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Changes in predation and parasitism of the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) populations in Spain following establishment of *Citrostichus phyllocnistoides* (Hymenoptera: Eulophidae)

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ABSTRACT

The citrus leafminer, *Phyllocnistis citrella*, is a pest native to Southeast Asia which threatened the citrus industry in the Mediterranean region upon its introduction in 1993. Immediately afterward, a classical biological control program was implemented in Spain. The exotic parasitoid *Citrostichus phyllocnistoides* was the only introduced parasitoid to become established. In 2006, data on both the incidence of *P. citrella* and the impact of its natural enemies were collected following the same protocols used in 1997–1999 when *C. phyllocnistoides* was not yet present. *C. phyllocnistoides* constituted 99.4% of the parasitoids collected in 2006 corresponding to a decrease in the incidence of *P. citrella* from 3.2–5.1 to 1.8–2.4 mines per leaf in 1997–1999 and 2006, respectively. Mortality caused by natural enemies on *P. citrella* in 2006 was 93.3% (18.0% parasitism, 40.8% feeding punctures and 34.5% predation). *C. phyllocnistoides*, which preferentially parasitizes *P. citrella* second instar larvae, has displaced most of the indigenous parasitoids that moved onto *P. citrella* mainly parasitizing third instar larvae, upon its introduction. Because *C. phyllocnistoides* is an idiobiont parasitoid and preferentially parasitizes *P. citrella* second instars, this stage has become dominant in the orchards. The shift in the relative abundance of *P. citrella* larvae has prompted generalist predators to prey mostly on second instars and has contributed to the displacement of the native non-specific parasitoids, which principally utilize third instars, from the system. Both indigenous predators and the introduced parasitoid are key players in the natural regulation of *P. citrella*.
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1. Introduction

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is a citrus pest native to southeastern Asia that has spread to most citrus growing areas in the Mediterranean and the Americas during the last decade of the 20th century (Urbaneja et al., 2000). Studies carried out between 1996 and 1999 showed that in Mediterranean areas, CLM damaged only 5–15% of the annual new leaf area of mature trees and therefore yield was not affected by the pest (Garcia-Mari et al., 2002). However, it is still regarded as an important pest in nurseries, as well as on young plants and top-grafted trees, where chemical control is necessary (Garcia-Mari et al., 2002).

As in similar situations of exotic pest outbreaks (Michaud, 2002), the rapid spread of CLM in Spain in 1995 prompted funding agencies to prioritize biological control projects based on the rearing and release of imported natural enemies. CLM became a target of the classical biological approach without a critical evaluation of whether such an approach was the best one. In Spain, pre-introduction studies of natural enemies of CLM (Lenteren and Woets, 1988; Barbosa and Segarra-Carmona, 1993; FAO, 1996; EPPO, 1999, 2000) were very limited (Urbaneja et al., 2000, 2003a), and six different parasitoids were introduced in less than 3 years (from 1996 to 1998) (Jacas et al., 2006). The host-specific endoparasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) successfully established in the Canary Islands, whereas the oligophagous facultative hyperparasitoid *Citrostichus phyllocnistoides* (Narayan) (Hymenoptera: Eulophidae) established in mainland Spain (Garcia-Mari et al., 2004). This species was also introduced and established in other Mediterranean countries (Argov and Rossler, 1996, 1998; Tsagarakis et al., 1999; Rizqi et al., 2003; Siscaro et al., 2003). Currently, CLM is no longer considered an important pest of citrus on mature bearing trees (Garcia-Mari et al., 2002).

Three years after the introduction of CLM in Spain, a 3-year survey (1997–1999) carried out in the region around the city of València (Fig. 1) – the main citrus growing area in Spain – showed that indigenous natural enemies of CLM, consisting of 11 generalist

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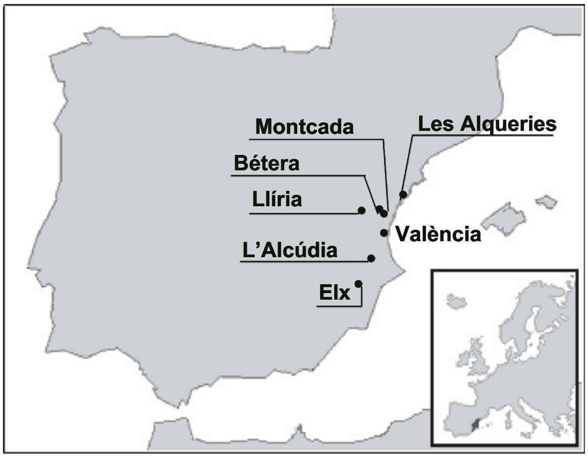

Fig. 1. Location of the clementine orchards considered in this study (Bétera, L'Alcúdia, Les Alqueries, Llíria, Montcada) and in the 1997–1999 survey (Montcada, Elx).

parasitoids and several unidentified polyphagous predators (including lacewings, spiders), fed on CLM populations resulting in up to 70% mortality of mature larvae by the end of the summer (Urbaneja et al., 2000). Predation was the main mortality factor (35.4%), followed by parasitoid host feeding (20.3%) and parasitism (16.9%). Composition of the parasitoid species complex changed during the 3 years of the study. However, the indigenous eulophids *Cirrospilus brevis* (=ca. *lyncus*) Zhu, LaSalle and Huang and *Pnigalio pectinicornis* L. were consistently the predominant species whereas the exotic parasitoids introduced up until then either had no significant effect on CLM populations (the eulophids *Quadrastichus citrella* Girault and *Galeopsomyia fausta* LaSalle) or did not succeed in overwintering (*A. citricola*). Studies carried out during the release and establishment of *C. phyllocnistoides* from 1998 to 2001 (Garcia-Mari et al., 2004) showed a shift in parasitoid species composition: *C. phyllocnistoides* represented 97.1% of parasitoids in 2001. This change affected percentage parasitism, which according to that study increased from 20–25% in 1995–1998 to near 60% in 1999–2001. Such changes apparently affected mean CLM densities (34% and 72% reductions for eggs and adults, respectively) and foliar damage (56% reduction). However, no comparison of total natural enemy-caused mortality before and after the establishment of *C. phyllocnistoides* has been undertaken. Successful control of a pest by natural enemies (either exotic or indigenous) must be determined by quantitative evaluation both before and after release (DeBach et al., 1976; Luck et al., 1988). For this reason the present study was carried out. Our aims were to monitor the incidence of CLM 12 years after its arrival, to quantify the impact of its natural enemies (both indigenous and exotic parasitoids and predators) in citrus orchards of the València region and to compare these results to those obtained before the establishment of *C. phyllocnistoides*. This should allow us to evaluate the biological control program against CLM in Spain.

2. Materials and methods

The methodology used was based on the survey carried out from 1997 to 1999 (Urbaneja et al., 2000). Hence, leaf flushing patterns and incidence of both the CLM and its parasitoids were recorded at 7–15 day intervals from early June until December 2006 in three citrus orchards at three different locations in the region of València: L'Alcúdia, Bétera and Llíria (Fig. 1). These orchards were 1 ha each and consisted of mature bearing clementine trees [*Citrus reticulata* Blanco] grafted on Carrizo Citrange rootstock [*Poncirus trifoliata* (L.) Rafinesque-Schmaltz × *Citrus sinensis* (L.) Osbeck]. The orchards at L'Alcúdia and Bétera received no chemical treatments during the sampling period except for a mineral oil application at L'Alcúdia against *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) on August 24, 2006. The orchard at Llíria followed IPM guidelines and was treated with pirimicarb against *Aphis spiraecola* Pagensteche (Hemiptera: Aphididae) on August 3 and with methylpyrimiphos against *A. aurantii* on August 10. All three orchards were drip irrigated with a ground cover of *Festuca arundinacea* Schreber (Poaceae) that was periodically mowed and were surrounded by similar citrus orchards.

Twenty trees were randomly chosen at each sampling date. A ring made of flexible polyethylene irrigation tubing, 56 cm in diameter, was thrown onto the tree and the number of total leaf flushes, egg-receptive flushes (those exhibiting leaves up to 2 cm long) and flushes containing CLM larvae or pupae found within the ring were counted. Three additional flushes, or suckers when no new shoots were available, were collected from each tree, put into a plastic bag and transported to the laboratory in a cooler for observation of their infested leaves under a stereoscopic binocular microscope. Leaves were examined for the presence of mines (either occupied or abandoned), CLM larvae (first to fourth instars based on their morphology) or pupae and parasitoid immature stages. Observations were terminated once 100 mines had been inspected. Dead CLM stages exhibiting black spots were considered the product of either host feeding by parasitoids or predation by insects with sucking mouthparts. Predation was assumed to be the reason for incomplete mines or mines where mutilated CLM larvae were found. Because both indigenous and naturalized parasitoids of CLM are idiobiont species that cause permanent paralysis when stinging the host, the instar of parasitized hosts was recorded to establish parasitoid ovipositional preferences. Based on these data, percentage parasitism, host feeding and predation were calculated (number of individuals either parasitized, pierced or preyed upon, respectively, divided by the total number of individuals inspected). Data for stage-specific natural enemy-caused mortality were combined with the actual distribution of the different stages observed in the field and used to estimate average survival of CLM immature stages. When possible, results were compared to those collected from 1997 to 1999 in Elx and Montcada (Fig. 1) by Urbaneja et al. (2000) although these authors did not consider CLM first instars in their study.

About 100 supplementary fully grown infested flushes were collected at the three orchards described above at each visit and at two additional clementine orchards located at the Instituto Valenciano de Investigaciones Agrarias (IVIA, Montcada; 10 samples between 4 August and 4 December 2006) and Les Alqueries (3 samples during August 2006) (Fig. 1) at different visits. These flushes were transported to the laboratory in a cooler where they were further enclosed in cardboard cages (22 × 30 × 22 cm). A clear PVC vial (1 cm diam 10 cm long) was inserted in one of the sides of the cages and used as an emergence trap for parasitoids, which were prepared for taxonomic determination after emergence.

3. Results

The three orchards were sampled 16 times between June 15 (date when infested leaves were first observed in Bétera) and December 28 (when infested leaves were last observed in both Bétera and L'Alcúdia). A total of 3548 mines were found in 2142 infested leaves inspected. Of the mines found, 1609 mines were complete (including empty mines left behind by emerged CLM) and therefore were considered to house apparently healthy CLM larvae and pupae. Additionally, 545 parasitized individuals

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