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Orijinal araştırma (Original article)

Determination of the natural mortality factors of Citrus leafminer [*Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)] in Adana Province, Turkey[1]

Adana'da Turunçgil yaprak galerigüvesi, [*Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)]'nın doğal ölüm faktörlerinin belirlenmesi

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Summary

In this study, natural mortality factors of Citrus Leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) were investigated under field conditions. The studies were carried out in a lemon orchard in Adana, Turkey during 2007-2008. Ten trees were selected and weekly one shoot from each tree, and a total of ten shoots, were collected randomly during May-November. In the laboratory, the first 15 leaves were checked using a binocular microscope. All biological stages of the pest and the parasitoids, dead or infected individuals, and empty mines, were counted. According to the method and symptoms of predation, the mortality factors of the pest were recorded. In 2007 and 2008, large numbers of larvae were parasitized (46.25% and 48.12%, respectively). The larval parasitoid *Citrostichus phyllocnistoides* Narayanan (Hymenoptera: Eulophidae) was the abundant species in both years. The third instar of the host had the highest percent of parasitized individuals. In the first year, 15.33% of the pest were consumed by spiders, 10.07% by *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and 3.14% by ants, whereas in the second year these ratios were 16.61%, 8.57% and 3.78%, respectively. Second instar larvae were the most preferred stage for predation. In 2007, in 13.66% of the mines no pests were recorded, whereas the mortality source of 5.19% of the larvae and pupae was not detected. In 2008, these ratios were 10.21% and 6.87%, respectively. It was determined that predators are as effective as the parasitoids in the biological control of the pest.

Key words: Citrus leafminer, *Phyllocnistis citrella*, *Citrostichus phyllocnistoides,* spider, *Chrysoperla carnea*

Özet

Bu çalışmada, Turunçgil Yaprak Galerigüvesi, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)'nın arazi koşullarında doğal ölüm faktörleri araştırılmıştır. Çalışma Adana'da bir limon bahçesinde 2007-2008 yıllarında gerçekleştirilmiştir. Bahçede 10 ağaç belirlenmiş, Mayıs-Kasım aylarında haftalık olarak her ağaçtan birer tane olmak üzere tesadüfi toplam 10 sürgün toplanmıştır. Laboratuvarda, sürgünler üzerindeki ilk 15 yaprak binoküler altında kontrol edilmiştir. Zararlının ve parazitoitlerin tüm biyolojik dönemleri, ölü veya zarar görmüş bireyler ile boş galerilerin sayımı yapılmıştır. Predatörlerin beslenme şekillerine ve beslenme simptomlarına göre zararlının ölüm nedenleri kaydedilmiştir. Buna göre 2007 ve 2008 yıllarında sırasıyla, larvaların büyük bir bölümü (% 46.25 ve % 48.12) parazitlenmiş olup her iki yılda da larva parazitoiti *Citrostichus phyllocnistoides* Narayanan (Hymenoptera: Eulophidae) en yoğun tür olarak gözlenmiştir. Parazitlenme oranı en yüksek üçüncü dönemdeki larvalarda olmuştur. İlk yıl zararlının %15.33'ü örümcekler, % 10.07'si *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) ve % 3.14'ü de karıncalar tarafından tüketilirken bu oranlar ikinci yıl sırasıyla % 16.61, % 8.57 ve % 3.78 olmuştur. Avlanmada ikinci dönem larvalar diğer dönemlerden daha çok tercih edilmiştir. 2007'de galerilerin % 13.66'sında zararlı saptanmazken, larva ve pupaların % 5.19'unun ölüm nedeni belirlenememiştir. 2008'de bu oranlar sırasıyla % 10.21 ve % 6.87 olmuştur. Zararlının biyolojik mücadelesinde predatörlerinde parazitoitler kadar etkili olduğu belirlenmiştir.

Anahtar sözcükler: Turunçgil yaprak galerigüvesi, *Phyllocnistis citrella*, *Citrostichus phyllocnistoides,* örümcek, *Chrysoperla carnea*

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Introduction

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) invaded Adana/Turkey in 1994, spreading rapidly throughout the commercial citrus growing areas of the east Mediterranean region (Uygun et al., 1995). The eggs of the pest are deposited individually on the adaxial and abaxial sides of young leaves. Larvae destroy the epidermis of these 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). Biological control is considered the most economical and environmentally sound long-term solution for managing *P. citrella* (Knapp et al., 1995; Hoy & Nguyen, 1997). Shortly after the invasion by *P. citrella*, native parasitoids attacking the pest werefound. In a previous study, ten species of parasitoids belonging to Eulophidae and unidentified individuals belonging to 5 genera were determined in the eastern Mediterranean region in Turkey. Among these parasitoid species, *Citrostichus phyllocnistoides* Narayanan (Hymenoptera: Eulophidae) comprised 61% of the parasitoids. Several predators, among them spiders, lacewing larvae and ants also have been found feeding on *P. citrella* (Elekçioğlu, 2001). There is increasing evidence indicating that generalist predators can reduce pest populations in agroecosystems (Wise, 1993; Rosenheim et al., 1993). Neverthless, little is known about the impact of these predators on citrus leafminer in Turkey. Therefore, the investigation of the natural enemy complex associated with *P. citrella* and its effect on the pest populations should include all possible natural mortality factors. In this study, the survival of some developmental host stages of *P. citrella* and the proportion attacked by natural enemies were determined.

Materials and Methods

Population dynamics of *Phyllocnistis citrella* and parasitoids

The population dynamics of *P.clitella* and its parasitoids were studied in a lemon orchard (Kütdiken variety) with 85 trees, established in 2002 and located in the Biological Control Research Station (37° 00.533´ N; 35° 33.186´ E), Adana, during 2007-2008. The orchard was drip irrigated and surrounded of citrus orchards with different varieties. No sprays were applied during the period of the experiment. The density of *P. citrella* and its parasitoids were determined by randomly picking 1 shoot from the middle region of 10 randomly selected trees. Each shoot was placed in a plastic bag, with a sheet of absorbant paper to absorb condensation, kept inside an icebox, and transported to the laboratory. The first 15 leaves within a shoot were examined with the aid of a binocular microscope by starting with the first apical leaf and continuing to the terminal leaf at the base of the shoot (Knapp et al., 1995). The numbers of larvae and pupae (including prepupae) of the pest were counted. Seasonal trends of the population dynamics of *P. citrella* and its natural enemies were assessed by using the numbers of living, dead and parasitized larvae and pupae of the pest. Samplings were taken weekly from May to November. From December to April, no shoots were picked buthey were checked visually since the shoots were free from any leafminer infestation.

Seasonal mortality

Dead and missing *P. citrella* were classified according to the cause of death, using the descriptions listed in Table 1 (Pomerinke, 1999; Amalin et al., 2002). Data on parasitism was classified according to the presence or absence of host and parasitoid. If no parasitoid stage was observed, the *P. citrella* was classified as non-parasitized. Presence of a larval or pupal parasitoid inside the mine or *P. citrella* pupal chamber indicated parasitism. Predation showing empty larval mines and necrotic marks on larvae is included in the inactive complete mines with unknown mortality since these predation marks are shared by ants and hunting spiders, and by ectoparasitoids and hunting spiders, respectively. If the *P. citrella* stage was necrotic or flaccid with symptoms of parasitoid or predator feeding, the *P. citrella* was recorded as dead. Only recently dead mines on newly flushed leaves accounted for the mortality data for all the sampling dates.

Table1. Identification of predation marks of different natural enemies of *Phyllocnistis citrella*

Species	Method of predation	Symptom of predation	Prey stages attacked
Lacewing	puncture the mine	dead prey larval form still visible	larval stage
Ant	slit open the mine or pupal chamber to pull out the prey	empty mine	larval and pupal stage
Hunting spiders	puncture the mine	necrotic marks from incomplete feeding	larval and pupal stage
		empty mine with the prey's crumpled skin from complete feeding	larval and pupal stage
	slit open the mine	empty mine	larval and pupal stage
Ectoparasitoids	puncture the mine	necrotic marks from incomplete feeding	larval stage
	presence of a parasitoid inside the mine		larval and pupal stage

Note: Predation symptom shared by two or more predators is classified as inactive incomplete mine with unknown mortality.

Statistical analysis

Parasitism percentage was calculated as the ratio of the number parasitized to the total number of hosts (Van Driesche, 1983). A correlation analysis was applied to determine the relationship between *P. citrella* larval and pupal density, and parasitization (%) ($P<0.05$). Analysis of variance (ANOVA) was applied to data on percentage parasitism and predation among stages and differences between stages were evaluated with Duncan's test at $P<0.05$. All analyses were performed by using the Microsoft statistical package program SPSS 15.0.(SPSS, 2006).

Results and Discussion

Population dynamics of *Phyllocnistis citrella* and parasitoids

The seasonal population patterns of *P. citrella* and its parasitoids in 2007 and 2008 are shown in Figures 1 and 2. In 2007, the first leaf infestation was observed in mid-May and proceeded till October. There were 3-4 peaks of highest immature CLM densities in June-August. The maximum peak of the CLM population was 513 individuals of larval and pupal instars on 3 July, 2007 The maximum peak of parasitized larvae and pupaewas on 14 August, 2008 and was comprised of 534 individuals with the highest parasitization rate (76.83%) for the season (Figure 1).

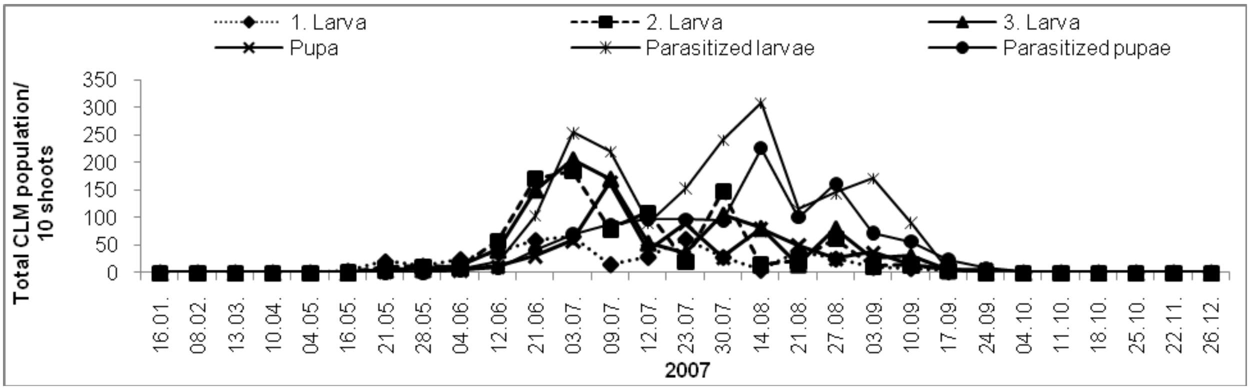

Figure 1. Average density of *Phyllocnistis citrella* immature stages in lemon orchard in 2007.

In 2008, the first leaf infestation was observed at the beginning of May and proceeded until the beginning of October. There were 4-5 peaks of high immature CLM densities from June to September. The maximum peak of 451 larval and pupal instars was on 2 July, 2007. The maximum peak of parasitized larvae and pupa population was on 18August, with 451 individuals, with 75.80% parasitization rate (Figure 2).

The percentage parasitism appeared to be related to *P. citrella* numbers. Correlation analysis indicated that there was a significant relationship between *P. citrella* population and parasitization ($r=0.990$, $r^2=0.980$, $P=0.009$ in 2007; $r=0.988$, $r^2=0.976$, $P=0.012$ in 2008). The parasitization rate was higher when the pest population increased in both years.

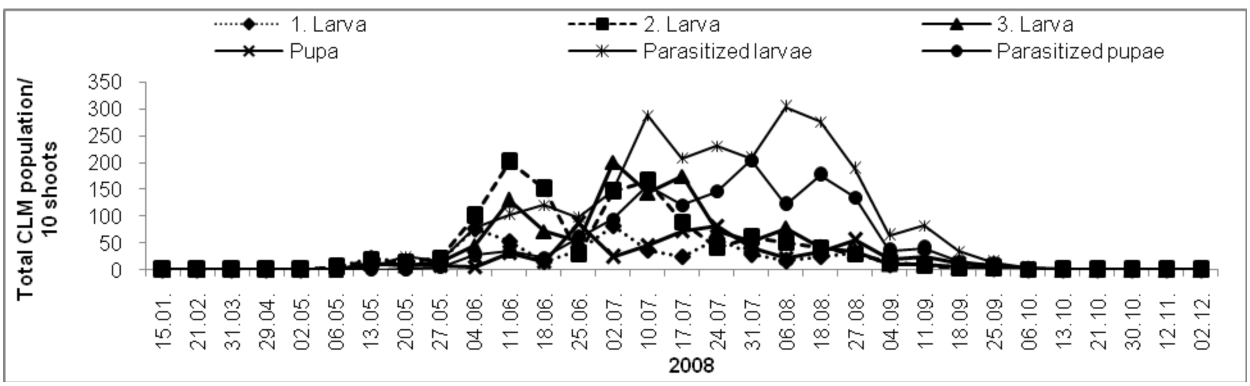

Figure 2. Average density of *Phyllocnistis citrella* immature stages in the lemon orchard in 2008.

The *P. citrella* population declined to zero during winter and the early spring months, including November through to April, and increased in the summer flushes in 2007 and 2008. The most likely reason for the increase and decrease in population was related to both the development of new shoots and increase in favorable temperatures for the pest's development. The seasonal pattern for *P. citrella* was similar to observations in Florida (Pena et al., 1996) and in southern Texas (Legaspi et al., 1999), where population densities increase from spring to fall and decline during the winter months. Other reports on the pest population are in agreement with the observations described here (Diez et al., 2006; Hoy et al., 2007).

Seasonal mortality

Percentages for different mortality factors of CLM in 2007 and 2008 are given in Figures 3 and 4, respectively. In 2007, parasitism ranged between 0.00% and 76.83%, with an average of 46.25%. In addition, 15.33% of the pest were consumed by spiders, 10.07% by *Chrysoperla carnea* (Stephens)

(Neuroptera: Chrysopidae) and 3.14% by ants. In 13.66% of the mines, no pest was found (empty mine), where mortality sources of 5.19% of the larvae and pupae were not detected (Figure 3). In 2008, parasitism ranged between 0.00% and 75.80%, with an average of 48.12%. In total, 16.61% of the pest was consumed by spiders, 8.57% by *C. carnea* and 3.78% by ants. In 10.21% of the mines no pest was recorded, where mortality sources of 6.87% of the larvae and pupae were not detected (Figure 4). Generally, mortality due to predation by the lacewing, *C. carnea* was higher in May-July and by spiders in August-October of both years. Total predation ranged from 1 to 40% (Figure 3) in 2007, whereas it ranged from 4 to 47% in 2008 (Figure 4).

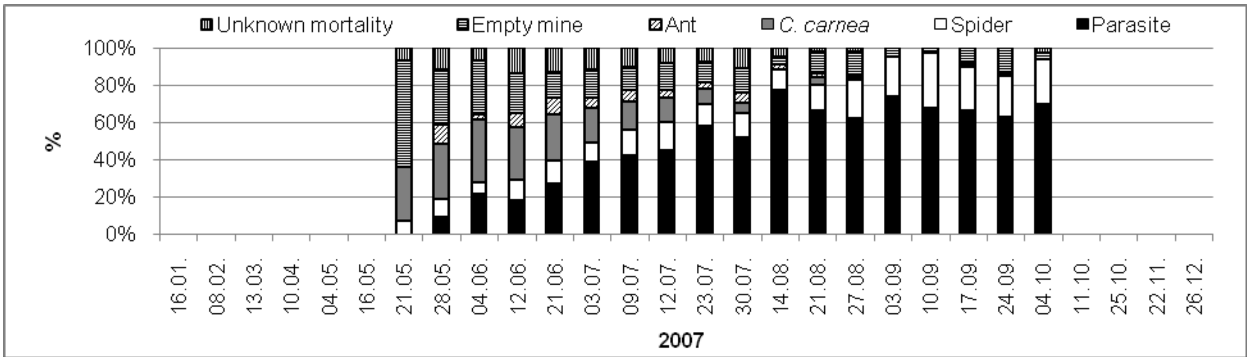

Figure 3. Percentage of different mortality factors of *Phyllocnistis citrella* in 2007.

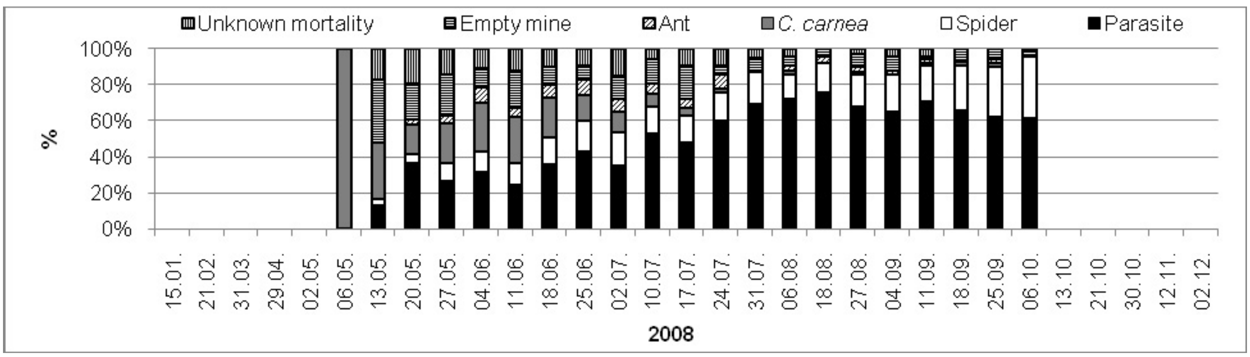

Figure 4. Percentage of different mortality factors of *Phyllocnistis citrella* in 2008.

Parasitism and predation were the main mortality factors for CLM. This agrees with Huang et al. (1989) who showed that parasitoids and predators in China were the most important factors influencing the most important late summer and autumn generations of CLM.

Parasitism played the major role in reducing the population of CLM in the present study. From the observations, it is concluded that the larval parasitoid *C. phyllocnistoides* was the most abundant parasitoid since its pupae can easily be distinguished from the pupae of other parasitoids of CLM. It is an ecto-parasitoid and specific to *P. citrella*. Several authors have reported low to high rates of parasitism due to the different parasitoid species complex of *P. citrella* (Pena et al., 1996; Legaspi et al., 2001; Amalin et al., 2002; Diez et al., 2006; Xiao et al., 2007). Chen & Lou (1990) reported that *C. phyllocnistoides* parasitized 54.38% of second to third instar larvae of CLM in the orchards of the Fuzhou district of China. Morakote and Nanta (1996) recorded parasitism of 25.42% in May, at the beginning of the season reached 91.93% in September in Thailand. In Valencia (Spain), the parasitism was 0-20% in summer and 30-60% in autumn (Garcia-Marí, 1996), but the percentage of parasitism increased from 20-25% to near 60% in a few years after the introduction of *C. phyllocnistoides* (Garcia-Marí, et al., 2004). The introduced parasitoid, *A. citricola* Logvinoskaya (Hymenoptera: Encyrtidae), was the dominant parasitoid recorded in southwest Florida, and accounted for 8-29% of the natural mortality of *P. citrella*

(Xiao et al., 2007). Marquez et al., (2003) detected 5 native parasitoid species, of which *Semielacher petiolatus* (Girault) (Hymenoptera: Eulophidae) was the most dominant species. However, the introduction of *C. phyllocnistoides* in 2000 has caused changes in the relative abundance of native parasitoids in Malaga province; in 1999, the percentage of 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 in Sicily. 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. The same situation has occurred in eastern Mediterranean region. No *C. phllocnistoides* specimens were recorded in the region before 1998. It was the most common parasitoid comprising 40% of all specimens recovered in 1998, the first year of its entry, and increased during the following years to 61% in 2001. After the introduction of this specialized parasitoid to the region, the population of the others decreased year by year. *Cirrospilus brevis* Zhu, LaSalle and Huang (Hymenoptera: Eulophidae) was the most frequently encountered species (69%) in 1997 in the region, but after the introduction of *C. phyllocnistoides*, the parasitism of *C. brevis* decreased to 25% in 2001 (Elekçioğlu & Uygun, 2006). The establishment and dispersal of *C. phyllocnistoides* from 1998 onwards showed how well this species adapted to the Mediterranean climate. It is a good biological control agent for *P. citrella* because of climatic adaptability and high specificity (it has only been cited on *P. citrella* (Noyes, 1998)) and high potential for increase (Vercher et al., 2003). As seen in other studies, parasitoid species and parasitization rate differ in different countries so it is thought that different ecological conditions in different regions affect the presence of the parasitoids and parasitization rate of CLM.

Percentages of dead 1st, 2nd and 3rd stage larvae and pupae of CLM due to parasitism in 2007 and 2008 are given in Figures 5 and 6, respectively. Parasitization differed significantly among stages ($F=114.569$, $df=3, 119$, $P<0.01$). The third instar host had the highest average of parasitized individuals (58.53±5.89%, 53.25±3.21%) followed by the second instar (34.18±5.48%, 37.80±2.80%) in 2007 and 2008, respectively. The highest percentage of parasitized third instar larvae (83%) was on 14th of August, 2007. All third instar larvae were parasitized in October, 2007 (Figure 5). In 2008, 81% of the parasitization was of the third instar larvae on the 18th of August (Figure 6). There were no significant differences in terms of parasitization among stages between the years ($F=0,468$, $df=3, 119$, $P>0.05$). In this study, the third instar larvae was the most preferred stage for parasitism, while the first instar had the lowest parasitization in both years which matched well with the findings of Argov and Rossler (1998), Ding et al. (1989) and Garcia-Marí et al. (2004). In Valencia (Spain), parasitism on second instars increased from almost 16% to 65% and on third instars from 35-38% to 59% and the mean percentage of parasitism increased from 20-25% to nearly 60% in a few years after the introduction of *C. phyllocnistoides* (Garcia-Marí et al., 2004). Wang et al. (2006) reported that the third instar larvae of CLM were the most preferred stage for parasitism by *C. phyllocnistoides.*

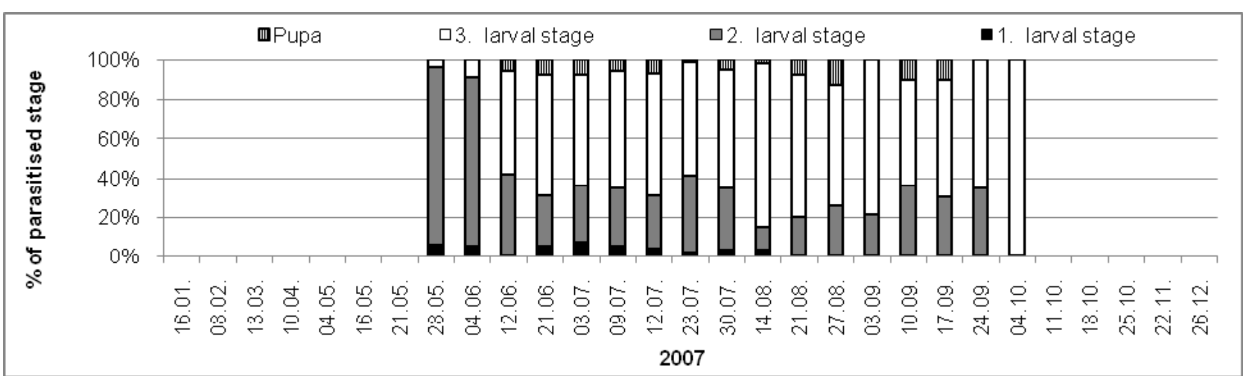

Figure 5. Percentages of dead 1st, 2nd, 3rd stage larvae and pupae of *Phyllocnistis citrella* due to parasitism in 2007.

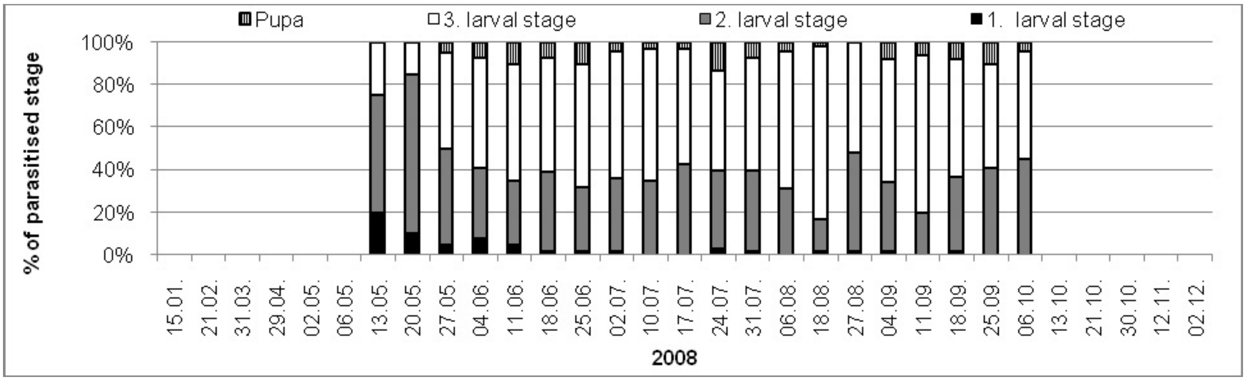

Figure 6. Percentages of dead 1st, 2nd, 3rd stage larvae and pupae of *Phyllocnistis citrella* due to parasitism in 2008.

Percentages of dead 1st, 2nd, 3rd stage larvae and pupae of CLM due to predation are presented in Figures 7 and 8. Predation differed significantly among stages (F=155.805, df=3, 126, P<0.01). Second instar larvae were preyed on at a significantly higher level, followed by the first instar larvae. An average of 47.16±2.41% and 46.76±2.19% of the predation occurred in the second instar larvae and 34.42±3.63% and 35.81±2.30% in the first instar larvae in 2007 and 2008, respectively. There were no significant differences in terms of predation among stages between the years (F=1.092, df=3,126, P>0.05).

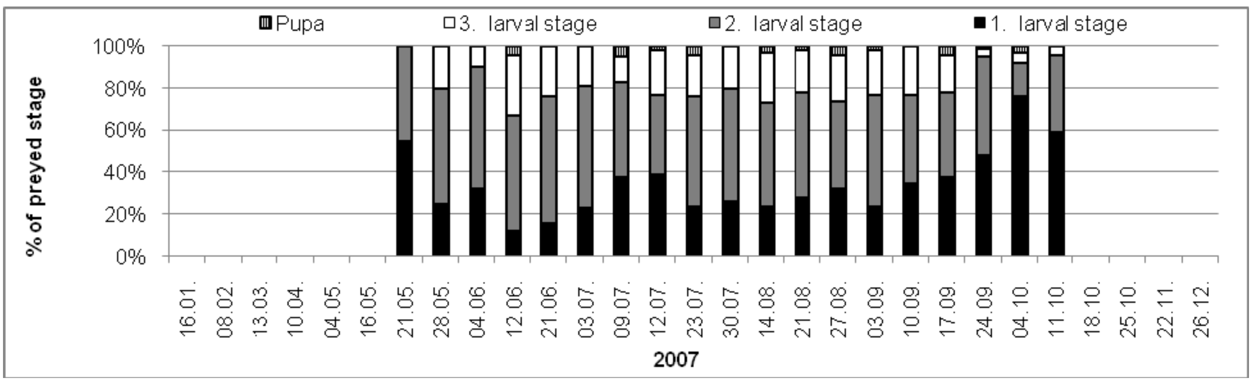

Figure 7. Percentages of dead 1st, 2nd, 3rd stage larvae and pupae of *Phyllocnistis citrella* due to predation in 2007.

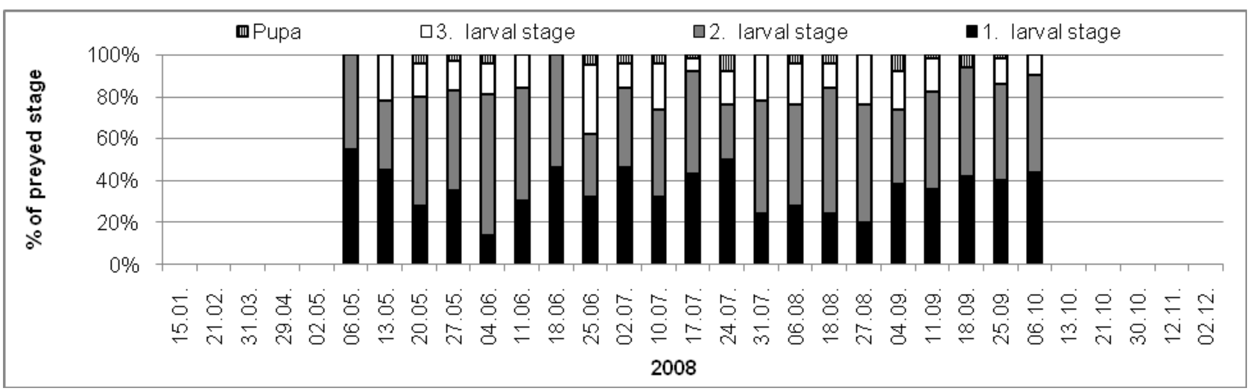

Figure 8. Percentages of dead 1st, 2nd, 3rd stage larvae and pupae of *Phyllocnistis citrella* due to predation in 2008.

Predation was the main mortality factor for the first instar larvae. Mortality due to parasitism was very low in the first stage (2.35±0.62% in 2007 and 3.40±1.06% in 2008), so the remainder of mortality was attributed to predation. Ant predation was observed especially in the first and second instars of *P.*

citrella. Ateyyat & Mustafa (2000) reported that first instar larval mortality was mainly due to predation. They also reported that most second instar larval mortality was due to predation in spring by *C. carnea,* which matches well with the present study's result that *C. carnea* predation was higher in spring than other months. Predation by spiders was higher in autumn than in other observation periods, as Ateyyat & Mustafa (2000) reported from Jordan.

A variety of predators has been documented to feed on *P. citrella*. In previous studies, green lacewing larvae, ants, thrips, hunting spiders and mirid bugs were reported as predators of CLM larvae in lime orchards in south Florida (Browning & Peña, 1995; Amalin et al., 1996; 2002). Chen et al. (1992) found that the larva of the lacewing, *Chrysoperla boninensis* Okamoto (Neuroptera: Chrysopidae) could consume 149.1 *P. citrella* larvae in its life time. Huang et al. (1989) concluded that abiotic factors and predation may be two major causes of *P. citrella* mortality during the first and second stages. These authors observed ants acting as important predators from late summer to fall during the dry and hot season. Amalin et al. (2002) listed lacewings, ants, hunting spiders and host feeding ectoparasitoids as predators of *P. citrella*. However, they attributed all predation observed in the course of their surveys to spiders and lacewings, even though they could not distinguish between these and the other predators.

Although predation of CLM by ants was lower than other predators in the present study, Xiao et al. (2007) stated that predation, particularly by ants, was the largest single cause of *P. citrella* mortality, accounting for more than 30% of all deaths by natural enemies, and 60% of all deaths by predators in Florida. They detected that first and second instars of *P. citrella* were most subject to ant predation. Pomerinke (1999) also reported ants as the major predators on *P. citrella* larvae, contributing an average 33% to total mortality.

In Alabama, predation was the dominant natural mortality factor acting on *P. citrella.* Predation accounted for 87-96% of all deaths on unprotected (control) Satsuma tree branches. In particular, predation by spiders was the single most important mortality cause, which accounted for 50-70% of all deaths. Predation by ants was second, accounting for 10-19% of all deaths. Predation by predatory insect larvae accounted for 3-27% of all mortalities (Xiao & Fadamiro, 2010). In contrast, Urbanaja et al. (2004) stated that neither ants nor lacewings could be identified as key-predators of *P. citrella* in Spain.

It is concluded from this study that the mortality of second instar larvae was mainly due to predation and that of third instar larvae to parasitization. The results showed that parasitization, as well as predation by spiders, lacewings and ants in earlier stages of *P. citrella,* are very important natural mortality factors acting on the pest in citrus orchards. Among the mortality factors, *C. phyllocnistoides* was the most important parasitoid and spiders were the most important predator. It is assumed that the individuals not affected by these mortality factors reached the adult stage. Nevertheless, even low contributions to the mortality of the pest by any natural enemy should not be ignored in the overall pest management. Enhancement and conservation of these natural enemies through the judicious use of pesticides and augmentation of field populations of key natural enemies are central to the development of a sustainable pest management strategy for the pests in citrus.

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