Arch. Biol. Sci., Belgrade, 58 (4), 239-244, 2006.

DATA ON PHENOLOGY, PARASITISM AND CONTROL OF CITRUS LEAF MINER, *PHYLLOCNISTIS CITRELLA* STAINTON (LEPIDOPTERA, GRACILLARIIDAE), IN GREECE

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Abstract – Research was conducted to determine whether *Phyllocnistis citrella* Stainton (Lepidoptera, Gracillariidae) has any preference in attacking either the upper or the lower surface of the leaves of citrus trees. This investigation took place during the year 1999 at Marathon (Attica, Central Greece) while studying the dispersal of *P. citrella* on the leaves of three different types of citrus trees. Data on the overwintering and parasitism of *P. citrella* from different areas in Greece are also presented during the years 1999-2002. In addition, the effect of *P. citrella* infestation on imidacloprid-treated and untreated tangerine trees was comparatively studied. The parasitoids collected were *Pnigalio* sp. (Hymenoptera, Eulophidae), *Cirrospilus* sp. (Hymenoptera, Eulophidae), *Neochrysocharis formosa* (Westwood) (Hymenoptera, Eulophidae) and *Citrostichus phyllocnistoides* (Narayanan) (Hym., Eulophidae).

Key words: Hymenoptera, *Phyllocnistis citrella*, *Pnigalio* sp., *Cirrospilus* sp., *Neochrysocharis formosa*, *Citrostichus phyllocnistoides*, dispersal, overwintering

UDC 632.78 (495)
595 (495)

INTRODUCTION

The citrus leaf miner, *Phyllocnistis citrella* Stainton, has become an important pest of *Citrus* spp. in Mediterranean countries since its introduction in 1994 (C a r i j o and G a r c i a, 1994). *Phyllocnistis citrella* was first detected in Greece in June of 1995 (A n a g n o u – V e r o n i k i, 1995), and since then it has spread rapidly to all citrus-growing areas of the country (A n a g n o u – V e r o n i k i *et al.*, 1995). The larvae of *P. citrella* mine in leaf tissues of any citrus and related species (H e p p n e r, 1999), and the larval feeding results in distorted and reduced young shoots of the citrus plants. C a r r i d o V i v a s and G a s c o n L o p e z (1995) studied the distribution of immature stages in three citrus species and reported that *P. citrella* seemed to prefer the lower surface when the leaves are shorter than 10 cm long. Measures for control of *P. citrella* consist of the application of chemical products and use of natural biocontrol agents.

The main object of this work was to ascertain whether *P. citrella* has any preference in attacking either the upper or the lower leaf surface of citrus trees, study overwintering of the pest, and identify the parasitoid species associated with *P. citrella* in Greece where no data is available so far. In addition, the effect of *P. citrella* infestation on imidacloprid-treated and untreated trees was comparatively studied.

MATERIALS AND METHODS

Dispersal of Phyllocnistis citrella

The dispersal of *P. citrella* between the upper and the lower leaf surface was studied in three citrus species (Orange: var. Common, Lemon: var. Common, and Tangerine: var. Clementine) in Marathon (Attica, Central Greece). Observations were carried out every 15 days (September 6, September 21, and October 6, 1999), and

100 infested leaves (10 leaves x 10 trees) of each citrus species were randomly collected at each sampling. The trees were not sprayed with any chemical product. The number of living larvae, pupae, empty pupal chambers, and dead larvae of *P. citrella* was counted.

Overwintering

To discover the way of overwintering of *P. citrella*, observations were made in four regions [Galatas (Trizina, Southern Greece), Skala (Laconia, Southern Greece), and Marathon and Kifissia (Attica, Central Greece)] during the years 1999-2002. Ten branches of 10 trees were collected every month from each area and the samples were examined in the laboratory.

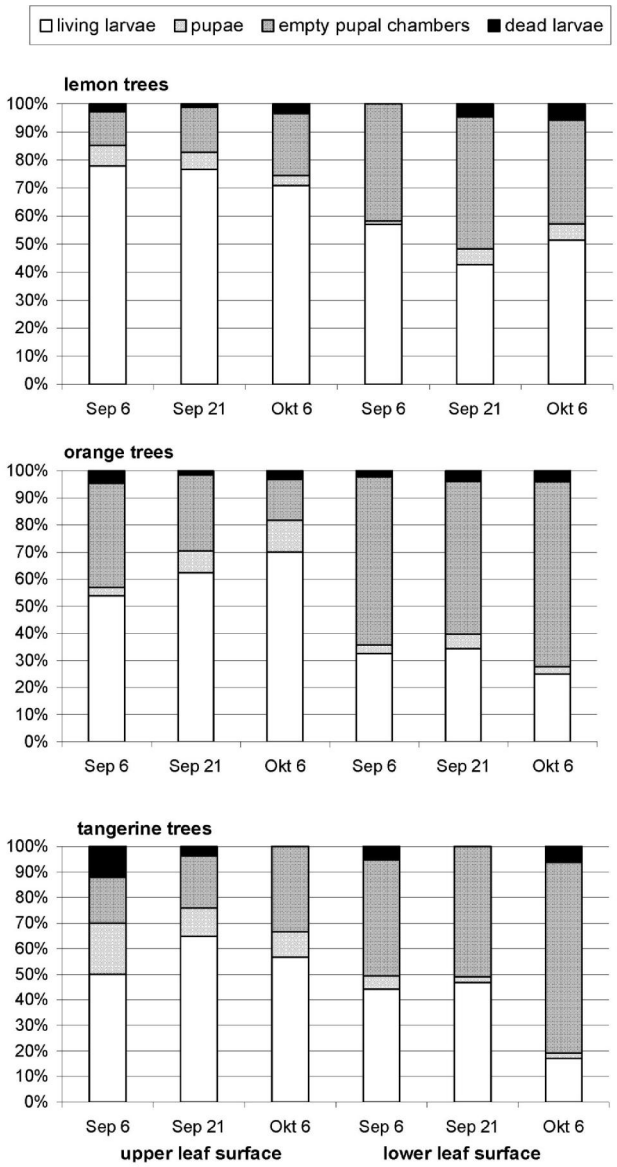

Fig. 1. Stages of *P. citrella* on the upper and lower leaf surface in three citrus species.

Parasitism

Data on the parasitism of *P. citrella* were collected from the areas of Marathon, Galatas, and Skala over the years 1999-2002. Each sample was composed of 25 branches, which were put in Plexiglas cylindrical cages (50 cm in height and 30 cm in diameter) and kept under laboratory conditions [25 ± 1°C, 65 ± 5 % RH, and photoperiod: 16:8 (L:D)] for 20 days until emergence of the parasitoids.

Comparison between treated and untreated tangerine trees

A comparison between treated and untreated groups of tangerine trees was made in order to verify whether there were visible effects of infestations on shoot growth and fruit diameter (preliminary measurements). Six shoots (each time) on four treated and four untreated trees were measured. The trees were 15 years old and were cultivated in a manner appropriate to reduce aestival shooting (avoidance of spring pruning and fertilization, reduction of summer irrigation). Measurements and sprayings were repeated every 15 days from July 13th to October 9th 1999 (Fig. 7). Six sprayings with Confidor 20 SC (imidacloprid 20%, Bayer Crop Science Hellas, Amarousion, Attica, Greece) in dose 0.075 % v/v , combined with Saf-T-Side 80 EW (fine oil 80%, Intrachem Hellas, Athens, Attica, Greece) in dose 0.3 % v/v were carried out every fifteen days, from July 13 to September 24.

RESULTS

Dispersal of Phyllocnistis citrella

During study of the dispersal of *P. citrella* between the upper and the lower leaf surface, the infestation level ranged between 0.68 and 1.03 per leaf. Younger stages of *P. citrella* were always found on the upper surface of the leaves (Fig. 1).

Overwintering

In examination of samples from four areas (Galatas, Skala, Marathon, and Kifissia), no presence of *P. citrella* was observed during the months of February, March, April, and May throughout the three years of the study.

Table 1. Composition of *P. citrella* infestation from October of 2000 to August of 2002.

Year	Month	Galatas			Marathon			Skala			Kifissia		
2000	October	LLL	PP	E	LLL	PP	E	LLL	PP	E	LLL	PP	E
	November	LLL	P	E	LLL	P	E	LLL	P	E	LLL	P	E
	December	LL	P	E	LL	P	E	LL	P	E	LL	P	E
	January	LL	P	E	L	P	E	L	P	E	L	P	E
	February	0	0	E	0	0	E	0	0	E	0	0	0
	March	0	0	0	0	0	0	0	0	0	0	0	0
	April	0	0	0	0	0	0	0	0	0	0	0	0
	May	0	0	0	0	0	0	0	0	0	0	0	0
2001	June	LLL	P	0	LL	P	0	LLL	P	0	L	P	0
	July	LLL	P	E	LLL	P	E	LLL	P	E	LL	P	E
	August	LLL	P	E	LLL	P	E	LLL	P	E	LLL	P	E
	September	LLL	PP	EE	LLL	PP	E	LLL	PP	EE	LLL	PP	E
	October	LLL	PP	EE	LLL	PP	E	LLL	PP	EE	LLL	PP	E
	November	LL	P	EE	LL	P	E	LL	P	E	LL	P	E
	December	LL	P	E	LL	P	E	L	P	E	0	0	E
2002	January	0	0	E	0	0	E	0	0	E	0	0	0
	February	0	0	0	0	0	0	0	0	0	0	0	0
	March	0	0	0	0	0	0	0	0	0	0	0	0
	April	0	0	0	0	0	0	0	0	0	0	0	0
	May	0	0	0	0	0	0	0	0	0	0	0	0
	June	L	0	0	L	0	0	L	0	0	L	0	0
	July	LL	P	E	LL	P	E	LL	P	E	LL	P	E
	August	LLL	P	E	LLL	P	E	LLL	P	E	LLL	P	E

L : living larvae < 0.2 per leaf
LL : 0.2 per leaf < living larvae < 0.4 per leaf
LLL : living larvae > 0,4 per leaf
P : pupae < 0.2 per leaf
PP : 0.2 per leaf < pupae < 0.4 per leaf
PPP : pupae > 0,4 per leaf
E : empty pupal chambers < 0.2 per leaf
EE : 0.2 per leaf < empty pupal chambers < 0.4 per leaf
0 : No presence

Similarly, no presence of *P. citrella* has was observed in January 2000 and 2002 whereas there was abundant infestation in January 2001 due to favorable weather conditions (Fig. 2). The composition of *P. citrella* infestation during October of 2000 and August of 2002 is presented in Table 1.

Parasitism

The parasitoids *Pnigalio* sp., *Cirrospilus* sp., *Neochrysocharis formosa* (Westwood), and *Citrostichus phyllocnistoides* (Narayanan) emerged after incubation of larvae collected from the samples. In 1999, the parasitoids *Pnigalio* sp., *Cirrospilus* sp., and *N. formosa* were found in Galatas, whereas the parasitoids *Cirrospilus* sp. and *N. formosa* were found in Skala. Finally, all four parasitoids were found at Marathon. In the years 2000 and 2002 all four parasitoids were found in all three areas (Fig. 3).

Comparison between treated and untreated tangerine trees

After the first spraying on 13th July 1999, the total length achieved by all the shoots in the experiments was 629 cm for the untreated and 635 cm for the treated trees. The two groups of trees thus had similar development (Fig. 4A). According to the first measurement on 13th July 1999, the percentage of infested leaves on the shoots of

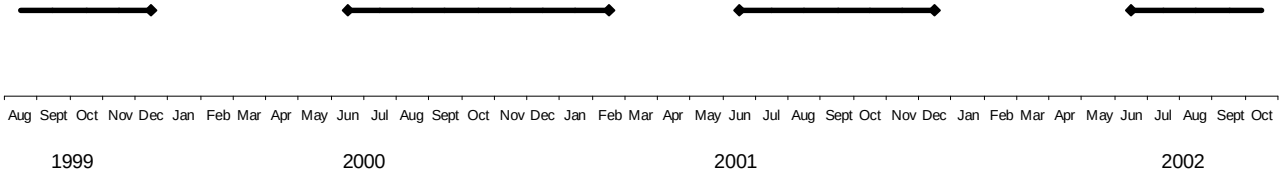

Fig. 2. Presence of living immature stages of *P .citrella* in citrus leaves during August of 1999 and October of 2002 in samples collected from Galatas, Skala, Marathon, and Kifissia.

untreated trees was 15.48 %, but it reached 98.04 % on 24th September 1999. On the other hand, the percentage of infested leaves in the case of treated trees was 18.52 % on 13th July 1999, but fell to lower levels due to sprayings (Fig. 4B). The fruit of the sprayed trees, as they were growing, seemed to be slightly larger in diameter; however, at maturity the fruit of the untreated trees had a higher average diameter, but the difference was not statistically significant (Table 2).

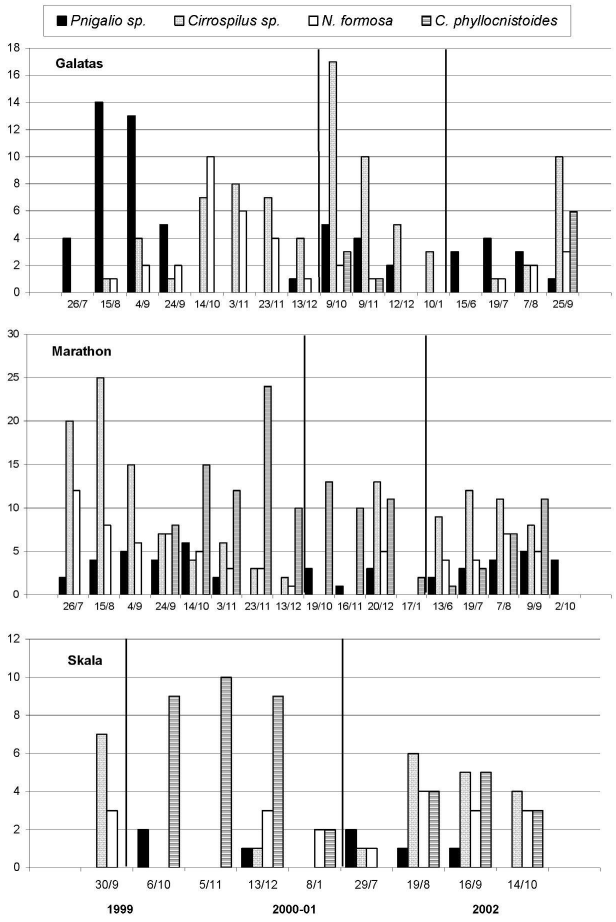

Fig. 3. Fluctuation of *P. citrella* parasitoid populations in three regions of Greece.

DISCUSSION

From observations on the dispersal of *P. citrella* between the two leaf surfaces in lemon, orange, and tangerine trees, it is concluded that its generations are overlapping. The observed stages of *P. citrella* on leaves (upper and lower surface) are the larvae, pupae, and the empty pupal chambers. It was also observed that the lower leaf surface was more heavily infested in all three examined citrus species. We may therefore assume that infestation

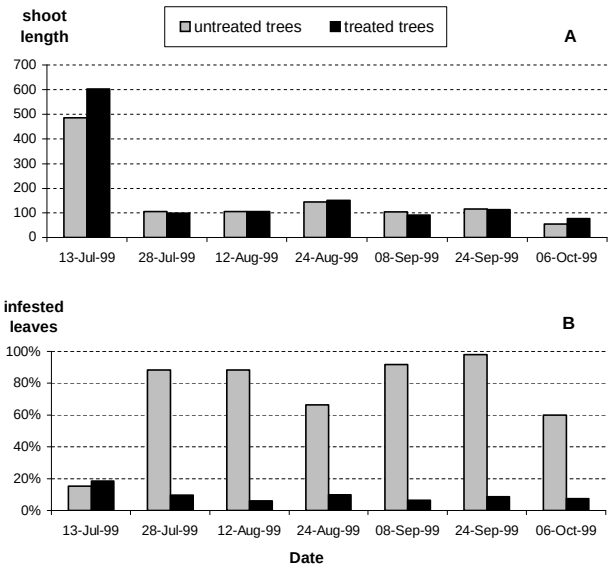

Fig. 4. Comparison between treated and untreated tangerine trees in Marathon: A. Length of new sprouting shoots, B. Proportion of infested leaves of new sprouting shoots in untreated tangerine trees and ones treated with imidachloprid + fine oil.

Table 2. Average diameter of tangerine fruit collected from untreated trees and ones treated with imidachloprid + fine oil trees

Observation date	Treated trees	Untreated trees
28/9/99	4.11 ± 0.39	4.03 ± 0.43
1/12/99	4.96 ± 0.36	5.16 ± 0.33

of the upper leaf surface follows that of the lower. This is also in agreement with previous studies (Carrido Vivas and Gascon Lopez, 1995; Das *et al*., 1998).

During the three years of the study, there was no presence of *P. citrella* during the months, January (except January of 2001, as a result of the fine weather conditions), February, March, April, and May in the sampling areas. This leads us to the hypothesis that reinfestations are caused by migrating populations of *P. citrella* from warmer areas or by populations that have overwintered in greenhouses or in well-sheltered places where they cannot be observed.

As far as the parasitism of *P. citrella* is concerned, since autumn of 2000 all four parasitoids were found in all three sampling areas. The study revealed good growth of populations of the parasitoids *Cirrospilus* sp. and *C. phyllocnistoides*, whereas *Pnigalio* sp. and *N. formosa* were found in smaller population densities.

Comparison between treated and untreated tangerine trees showed that sufficient control of *P. citrella* infestation can be achieved by sprayings with imidachloprid + fine oil. Satisfactory efficacy of imidachloprid against *P. citrella* has already been reported in Greece (Anagnou Veroniki *et al*., 1998). On the other hand, it is demonstrated that in trees cultivated in a manner appropriate to reduce summer shooting, there is no reduction of crop production.

Acknowledgements – This research was partially supported by the the Ministry of Science and Environment Protection of the Republic of Serbia (Grant 143006B). The authors thanks to Aleksandar Stojanović (Belgrade Natural History Museum) for help in parasitoids identification.

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ПОДАЦИ О ФЕНОЛОГИЈИ, ПАРАЗИТИЗМУ И КОНТРОЛИ ЛИСНОГ МИНЕРА ЦИТРУСА, *PHYLLOCNISTIS CITRELLA* STAINTON (LEPIDOPTERA, GRACILLARIIDAE) У ГРЧКОЈ

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Циљ истраживања је да ли *Phyllocnistis citrella* Stainton (Lepidoptera, Gracillariidae) показује већу преференцу према горњој или доњој површини листова цитрусних стабала. Ова истраживања су спроведена у периоду 1999-2002 на локалитетима Galatas (Trizina, Southern Greece), Skala (Lakonia, Southern Greece), Marathon и Kifissia (Attica, Central Greece). Такође на локалитету Marathon, ефекат *P. citrella*, на третираним (imidachloprid) и нетретираним цитрусним стаблима је истраживан. Ниво зараженост листова цитруса варира од 0.68 – 1.03 ларве *P. citrella* по листу и није нађена статистички значајна разлика у погледу преференце *P. citrella* на горњу и доњу површину листова. Међутим, значајне разлике су констатоване у зараженост доње и горње површине листова различитим ларвалним стадијумима. Детектовано је присуство млађих ларвалних стадијума на горњој лисној површини, а старијих на доњој. Са *P. citrella* сакупљени су и паразитоиди: *Pnigalio* sp. (Hym., Eulophidae), *Cirrospilus* sp. (Hym., Eulophidae), *Neochrysocharis formosa* (Westwood) (Hym., Eulophidae) и *Citrostichus phyllocnistoides* (Narayanan) (Hym., Eulophidae).