 % of total egg number issued by the female. The larvae develop in greater numbers on the underside of the leaf, with a rate of 61 % against 39 % on the upper surface. The young green fruits of grapefruit also attacked during September, a poor period in shoots. Three Hymenoptera Eulophidae associated to *Phyllocnistis citrella* were recorded: *Pnigalio* sp. and *Cirrospilus pictus* Nees, natives ectoparasitoids and *Ageniaspis citricola* Logvinovskaya, exotic endoparasitoid.

Key words: *Phyllocnistis citrella*, grapefruit, population dynamics, inventory, parasitoids, Algeria.

INTRODUCTION

The citrus leaf miner *Phyllocnistis citrella* is a serious pest of citrus and related species to the plant family Rutacea; it was first observed in Algeria in the summer 1994 (Berkani, 1995). It is a small moth that belongs to the family Gracillariidae.

This species is native to Asia and can be found throughout Asia, Taiwan, southern Japan, the Philippines, Indonesia, New Guinea, Australia, South Africa, parts of North, West and East Africa, the Mediterranean region of Europe, and from Saudi Arabia to India.

It can also be found throughout the Caribbean Islands, Central America, Mexico, and South America (Heppner, 1993).

In Africa it appeared in the North-Eastern Sudan in 1962 and Ethiopia in 1970, and in various countries of West Africa during the 70's: Ivory coast, Congo, Gabon, Cameroon, Niger, Benin and North Africa in 1994 (Quillici *et al*.,1995). It was reported during summer 1994 in the Algerian west, considered as the third important phytophagous parasite insect appearing during the last 15 years.

Considering the danger represented by the development of *Phyllocnistis citrella,* this pest has been the subject of several works around the world during these last years. Garijo & Garcia (1994) and Knapp (1996) studied its biology and its dynamics. Vercher *et al*., (2001) look an inventory of its natural enemies. Numerous chemical control attempts carried out in several countries invaded by the pest could not master its spread. The biocontrol remains a promising alternative that is why in Algeria many researchers focus on the study of *Phyllocnistis citrella* to look into its population dynamics and its natural enemies inventory to better control the development of *Phyllocnistis citrella* populations (Doumandji-Mitiche *et al*., 1999).

The present study was conducted on the population dynamics of *Phyllocnistis citrella* and its parasitoids on Marsh seedless grapefruit in the region of Tizi-Ouzou, Algeria. It contributes to numerous studies that were made in this country, on *Phyllocnistis citrella* on various varieties of citrus varieties (orange, Clementine, lemon and bitter orange), evaluating its importance on grapefruit, which has been less studied in the world, in general, and Algeria, in particular.

MATERIALS AND METHODS

The observations were done during the period 2005-2006, on a experimental plot of land of 2 ha, located near wadi Sebaou. This grapefruit orchard was chosen in order to follow the evolution of populations of *Phyllocnistis citrella*. The climate is this area is Mediterranean. Average temperatures in the region are between (25-30°C) and the humidity extends from (50-80%).The average rainfall varies from1 to 300mm per year.

The experimentation was conducted from May 2005 to January 2006 in a land of variety Marsh Seedless, 4-years old. We weekly took two shoots 10 to 15 cm long, in each direction of points cardinal of ten trees taken at random. After the collecting, samples were protected in plastic bags with information of date, the localization and direction of the tree.

The shoots of grapefruit were examined in the laboratory under the binocular microscope to count the alive, dead individuals and parasited leaf miner, for each development stage of *Phyllocnistis citrella*: egg, larvae and pupae.

We also measured the length of each leaf attacked by *Phyllocnistis citrella*, in order to evaluate if infestation degree depended on leaves size.

We established four lengths classes of from measurements obtained on all the infested leafs by *Phyllocnistis citrella* sampled. These are: Class1: 10-30 mm; Class 2: 30-50 mm; Class 3: 50-70 mm and Class 4: 70-90 mm. The number of eggs, larvae and pupae were awarded to each class.

The parasitoids identifications were made thanks to the assistance provided by the Pr Garcia Mari, Entomology Laboratory at the Polytechnic University of Valencia, Spain.

The statistical analysis of our results was realized by applying the following tests: test adjustment record is a test $\chi 2$. It helps determine whether the distribution of clutch *P. citrella* follows a particular distribution at the cardinal points.

The comparison between the percentages observed Po and P is based on the difference ε. If $\varepsilon \geq 1.96$, the difference is statistically significant (Dagnelie, 1975).

RESULTS

1. The description and biology of citrus leaf miner on grapefruit.

The adult moth of the citrus leaf miner, *Phyllocnistis citrella* measuring 2-4 mm of length, and approximately 6 mm of wingspan**.**

The adult oviposits on young leaves of the grapefruit near the rib of leave surface as noted by Knapp *et al.* (1995) and the larva (measuring up 3 mm long), immediately enters under the epidermis and forms a mine.

Each pale –green larva tunnels a characteristic, sinuous, silvery mine in the leaf, with a raised parchment-like skin lined centrally with dark excreta. Larvae never leave their mines to form other mines or move between lower and upper sides of leaves. Development of the first three stages takes about 5 to-6 days in summer. Mature third instar larvae are about 3mm long. The fourth stage (the prepupa) is yellowish brow and ressembles the third-stage larva but it does not feed (Gagreathe &Bradley, 1997). The prepupa prepares its pupa chamber at the edge of the leaf by folding the edge of the leaf down and binding it together with silk. Transformation into a pupa occurs within the chamber. After about 6 days, adults emerge and begin the cycle again.

The larval developmental period is short, generally spanning 3 to 5 days from the egg hatching to the pupation, as observed by Knapp (1996). The life cycle length varies with temperature, averaging about 17days at 25°C (Lo Pinto & Fucarino, 2000).

The duration of larval development depends on the temperature, with 10.5 ± 0.5 days at 40° C and 18.5 ± 2.5 days at 20° C (Ba-Angood, 1978; Sánchez *et al*., 2002).

2. The damages and economic relevance on grapefruit.

Our results show that the growth of shoots, thus the development of the grapefruit plant is negatively affected by the attack of *Phyllocnistis citrella*. In July, August and November 2005, we noted a high level of attack. There are various standard types of damages: visible mine on the underside of leaves, mines on the non lignified young stem. The epidermis is destroyed by the first and the third larval stage, the fourth embryonic stage called nymph, remains inside the mine where it builds its pupal chamber. We were able to observe during September 2005 several mines of *Phyllocnistis citrella* on young green grapefruit. In a period with very few young shoots, the female lays her eggs on green fruit of grapefruit, in accordance to the results of Gottwald *et al*., (1997). We noted that young fruits are particularly attacked, without these larvae finish their development.

We also noted a microbial development on the deteriorated leafs (data not shown) probably due to *Xanthomonas axonopodis pv citri* as observed by *(*Gottwald *et al.* (1997) and Chagas *et al.* (2001).

3. Parasitism of *Phyllocnistis citrella* on grapefruit.

We observed three hymenoptera parasitoides belonging to the family Eulophidae associated with *Phyllocnistis citrella*, in the studied orchard. Two of them are ectoparasitoids who parasitize the larval stages, prepupae and pupae: *Pnigalio* sp (Fig.1) *and Cirrospilus pictus* (Nees) (Fig.2).

These ectoparasitoids lay eggs on the insect, larval development of the parasite takes place entirely on the body of the host until the adult form.

The third parasitoid is *Ageniaspis citricola* (Logvinovskaya)*,* polyembryonic endoparasitoid feeding on eggs and immature larvae of citrus leaf miner (Fig. 3). This species of parasitoid specie was introduced with *Semialacher petiolatus* (Girault) and *Quadrastriatus sp* in Algeria for the biological control program of the citrus leafminer (Boualem & Berkani, 2006).

Figs. 1-3. Females 1. *Pnigalio* sp., 2. *Cirrospilus pictus,* 3. *Ageniaspis citricola*

The first foliage contamination by the eggs of *Phyllocnistis citrella* was noted in the sap push of July 2005, period which coincides with the summer thrust on grapefruit. It should be noted a total absence of eggs during spring and autumn. We observed an important infestation during autumn, which agree with Dhouibi and Wadoud (1997). Nucifora, (1996) also noted that the seedlings grown from the first sap push were avoided by *Phyllocnistis citrella*. According to Malausa, (1997), the inactivity of this insect during this period is due to the fact that the adults density is low during the winter, thus it is not able to provoke damages on orange. The absence of eggs during the month of October could be attributed to bad weather (Heavy rainfall). The number of eggs shows three spawning periods corresponding to three generations of *Phyllocnistis citrella*, key periods pontes important, as showed in Figure 4.

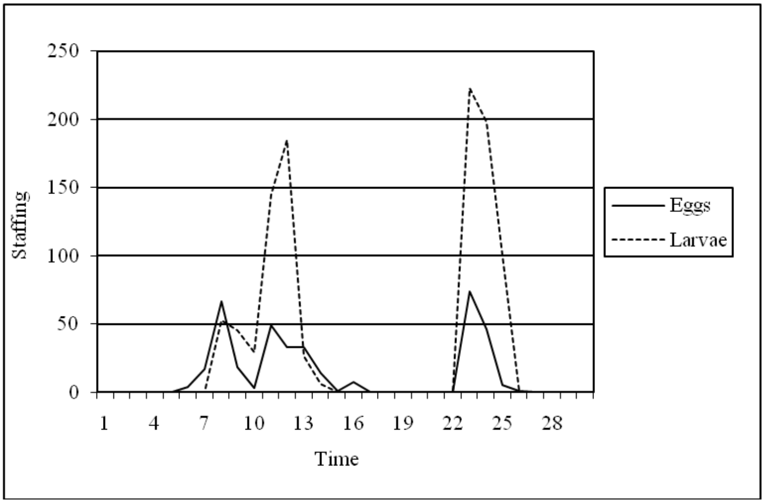

Fig. 4. The temporal distribution of eggs and *Phyllocnistis citrella* larvae on the grapefruit during year 2005-2006.

- The first generation is between mid-July and mid- August at Temperature (T°) average of 29 ° C, with 50 % of relative humidity (H R).

- The Second generation between late August until September with 29° C and 23° C, with 59% of H R.

- The third generation starts in November and ends in mid-December from 20° C and 15°C, with 75% of H R

The evolution of these generations agrees with the temperature and the relative relative humidity of the seasons as well as the development of vegetation of the host plant. According to Mingdo *et al.,* (1989) climatic conditions would act indirectly, causing stressed leaves, and this would be detrimental to larvae.

The examination of samples taken at the cardinal points and the center of the tree shows five distributions of eggs on the main directions. However, the center recorded the highest with 25% of the total number of eggs laid by the female (Fig.5).

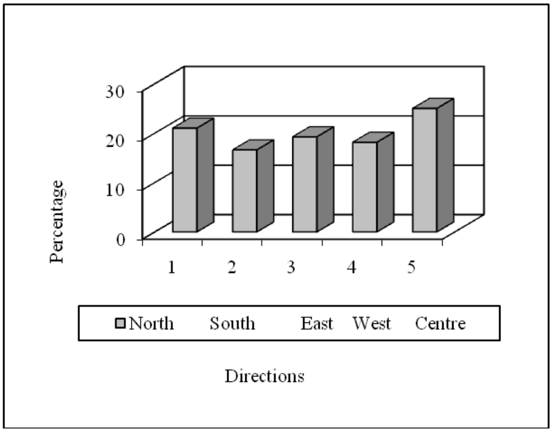

Fig. 5. The distribution *Phyllocnistis citrella* eggs by the orientation.

Our results are similar to that obtained by Sahraoui *et al*., (2001), which pointed out that females of *Phyllocnistis citrella* tend eggs on the branches arranged in the center of the tree which provides a shady and moist microclimate.

The localization of spawning would be determined by the physiological state, the female chooses the shoots in rapid growth phase, clear green, to deposit her eggs.

Statistical analysis of egg number distribution of *Phyllocnistis citrella* according to the guidance of the tree is determined by the test adjustment record.

The value of $\chi 2$ calculated is obtained by applying the following formula:

$$\chi^2 = \sum \frac{(f_o - f_e)^2}{f_e} = 1.42$$

We compare the value of $\chi 2$ calculated 1.42, with the critical value of the table. The number of relations between the two distributions is 1 since only the total frequency is supposed identical.

For a = 0.05 and d.l.= (5 – 1) = 4 $\chi 2$ critical = 9.49, calculed is below the critical value, we must maintain Ho: distribution uniform. The hypothesis is accepted. The distribution of eggs at the cardinal points is normal.

We compares a percentage observed Po on n case, based on the standard deviations ε. If $\varepsilon \geq$ 1.96, the difference is statistically significant.

The values calculated for comparison between the five different percentage of the cardinal points gives us a $|\varepsilon| < 1.96$ ====> the difference between the percentages is not significant.

Distribution of larval populations on leaf surfaces

An analysis of the distribution of development stages of *Phyllocnistis citrella*, on either side of the leaf, indicated that the larvae will develop in greater number on the lower side of the leaf, with a rate of 61% while the upper side registers only 39% (Fig 6).

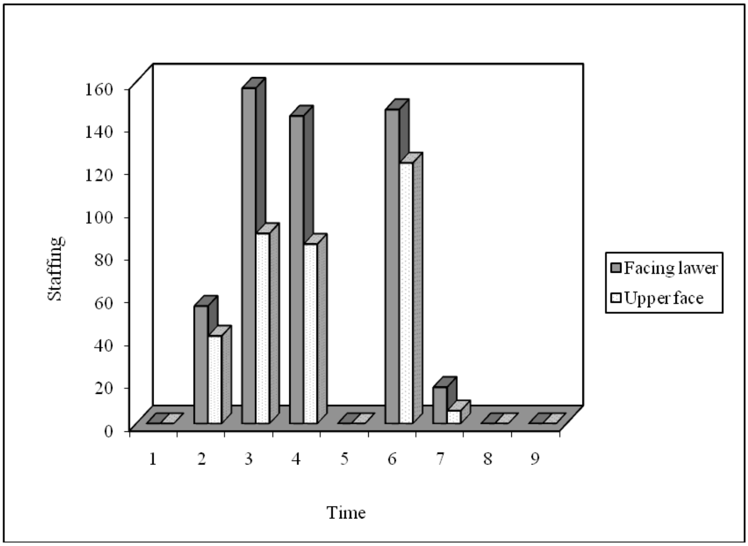

Fig. 6. The distribution of the larvae of *Phyllocnistis citrella* by leaf surface.

This affinity to the underside of the Grapefruit leaf could be explained as a defence mechanism against its natural enemies protection to the sunlight and also by the presence of epidermal hairs which allow a better fixing of the deposited eggs, as reported by Garrido and Gascon (1995) on orange.

The results of statistical analysis for sides leaf is to compare the two percentages obtained for on the lower side with 61% and the upper side with 39% thanks to the following.

formula: $$\varepsilon = \frac{P_o - P}{\sqrt{\frac{P \times Q}{n}}}$$

Po= 61% P+Q= 100

P= 39% n= 374

$|\varepsilon|$= 8.52 and 8.52 >1.96 ====> the difference is statistically significant.

Our results about the lengths classes show that the degree of infestation of *Phyllocnistis citrella* is inversely proportional to the Grapefruit leaf length (Fig.7).

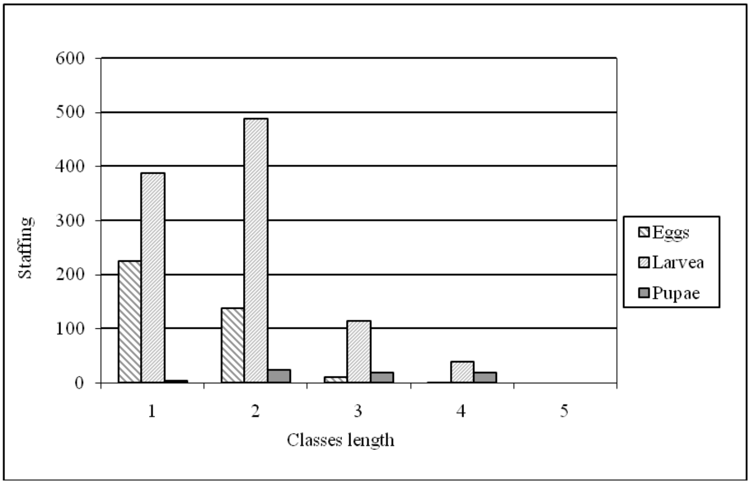

Fig. 7. The distribution of the larvae of *Phyllocnistis citrella* by class of leaves length.

This reflects the preference of the leaf miner to lay eggas on the young tender leaves, which are small, but rich in nutritious elements, as noted by Kharrat and Jarraya (2005) on citrus.

The pest population is relatively large at the late summer (August-September 2005) and the end of autumn (November to December 2005). The rates of parasitism were respectively 30 % and 25% for those two periods; but very low at the beginning of summer (2.5%- 3%) (Fig. 8).

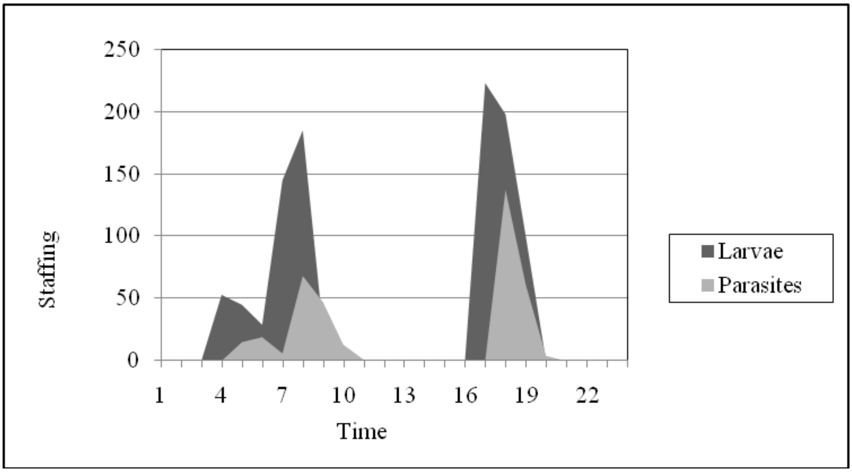

Fig. 8. The monthly variation of the number of larvae *Phyllocnistis citrella* of and their parasites on Grapefruit.

Pnigalio sp. was the most frequent species found in the orchard at the end of August, followed by the other species *Cirrospilus pictus* and *Ageniaspis citricola* which appeared in November.

Vercher *et al*., (1997) reported that in order to control *Phyllocnistis citrella* by biological means, the parasitoid *Ageniaspis citricola* was introduced in the citrus orchard. It was firstly predominant, but then it was gradually replaced in late autumn by both species of parasitoids *Cirrospilus pictus* and *Cirrospilus vittatus*. Those species here observed, were also reported on orange, lemon and clémentine in Spain (Garijo & Garcia,1994); in Morocco (Smaili *et al*., 1999); in Italia (Conti *et al*.,2001) and in Algeria (Berkani & Mouats, 1998; Doumandji -Mitiche *et al*., 1999; Sahraoui *et al*., 2001).

CONCLUSION

The evolution of the density of *Phyllocnistis citrella* on the seedlings from the Grapefruit tree has been very little studied around the world, including in Algeria.

We were interested at the population dynamics of the citrus leaf miner and its parasitoids in the Marsh seedless Grapefruit variety in Tizi-Ouzou during the period 2005-2006.

We noted the presence of three sap spurts, in the spring, summer and autumn. In our conditions *Phyllocnistis citrella* has developed three generations, one in the annual surge of summer and two generations in the autumn. We noted synchronization between the vegetative growth and the development of the insect. Egg-laying begins, in early July; with the maximum values recorded in autumn. No insects were found in the spring. The total absence of *Phyllocnistis citrella* on thrust of the sap during the spring is due to the inactivity of this insect during this period and the low density of adults in winter, not enough to provoke damages on grapefruit.

The female chooses the center of the tree to deposit her eggs, where the microclimate is shady and moist and the shoots are in rapid growth phase. We noted 25 % of total number eggs issued by the females at the center of the tree.

Our results show that the infestation degree of *Phyllocnistis citrella* is inversely proportional to the grapefruit leaf length. The larvae will developed in greater numbers on the face of the lower leaf, with a rate of 61% while the upper face registers only 39%, caused by the protection and the structure of the leaf. We also noted that the young fruits of Grapefruit were occasionally attacked, rendered unfit for marketing, without that larvae reach term.

We observed three hymenoptera parasitoids associated with *Phyllocnistis citrella* which belong to the family Eulophidae, *Ageniaspis citricola*, *Cirrospilus pictus* and *Pnigalio* sp.

Their population are relatively high at the late summer (August-September 2005) and the end of autumn (November to December 2006), corresponding to periods of severals infestations of *Phyllocnistis citrella*.

All these results can contribute to better understanding of the dynamics of *Phyllocnistis citrella* on the grapefruit plant in Algeria, to fight this phytophagous, insect and to improve *Citrus* production.

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