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The Parasitoid Complex of the Citrus Leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in the East Mediterranean region of Turkey and Their Role in Biological Control

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Abstract: A survey of parasitoids of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was carried out in the citrus orchards in the east Mediterranean region of Turkey during 1995-2001. Their relative abundance and rate of parasitism were also determined. Ten species of parasitoids were identified: *Cirrospilus* sp. nr. *lyncus* (Walker), *C. pictus* (Nees), *C. variegatus* (Masi), *C. vittatus* Walker, *C. ingenuus* Gahan, *Ratzeburgiola incompleta* (Boucek), *Diglyphus isaea* (Walker), *Semielacher petiolatus* (Girault), *Citrostichus phyllocnistoides* (Narayanan), *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae). *Neochrysocharis* sp., *Chrysocharis* sp., *Barycapus* sp., *Pnigalio* sp. (Hymenoptera: Eulophidae) and the *Pteromalus* sp. (Hym.: Pteromalidae) were also identified. Among these species, *R. incompleta* in 1995-1996 and *C.* sp. nr. *lyncus* in 1997 were the most dominant of all encountered species. In 1998, a new parasitoid species, *C. phyllocnistoides*, expanded to all citrus growing orchards in the region, becoming the most abundant parasitoid species between 1998 and 2001. The parasitization rate was very low during the main peak of the pest in early summer, but increased considerably later in the season. It is determined that these parasitoids play a significant role in reducing the *P. citrella* population during the late summer and autumn flushes.

Key Words: *Phyllocnistis citrella*, citrus leafminer, biological control, parasitoid, relative abundance

Turunçgil Yaprak Galerigüvesi, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)'nın Doğu Akdeniz Bölgesi'ndeki Parazitoit Kompleksi ve Bunların Biyolojik Mücadeledeki Rolleri

Özet: Turunçgil yaprak galerigüvesi, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)'nın parazitoitlerini saptamak amacıyla 1995-2001 yıllarında Doğu Akdeniz Bölgesi'nde turunçgil bahçelerinde örneklemeler yapılmıştır. Ayrıca bu parazitoitlerin oransal bulunma sıklığı ve parazitleme oranları da belirlenmiştir. Örneklemeler sonucunda toplam 10 parazitoit türü saptanmıştır. Bunlar; *Cirrospilus* sp. nr. *lyncus* (Walker), *C. pictus* (Nees), *C. variegatus* (Masi), *C. vittatus* Walker, *C. ingenuus* Gahan, *Ratzeburgiola incompleta* (Boucek), *Diglyphus isaea* (Walker), *Semielacher petiolatus* (Girault), *Citrostichus phyllocnistoides* (Narayanan), *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae)'dır. Ayrıca *Neochrysocharis* sp., *Chrysocharis* sp., *Barycapus* sp., *Pnigalio* sp. (Hymenoptera: Eulophidae) ve *Pteromalus* sp. (Hym.: Pteromalidae) de cins düzeyinde teşhis edilmiştir. Bu türlerden *R. incompleta* 1995-1996'da, *C.* sp. nr. *lyncus* ise 1997'de en sık rastlanan türler olarak belirlenmiştir. 1998 yılında yeni bir parazitoit türü, *C. phyllocnistoides* bölgedeki tüm turunçgil alanlarına yayılmış ve 1998-2001 yıllarında en sık rastlanan tür olmuştur. Parazitlenme oranının zararlı popülasyonunun yüksek olduğu yaz başlarında düşük ancak mevsim sonuna doğru arttığı belirlenmiştir. Yaz sonu ve sonbahar sürgünlerinde zararlı populasyonunu düşürmede bu parazitoitlerin önemli rol oynadıkları kanısına varılmıştır.

Anahtar Sözcükler: *Phyllocnistis citrella,* turunçgil yaprak galerigüvesi, biyolojik mücadele, parazitoit, oransal bulunma sıklığı

Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillaridae) (CLM), was first determined in the east Mediterranean region of Turkey in June 1994 (Uygun et al., 1995). It spread quickly and was present in very high numbers in almost every orchard, except for a very small area in the east Black Sea region, by the summer of 1995. The first attempt to control the new pest was via broad-spectrum insecticides, but chemical control appeared to be a costly and short-term solution (Yumruktepe et al., 1996). To prevent damage in the countries where the pest originated, it was usually biologically controlled by natural enemies, especially by hymenopterous parasitoids (Knapp et al., 1993). *Ageniaspis citricola* Logvinovskaya (Hym.: Encyrtidae), *Semielacher petiolatus* (Girault) and *Cirrospilus ingenuus*

(Subba Rao and Ramamani) (Hym.: Eulophidae) were imported and mass reared (Uygun et al., 1996a). Some release experiments were performed several times.

Indigenous parasitoids in some environments have been reported as effective for *P. citrella* control, and have been able to control the host population. In this paper, continuous studies on the native parasitoids of *P. citrella* and their relative abundance, and the level of pupal parasitism over the years and its effect on the pest population are documented.

Materials and Methods

For the determination of *P. citrella* parasitoids, sampling was performed in commercial citrus orchards in the east Mediterranean region. Citrus shoots infested with *P. citrella* were collected from different sites during 1995-2001. In each orchard 25-30 shoots were collected. In the laboratory, all the other pests on the leaves were discarded. The shoots were put into plastic bags and aerated. In order to lower the humidity, blotting paper was put into the bags. The bags were checked each day and the hatched parasitoids and *P. citrella* adults were collected by suction tube. All the parasitoid species were identified by Dr. LaSalle (Australian National Insect Collection, CSIRO Entomology, Australia) and by the first author, and according to the results their relative abundance was constructed. Furthermore, from either the observations or the literature, it was determined whether the parasitoids are endoparasitic, ectoparasitic, solitary or gregarious. To evaluate the level of parasitism, 20 newly developed shoots were taken to the laboratory. The leaves with pest pupae were discarded and checked under a binocular microscope for the presence of parasitoids.

Results and Discussion

In surveys conducted in the east Mediterranean region during 1995-2001, 10 indigenous hymenopterous parasitoid species and individuals belonging to 5 genera attacking *P. citrella* were identified (Table 1).

Table 1. Indigenous hymenopterous parasitoids of Phyllocnistis citrella in the east Mediterranean region of Turkey during 1995-2001.

Family	Species	Specificity of the parasitoid
Eulophidae	*Cirrospilus ingenuus* Gahan	Ectoparasitoid
"	*Cirrospilus pictus* (Nees)	"
"	*Cirrospilus variegatus* (Masi)	"
"	*Cirrospilus vittatus* Walker	"
"	*Cirrospilus* sp. nr. *lyncus* (Walker)	"
"	*Ratzeburgiola incompleta* (Bouce)	"
"	*Citrostichus phyllocnistoides* (Narayanan)	"
"	*Semielacher petiolatus* (Girault)	"
"	*Diglyphus isaea* (Walker)	"
"	*Pnigalio* sp.	"
"	*Baryscapus* sp.	"
"	*Neochrysocharis formosa* (Westwood)	Endoparasitoid
"	*Neochrysocharis* sp.	"
"	*Chrysocharis* sp.	"
Pteromalidae	*Pteromalus* sp.	"

Five species belonged to the genus *Cirrospilus* and 2 to the genus *Neochrysocharis* and the others were distributed singly among 7 genera. All the parasitoids were solitary and parasitize the second and third larval stages, prepupa or pupa. No egg parasitoids were found. Among all the parasitoids *C. ingenuus*, *Semielacher petiolatus* and *Citrostichus phllocnistoides* are specific to *P. citrella* (LaSalle and Parrella, 1991). The nonspecific *Cirrospilus* spp. are parasitoids, facultative hyperparasitoids or rarely obligatory hyperparasitoids of a variety of insects concealed within plant tissue. They are often polyphagus and commonly found on invading *P. citrella*. *Diglyphus isaea* (Walker) is a common parasitoid of leafmining Diptera on herbaceous and cultivated plants used in biological control programs against various agromyzids. Schauff et al. (1998) reported that *Ratzeburgiola incompleta* is a species that attacks leafmining Lepidoptera. *Neochrysocharis* spp. are parasitoids of a variety of insects (mainly leafminers and gall formers) concealed in plant tissue. *Baryscapus* spp. have a wide host range including many species that are hyperparasitoids. *Chrysocharis* spp. and *Pteromalus* spp. are endoparasitoids of leafmining insects in Diptera, Lepidoptera, Coleoptera and Hymenoptera. *Pnigalio* spp. are generally polyphagous parasitoids of leafmining and gall-making insects. *A. citricola*, which is known worldwide as the most effective parasitoid of *P. citrella*, could not found in the samples at the release year or the following years. Hoy and Nguyen (1997) recorded that this parasitoid was climatically adapted to humid tropical and suptropical climates. Siscaro et al. (2003) detected that the high mortality of adults at 25-35 ºC showed that the parasitoid was unsuitable for the biological control of *P. citrella* in Mediterranean citrus orchards. It is thought that because of the high temperature (above 35 ºC) in July and August, *A. citricola* could not become established in the east Mediterranean region of Turkey.

Heppner (1993) recorded about 39 species of Asian parasitoids of *P. citrella*. Ujiye (1988) identified 13 parasitoids of the pest in Honshu (Japan), 11 of which belonged to the family Eulophidae and the others to the families Braconidae and Elasmidae. Browning et al. (1996) recorded 13 parasitoids in Florida in 1993. Liotta (1997) recorded 11 parasitoids belonging to the family Eulophidae in Sicily and Quilici et al. (1997) found 4 parasitoid species in France. Schauff et al. (1998) recorded over 80 species of chalcidoid parasitoids reared from *P. citrella* throughout the world. As it seen from the other studies different parasitoid species were found in different countries. It is thought that different ecological conditions and different hosts in different regions affected the presence of the parasitoids.

The relative abundance of native parasitoids changed during the study (Figure 1). In 1995 and 1996, the

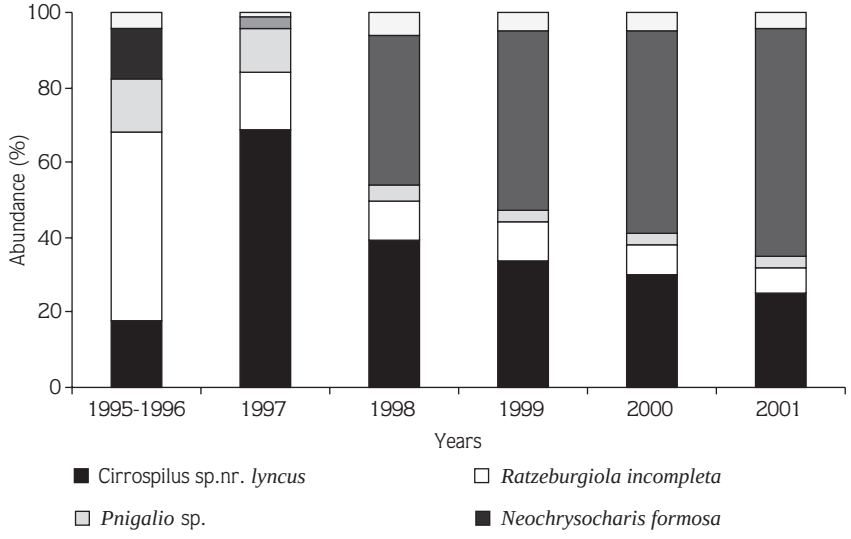

Figure 1. Relative abundance of native parasitoids of Phyllocnistis citrella in citrus orchards in the east Mediterranean region during 1995-2001.

predominant species was *R. incompleta*, which made up 50% of all parasitoid specimens recovered. In 1997, *Cirrospilus* sp. nr. *lyncus* was the most frequently encountered species (69%), whilst *R. incompleta* dropped to a rate of 15%, and only a few specimens of *N. formosa* were recovered. An interesting finding was the recovery of *S. petiolatus* in our samples. This parasitoid was imported from Australia and released twice—100 individuals each time—in 1996 (Uygun et al., 1997). No *S. petiolatus* specimens were recorded in 1996, even at the release sites. However, 100 individuals emerged from *P. citrella* samples collected at various locations in the east Mediterranean region in 1997, even far from the release site. This result indicated that the parasitoid became established in the main citrus growing area of the east Mediterranean region of Turkey. However, in the following years, this parasitoid was encountered in very low numbers. A new parasitoid species, *C. phyllocnistoides*, was the most common parasitoid: 40% of all specimens recovered in 1998. It increased even more during the following years and reached 61% in 2001. Since this specialized parasitoid was not found before 1998, it is possible that it has entered the eastern Mediterranean area of Turkey recently. After the introduction of *C. phyllocnistoides* to the region, the population of *C.* sp. nr. *lyncus* decreased year by year to a rate of 25% in 2001. There is no clear explanation for the decrease or increase in these parasitoids' rates. More studies must be done on the other hosts or on the ecological factors affecting these parasitoids. Vercher et al. (2003) reported that native parasitoids of *P. citrella* appeared to be parasitizing leaf miners in herbaceous and woody plants situated close to the target orchards.

Vercher et al. (2003) reported 10 different parasitoid species in Spain, and *A. citricola* was established but unable to overwinter. Between 1999 and 2001 *C. phyllocnistoides* spread to all citrus orchards, becoming the most abundant parasitoid and displacing native and other introduced parasitoids. Márquez et al. (2003) found 5 native parasitoid species in a 3-year study between 1999 and 2001. In their study, *S. petiolatus* was the most dominant species, but the introduction of *C. phyllocnistoides* in 2000 has caused changes in the relative abundance of native parasitoids in Malaga province; in 1999, the leafminer parasitized by *C. phyllocnistoides* was lower than 10%, but in 2001 this rate was 60%. The most dominant species in 2000, *S. petiolatus*, decreased to 22% in 2001. Liotta et al. (2003) reported that after the introduction of the exotic parasitoids, *C. phyllocnistoides* and *S. petiolatus,* they have displaced the indigenous parasitoids in Western Sicily. As seen from the literature, like in other Mediterranean citrus cultivated areas, in the east Mediterranean region of Turkey the introduction and spread of *C. phyllocnistoides* led to a progressive reduction in other parasitoids' relative abundance in the parasitoid complex of *P. citrella.*

At one location in Adana the effectiveness of indigenous parasitoids in controlling of *P. citrella* was also examined during 1997-2001 (Figure 2). According to the 5-year study, the parasitization rate was lower in June and July, being higher from August onwards. The maximum parasitization rate was observed in September, being 38% in 1997, 63% in 1998, 54% in 1999, 74% in 2000, and 72% in 2001. In the last 2 years, the parasitization rate was increased. It is thought that non-spraying of the trees older than 5 years old and not using broad spectrum insecticides in the young orchards for pest control (Yumruktepe et al., 1996) positively affected the natural biocontrol. It is concluded that indigenous parasitoids are partly effective in controlling the citrus leafminer and the enhancement of these parasitoids may be a promising approach in integrated pest management programs directed against *P. citrella*.

Conclusion

Ten parasitoid species and 5 genera attacking the pest were determined during 1995-2001 in the east Mediterranean region of Turkey. Different parasitoid species were dominant at the beginning and, according to the surveys, the parasitization rate was very low during the main peak of the pest in early summer, but increased considerably later in the season. As experienced to date, these parasitoids played a significant role in regulating and stabilizing the *P. citrella* population during the late summer and autumn flushes but they are not thought to be high enough to reduce the number of *P. citrella* below the economic threshold. However, the limited knowledge

on the biology and ecology of these parasitoids makes further studies necessary to understand their specific role in controlling *P. citrella* and to enhance their efficiency in citrus orchards. It is thought that the tri-trophic interactions between plants, hosts and parasitoids must be studied to find out the spontaneous plants acting as a reservoir. In addition, native vegetation sustaining parasitoid populations and providing them with alternative hosts, especially in periods of scarce availability of *P. citrella* larvae, must be detected. More attention should be given to the knowledge of the biology and ecology of parasitoid species, both to better use them in biological control programs and to enhance natural biocontrol.

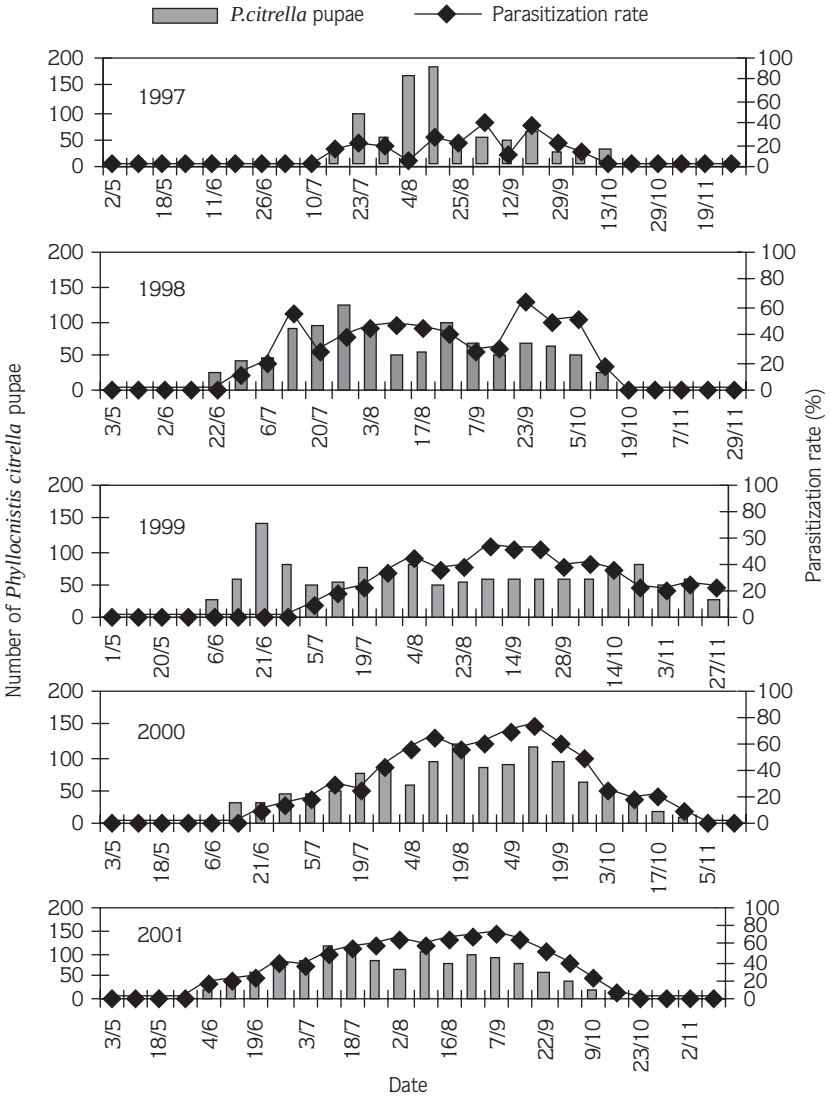

Figure 2. Parasitization rate of Phyllocnistis citrella pupae by indigenous parasitoids in Adana during 1997-2001.

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