Population dynamics of *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera, Gracillariidae) and impact of its parasitism on clementine trees at the Boufarik-Blida station (Algeria)

https://doi.org/10.56238/sevened2024.018-050

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ABSTRACT

A study was conducted for eight months in 2021 in the Boufarik-Blida region (Algeria) to analyse the population dynamics of *Phyllocnistis citrella* and the incidence of the parasite on citrus fruit, inparticular clementines. The main data showed a significant increase in the rate of parasitism, with peaks of 73% and 72% during the first outing. After 28 June, fluctuations in the rate of parasitism were observed, highlighting the importance of the activity of beneficials, particularly those reared in a greenhouse close to the orchards, such as *Semielacher pesiolatus*. The relationship between rates of infestation and parasitism is increasing, with parasitism exceeding infestation. This dynamicis influenced by several factors, including temperature variations, and highlights the effectiveness of biological control using parasitoids. The installation of greenhouses for rearing beneficials near infested orchards, combined with other integrated pest management methods, represents a promising and sustainable solution for combating citrus leafminer.

Keywords: *Phyllocnistis citrella*, Clementine, Parasitoids, Integrated pest management, Boufarik-Algeria.

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INTRODUCTION

Among the countries of the Mediterranean basin, Algeria is a major citrus producer and exporter (Loussert, 1989; Khechna, 2011; Khechna *and al*., 2017; Mahmoudi *and al*., 2017; Bendoumia *and al*., 2023). In fact, Algerian citrus growing is particularly widespread in the Mitidja plain, where it represents a strategic segment (Khechna, 2018). However, according to Khechna, citrus growing is subject to pressure from a multitude of bio-aggressors, including fungi, bacteria, viruses, nematodes and insects, which can either cause severe yield losses or alter the quality of produce or plants, resulting in downgrading or even failure to market.

One of these pests is *Phyllocnistis citrella* (Stainton, 1856), which appeared in Algeria inthe summer of 1994 (Berkani, 1995; Berkani, 2003 and Berkani *and al*., 1996). This insect is considered to be a major enemy of citrus plantations because of its great climatic adaptability and the damage it causes, since it affects the photosynthetic function, resulting in a drop in yield in oldertrees and slower growth in young plants (Saharaoui, 1997; Smith *and al*., 1997; Saharaoui *and al*., 2001; Khechna *and al*., 2017; Khfif, 2022).

The excessive and inappropriate use of conventional pesticides (broad spectrum of action, low selectivity) against harmful organisms has disrupted the natural balance of the agricultural ecosystem (effect on parasitoids and predators, infestations of secondary pests, phenomenon of pestresistance). In addition, they present serious health risks for farmers and negative repercussions forthe environment (contaminating the air, soil and groundwater) (Soltani, 2013). In fact, the use of biological control with other control techniques can be a solution for integrated protection against pests (Argov and Rossler, (1998); Schauff *and al*. (1998) ; Khfif 2022 ; Fazekas (2023) ; Ullah *andal*. (2023)). According to these authors, biological control plays a vital role in reducing the population of this leafminer and its success depends on continuous monitoring of orchards and of the populations actually present in order to intervene at the right time.

In the light of the preceding bibliographical data, the problem of the present study is constructed. The aim of this study is to investigate the bio-ecology of the citrus leafminer *Phyllocnistis citrella* and to identify the beneficials that can play an important role in the biological control of the clementine varietie in the Boufarik-Blida region (Algeria), which is very rich in citrus fruits, during the national containment period as a preventive measure against the Corona virus 'Covid-19' pandemic. These studies should lead to the limitation of losses in terms of quantity and quality and propose effective and non-polluting control techniques (Bendoumia, 2018).

MATERIALS AND METHODS

The present study was carried out at the Boufarik station (36° 59′ 49″ 42 N., 2° 91′ 98″ 26.7L.) on the Mitidja plain in Algeria. It covers an area of 3 ha and is bordered to the north, west and south by citrus orchards and to the east by the motorway. The station is located at the entrance to the town of Boufarik in the semi-urban zone, on the edge of the road leading to Tessala El Merdja.Its work is technical and administrative, and it is affiliated to the I.N.P.V. in El Harrach.

A clementine orchard (*Citrus clementina*) was chosen to carry out the required observations.It should be noted that this clementine orchard is located next to a greenhouse where parasitoids ofthe *Citrus leafminer*, *Semielacher pesiolatus* in particular, are reared. In addition, the health of the trees in this orchard is clean : no pesticides are used in the orchard.

Sampling was carried out several times a month for 08 successive months in 2021 (Januaryto August). It should be noted that this is a very difficult period with national containment as a preventive measure against the 'Covid-19' corona virus pandemic. In addition, periodic and random population counts were carried out in order to monitor variations in the infestation rate and the parasitism rate of the pest *Phyllocnistis citrella*. Samples were taken at ground level from two treesin each of these blocks, at different cardinal points of the tree, on branches containing young, medium-sized or old shoots. The samples, made up of 100 leaves for each sampling, were placed inpaper bags with all the information needed to identify the sample, in particular the date taken, the location, the variety and the orientation of the branches on the tree.

In the laboratory, the various stages of the leafminer - living, dead or parasitized - were identified under a binocular microscope and recorded for each leaf surface. In addition, the nymphsof the various species of beneficial insects were collected. It should be noted that the stages of development of the insects were precisely identified on the basis of scientific criteria reported in the literature, in particular the criteria mentioned by (Berkani, 2003).

RESULTS AND DISCUSSION

The rate of infestation and parasitism and the effect of parasitoid use obtained during the period January-August 2021 are developed.

FLUCTUATION OF THE *PHYLLOCNISTIS CITRELLA* POPULATION

Table 1 and Figure 1 show the fluctuation in the *Phyllocnistis citrella* population over the period June-August 2021.

Fig. 1 - Total results for variations in the various stages of parasitism Source : Prepared by the authors (2024)

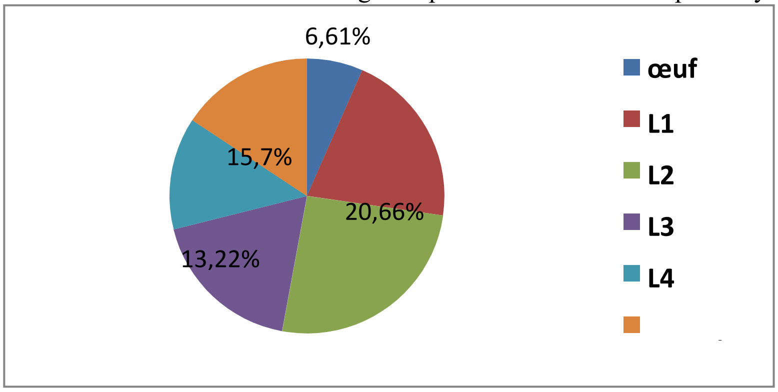


Table 1 - Different stages of parasitism during the study period Source : Prepared by the authors (2024)

The dates	Stadiums and numbers						
	egg	L1	L2	L3	L4	Nymphe	Total
January - May	0	0	0	0	0	0	0
07-06-2021	0	0	0	0	0	0	0
14-06-2021	0	0	2	4	2	8	16
21-06-2021	3	1	6	1	6	1	18
28-06-2021	1	3	2	5	1	1	13
04-07-2021	2	10	2	1	3	3	21
12-07-2021	2	2	13	4	1	2	24
19-07-2021	0	5	4	5	2	4	20
09-08-2021	1	4	2	2	1	0	18
The Total	08	25	31	22	16	19	122

Analysis of the results of fluctuations in the population of *Phyllocnistis citrella*

Table (1) shows a fluctuation in the number of *Phyllocnistis citrella* populations on clementines in the Boufarik region during the period (January-August 2021).

The total number of eggs laid during this period was very low, fluctuating between 8 and 122 individuals.

The delay in the establishment of *Phyllocnistis citrella* was due to thermal conditions duringthe month of January and the first week of June when the temperature was below 18°C and there was a lack of food. After this period there was an increase in vegetation under favourable climatic conditions.

In July 2021, *Phyllocnistis citrella* reached the peak of its development under climatic conditions of (T=24.7 to 28.9° and humidity of 45%).

The number of larvae considered to be the greatest in relation to the other 94 stages was 122. We reported a number of 25 individuals of L1, 31 individuals of L2, 22 individuals of L3, 16 individuals of L4. For the number of nymphs is presented with a very important number 19 to 122 individuals. It should be pointed out that for the first few months of the study, *Phyllocnistis citrella* had zero activity or no activity at all.

The results of this study show that the leafminer is subject to various mortality factors. Among the factors influencing its development, climatic conditions are essential ecological parameters in the development of any living being. Another factor affecting parasitism is competition between species. A very important factor is the acclimatisation of parasitoids.

Discussion on population fluctuations of *Phyllocnistis citrella*

In citrus fruit, sap flow corresponds to the emission of tender young leaves, the duration of which depends on climatic conditions, the nature of the orchard (age, maintenance, etc.) and the

species. The tree emits three sap bursts a year : the first in spring (PS1), the second in summer (PS2)and the third in autumn (PS3). The first leaves of PS1 generally appear at the end of February, andfinish in mid-June. A week to ten days later, the first leaves of the second, very short-lived sap burstappear, followed by the third and final sap burst (PS3). Flowering occurs during SP1. However, thephrenological development of citrus trees is more or less complex depending on other parameters such as the site and the health of the tree (Boualem, 2009).

Berkani (1995), shows that in summer the pest *Phyllocnistis citrella* is very active and attacks sap-laying seconds. Attacks by *Phyllocnistis citrella* on lemons are higher than on oranges and clementines ; during the months of December, January and February, attacks are low on all three hosts. This is due to the slower vegetative stage, which lengthens the cycle.

Zouaoui (1997), points out that the availability of abundant and fresh vegetation added to favourable climatic conditions from the end of May until the end of June under conditions of (average T = 18.06 to 21.15°C and humidity from 65.55 to 69.18%) the leafminer reaches a maximum development following to the availability of food (the summer posse) and prolongation of favourable climatic conditions one even sees a fast succession during the second dekad of July until the end of September. From the third dekad of July to the first dekad of August, the maximum temperature frequently exceeding 30° with a slightly low humidity of 57% affects the developmentof the pest and the population density decreases.

Zouaoui (1997), shows that during the third dekad of July and the whole month of August and under conditions of (T is higher than 30° and minimum humidity of 50%) negatively affect the development of *Phyllocnistis citrella*.

Saharaoui *and al.* (2001), after three successive years of observations in 1996, 1997 and 1998, show that heavy outbreaks of the leafminer were observed on both citrus varieties. In winterand in the plain of Mitidja the minimum and maximum temperatures vary between 4 and 10°C and14 and 20°C, the relative humidity of the air oscillates between 68% and 87.9% and the pluviometryof 6.7mm in this period the leafminer maintain at a very weak level on some young growth of the lemon trees and on the orange trees one will maintain a complete absence of the leafminer during the first two months of spring. From the second sap flow at the end of June, the insect will recover its development on the young shoots. In summer, when conditions are favourable and humidity high,the embryonic and larval stages explode and are prolonged during the summer period (end of June-end of August). The availability of abundant fresh vegetation, favoured by very favourable climatic conditions and by irrigated intercropping.

According to Dr. Khechna (2011), the population of *Phyllocnistis citrella* is almost negligible or non-existent during the winter and spring periods. The summer period is the most favourable for leafminer populations due to favourable temperatures and the presence of tender young leaves.

Mechelany and Matny (1998) show that *Phyllocnistis citrella* attacks on lemons are higher than on oranges and clementines. Attack is very low on the three hosts, which is due to the slow vegetative stage of the insect in diapause, which means that the cycle is longer, and to the climatic conditions.

According to Boualeme (2009), many countries infested by the citrus leafminer have optedin particular for the introduction and acclimatisation of entomophagous species. Populations of the leafminer are more or less regulated by the action of the various mortality factors present, which makes it possible to control the pest. mortality factors, which helps to mitigate its harmful effect on citrus yields and production.

Saharaoui *and al.* (2001) report that the impact of the leafminer parasite complex was felt much more on pre-pupae and L3 larval stages than on pupae. On lemon trees, the annual parasitism rates on pre-pupae were 15.78% in 1996, 38.90% in 1997 and 28.66% in 1998. They fluctuated between 12.45% and 22.24% on the L3 stages over the three years and never exceeded 8% on pupaein any year. On orange trees, the pre-pupal stage was the most receptive with a parasitism rate of 19.22% in 1996, 41.66% in 1997 and 20.17% in 1998. In 1998, there were no significant differences between the parasitism rates of pre-pupae (20.17%) and larvae at the L3 stage (21.11%). A decreasein the number of larvae affected by climatic changes.

Saharaoui *and al.*, (2001), shows that mortality, largely linked to climatic conditions, is likely to cause high mortality in insect populations, particularly in the larval stage, whereas the pupaland chrysalis stages, protected by their cocoons, are less exposed to these factors. The high mortality recorded during the summer period was partly caused by significant spatial competition due to high larval density. On lemon trees in 1997, the rate of parasitism ranged from 10.48% (15 August) to 28.09% (30 August). In summer 1998, parasitism combined a maximum rate of 34.59% in midsummer (30 July) with a minimum rate of 0.64% at the beginning of winter (15 December) and was 7.76% in autumn (30 September). On orange trees, parasitism activity was practically nil in winter during the three years (1996, 1997, 1998) due to the lack of young shoots. The first parasite was observed around 15 May. The first parasite was observed around 15 May, when the parasitism peak was of the order of 26.03%. This rate varied from 2.21% (15 July). The parasite index was high during the first two months of the summer of 1998, when parasitism rates reached 45.66% on30 July before dropping to 25.32% on 15 August. The hot weather caused the rate of parasitism to fall to 9.61% at the end of August. The impact of the leafminer parasite complex was felt much more on pre-pupae and L3 larval stages than on pupae. The mortality of the organisms is linked to the effect of the parasitoids.

ESTIMATING THE INFESTATION RATE

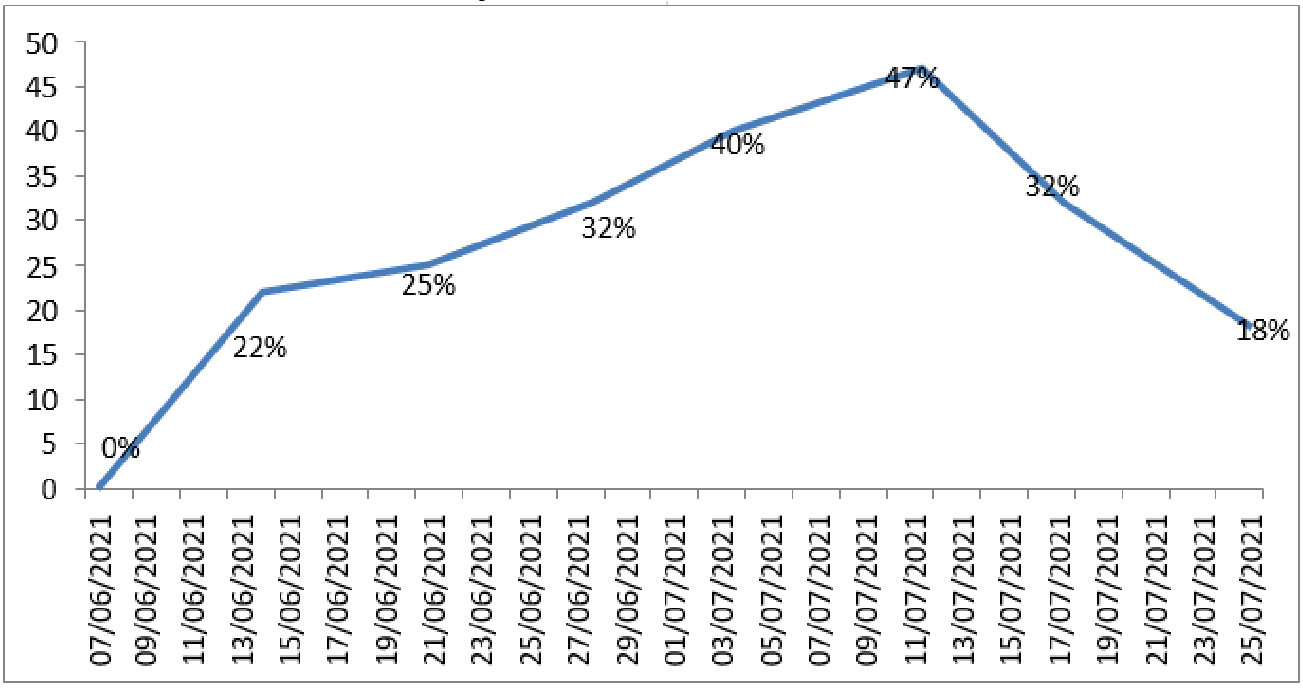
Table 2 and Figure 2 show the estimated infestation rate for June-August 2021.

Table 2 - Estimated infestation rate for June-August 2021

The dates	The rate of infestation
07-06-2021	0%
14-06-2021	22%
21-06-2021	25%
28-06-2021	32%
04-07-2021	40%
12-07-2021	47%
19-07-2021	32%
09-08-2021	18%

Source : Prepared by the authors (2024)

Fig. 2 - Variations in infestation rates



Source : Prepared by the authors (2024)

Analysis of infestation rate estimation results

Table 2 and Figure 2 show that a significant increase in the infestation rate was recorded during the period 07 June-14 June 2021, reaching 22%. This increase continued gradually until it reached its peak in the second week of July (12-07-2021) with a rate of 47% at an average temperature of 28.9°C and humidity of 57%. The infestation rate then fell, reaching a low point in the second week of August (09-08-2021). This variation is due to the summer push period, especially in (June-August 2021).

Discussion on estimating infestation rates

Khechna *and al*. (2017) found high infestation rates for the Wachinthon variety, with 50% observed on 13 July and 60% on 24 August in 2011. An increase in the infestation rate can be explained by the appearance of the second sap burst in the second dekad of June. However, from the third sap burst onwards, the infestation rate fell. In 2012, she reported two peaks in plot 1. The first occurred on 18 July 2012 with a rate of 35% and the second, of lesser importance, was reportedon 12 September 2012 with a rate of 20%. For plot 2, three peaks of almost equal importance werenoted. The first was observed on 18 July 2012 with a rate of 37%, the second peak on 15 August ofthe same year with a rate of 35% and the third peak on 12 September with a rate of 38%.

Boualem (2009) in 2003 found that infestation rates on the second sap flush varied from 67.5% (minimum) on 9 July to 97.4% (maximum) on 26 August. On PS2. Temperatures varied, with a maximum of 38.6°C recorded on 19 July and a minimum of 16°C on 26 August. In 2004, thetrend in contamination was similar to that of the previous year, with maximum rates of 96.6% on the summer shoot in July and 95% in August, while temperatures reached minimums of 21.8°C (19/07) and 20°C (30/08) and maximums of 32.1°C (26/07) and 37°C (23/08). With the same author,and during 2005, *P. citrella* showed low activity at the start of the second sap flow. Infestation rates,which reached 10 to 13% during the month of June at an average temperature of 23.6°C and 63% humidity on 19 June, rose sharply during the summer. In fact, they reached a maximum of 89.4% in July and 99% in August, with maximum temperatures and RH of 34.9°C and 98% in July and 33.9°C and 98% in August. Infestation rates were also high in 2006. On PS2, for example, a maximum of 96% was observed in July and a minimum of 71.2% in September.

ESTIMATION OF PARASITISM RATES

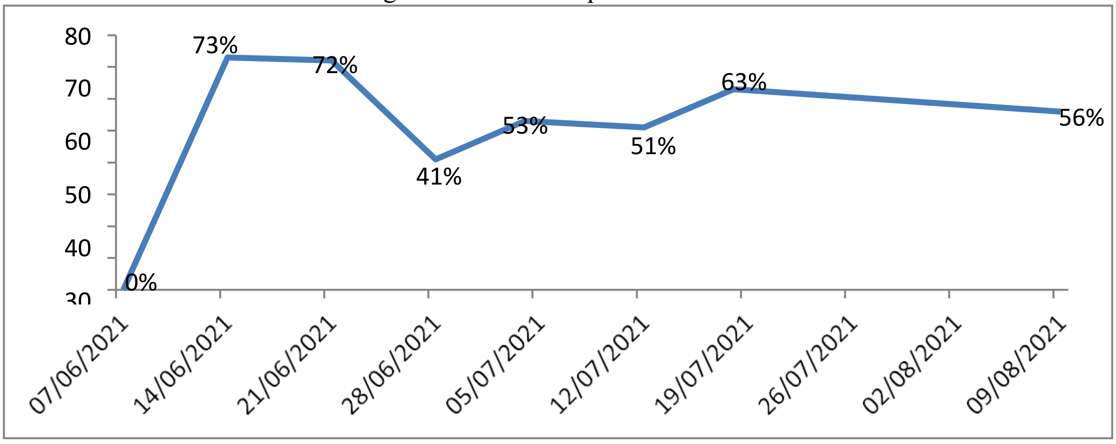
Table 3 and Figure 3 show the estimated infestation rate over the period June-August 2021.

Table 3 - Estimated rate of parasitism

The dates	The rate of parasitism
07-06-2021	0%
14-06-2021	73%
21-06-2021	72%
28-06-2021	41%
04-07-2021	53%
12-07-2021	51%
19-07-2021	63%
09-08-2021	56%

Source : Prepared by the authors (2024)

Fig. 3 - Variations in parasitism rates



Source: Prepared by the authors (2024)

Analysis of parasitism rate results

Table 3 and Figure 3 show a significant increase in the rate of parasitism, reaching two peaksof 73% and 72% for the first outing. After 28 June, there was a fluctuation in parasitism rates. This underlines the importance of the activity of beneficials. Near the clementine orchard is a greenhouse where parasitoids of the citrus leafminer, Semielacher pesiolatus in particular, are reared.

The discussion on the rate of parasitism

Khechna *and al*. (2017), show that the first rate of parasitism is noted on 29 June 2011 witha rate of 15% and the second with a rate of 20% reported on 27 July. These parasitism percentageswere recorded after the first release of citrus leafminer parasitoids (Semielacher petiolatus and Citrostichus phyllocnistoides), which was carried out after the second sap burst.

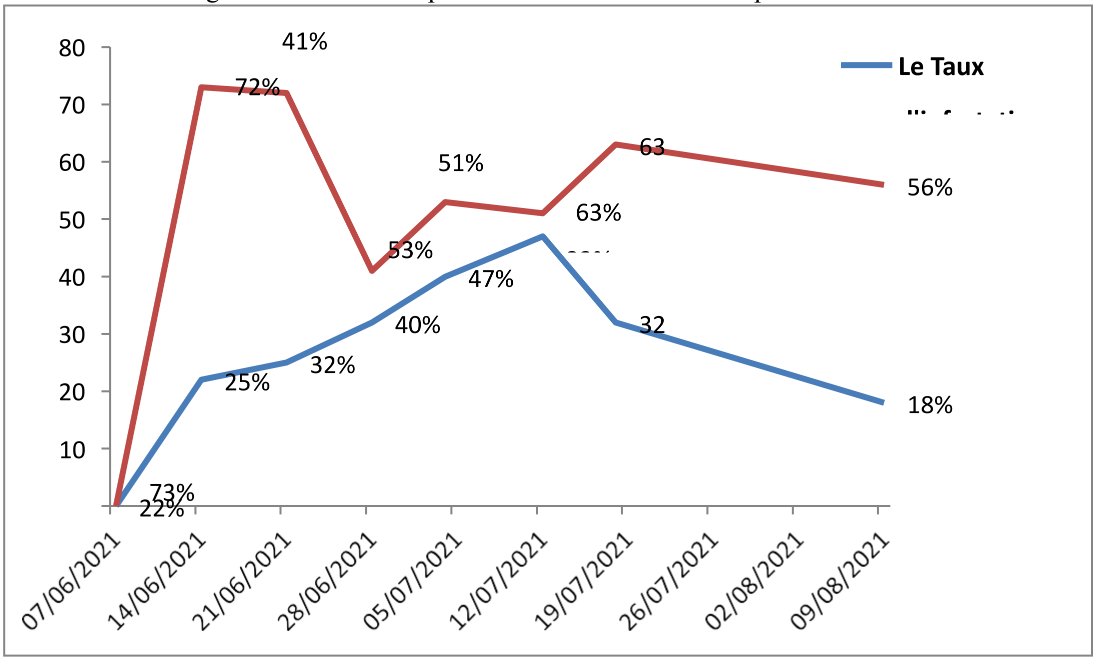
Mechelany and Matny (1998) show that the maximum level of parasitism on lemon trees is 4% in June, with an absence of parasitism from January to mid-March. On orange trees, the percentage is higher at 5%. Absence was noted in October and November and also between July/August and December/February in April. On clementines, the maximum level of parasitism is 6%, with an absence of parasitism noted from June to the end of September.

THE RELATIONSHIP BETWEEN THE RATE OF INFESTATION AND THE RATE OF PARASITISM

Table 4 and Figure 4 show the relationship between the infestation rate and the parasitism rate.

Release dates	The rate of infestation	The rate of parasitism
07-06-2021	0%	0%
14-06-2021	22%	73%
21-06-2021	25%	72%
28-06-2021	32%	41%
04-07-2021	40%	53%
12-07-2021	47%	51%
19-07-2021	32%	63%
09-08-2021	18%	56%

Fig. 4 - The relationship between infestation rate and parasitism rate



Source: Prepared by the authors (2024)

Interpretation of the relationship between the rate of parasitism and the rate of infestation

In Figure 4, the two curves plotted are those obtained in the same plot during monitoring of the crop over the period January-August 2021.

According to the results obtained in Table 4 and Figure 4, it should be noted that the parasitism rate is higher than the infestation rate. In fact, the value of the infestation rate is lower than the value of the parasitism rate.

There were two peaks in parasitism on 14 and 21 June 2021. On the other hand, for the infestation rate there is a peak on '12 July 2021'.

The relationship between the infestation rate and the parasitism rate is increasing, but the value of the parasitism rate exceeds that of the infestation rate.

The significant relationship between the rate of parasitism and the rate of infestation is due to several factors, in particular thermal variations. This can be explained by the good management and success of the biological control method using parasitoids.

Irrigation is another factor affecting the establishment of Phyllocnistis citrella. Trees that are well irrigated have a very high number of parasitized organisms.

Once the pest has settled in, there are other parameters that influence its distribution.

The distance between the clementine trees and the greenhouse. Trees closest to the greenhouse have a limited number of parasitized organisms compared with trees further from the greenhouse, which contain a very large number of parasitized organisms.

Another parameter that affects infestation is the proximity of crops. Neighbouring sides of Thomson trees contain a very large number of parasites, whereas neighbouring sides of Rosasser stone trees contain a very small number of parasites.

Another parameter that affects the distribution of harmful organisms is temperature variations, which play a very important role in the development of this pest. The peak of the parasitism rate is reached when the average temperature is 28.9°, which is a favourable temperaturefor the production of parasitism.

The increase in the rate of parasitism in relation to the rate of infestation is a result of the absence of the use of chemical treatments in the experimental orchard.

Discussion of the relationship between the rate of parasitism and the rate of infestation

KHECHNA *and al*. (2017) found in 2011 that the infestation rate is higher than the parasitism rate. This is due to pesticide treatments. The parasitism rate increased from 15 August 2012 compared with the infestation rate, which highlights the importance of the activity of beneficials. Similarly, the parasitism rate is the highest in relation to the infestation rate, increasing and reaching a maximum of 68% in summer sap shoots on 21 June 2021 (Bendoumia *and al*., 2023).

The importance of the parasitism rate in relation to the infestation rate is due to the activityof the beneficials.

CONCLUSION AND OUTLOOK

For the present study, the parasitism rate is high in this orchard because the sanitary conditionof the trees is clean ‘no pesticides are used in this orchard’. Also, the presence of greenhouses for rearing citrus leafminer parasitoids next to clementine trees favours and increases the rate of parasitism. In fact, it is interesting to install citrus leafminer parasitoid rearing greenhouses next toor in the middle of citrus trees in order to increase the rate of parasitism.

In addition, the orchard is located in the Boufarik region, where there is general containmentas a preventive measure against the Corona virus 'Covid-19' pandemic. This site offers climatic conditions conducive to the development of citrus leafminer parasitoids. Containment also reducesthe atmospheric pollution that encourages pest outbreaks in general, and citrus leafminers in particular.

Finally, regional containment is a very important future solution for long-term control of the dreaded citrus leafminer as part of an integrated pest management programme. In fact, we need to propose to those responsible for the region where citrus trees are grown that a regional containment system be introduced for a period of time to be studied, in order to minimise the use of pesticides against the citrus leafminer. In addition, we need to study the bio-ecology of natural enemies in greenhouses near citrus leafminer-infested yards and incorporate them with other integrated pest management methods.

ACKNOWLEDGEMENTS

The authors would like to thank all those who contributed to this scientific article for their help and encouragement.

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