# Population Fluctuation of Citrus Leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and its Parasitoids in the Eastern Mediterranean Region of Turkey

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**Abstract-** The seasonal abundance, parasitoid complex and percentage of parasitism of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) were investigated in three grapefruit orchards in the eastern Mediterranean region in 2005-2007. *Phyllocnistis citrella* populations increased during summer months and declined during fall. During the study period, 2-3 peaks of both pest and parasitoid populations were detected on the summer shoots and 1-2 peaks on the fall shoots. At the experimental sites, 10 parasitoid species and unidentified individuals belonging to 2 genera were determined to attack *P. citrella. Citrostichus phyllocnistoides* Narayanan (Hymenoptera: Eulophidae) was the most abundant parasitoid (72.8%), followed by *Cirrospilus brevis* Zhu, LaSalle & Huang (11.2%) and *C. ingenuus* Gahan (Hymenoptera: Eulophidae) (7.1%). *Sympiesis striatipes* (Ashmead) (Hymenoptera: Eulophidae), was determined for the first time as a parasitoid of *P. citrella* in Turkey. Parasitism ratio (%) ranged between 39.56-50.67% in Hatay, 42.60-47.61% in Adana and 35.60-41.25% in Mersin during the study years. The highest rates of parasitism were observed at the end of summer and in the fall. It was determined that percent parasitism was significantly correlated with increases of *P. citrella* density, but did not significantly differ among the study sites and years. The results show that *C. phyllocnistoides* has an important role among the *P. citrella* parasitoids present in Turkey due to its frequency.

Key words: *Phyllocnistis citrella*, population fluctuation, parasitoid composition, parasitism, *Citrostichus phyllocnistoides* 

# **INTRODUCTION**

Citrus leafminer (CLM), Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae) is a serious pest in nurseries and top grafted trees in citrus-producing areas of the World (Clausen, 1931). All citrus growing countries in the Mediterranean Basin were invaded by the pest between 1994 and 1995 (García-Marí et al., 1997). In Turkey, the pest was reported for the first time in June 1994 in the eastern Mediterranean region (Uygun et al., 1995). Shortly after the invasion, native parasitoids (primarily eulophids) attacked the pest, as well as generalist predators (Uygun et al., 1996), but growers considered CLM densities too high and treated both mature and young groves multiple times per season (Yumruktepe et al., 1996). Subsequently, P. citrella has spread to all areas where citrus is planted in the Mediterranean and Aegean regions and must be controlled in nurseries

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in Turkey (Elekcioğlu, 2001).

Larvae of the pest destroy the epidermis of young leaves by mining through the leaf surfaces and the damaged leaves curl and become sclerotic and necrotic. Heavy infestations can seriously affect plants from nurseries and recently planted trees, although the damage is less significant in mature trees (Uygun et al., 2000). Current control of CLM by growers is primarily based on repeated application of insecticides during the flush of young leaves on trees younger than five years old. Although they have effectively controlled the pest, the continued use of pesticides for several decades has disrupted biological control by natural enemies, led to resurgence in P. citrella populations, and led to widespread resistance to various types of insecticides. Decreasing efficacy and increasing concern over adverse environmental effects have brought the need for the development of new types of selective control alternatives or methods of crop protection with or without reduced use of synthetic insecticides. Biological control is one of the best options for controlling this pest (Hoy et al., 2007). Nearly 80 species of parasitoids have been obtained

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from citrus leafminer worldwide (Schauff et al., 1998). Many of these species do not provide much effective control of the pest. Some, however, play a significant role in the regulation of the pest levels, such as species of Cirrospilus sp. (LaSalle and Pena, 1997). One of the first efforts on the biological control of the citrus leafminer in the Mediterranean area was initiated in Israel in 1994-1995 (Argov et al., 1998) and after that biological control programs were successively developed in most of the countries in this region, such as Spain (Garcia-Mari et al., 1997), Italy (Siscaro et al., 1999), France (Malausa, 1997), Cyprus (Orphanides et al., 1999), Syria and Tunusia (FAO, 1996), in which programs, except the indigenous species recorded, a number of exotic parasitoids was used. After the introduction of the pest, a biological control program also was initiated in Turkey. Exotic hymenopteran parasitoid including Ageniaspis species, citricola Logvinovskaya (Encyrtidae), Cirrospilus ingenuus Gahan (C. ingenuus was called C. quadristriatus at that time) and Semielacher petiolata (Girault) (Eulophidae), were introduced from Australia. They were reared in the insectary of the Institute of Plant Protection (Adana) in small numbers and released in citrus orchards in various areas of Adana. Among these introduced parasitoid species, C. ingenuus and S. petiolata were established (Uygun et al., 1997). As well, ten native species (Eulophidae) belonging to five genera have been recorded in Turkey. Among these. Citrostichus phyllocnistoides Narayanan (Eulophidae) was considered as a potential biological control agent. It was first detected on CLM in 1998, and by 2001 accounted for 61% of the encountered parasitoids (Elekcioğlu and Uygun, 2006).

Knowledge of the population dynamics and rate of parasitism are especially critical in evaluating the potential effectiveness of biological control agents. This study was undertaken to investigate the population dynamics of CLM and its parasitoids at three sites in eastern Mediterranean region and evaluate the importance of the latter for biological control of the pest.

# MATERIALS AND METHODS

The seasonal occurrence of P. citrella and its

parasitoids were studied from 2005 through 2007, in three grapefruit (Mars Seedless) orchards in the eastern Mediterranean region. The first orchard was 12 years old in Dörtyol/Hatay, the second was 16 years old in Balcalı/Adana (Çukurova University Research Farm), and the third was 18 years old in Erdemli/Mersin. All plots were drip irrigated and surrounded with different varieties of citrus orchards. At one edge of each orchard, 25 trees were selected and during the study, these trees remained insecticide-free. The density of P. citrella and its parasitoids was determined by randomly picking 1 shoot (10-15 leaves per shoot) from 10 randomly selected trees among the 25 trees. Ten shoots were collected at every census date. Samplings were taken weekly from May to November and monthly from December to April. Leaves with larvae and pupae were isolated and kept inside 12 cm petridishes containing moistened cotton to observe the emergence of adults and parasitoids of P. citrella. Dish covers were perforated with fine pores for ventilation. Leaves were held under temperature 25±1°C and photoperiod 16h/day until emergence of the parasitoids. All emerging adults of P. citrella and parasitoids were counted and parasitoids preserved in glass vials and labeled. From December to April, the shoots were checked visually since the shoots were free from any leafminer infestation. Weather data were obtained from the nearest weather stations to the trial orchards and averaged each month over the experiment.

#### Statistical analysis

Seasonal trends of the dynamics of *P. citrella* and its parasitoids were assessed using the numbers of larvae and pupae of the pest and immature stages of the parasitoids. The percentage parasitism of the samples was calculated as the ratio of the number parasitized host larvae and pupae to the total number of all host stages (van Driesche, 1983). Data for parasitism in the years (2005, 2006, and 2007) and at the study sites (Hatay, Adana, Mersin) were subjected to one-way ANOVA to compare the percentage of parasitism among the years and study sites. A correlation analysis was applied to determine the relation between *P. citrella* population density and parasitoid density (%)

(P $\leq$ 0.05) in the study sites. Relationships among the variables were determined by Pearson's correlation coefficient. In order to test the effect of host density on percentage parasitism for all and most common parasitoid species, regression analysis were performed on percentage parasitism (independent variable) and number of *P. citrella* individuals per leaf (dependent variable) (%) (P<0.05). All analyses were performed using the Microsoft statistical package program SPSS 15.0. (SPSS, 2006).

#### RESULTS

#### *Population dynamics*

In total, 25,441 leaves were inspected between May 2005 and December 2007 to determine presence or absence of P. citrella immature stages in the three orchards. In total 6,476 from 44 samples, 7,804 from 49 samples, 5,412 from 44 samples of CLM individuals were recorded in Hatay, Adana and Mersin, respectively. The first leaf infestation was observed in May and June at all study sites and continued in the summer flush till the end of the autumn flush in September/October (Figs. 1-3). At all of the sampling sites, P. citrella populations declined in November and December through April. In general, parasitoids exhibited a population fluctuation similar to that of the pest and also a similar behavior throughout the spring to winter period. The highest abundance of parasitoids coincided with the highest pest density (early and mid-summer) in all sampling periods. Parasitism of CLM ranged from 0% and 100% across years in Hatay and Adana and 0% and 76% across years in Mersin.

In Hatay, the first infestation was observed in flush starting at the beginning of June in 2005 and 2006, and at the end of May in 2007, with the infestation proceeding until autumn flush (Fig. 1). In all, there were 2-3 peaks of highest immature CLM densities (all larvae and pupae) in July, August and September. The average number of CLM per leaf was 1.78, 0.33 and 1.65 individuals in 2005, 2006 and 2007, respectively. The peak of CLM activity was in 2005 with an average of 6.75 individuals at the beginning of July. In general, the parasitoid populations fluctuated similarly to that of CLM, with the population attaining a maximum of 2.10 individuals per leaf in July in 2007. Parasitization rate of CLM was averagely 39.56%, 48.15% and 50.67% in 2005-2007, respectively. The percentage parasitism did not significantly differ among the years (F=0.494, df=2, P=0.613).

In Adana, the first infestation was observed at the beginning of June in 2005 and 2006, but in May in 2007 (Fig. 2). During July to October, 3-4 population peaks occurred, varying between 0.01 and 3.87 citrus leafminer per leaf with an average number of CLM per leaf of 1.62, 0.75 and 1.77 individuals in 2005, 2006 and 2007, respectively. The parasitoid population peaked at an average of 2.13 individuals per leaf in the second part of August in 2007. Average percent parasitism was 47.61%, 42.60% and 47.56%, respectively from 2005-2007 and parasitism (%) did not significantly differ among years (F=0.232, df=2, P=0.794).

In Mersin, the first infestation was observed at the beginning of June and proceeded till October in 2005-2007 (Fig. 3). There were 3-4 peaks of highest immature CLM densities each year. The average number of CLM per leaf was 0.98, 0.64 and 1.45 individuals in 2005, 2006 and 2007, respectively. CLM and the parasitoid population reached a peak of 4.70 and 1.77 individuals per leaf, respectively in July in 2007. The parasitized CLM larvae and pupae were averagely 35.60%, 37.31% and 41.25% in 2005, 2006, 2007, respectively. The percentage of parasitism did not significantly differ among the years (F=0.318, df=2, P=0.729).

When we compare the study sites among years, percentage of parasitism did not significantly differ (F=0.883, df=2, P=0.441 in 2005, F=0.474, df=2, P=0.626 in 2006 and F=0.672, df=2, P=0.515 in 2007). The parasitoid population coincided with *P. citrella*. Correlation analysis indicated that there was a significant relationship between *P. citrella* and parasitoid population (R=0.694, n=74, P<0.01 in Hatay; R=0.879, n=77, P<0.01 in Adana and R=0,861, n=69, P<0.01 in Mersin).

#### Parasitoid composition

During the study, ten species of parasitoids [Citrostichus phyllocnistoides (Narayanan), Cirrospilus ingenuus Gahan, Cirrospilus pictus (Nees), Cirrospilus vittatus Walker, Cirrospilus brevis Zhu, LaSalle & Huang, C. variegatus (Masi),



Fig. 1. Average density of *P. citrella* and its parasitoids in grapefruit orchard in Dörtyol/Hatay between 2005 and 2007. Average monthly temperature (°C), relative humidity (% RH), and rainfall ( $kg/m^2$ ) in Hatay between 2005-2007.

Pnigalio incompletus (Bouček), Neochrysocharis formosa (Westwood), Semielacher petiolata (Girault). **Symplesis** striatipes (Ashmead) (Hymenoptera: Eulophidae)], and unidentified individuals belonging to 2 genera [Barycapus sp., Pnigalio sp. (Hymenoptera: Eulophidae)] were recorded (Table I). A total of 7,257 parasitoids emerged. The most abundant parasitoid during all seasons was C. phyllocnistoides, accounting for 72.8% of the parasitoids observed. Cirrospilus brevis was the second (11.2%) and C. ingenuus (7.1%) the third most encountered species in this study. Pnigalio incompletus (3.9%) and Pnigalio sp. (1.5%) were observed in May and June. All the other parasitoid species were in a ratio of lower than 1.5%. Symplesis striatipes was found for the first time as the parasitoid of *P. citrella* in Turkey. Among the parasitoid species, *Barycapus* sp. was detected only in Mersin, while the others were recorded in all study sites.

#### Parasitism rate

Parasitism in the orchards generally followed a trend similar to that of P. citrella density throughout the spring to winter during the study period. In general, a positive relationship was evident between P. citrella density and abundance of parasitoids, the highest abundance of parasitoids in summer corresponded to an increase in the host population. The percent parasitism was similar in Hatay (46.34%) and Adana (46.67%), with both higher than Mersin (39.14%). Throughout the study, the highest rates of parasitism were observed in August and in the autumn. Analysis of the data showed a clear dependence between percentages of parasitism and citrus leafminer population density for all parasitoid species (Fig. 4) and for the most frequent parasitoid populations (C. phyllocnistoides, C. brevis, C. ingenuus) (Fig. 5).

A positive regression was evident between the number of *P. citrella* and percent parasitism at





Fig. 2. Average density of *P. citrella* and its parasitoids in grapefruit orchard in Balcah/Adana between 2005 and 2007. Average monthly temperature (°C), relative humidity (% RH), and rainfall ( $kg/m^2$ ) in Adana between 2005-2007.

 Table I.
 Abundance and frequency of *Phyllocnistis citrella* parasitoids found on citrus in eastern Mediterranean region of Turkey in 2005-2007,

Species	Abundance per year			<b>Relative frequency</b>		No. of percentage of different taxa	
	2005	2006	2007	No.	%	No.	%
Citrostichus phyllocnistoide	1,388	1,745	2,148	126	92.0	5,281	72.8
Cirrospilus brevis	301	242	271	82	59.9	814	11.2
Cirrospilus ingenuus	209	145	159	60	43.8	513	7.1
Pnigalio incompletus	94	102	86	42	30.7	282	3.9
Neochrysocharis Formosa	38	28	21	21	15.3	87	1.2
Semielacher petiolata	14	19	11	9	6.6	44	0.6
Sympiesis striatipes	17	16	14	10	7.3	47	0.6
Cirrospilus vittatus	9	12	15	12	8.8	36	0.5
Cirrospilus pictus	10	7	5	11	8.0	22	0.3
Cirrospilus variegatus	5	4	6	6	4.4	15	0.2
Barycapus sp.	4	2	1	2	1.5	7	0.1
Pnigalio sp.	39	29	41	26	19.0	109	1.5
Total	2,128	2,351	2,778	137 (a)	(b)	7,257	100

a Total number of samples exceed this figure since one sample may contain several species

b Total of percentages exceeds 100



Fig. 3. Average density of *P. citrella* and its parasitoids in grapefruit orchard in Erdemli/Mersin between 2005 and 2007. Average monthly temperature (°C), relative humidity (% RH), and rainfall (kg/m<sup>2</sup>) in Mersin between 2005-2007.



Number of citrus leafminer larvae and pupae per leaf

Fig. 4. Relation between percentage parasitism of all parasitoid species and average density of *Phyllocnistis citrella* in Hatay (A), Adana (B) and Mersin (C) between 2005 and 2007.

all study sites (F=81.867,  $R^2$ =0.532, df=1, 73, P=0.05 in Hatay, F=133.151,  $R^2$ =0.639, df=1, 76, P=0.05 in Adana and F=106.942,  $R^2$ =0.614, df=1, 68, P=0.05 in Mersin) (Fig.4). Average percent parasitism of *C. phyllocnistoides* was highest in

Hatay (44.15%). Percent parasitism by *C*. *phyllocnistoides* correlated more with host density (R=0.726, n=74, P $\square$ 0.01) (Fig. 5 A) than for *C*. *brevis* and *C*. *ingenuus* (Fig. 5). There was a weak correlation between the pest population and



Number of citrus leafminer per leaf

Fig. 5. Relation between percentage parasitism of *Citrostichus phyllocnistoides* (A), *Cirrospilus brevis* (B), and *Cirrospilus ingenuus* (C) and average density of *Phyllocnistis citrella* in Hatay, Adana and Mersin between 2005 and 2007.

C. ingenuus in Hatay (R=0.476, n=74, P<0.01) (Fig. 5C). The percent parasitism of C. phyllocnistoides in Adana and Mersin was approximately 39.43% and 31.12%, respectively in 2005-2007. Cirrospilus brevis populations significantly correlated to host density in Adana and Mersin, more so than the other two parasitoid species (Fig. 5B).

#### DISCUSSION

The population of *P. citrella* increased during summer flushes and declined in the autumn. The most likely reason of the increase and decrease in population was related to both the development of the new flushes and presence of favorable temperatures for the pest development. Besides temperature, rainfall and humidity were also believed to be direct factors which influenced the flushing leaves due to the infestation of CLM. During June through July, CLM populations increased and parasitoids appeared, then declined during winter period depending on the pest population. Other reports on the pest population are in agreement with the observations previously described; the seasonal pattern of *P. citrella* in the region was similar to observations in Florida (Peña *et al.*, 1996; Hoy *et al.*, 2007), in southern Texas (Legaspi *et al.*, 1999), in Argentina (Diez *et al.*, 2006) and in İzmir (Güncan *et al.*, 2009) where population densities increase from spring to fall and decline during the winter.

The parasitoid species reared confirm records obtained for previous the eastern Mediterranean region (Uygun et al., 1997; Elekcioğlu, 2001; Elekcioğlu and Uygun 2006). In 1995 and 1996, the predominant species was R. incompleta (syn. of Pnigalio incompletus), which made up 50% of all parasitoid specimens recovered. In 1997, Cirrospilus sp. nr. lyncus (Cirrospilus brevis) was the most frequently encountered species (69%) in the region (Elekcioğlu and Uygun, 2006). After the introduction of C. phyllocnistoides in 1998, the population of C. brevis decreased to 25% in 2001. The same situation occurred in western Sicily after the introduction of the exotic parasitoids C. phyllocnistoides and S. petiolatus, which displaced the indigenous parasitoids (Liotta et al., 2003). Citrostichus phyllocnistoides was synchronized with the pest, spending the winter in diapause on P. citrella, with delayed development in the spring in Tucuman, Argentina (Diez et al., 2006). Among the observed parasitoids, C. phyllocnistoides, C. ingenuus and S. petiolata were specific to P. citrella, whereas the others attack other lepidopteran miners (LaSalle and Parrella, 1991). Symplesis striatipes was determined for the first time as a parasitoid of P. citrella in Turkey. It is an ectoparasitoid, polyphagous eulophid that is also found on other gracillariid leafminers such as Acrocercops spp. and Phyllonorycter sp. in several Asian countries (Schauff et al., 1998). It was found in low numbers, hence, it is thought to have a very low impact on the reduction of pest populations. Despite its low numbers in Turkey, S. striatipes has been reported as an important control agent of P. citrella in other countries (Ujive and Adachi, 1995). The structure of the parasitoid complex of P. citrella varies according to the region under study. Cirrospilus lyncus Walker, P. mediterraneus Ferriere and Chrysonotomia sp. were detected as parasitoids of CLM in Antalya, west Mediterranean region of Turkey, where C. lyncus was the dominant, but P. mediterraneus was the most frequently encountered species (Ciftçi, 1995). In the Aegean region of Turkey, R. incompleta, C. sp. nr. lyncus and Pnigalio sp. were recorded (Baspinar et al., 1996). In eastern Spain, after the introduction of C. phyllocnistoides in 1999, the native parasitoids found on CLM decreased dramatically, representing 1% of the total parasitoid assemblage where Pnigalio sp. and Cirrospilus brevis Zhu, LaSalle and Huang, were the most two abundant species representing >90% of the parasitoids in 1995 (Vercher et al., 2005). Citrostichus phyllocnistoides constituted 99.4% of the parasitoids collected in 2006 in Spain (Karamaouna et al., 2010). Five species of parasitoids, one introduced (Ageniaspis citricola Logvinovskaya) and four native species were observed in Tucuman, Argentina (Diez et al., 2006). Citrostichus phyllocnistoides was a potential biological control agent in Fuzhou region of southeast China (Wang et al., 2006). In Western Crete, five parasitoid species were recorded out of which C. phyllocnistoides was the most abundant one (80%) followed by S. petiolatus (Kalaitzaki et al., 2011). In Greece, P. citrella parasitoid complex included the native species N. formosa (Westwood), P. pectinicornis L. (Hym.: Eulophidae) and the introduced C. phyllocnistoides. The most abundant of those was N. formosa (Tsagkarakis et al., 2013). As seen from other studies, different parasitoid species were found in different countries. It is thought that different ecological conditions and alternative hosts inhabiting nearby citrus plantations acting as reservoirs for the parasitoids in different regions, affected the presence and assortment of different parasitoid species.

The higher parasitism in late summer and fall in our study sites is likely due to declines in the population of *P. citrella*. A similar situation was observed in Veracruz, Mexico, with high parasitism rates accompanied by low numbers of *P. citrella*  (Bautista-Martínez et al., 1998). It is determined that a clear dependence existed between percentages of parasitism and CLM population density for the parasitoid most frequent populations (*C*. phyllocnistoides, C. brevis and C. ingenuus). This means that the efficacy of the parasitoid increases with decrease in population density of the pest. In general, a positive relationship was evident between P. citrella and parasitoid abundance at all study sites and all years. This is in agreement with previous studies, in which the spatial distribution of host and its natural enemies has a great influence on the dynamics of both populations (Jahnke et al., 2008). In Hatay, C. phyllocnistoides densities correlated more to the pest densities than did C. brevis and C. ingenuus due to the higher number of the parasitoid specimens at this site. The results show that C. phyllocnistoides has an important role among parasitoid species present in Turkey. In this study, percent parasitism by all parasitoid species was lower than those observed in other countries. Parasitism of CLM was averagely 60% in Florida (Hoy and Nguyen, 1997) and 70% in Mexico (Bautista-Martínez et al., 1998). Five native parasitoid species were found in the Malaga province (Spain); the leafminer parasitized by C. phyllocnistoides was lower than 10% in 1999, but in 2001 this rate was 60% (Marquez et al., 2003). The percent parasitism recorded for C. phyllocnistoides in this study was lower than that recorded in some countries. Studies conducted in Fuzhou (South China), showed 54.38% parasitism for this parasitoid species and the results in Guangzhou showed values of 67.6% parasitism, where C. phyllocnistoides was the dominant parasitoid of CLM in citrus orchards in both regions (Ding et al., 1989). In India similar results were detected; parasitism by C. phyllocnistoides started at the end of July, increased after August on with a percentage parasitism of 40-50% (Subba Rao and Ramamani, 1965). In Texas, the percentage of parasitism by native parasitoids ranged between 0 and 42% (French and Legaspi, 1996) which is also similar to the results here. In Greece, the average percentage of parasitism was lower (13.1%, 13.8% and 11.7% on orange, mandarin and lemon, respectively) with the most abundant species N. formosa (Tsagkarakis et al., 2013).

Damage caused by P. citrella is a major problem (especially for nurseries) in Turkey. High pest populations occurred during the summer. C. phyllocnistoides has an important role among the parasitoid species due to the frequency with which it is observed on the pest. Despite their lower number in the region, other parasitoid species such as C. brevis and C. ingenuus have also been important control agents of P. citrella. These species were undoubtedly responsible, in part, for the decrease in the population densities of the pest since their introduction, and to current levels. It is thought that with proper cultural practices and the application of specific acaricides and insecticides. the enhancement and conservation of all the parasitoid species is possible.

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