

Which role do lacewings and ants play as predators of the citrus leafminer in Spain?

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Abstract

In a study about the impact of natural enemies of *Phyllocnistis citrella* Stainton conducted in Spain, predation appeared as the most important mortality factor. Predators responded to changes in flushing, and this was attributed to continuous availability of preferential prey feeding on tender flushes. In this paper the role of aphid-mutualists and generalist predators feeding on aphids as well as on *P. citrella* (ants and lacewings, respectively), has been investigated. In the laboratory, *Chrysoperla carnea* Stephens could complete its development when fed on *P. citrella* third instars only. In the field, highly statistically significant regressions were obtained between prey indicators (aphid density and flush) and *C. carnea* densities. Nevertheless, using *P. citrella* density resulted in a negative relationship. In another field trial, predation rates did not change when ants were excluded from *P. citrella*-infested trees. Therefore, neither ants, nor lacewings could be identified as key-predators of *P. citrella*. Although we could not prove the efficiency of these predators, these studies have given us the chance to become aware of the existence of an important guild of unidentified generalist natural enemies.

Key words: biological control, *Chrysoperla carnea*, *Phyllocnistis citrella*, predators.

Resumen

¿Qué papel juegan las crisopas y las hormigas en la depredación del minador de las hojas de los cítricos en España?

En un estudio previo realizado en España, donde se investigó el impacto de los enemigos naturales sobre los estadios inmaduros de *Phyllocnistis citrella* Stainton, se observó que el factor de mortalidad más importante era la depredación. Este factor respondía a los cambios de brotación, y ello se atribuyó a la disponibilidad de presas sobre los brotes tiernos. En este trabajo se investiga el papel de las hormigas y las crisopas como depredadores del minador. En condiciones de laboratorio, *Chrysoperla carnea* Stephens pudo completar la totalidad de su ciclo biológico alimentándose exclusivamente de larvas de tercer estadio de *P. citrella*. En el campo se consiguió relacionar estadísticamente las poblaciones de este depredador con las densidades de pulgones y brotes jóvenes. Sin embargo, no fue posible establecer estas relaciones con las poblaciones del minador. En otro ensayo de campo, los porcentajes de depredación de *P. citrella* no se vieron afectados cuando se compararon árboles con un alto nivel de infestación con y sin la presencia de hormigas. Por tanto, ni las hormigas ni las crisopas pudieron ser identificadas como depredadoras importantes de *P. citrella*. Aunque no se pudo probar la eficacia de estos depredadores, el presente trabajo nos ha permitido poner de manifiesto la existencia de enemigos naturales generalistas aún sin determinar y que están presentes en el ecosistema de los cítricos.

Palabras clave: control biológico, *Chrysoperla carnea*, *Phyllocnistis citrella*, depredadores.

Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton, (Lepidoptera: Gracillariidae) is a pest native to Southern

Asia which spread to all citrus-growing areas worldwide during the last decade of the XX century (Urbaneja *et al.*, 2003). Classical biological control programs against this pest were extensively developed in Mediterranean countries (Argov and Rössler, 1996, 1998; Lekchiri, 1996; Siscaro *et al.*, 1997; Urbaneja *et al.*, 2000, 2003). From 1996 to 1999, two orchards

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located in the Valencia region (the major citrus-growing area of Spain) were monitored for incidence of the leafminer and its natural enemies, both indigenous and introduced (Urbaneja *et al.*, 1998, 2000). It was found that the contribution of parasitoids to overall natural enemy-caused mortality (parasitism and host feeding) was lower than that from predators. As in Florida (Pomerinke, 1999; Amalin *et al.*, 2002), predation was the most important mortality factor caused by natural enemies. Predation levels recorded averaged 25% (expressed as percentage of susceptible leafminer stages) and reached a peak of as much as 63% in one of the orchards studied. Regressions relating density-dependent mortality caused by natural enemies to *P. citrella*-indicators (density and number of infested flushes) showed that predation was significantly related to densities of young receptive flushes when summer-fall flushing was continuous. Therefore, it appeared that predators responded to changes in flushing when this was regular, and this was attributed to continuous availability of preferential prey feeding on tender flushes, such as aphids. It was suggested from that study that generalist predators feeding on aphids, such as green lacewings, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), and aphid-mutualists, such as ants, which also feed on *P. citrella* (Pomerinke, 1999), and were very commonly seen in *P. citrella*-infested orchards, were probably responsible for the high predation rates observed. In order to check whether this hypothesis was correct, we undertook the following investigation, in which the role of both green lacewings and ants as *P. citrella* predators was directly measured.

Material and Methods

Colonies of both *P. citrella* and *C. carnea* were initiated with insects collected from citrus plants grown in commercial groves around the city of Valencia (39° 27' N, 0° 22' W). Field-collected insects were regularly added to the colonies.

Rearing of *P. citrella*

P. citrella was reared as described by Urbaneja *et al.* (1998). Two-year old potted sour orange trees were pruned to obtain homogeneous young flushes. The optimal stage for introducing these plants into the *P.*

citrella rearing units was attained when oldest leaves of the new flushes measured about 4 mm long. Adult moths used for the infestation of new plants were obtained from a continuous culture. Groups of 4 plants were introduced weekly into a screened cage (80 × 110 × 120 cm) where the rearing took place. Plants were left undisturbed for 3 weeks, allowing adults to emerge within the cage so that artificial reinfestation was not necessary. Adult moths were fed a mixture of honey and water (1:3, v v⁻¹) directly sprayed onto the plants. After the three week-period, plants were recycled.

P. citrella larvae for feeding *C. carnea* larvae were reared in another cage (same dimensions as above). Groups of 16 small trees were exposed to approx. 300 moths. One day later, plants were removed from those cages and kept for about 7 d. By then, most of the leaves were housing *P. citrella* third instars. Under field conditions, this instar was the preferred one for predation (Urbaneja *et al.*, 2000).

Both colonies and plants were maintained in a glasshouse at 25 ± 5°C and 60 ± 10% of relative humidity (R.H.), under natural light conditions. This experiment was conducted from July to October 1999.

Rearing of *C. carnea*

Adult *C. carnea* collected in citrus groves were kept in a Perspex cage (30 × 25 × 20 cm) in a climatic chamber at 28 ± 1°C, 70 ± 5% R.H. and a photoperiod of 16:8 (L:D). These were fed a mixture of honey and broad bean pollen (1:1, v v⁻¹), as well as broad bean stems infested with *Aphis craccivora* Koch (Homoptera: Aphidae) as prey in order to promote oviposition. These aphids were obtained from a laboratory colony (Hermoso de Mendoza *et al.*, 1984).

Development of *C. carnea* on *P. citrella*

Less than 1-d old eggs were collected and individually deposited on a layer of agar (2% weight) in petri dishes (55 mm Ø). Emerged larvae were offered sour orange leaves containing *P. citrella* third instar larvae obtained as previously described. Ten larvae were offered each day. Leaves were changed daily and checked for predation evidence. Cages were kept in a climatic chamber at 28 ± 1°C, and a photoperiod of 16:8 (L:D). To facilitate pupation of *C. carnea*, 4 days after molting into the third instar, a piece of corrugated

cardboard was introduced into each cage. Development and predation were checked daily until adult emergence. Fifty replicates (eggs) were considered.

Chrysoperla carnea*. Field: monitoring and incidence on *P. citrella

Flushing patterns and incidence of aphids, *P. citrella* and *C. carnea* were followed from spring to fall 1999 at 15-day intervals at two different locations: Elx (38° 16' N, 0° 41' W, 86 m altitude) and Montcada (39° 35' N, 0° 23' W, 33 m altitude). The orchard at Elx (0.6 ha) consisted of 6-year-old Navelina sweet orange trees, *Citrus sinensis* (L.) Osbeck, and was surrounded by other citrus orchards and date palm and pomegranate groves. The orchard at Montcada (0.4 ha) consisted of 11-year-old Verna lemon trees, and was surrounded by other citrus orchards. Both orchards had not received any chemical treatment since 1997 and remained untreated during the study period. They were drip-irrigated on bare soil and had been monitored since 1996 (Urbaneja *et al.*, 1998, 2000).

In each orchard, twenty trees were randomly chosen and sampled at 15-d intervals. A ring, 40 cm in diameter, was thrown onto each tree four times, and the number of total flushes, aphid-infested flushes and flushes housing any immature stage of *P. citrella* found within the ring were counted. Additional flushes were also collected, put in a plastic bag and refrigerated until reaching the laboratory, where all leaves from each flush were observed under a stereoscopic binocular microscope. The number of aphids, *P. citrella* mines (either occupied or empty) were counted. Mortality, scored by counting incomplete mines or mines in which mutilated *P. citrella* larvae were found, was assumed to be the result of predation. The stage of mutilated larvae (from second to fourth) was recorded to establish feeding preferences of predators.

The number of adults of *C. carnea* was scored at the same orchards. Adults were monitored by means of sticky yellow traps (14 × 14 cm) (Tangletrap, Tanglefoot Co., Grand Rapids, MI, USA). Eight trees were randomly chosen at each visit and one trap put on each of them at 1.5 m high. Traps were replaced at each sampling date.

Density dependence of predation was studied by plotting predation against mean population densities. Corresponding regression equations were calculated using the Statgraphics software package (STSC, 1987).

Ants. Field: impact on *P. citrella*

Incidence of ants on immature stages of *P. citrella* was measured in a 1-ha Navelina orange orchard located at Bétera (39° 41' N, 0° 27' W, 125 m altitude) in summer 1999. In a preliminary survey carried out in this orchard, very high predation rates (almost 50% of *P. citrella* larvae and pupae), and an important presence of ants, mainly *Lasius niger* (Latreille) (Hymenoptera: Formicidae), the most abundant ant species in Spain (Palacios *et al.*, 1999), were detected. Twenty trees were randomly selected. Ten trees were used as control. On July 5, the remaining ten received a dust treatment of methiocarb (Mesurol, WP, 50% a.i.; Bayer Hispania, S.A.) around their trunks, which resulted in a complete elimination of ants from their canopies. From July 6 to August 16, trees were sampled weekly. Samples consisted of 4 randomly chosen flushes from each tree. These were kept in a plastic bag and refrigerated until reaching the laboratory, where they were observed under binocular microscope as described for the *C. carnea* field assay. In this case, in addition to predation, both parasitized hosts and hosts showing black stings, a typical symptom of having been fed upon by parasitoids, were scored.

Results were subjected to one-way analysis of variance and LSD was used for mean separation at $P < 0.05$ (STSC, 1987). If necessary to achieve normality, data were transformed (reciprocal and square root transformations) before the analyses.

Results

Development of *C. carnea* on *P. citrella*

Complete development of *C. carnea* when reared on *P. citrella* third instars was possible, but survival was very low (Table 1). Only one larva out of the 50 tested could successfully reach adulthood in 42 days. As expected, the number of *P. citrella* third instars eaten increased with each larval stage. On average, one larva of *C. carnea* consumed 77.3 ± 22.7 ($n = 10$) *P. citrella* third instars to complete its development.

Chrysoperla carnea*. Field: monitoring and incidence on *P. citrella

Spring flush in Elx (Fig. 1A) appeared from February to May (1,065.2 flushes m⁻²), and represented about

Table 1. Mean development times (days, mean ± SE), survival percentages, and predation (number of larvae consumed per larva per day) of *C. carnea* when reared at 28°C and a photoperiod of 16:8 (L:D) on third instars of *P. citrella*. Initial numbers were 50 eggs

Stage	Development time	% survival	Predation
Egg	4.65 ± 0.73	76.0	—
LI	4.60 ± 0.60	60.5	0.8 ± 0.3
LII	4.00 ± 1.08	60.9	2.1 ± 0.4
LIII	11.71 ± 2.59	71.4	4.9 ± 1.0
Pupa	13.00	10.0	—
Total	42.00	2.0	

90% of total flush produced during the whole season. In contrast, in Montcada spring flush was less abundant and appeared in April. Summer and fall flushes were very similar at both Elx and Montcada (Fig. 1A and

2A), and paused in September. Spring flush was not affected by *P. citrella*, but almost all flushes appearing from late May were infested by the leaf miner (Fig. 1A and 2A). Numbers of *P. citrella* recorded in lemon at Montcada were higher than those recorded in orange at Elx (Fig. 1B and 2B).

In Elx, there was an important attack of aphids, with a maximum of 17.8% of flushes infested (2,446 aphids m⁻²) in April. Two secondary peaks were observed in July and October (Fig 1B), and these were also recorded in Montcada (Fig. 2B). Because flushing was not so intense during the secondary peaks, percentage of aphid-infested flushes reached higher values than in spring (86.1% at Elx), although infestations were much lower (179 and 118 aphids m⁻² at Elx and Montcada, respectively). Navelina orange trees at Elx supported much higher aphid populations than Verna lemon trees at Montcada.

Predation upon *P. citrella* immature stages is presented in Figures 1C and 2C. In Elx, predation averaged

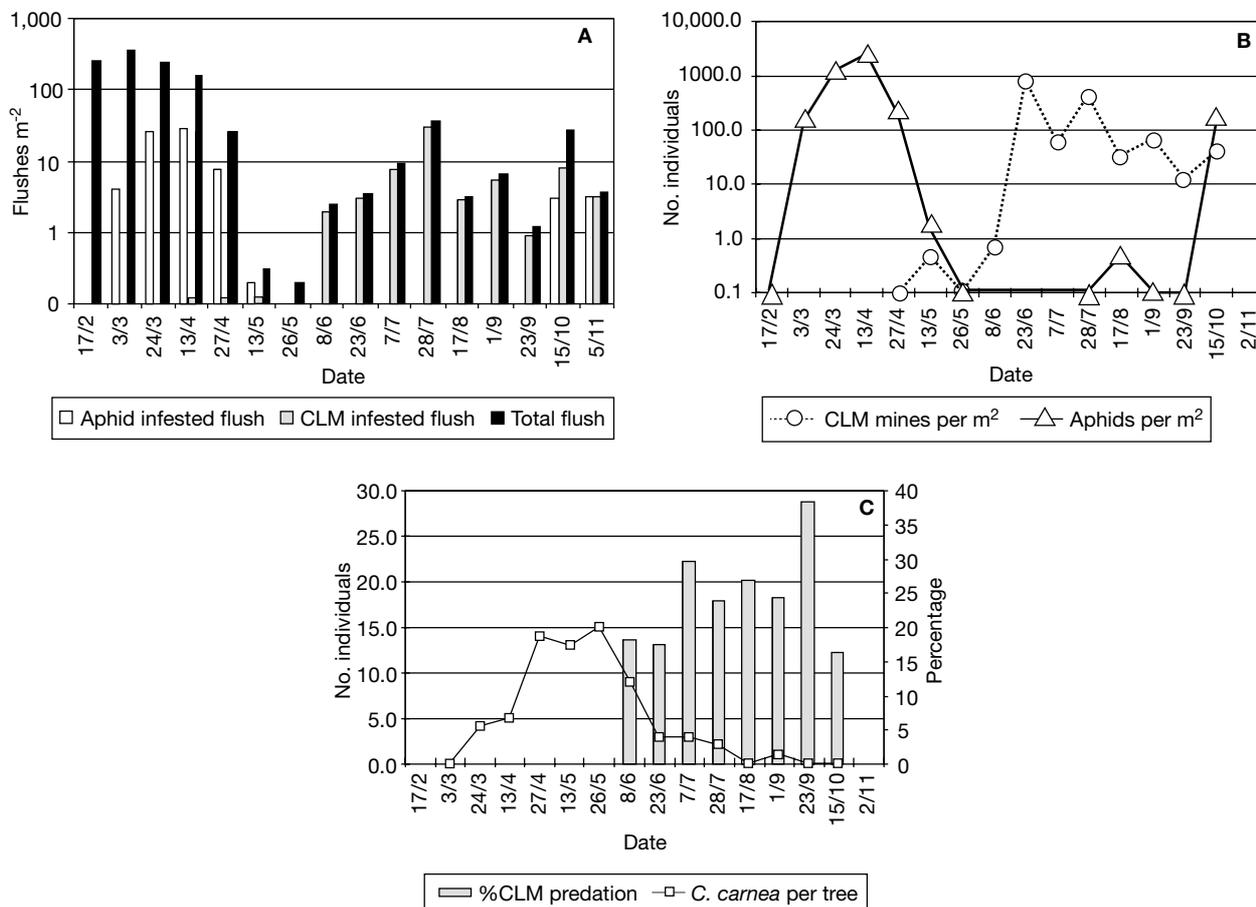


Figure 1. Elx. A: Flushing patterns: total number of flushes m⁻², aphid-infested flushes m⁻² and CLM (citrus leaf miner) infested flushes m⁻². B: Incidence of *P. citrella* (mines m⁻²) and aphids (individuals m⁻²). C: Percentage of predation (% of susceptible *P. citrella* stages) and *C. carnea* (adults per trap).

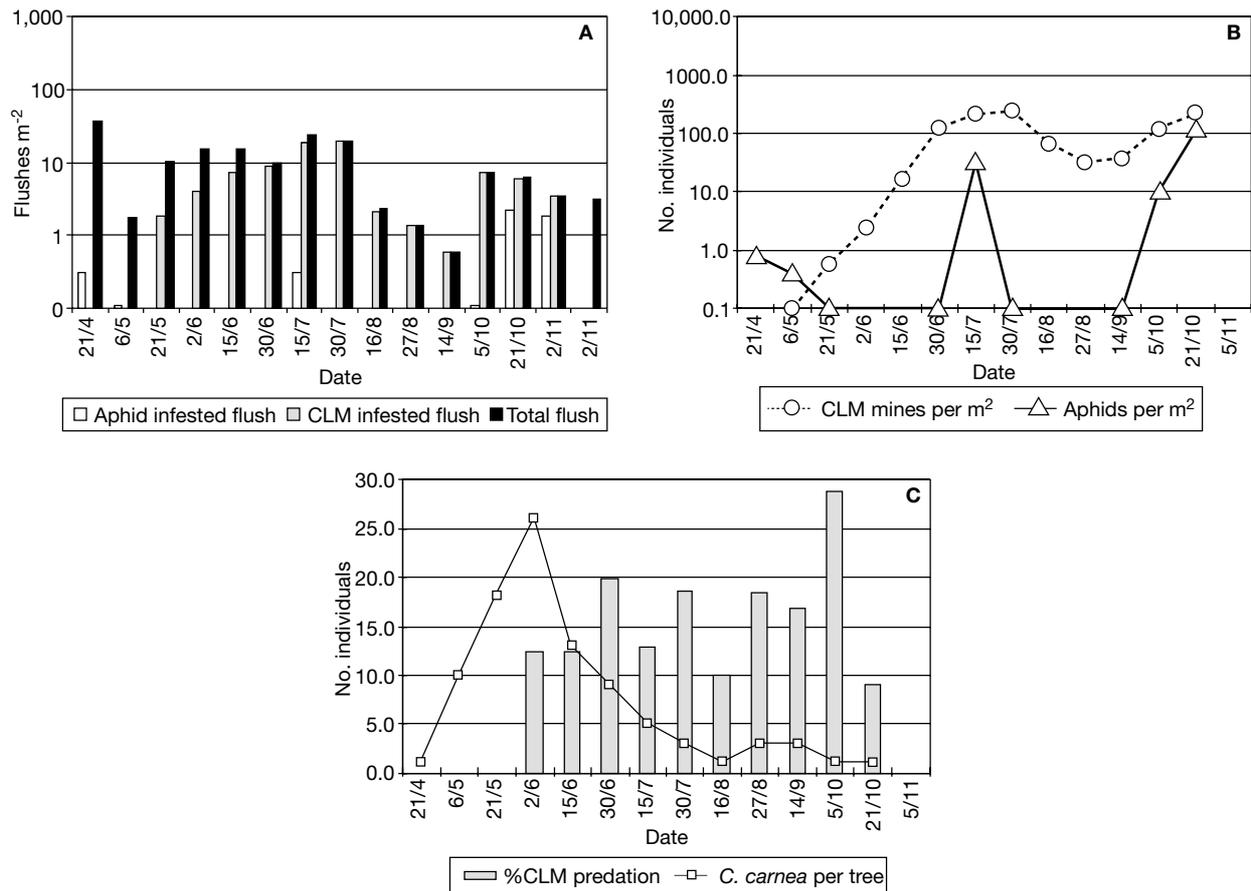


Figure 2. Montcada. A: Flushing patterns: total number of flushes m⁻², aphid-infested flushes m⁻² and CLM (citrus leaf miner) infested flushes m⁻². B: Incidence of *P. citrella* (mines m⁻²) and aphids (individuals m⁻²). C: Percentage of predation (% of susceptible *P. citrella* stages) and *C. carnea* (adults per trap).

23.7%, with a maximum of 38.3% at the end of September. In Montcada, predation reached lower values than in Elx (average of 15.7%, with a maximum of 28.9%) in October. Preferences of *P. citrella* predators were similar at both locations. From 477 and 520 immatures exhibiting evidence of predation (mutilated larvae) at Elx and Montcada, respectively, it appeared that the third instar was the most commonly attacked (59.1 and 53.4 %, respectively), followed by the fourth one (27.8 and 28.2 %, respectively), the second one (10.4 and 8.5, respectively), and the pupa (2.7 and 9.9, respectively).

Figures 1C and 2C show the numbers of *C. carnea* adults caught in a trap between two sampling dates. At both locations, maximum populations were observed between late May and early June (15 and 26 individuals per tree at Elx and Montcada, respectively). Afterwards, they decreased and did not increase again until fall, reaching much lower values than in spring (3 individuals per tree at both locations).

Relationships between *C. carnea* captures and flushing and both *P. citrella* and aphid densities were investigated at two different times, synchronous (Table 2) and 4-week delayed (Table 3). When synchronously studied, *C. carnea* numbers could be satisfactorily related to flushing (total number of flushes m⁻²) at Elx ($y_i = 3.095 + 2.457 (1/x_i)$; $r = 0.6704$), but not at Montcada. All other possible relationships investigated were not significant ($P > 0.05$), although some of them were close to statistical significance (Table 2). *P. citrella*-predation was not related to *C. carnea* numbers either ($F = 1.74$; $df = 1,7$; $P = 0.228$ and $F = 0.42$; $df = 1,9$; $P = 0.535$, at Elx and Montcada, respectively). Because only adults were counted, a 4-week delay was introduced in order to reflect the effect of the progeny of those adults on predation. Results changed considerably. *C. carnea* numbers responded to total flush ($y_i = 1.047 + 0.680 (x_{i-4})^{1/2}$, $r = 0.778$, and $y_i = 1.237 + 0.382 (x_{i-4})$, $r = 0.592$, at Elx and Montcada,

Table 2. Regression analyses between *C. carnea* densities (number of individuals per tree) and synchronous numbers of flush (flushes m⁻²), aphids, and *P. citrella* (individuals m⁻²), and aphid-infested and *P. citrella*-infested flushes (flushes m⁻²)

x_t y_t	Total flush	Aphids	Aphid-infested flushes	<i>P. citrella</i>	<i>P. citrella</i> -infested flushes
<i>C. carnea</i> orange-Elx	$F = 10.61$ $df = 1,13$ $P = 0.0062$	$F = 0.50$ $df = 1,13$ $P = 0.4907$	$F = 0.47$ $df = 1,13$ $P = 0.5042$	$F = 3.64$ $df = 1,13$ $P = 0.0789$	$F = 3.27$ $df = 1,13$ $P = 0.0939$
<i>C. carnea</i> lemon-Montcada	$F = 0.96$ $df = 1,13$ $P = 0.3452$	$F = 3.20$ $df = 1,13$ $P = 0.0968$	$F = 3.24$ $df = 1,13$ $P = 0.0950$	$F = 4.45$ $df = 1,12$ $P = 0.0567$	$F = 0.32$ $df = 1,13$ $P = 0.5785$

respectively), to *P. citrella*-infested flush ($y_t = 10.289 - 5.355 (x_{t-4})^{1/4}$, $r = -0.694$, and $y_t = 19.303 - 14.620 (x_{t-4})^{1/16}$, $r = -0.872$, at Elx and Montcada, respectively), to aphid-infested flush ($y_t = 1.133 - 5.663 (x_{t-4})^{1/4}$, $r = 0.908$, and $y_t = 3.381 + 37.109 (x_{t-4})^{1/16}$, $r = 0.593$, at Elx and Montcada, respectively), and to *P. citrella* numbers ($y_t = 13.793 - 10.048 (x_{t-4})^{1/16}$, $r = -0.937$, and $y_t = 26.007 - 18.010 (x_{t-4})^{1/16}$, $r = -0.970$, at Elx and Montcada, respectively). Although *C. carnea* numbers responded to aphids at Elx ($y_t^{1/2} = 0.542 + 1.133 (x_{t-4})^{1/8}$, $r = 0.874$, $F = 29.22$, $df = 1,19$, $P = 0.004$), such a correlation could not be established at Montcada.

Ants. Field: impact on *P. citrella*

Results displayed in Table 4, show that there were almost no differences in any of the parameters considered (numbers of *P. citrella* per leaf, % predation, % parasitism and % host feeding) between control and ant-excluded trees. Only on July 20, predation was significantly higher in ant-excluded trees than in control (32.6 vs 17.2%). Both parasitism and host feeding were very low and in this orchard, sometimes we could not even find larvae exhibiting the typical symptoms of these mortality factors. Parasitoids recovered from the samples taken at Bétera were the eulophids *Pnigalio pectinicornis* L. and *Cirrospilus brevis* (= *C. sp.* near

lynxus) Zhu, LaSalle and Huang. During sampling, specimens of the nocturnal spider *Chiracanthium mildei* L. Koch. (Araneae: Clubionidae) were often observed resting in their nests, which were commonly built on *P. citrella*-distorted leaves. Some individuals were taken to the laboratory and offered *P. citrella* third instars as described for *C. carnea*. Spiders could actually be observed feeding on *P. citrella*.

Discussion

As in previous studies (Urbaneja *et al.*, 1998, 2000), mortality inflicted to larvae of *P. citrella* by its natural enemies was mainly due to predation. On average, predation represented about 30% of total mortality on susceptible leaf miner stages, and this mortality factor alone accounted for a maximum value of 100% of total mortality on one sampling date at Bétera (20th July in ant-excluded trees). Relative predation figures obtained in this orchard (Table 5) were very similar to those obtained both at Elx and Montcada (Urbaneja *et al.*, 2000). Likewise, parasitoids recovered from the orchard at Bétera corresponded to the most abundant indigenous parasitoids reported in the other two orchards, as well as in our region as a whole at that time (Urbaneja *et al.*, 2000).

Our laboratory studies demonstrated that *C. carnea* could complete its development feeding on *P. citrella*

Table 3. Regression analyses between *C. carnea* densities (number of individuals per tree) and numbers of flush (flushes m⁻²), aphids, and *P. citrella* (individuals m⁻²), and aphid-infested and *P. citrella*-infested flushes (flushes m⁻²) 4 weeks earlier

x_{t-4} y_t	Total flush	Aphids	Aphid-infested flushes	<i>P. citrella</i>	<i>P. citrella</i> -infested flushes
<i>C. carnea</i> orange-Elx	$F = 16.84$ $df = 1,11$ $P = 0.0018$	$F = 29.22$ $df = 1,9$ $P = 0.0004$	$F = 46.76$ $df = 1,10$ $P < 0.0001$	$F = 71.32$ $df = 1,10$ $P < 0.0001$	$F = 9.31$ $df = 1,10$ $P = 0.0122$
<i>C. carnea</i> lemon-Montcada	$F = 5.39$ $df = 1,10$ $P = 0.0426$	$F = 0.24$ $df = 1,10$ $P = 0.6350$	$F = 5.41$ $df = 1,10$ $P = 0.0423$	$F = 159.18$ $df = 1,10$ $P < 0.0001$	$F = 31.72$ $df = 1,10$ $P = 0.0002$

Table 4. Incidence of *P. citrella* and relative predatory, oviposition, and feeding preferences on *P. citrella* (percentages) in a Navelina orchard at Bétera

Date	<i>P. citrella</i> mines per leaf		% predation		% parasitism		% host feeding	
	Control	Ant-excluded	Control	Ant-excluded	Control	Ant-excluded	Control	Ant-excluded
6 th July	2.08 ± 0.33a	1.75 ± 0.15a	32.1 ± 6.3a	38.4 ± 10.9a	0.0 ± 0.0a	0.0 ± 0.0a	1.3 ± 1.3a	1.0 ± 1.3a
13 th July	1.89 ± 0.30a	1.68 ± 0.14a	45.9 ± 10.8a	36.0 ± 9.8a	0.0 ± 0.0a	0.4 ± 0.8a	2.9 ± 3.2a	1.6 ± 1.7a
20 th July	2.25 ± 0.74a	2.09 ± 0.31a	17.2 ± 4.6b	32.6 ± 11.3a	0.0 ± 0.0a	0.0 ± 0.0a	1.6 ± 2.4a	0.0 ± 0.0a
29 th July	2.00 ± 0.21a	2.28 ± 0.42a	38.9 ± 14.8a	23.9 ± 7.9a	2.1 ± 2.4a	0.3 ± 0.5a	2.7 ± 3.6a	0.7 ± 0.9a
16 th August	2.81 ± 0.36a	2.68 ± 0.37a	12.2 ± 5.6a	12.6 ± 3.6a	0.3 ± 0.6a	1.3 ± 1.1a	0.9 ± 1.3a	1.3 ± 1.1a

For each date, within paired columns, means followed by the same letter are not significantly different ($P < 0.05$, LSD test).

only. Nevertheless, immature mortality was very high. In China, Runtian *et al.* (1989) studied the predatory potential of the chrysopid, *Chrysopa boninensis* Okamoto on *P. citrella* in the laboratory. Life cycle was shorter (28.8 days) and predation higher (149.1 larvae) than those obtained for *C. carnea*. Life cycle of *C. carnea* was also shorter when reared under optimal conditions (Ru *et al.*, 1975; Tauber and Tauber, 1983; Tauber *et al.*, 1987). Therefore, our results could be attributed to the strict monophagy *C. carnea* was forced into during the assay. Maximal abundance of *C. carnea* in the field corresponded to periods of minimal flushing (Fig. 1A and 1C) and the regression found in Elx reflected this situation. Nevertheless, predation upon *P. citrella*, which had been satisfactorily correlated to flushing in Elx when this was regular (Urbaneja *et al.*, 2000), could not be related to numbers of *C. carnea* in any case. When synchronous densities were considered, the latter could not be related to either aphid-infested flushes or *P. citrella*-infested ones, but regressions were close to statistical significance when numbers of *P. citrella* (both at Elx and Montcada) and aphids (at Montcada only) were considered. However, highly statistically significant regressions were obtained when a 1-month delay between presumed prey indicators (either densities or flush) and predator densities were considered. *C. carnea* numbers peaked

following both flush and aphid peaks at both locations (Table 3), with aphids at Montcada Verna lemon orchard being the only exception. As established in previous studies (Hermoso de Mendoza *et al.*, 1986), Navelina orange trees supported aphid populations almost ten times higher than those observed in Verna lemon, and this could explain why the regression could not be established at Montcada. Contrary to these results, relating *C. carnea* numbers to *P. citrella* indicators resulted in a negative relationship. Therefore, increasing numbers of this pest did not lead to increases of *C. carnea*. The green lacewing is a polyphagous predator, and these results demonstrate that although it can feed on *P. citrella*, it can not be considered a key *P. citrella*-predator. *C. carnea* has a long reputation as aphid predator in Mediterranean citrus (Llorens, 1990; Garrido and Ventura, 1993; Katsoyannos, 1996). Although results from Elx point at this predatory behavior, it remains unclear what *C. carnea* was responding to at Montcada (Fig. 2B and 2C). The same conclusion about ants can be drawn from our exclusion field assay at Bétera. Although ant predation was the form of mortality most often observed in Florida (Pomerinke, 1999; Amalin *et al.*, 2002), predation rates in our orchard did not change when ants were excluded from *P. citrella*-infested trees. Furthermore, predation was even higher in ant-excluded trees at one sampling date (20th July), and this could be attributed to the aphid-defending behavior often observed in ants (Palacios *et al.*, 1999). Ant-species composition in Spain and in Florida is different (Palacios *et al.*, 1999) and this could also account for the differences detected. Therefore, neither ants, nor green lacewings could be identified as key sources of predation on *P. citrella*.

In addition to lacewings and ants, other species have been cited in the Mediterranean as possible predators of *P. citrella*. These include spiders, minute pirate bugs, thrips, and ladybirds. We could observe how the spider

Table 5. Relative oviposition, feeding and predatory preferences on the citrus leafminer (percentages and actual counts) in the Navelina orchard at Bétera

Host stage	Oviposition		Feeding		Predation	
	%	Count	%	Count	%	Count
LII	—	—	6.2	41	4.6	5
LIII	71.4	35	56.7	373	58.7	64
LIV	14.3	7	33.1	218	6.4	7
Pupa	14.3	7	4.0	26	30.3	33

C. mildei fed on larvae and pupae of *P. citrella*, and we have sometimes seen Anthocoridae and thrips feeding on larvae of *P. citrella* in our orchards. Although we have not been able to prove the efficiency of any of these predators, the establishment of *P. citrella* in our orchards has given us the chance to become aware of the existence of an important guild of undetermined generalist predators, and opportunistic parasitoids (Urbaneja *et al.*, 2000). These results should make citrus entomologists consider conservation biological control not as a mere complement of necessary classical biological control programs, but as the cornerstone of future Integrated Pest Management in citrus systems.

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