# Population fluctuation of *Phyllocnistis citrella* and its parasitoids in two citrus species in Western Crete (Greece)

## A.P. KALAITZAKI<sup>1\*</sup>, A.E. TSAGKARAKIS<sup>2</sup> AND D.P. LYKOURESSIS<sup>2</sup>

<sup>1</sup>Institute of Olive Tree and Subtropical Plants, Agrokipio, 731 00 Chania, Greece <sup>2</sup>Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, 75 Iera Odos str., 118 55 Athens, Greece

## ABSTRACT

Population fluctuation, percentage of parasitism and parasitoid species composition of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) were studied in a citrus orchard with orange and mandarin trees in Chania (Greece), in 1999, three years after the release of the introduced parasitoids *Citrostichus phyllocnistoides* (Narayanan), *Quadrastichus* sp., *Semielacher petiolatus* (Girault), *Cirrospilus quadristriatus* (Subba Rao and Ramamani) and *Ageniaspis citricola* Logvinovskaya. Results showed that significantly more *P. citrella* individuals per leaf were recorded on orange than on mandarin trees. Regarding the parasitoid complex composition, the exotic species *C. phyllocnistoides*, *S. petiolatus* and *Quadrastichus* sp., as well as the natives *Pnigalio pectinicornis* L. and *Neochrysocharis formosa* (Westwood) were recorded. Among them, *C. phyllocnistoides* was the most abundant, followed by *S. petiolatus*. The average percentage of parasitism reached 17% and 22% on orange and mandarin, respectively.

KEYWORDS: Phyllocnistis citrella, biological control, Citrostichus phyllocnistoides, Semielacher petiolatus, Pnigalio pectinicornis, Neochrysocharis formosa, Quadrastichus sp., citrus.

### Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), having origin from eastern and southern Asia, after 1993 was rapidly spread to all citrus growing regions of America and in the Mediterranean basin. In Greece it was first recorded in June 1995 on the island of Rhodes and in Crete (Anagnou 1995, Michelakis 1997), and within a few months, it was spread in most of the citrus growing areas of Greece (Tsagkarakis et al. 1999).

Heavy infestation of *P. citrella* can severely damage young citrus trees in the field or in nurseries, while the damage is less significant

in mature trees (Garcia-Marí et al. 2002).

According to Knapp et al. (1995), chemical control of the citrus leafminer consists a short term and costly solution, with additional unfavorable consequences to the existing IPM programs in citrus. Therefore, classical biological control, through the introduction of hymenopteran parasitoids, became soon the most promising method to manage *P. citrella* in orchards with mature trees. This was supported by the fact that over 80 hymenopteran species, mostly chalcidids, have been recorded to parasitize citrus leafminer throughout the world (Schauff et al. 1998) and, consequently, they have been included in a number of IPM programs

\*Corresponding author, e-mail: akalaitzaki@nagref-cha.gr; argkalaitzaki@gmail.com

(Hoy et al. 1995, Neale et al. 1995, Agrov and Rössler 1996. Smith and Beattie 1996). One of the first efforts on the biological control of the citrus leafminer in the Mediterranean area was initiated in Israel in 1994-1995 (Agrov and Rössler 1996, Agrov et al. 1998), in which 30 indigenous parasitoid species were recorded (FAO 1996, Schauff et al. 1998). After that, biological control programs were successively developed in most of the countries in that region, such as Cyprus (Orphanides et al. 1999), Spain (Garcia-Mari et al. 1997), Italy (Siscaro et al. 1999), France (Malausa 1997), Algeria (Berkani and Mouats 1997), Morocco (Nia et. al. 1997), Syria, Tunisia and Turkey (FAO 1996), in which programs, except the indigenous species recorded, a number of exotic parasitoids was used. From those species, the most common were the eulophids Citphyllocnistoides rostichus (Naravanan). Quadrastichus sp., Semielacher petiolatus (Girault) and Cirrospilus quadristriatus (Subba Rao and Ramamani) (Hymenoptera: Eulophidae), as well as the encyrtid Ageniaspis citricola Logvinovskava (Hymenoptera: Encyrtidae).

In Greece, a similar biological control program was initiated one year after the first record of *P. citrella*, with the introduction of exotic hymenopteran parasitoid species consist part of it. These species were the eulophids *C. phyllocnistoides, Quadra-stichus* sp., *S. petiolatus* and *C. quadric-striatus* and the encyrtid *A. citricola.* The parasitoids were introduced from Cyprus and subsequently mass reared in the insectary of the Institute of Olive Tree and Subtropical Plants in Chania and systematically released, in citrus orchards in various areas of Greece (Kalaitzaki et al. 1999).

The aim of this study was to monitor the seasonal abundance of citrus leafminer and its parasitoids (native and exotic) on orange and mandarin, in Western Crete as well as to calculate the percent parasitism caused by them.

### **Materials and Methods**

In order to estimate the population fluctuation of citrus leafminer and the incidence of its parasitoids, weekly samplings were conducted, from 28 May until 9 December 1999, in a 4.3-ha organic citrus orchard in Chrysopygi region, located about 4.2 Km north east from the city of Chania (Greece). This orchard contained 270 orange trees (Citrus sinensis (L.) Osbeck cv. Washington Navel), 7 years-old, and 418 mandarin trees (Citrus deliciosa Tenore cv. Mediterranean common), 30 years-old. No insecticide sprays were applied during the period of the study. In each sampling, five trees were randomly selected from each citrus species. The canopy of each tree was divided in two sides (north and south) and two lavers (one and two meters above the ground) and one flush, contained 6-25 young leaves, was collected from each of these four divisions. Therefore, a total of 130-500 young leaves per citrus species were collected in each sampling, which were placed into plastic bags. Leaves were examined in the laboratory under a binocular stereo-microscope for presence of mines (either occupied or abandoned), eggs, larvae (first to fourth instar based on their morphology) and pupae of the citrus leafminer, live and dead, as well as parasitoid immature stages (eggs, larvae, pupae). Leaves contained parasitized individuals of P. citrella were placed individually in plastic Petri dishes with water soaked cotton. Each Petri dish was firmly closed by laboratory film (parafilm) and placed in a growth chamber under conditions of 25  $\pm$  $1^{\circ}$ C,  $65 \pm 5\%$  RH and 16:8h L:D photoperiod, until adult emergence of the parasitoids. Adult parasitoids were collected in plastic vials and kept for systematic taxonomy. Parasitoids were indentified using the key by Schauff et al. (1998). Two of the species (Pnigalio pectinicornis L. and Neochrysocharis formosa (Westwood)) were sent to Dr. J. LaSalle (British Museum of Natural History, London), who confirmed the identification.

The percentage parasitism of the samples was calculated as the ratio of the number of the parasitized host larvae and pupae to the total number of all host stages (van Driesche 1983).

Wilcoxon rank-sum tests were used to compare the population abundance of P. citrella and its parasitoids (as number of live individuals per leaf) on orange with the population abundance on mandarin trees. Data on the fluctuation of P. citrella on orange and mandarin within the sampling date were Student's t- test, analvzed by after log(x+0.5) transformation. Wilcoxon ranksum tests were also used for comparing the percentage of parasitism on orange with the percentage of parasitism on mandarin trees as well as to compare the percentage of parasitism from each parasitoid species to percentage parasitism from C. phyllocnistoides within the citrus species.

In order to test the effect of host density on percentage parasitism, regression analyses were performed on percentage parasitism (dependent variable) and numbers of *P. citrella* individuals per leaf (independent variable). Analyses were conducted using the statistical package JMP 7.0.1 (SAS Institute 2007).

### Results

The citrus orchard was sampled for 28 times between May 28 and December 9 and in 11,348 leaves examined, 7,624 healthy and 2,007 parasitized *P. citrella* larvae and pupae were found.

### Population fluctuation of the citrus leafminer on two citrus species

Citrus leafminer healthy eggs, larvae and pupae ranged from 0.14 to 3.9 individuals per leaf on orange (mean:  $1.29 \pm 0.12$ ) (Fig. 1A) and from 0.02 to 1.06 on mandarin (mean:  $0.35 \pm 0.05$ ) (Fig. 1B). Significant differences were observed in the total number of live citrus leafminer individuals (eggs, larvae and pupae) per leaf between the two citrus varieties (Wilcoxon rank sum test, P < 0.01). Specifically, more individuals/leaf were recorded on orange than on mandarin trees (Table 1; Fig. 2).

On orange, two peaks of the live immature of the citrus leafminer / leaf were recorded: the first in the summer, early June, and the second in the autumn, end of October. Immature population was peaked on June 3 (3.9 individuals / leaf) (Fig. 1A). Respectively, on mandarin trees three peaks were recorded (mid June, end of October and early December) with maximum values occurred on June 17 (1.1 individuals / leaf) and on October 25 (1.01 individuals / leaf) (Fig. 1B).

#### Incidence of citrus leafminer parasitoids

Parasitoid population density ranged from 0.02 to 0.56 individual per leaf on orange (mean: 0.23  $\pm$  0.03) (Fig. 1A) and from 0.00 to 0.370n mandarin (mean: 0.15  $\pm$ 0.02) (Fig. 1B). Significant differences were observed in the number of parasitized larvae between the two species, since it was higher on orange compared with mandarin (Wilcoxon rank sum test, P < 0.01) (Table 1).

The percent parasitism ranged from 1.66% to 51.15% on orange (mean: 16.74%  $\pm$  2.59) and from 0.00% to 47.05% on mandarin (mean: 22.00%  $\pm$  2.44) (Table 1; Fig. 1). No significant differences in the overall percentage of parasitism were observed between the two citrus species (Wilcoxon rank sum test, P = 0.072).

Percent parasitism reached its maximum (ranged from 45-51%) in early September, late November and early December on orange, and in mid September and early December on mandarin trees. Contrariwise, the lowest parasitism (below 7%) was observed in the beginning of the sampling period, in late May. The respective values for the rest of the sampling period ranged from 10% to 25% on orange and from 13% to 35% on mandarin trees (Fig. 1). In general, a positive regression was evident between the number of *P. citrella* and percent parasitism on both citrus species (F = 16.38,  $R^2 =$ 

0.386, df = 1,26, P < 0.0004, y = 26.721-7.681x and F = 31.821,  $R^2 = 0.533$ , df = 1,26, P < 0.0001, y = 0.707-0.016x on orange and mandarin trees, respectively).

# Parasitic complex composition and citrus leafminer stage incidence

The collected parasitoids were the native ones *P. pectinicornis* and *N. formosa*, and the introduced *C. phyllocnistoides*, *S. petiolatus* and *Quadrastichus* sp. (Table 2). *C. quadristriatus* and *A. citricola* were not recovered since their colonies were collapsed one year after establishment.

The most abundant was C. phyllocnistoides on both citrus species, since it represented the 86.78% and 93.60% of all the species on orange and mandarin trees, respectively (Table 2), with continuous presence in the samples from June until December (Figure 3A; Figure 3B). C. phyllocnistoides was followed in abundance by S. petiolatus and P. pectinicornis on both citrus species (Table 2). S. petiolatus was present from mid June to early December, while its population was very low on fall (Fig. 3). P. pectinicornis was found only early in the season, from May to early July, on both citrus species. Ouadrastichus sp. appeared only once in the sampling period, with no apparent impact on overall parasitism rates, since its contribution to the percent parasitism was less than 0.1% (Figure 3).

The parasitism rate by *C. phyllocnistoides* was significantly higher than those by *S. petiolatus*, *P. pectinicornis, Quadrastichus* sp. and *N. formosa* (Wilcoxon rank sum test, P < 0.0001).

The parasitism rate by *C. phyllocnistoides* as well as by *S. petiolatus*, *P. pectinicornis* and *N. formosa* on orange was not significantly different from the parasitism rate of these parasitoids on mandarin trees (Wilcoxon rank sum test, P = 0.062; P = 0.146; P = 0.083 and P = 0.081, respectively) (Table 1).

The total number of the recovered parasitoids was almost the double on orange trees compared with mandarin, since 1203 and 651 individuals respectively, were emerged. Parasitoid host preference on different immature stages of the leafminer was similar on both the citrus species. The highest percentage of parasitism was recorded on second instar larvae, reached 72% and 76% on orange and mandarin trees, respectively (Table 3). Third instars followed in parasitism, reached 22% and 21% on orange and mandarin trees, respectively (Table 3).

### Discussion

Results of this study showed that significantly more P. citrella individuals/leaf were recorded on orange than on mandarin trees. Similar results have been recorded by Bermudez et al. (2004). The reason for the lower preference of P. citrella on mandarin could be owed that mandarins do not develop intense vegetative flushes and consequently the microclimate in their canopy seems to be not ideal for the citrus leafminer compared to other citrus species (Latif and Yunus 1951). Similarly, the limited flush during summer and autumn which was observed in mandarin compared to orange trees during this study (Kalaitzaki et al. unpublished data) could also affect the infestation level from the citrus leafminer. Also, as pointed out by Knapp et al. (1995) and Jacas et al. (1997), differences in susceptibility among different citrus species seem to be related to the flushing patterns of the trees.

The parasitoid complex of *P. citrella* in our study was composed by the introduced *C. phyllocnistoides, S. petiolatus* and *Quadrastichus* sp., and the native ones *P. pectinicornis* and *N. formosa.* The most abundant was *C. phyllocnistoides* on both citrus species, since constituted higher than 80% of the parasitoid complex. In general, composition of parasitoid fauna in Chania was almost the same between the two citrus species.

The total number of parasitoids was higher on orange trees compared with mandarin, probably due to the higher leafminer population registered in the former species, as mentioned above.



FIG. 1. Average number ( $\pm$  SE) of *Phyllocnistis citrella* individuals (gray bars) and its parasitoids per leaf (black solid line) and percentage parasitism of *P. citrella* (black dotted line) in a citrus orchard with orange (A) and mandarin trees (B) in Chania, Greece, in 1999.

	Citrus species			
	Orange	Mandarin		
Phyllocnistis citrella infestation				
No of individuals of <i>P.citrella</i> / leaf	1.29 (± 0.12)	0.35 (± 0.05)*		
P. citrella parasitism				
No of parasitized larvae of <i>P.citrella</i> / leaf	0.23 (± 0.03)	0.15(± 0.02)*		
Overall parasitism (%)	16.75 (± 2.59)	22.00 (± 2.44)		
Citrostichus phyllocnistoides (%)	15.21 (± 2.54)	20.81 (± 2.49)		
Semielacher petiolatus (%)	1.11 (± 0.31) **	0.79 (± 0.27)**		
Quadrastichus sp. (%)	0.05(±0.05) **	0.00 (± 0.00) **		
Pnigalio pectinicornis (%)	0.30 (± 0.11) **	0.25 (± 0.22) **		
Neochrysocharis formosa (%)	0.03 (± 0.01) **	0.00 (± 0.00) **		
Unknown (%)	0.04 (± 0.02) **	0.15 (± 0.14) **		

TABLE 1. Average number ( $\pm$  SE) of individuals / leaf and parasitism rates (mean  $\pm$  SE) for *Phyllocnistis citrella* growing on orange and mandarin plants from citrus orchard in Chania region, Greece, in1999.

\*Significant difference between citrus species within a row. \*\* Significant difference from *Citrostichus phyllocnistoides* within a column (Wilcoxon rank sum test, P < 0.01).



FIG. 2. Average number ( $\pm$  SE) of *Phyllocnistis citrella* individuals (number of eggs larvae and pupae per leaf) on orange (white bars) and mandarin trees (black bars) in Chania region, Greece, in 1999. Different letters within each date indicate significant differences (t test; P < 0.05).

	Orange	e trees	Mandarin trees	
Parasitoid species	n	%	n	%
Citrostichus phyllocnistoides	1044	86.78	644	93.60
Semielacher petiolatus	93	7.73	34	4.94
Quadrastichus sp.	2	0.16	0	0.00
Pnigalio pectinicornis	54	4.48	7	1.02
Neochrysocharis formosa	3	0.25	0	0.00
Unknown	7	0.58	3	0.44

TABLE 2. Composition of citrus leafminer parasitoid guilds (actual counts and percentages) on orange and mandarin trees in 1999 in Chania, Greece.

TABLE 3. Mean percentage of parasitism per citrus leafminer instar on orange and mandarin trees in 1999 in Chania, Greece. (n = actual counts)

	Citrus leafminer instars								
Citrus spe- — cies	Seco	Second		Third		Fourth		Pupa	
	n	%	n	%	n	%	n	%	
Orange	855	72.46	263	22.29	57	4.83	5	0.42	
Mandarin	518	76.06	146	21.44	14	2.05	3	0.44	



FIG. 3. Percentage parasitism of *Phyllocnistis citrella* of different parasitoids in citrus orchard with orange (A) and mandarin trees (B) in Chania, Greece, in 1999.

Regarding the introduced parasitoids, the three systematically released species, *C. phyllocnistoides*, *S. petiolatus* and *Quadrastichus* sp., were established. *C. phyllocnistoides* appeared for the first time in the samples in late May, having low rates of parasitism. Later in the season, parasitism by this parasitoid was increased from early September (44%) on orange and mid August (36%) on mandarin trees, reaching its maximum in early December (50% on orange and 45% on mandarin). Therefore, parasitoid guild composition changed entirely one year after the introduction of *C. phyllocnis*- toides, which became dominant, displacing all the indigenous parasitoids. C. phyllocnistoides, originated from Asia, is reported as one of the most important parasitoids in China (Wang et al. 1999) and in Japan (Uiive 2000). Apart from Chania, it is referred to displace the native non-specific parasitoids also in Spain (Garcia-Marí et al. 2004, Karamaouna et al. 2010). The establishment, dispersal and the high rates of parasitism that achieved were also observed in Israel (Argov et al. 1998), Italy (Siscaro et al. 1997), Morocco (Rizqi et al. 2003), Cyprus (Orphanides et al. 1999) and in Turkey (Elekçioğlu and Uygun 2006). This fact leads to the conclusion that the C. phyllocnistoides is effective and adapted well in the Mediterranean climate

Our results showed that the introduced *S. petiolatus* was also established in the region of our study but, compared with *C. phylloc-nistoides*, was found in lower numbers, less frequently and its contribution in the rate of parasitism was low. It has been also established in other Mediterranean countries, as in Israel (Argov et al. 1998), Italy (Mineo and Mineo 1999, Viggiani 2001, Siscaro et al. 2003), Morocco (Abbassi et al. 1999, Rizqi 2003), Syria (Al-Khateeb 1999), Spain (Garcia-Marí et al. 2004), Cyprus (Orphanides et al. 1999) and in Turkey (Elekçioğlu and Uygun 2006).

Although *Quadrastichus* sp. was recorded in the samples, it was found only once and in extremely low number, hence, it had no effect on the reduction of leafminer population. Similarly, low parasitism rates by *Quadrastichus* sp were observed in Israel (Argov et al. 1998), whereas it was failed to establish in Italy (Siscaro et al. 2003), Cyprus (Orphanides et al. 1999) and Morocco (Rizqi 2003).

The native *P. pectinicornis* was presented until mid July. Afterwards, it possibly migrated to the neighboring olive trees (which was the major crop of the area), since it is referred to have a wide host range as the olive fruit fly *Bactrocera oleae* (Gmelin) (Diptera: Tephritidae) and the olive moth *Prays oleae* (Bernard) (Lepidoptera: Yponomeutidae) (Neuenschwander et al. 1983).

The number of indigenous parasitoid species found to attack citrus leafminer in Chania region, four years after the invasion of P. citrella, was very low compared to 21 in Italy (Liotta 1997, Longo and Siscaro 1997, Nucifora and Nucifora 1997, Mineo and Mineo 1999, Conti et al. 2001), 16 in Spain (Urbaneja et al. 2000), 10 in Turkey (Uygun et al. 1997), 6 in Cyprus (Orphanides et al. 1999) and 5 in France (Quilici et al. 1997). Moreover, previous records in 1996, 1997 and 1998, in a citrus orchard near the sampling region of this study, showed that only 3 native parasitoid species were found to parasitize the citrus leafminer i.e. P. pectinicornis, N. formosa and Cirrospilus pictus (Nees) (Kalaitzaki et al. 2000) while in the areas of Galatas, Skala (Southern Greece) and Marathon (Central Greece) only four parasitoid species, Pnigalio sp., Cirrospilus sp., N. formosa and C. phyllocnistoides were found, during 1999-2002 (Anagnou et al. 2006).

This study also showed variability in P. citrella parasitism on both citrus species, with its average percent low in general, since it was found 16.75% and 22.0 % on orange and mandarin trees, respectively. However, it was significantly higher compared with 1.15, 4.4 and 9.3%, which were the mean percent parasitism found in a previous study, in a citrus orchard near the sampling region of this study, in 1996, 1997 and 1998, respectively (Kalaitzaki et al. 2000). Impact of the beneficial fauna, expressed as rate of parasitism on P. citrella population was highest in fall on both citrus species. This happened mainly due to a decline of P. citrella population during fall, when parasitoids were already well established.

In general, a positive relationship was evident between *P. citrella* and parasitoid abundance on both citrus species. 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) and most natural enemies react to the spatial distribution of their prey (Pedigo 1996).

Our results also showed that, in both citrus species, the highest percentage of parasitism was found on second instar larvae, since C. phyllocnistoides, as well as S. petiolatus parasitize mostly on second and less on third instar. P. pectinicornis parasitizes mostly on third and fourth instar and less on pupae, and N. formosa mostly on second and third, and less on fourth instar. Competition among the most abundant native parasitoid P. pectinicornis and the exotics C. phyllocnistoides and S. petiolatus does not exist directly, since their host-instar-preference does not overlap. However, C. phyllocnistoides was able to compete with S. petiolatus and *Ouadrastichus* sp., which show a similar host stage preference. Unfortunately, eggs and young larvae remain largely ignored by both the native and exotic parasitoids.

In summary, in Chania region the release program for classical biological control of *P. citrella* resulted in the establishment of the three systematically released exotic parasitoids i.e. *C. phyllocnistoides*, *S. petiolatus* and *Quadrastichus* sp., the most abundant of which was *C. phyllocnistoides* on both citrus species. Parasitoid guild composition changed entirely after the introduction of *C. phyllocnistoides*, which displaced the indigenous parasitoids.

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# Πληθυσμιακή διακύμανση του *Phyllocnistis citrella* και των παρασιτοειδών του στη Δυτική Κρήτη

## Α.Π. ΚΑΛΑΪΤΖΑΚΗ<sup>1</sup>, Α.Ε. ΤΣΑΓΚΑΡΑΚΗΣ<sup>2</sup> ΚΑΙ Δ.Π. ΛΥΚΟΥΡΕΣΗΣ<sup>2</sup>

<sup>1</sup>Ινστιτούτο Ελιάς & Υποτροπικών Φυτών Χανίων, Αγροκήπιο 73100, Χανιά <sup>2</sup>Εργαστήριο Γεωργικής Ζωολογίας και Εντομολογίας, Γεωπονικό Πανεπιστήμιο Αθηνών, Ιερά Οδός 75, 118 55 Αθήνα

### ΠΕΡΙΛΗΨΗ

Μελετήθηκαν η διακύμανση του πληθυσμού, το ποσοστό παρασιτισμού και η πληθυσμιακή σύνθεση των παρασιτοειδών του φυλλορύκτη των εσπεριδοειδών Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae), ιθαγενών και εισαγόμενων, που βρέθηκαν σε δύο από τα σπουδαιότερα είδη εσπεριδοειδών που καλλιεργούνται στην Ελλάδα την πορτοκαλιά (Citrus sinensis) και τη μανταρινιά (Citrus reticulata). Η μελέτη πραγματοποιήθηκε το 1999 σε βιολογικό εσπεριδοειδώνα του νομού Χανίων. Από τα αποτελέσματα της μελέτης προκύπτει ότι σημαντικά υψηλότερος πληθυσμός του φυλλορύκτη των εσπεριδοειδών ανευρέθηκε στην πορτοκαλιά σε σχέση με την μανταρινιά. Από τα πέντε είδη εξωτικών παρασιτοειδών (Citrostichus phyllocnistoides, Quadrastichus sp., Semielacher petiolatus, Ageniaspis citricola, Kon Cirrospilus quadristriatus), που είχαν στο παρελθόν εισαχθεί και εξαπολυθεί, βρέθηκαν στα δείγματα μόνο τα τρία πρώτα, εκ των οποίων σημαντικά πολυπληθέστερο βρέθηκε να είναι το C. phyllocnistoides σημειώνοντας τα υψηλότερα ποσοστά παρασιτισμού σε σχέση με τα υπόλοιπα. Το S. petiolatus όμως βρέθηκε σε μικρότερους πληθυσμούς σημειώνοντας σημαντικά χαμηλότερο ποσοστό παρασιτισμού σε σχέση με αυτό του C. phyllocnistoides. Αμφότερα τα παραπάνω εξωτικά είδη προτιμούν να παρασιτούν κυρίως προνύμφες  $2^{\eta_s}$  και  $3^{\eta_s}$  ηλικίας του φυλλορύκτη, γεγονός που πιθανώς οδηγεί σε ανταγωνισμό μεταξύ τους με αποτέλεσμα την επικράτηση του C. phyllocnistoides. Από τα δύο ιθαγεγή παρασιτοειδή που αγευρέθησαν, τα Pnigalio pectinicornis και Neochrysocharis formosa, πολυπληθέστερο ήταν το πρώτο, ενώ η εν γένει δράση τους συνέβαλε ελάγιστα στη μείωση του πληθυσμού του φυλλορύκτη.