BioControl (2008) 53:473–487
DOI 10.1007/s10526-007-9084-2

Parasitoid complex of black scale *Saissetia oleae* on citrus and olives: parasitoid species composition and seasonal trend

Alejandro Tena · Antonia Soto · Ferran Garcia-Marí

Received: 24 January 2007 / Accepted: 13 April 2007 / Published online: 3 May 2007

Abstract The parasitoid complex of black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) was studied on citrus and olives to determine their relative abundance, seasonal trend, geographical distribution, and their incidence on black scale populations. Branches and leaves of ten citrus and four olive groves infested with black scale were periodically collected over the period March 2003–December 2005 in eastern Spain, covering an area of 10,000 km^2. Adult parasitoids were also sampled with a portable engine-powered suction device. Black scale females were attacked by *Scutellista caerulea* (Fonscolombe) (Hymenoptera: Pteromalidae), which was found beneath 35.4 ± 7.5% and 22.4 ± 3.5% female scale's body in citrus and olive groves, respectively. However, *S. caerulea* attacked the scales when most of their eggs had already hatched. The parasitic mite *Pyemotes herfsi* (Oudemans) (Prostigmata: Pyemotidae) fed on all development stages of *S. caerulea*. The gregarious female's endoparasitoid *Metaphycus lounsburyi* (Howard) (Hymenoptera: Encyrtidae) was common in citrus and olive trees, but the parasitism rates it reached was low. Second and third instars of black scale were mainly parasitized by the solitary endoparasitoid *Metaphycus flavus* (Howard), and secondarily by *Metaphycus helvolus* (Compere) which was much less abundant and limited in distribution. Thus, *M. helvolus*, introduced 30 years ago, has not displaced *M. flavus* as in other Mediterranean areas. According to their abundance, distribution and incidence, *M. flavus* and *S. caerulea* appeared as the main parasitoids of black scale in eastern Spain, whereas *M. helvolus* and *M. lounsburyi*, considered the main parasitoids in other citrus and olive areas of the world, had a limited incidence. Recommendations for improving the level of biological control are discussed.

Keywords Biological control · Citrus · *Coccophagus* · *Metaphycus* · Olives · Parasitoids · *Scutellista caerulea*

A. Tena · A. Soto · F. Garcia-Marí (✉)
Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camí de Vera 14, Valencia 46022, Spain
e-mail: fgarciam@eaf.upv.es

Introduction

Black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) is a cosmopolitan and polyphagous soft scale pest of more than 60 plant species in the Mediterranean region, including citrus and olives (Morillo 1977; Carvalho et al. 2003). The damage black scale causes to these plants varies annually because of variation in the combined effect of mortality factors such as those arising from natural enemies, climatic effects, and/or broad-spectrum insecticide applications (Panis 1977; Mendel et al. 1984). Satisfactory biological control of black scale has been achieved through the releases of parasitoids reared in the laboratory (Graebner et al. 1984) or through the introduction of a complex of parasitoids and predators (Mendel et al. 1984; Waterhouse and Sands 2001). In eastern Spain, different parasitoids have been introduced (Carrero 1981; Meliá and Blasco 1981) but their establishment and incidence on black scale remain unclear.

Previous studies of the parasitoid complex of black scale around the world have identified four dominant parasitoid species associated with black scale. These include *Metaphycus lounsburyi* (Howard) (= *M. bartletti* Annecke & Mynhard), *M. helvolus* (Compere) (Hymenoptera: Encyrtidae), *Coccophagus lycimnia* (Walker) (Hymenoptera: Aphelinidae) and *Scutellista caerulea* (Fonscolombe) (= *S. cynea* Motschulsky) (Hymenoptera: Pteromalidae) found in citrus (Mendel et al. 1984; Lampson and Morse 1992) and olives (Kennett 1986; Daane et al. 1991; Pereira 2004).

Metaphycus helvolus and *M. lounsburyi* are parasitoids of black scale in South Africa where they and the scale are endemic. These parasitoids have been introduced into citrus and olive growing regions world wide (including Australia, California (USA), Cyprus, France, Greece, Israel, Italy and Spain), to reduce black scale populations to non economic densities (Argov and Rössler 1993; Guerrieri and Noyes 2000; Malipatil et al. 2000). *Metaphycus helvolus* is a solitary, primary endoparasitoid that attacks 2nd and 3rd instar black scale (Lampson et al. 1996). It has been augmentatively released against black scale in an inland coastal valley of southern California where it effectively suppressed the scale as part of an integrated citrus pest management program (Graebner et al. 1984). Such releases have also shown promise in California olive groves (Daane et al. 1991). In Crete (Greece) *M. helvolus* displaced the native parasitoid *M. flavus* (Howard) (Hymenoptera: Encyrtidae) (Argyriou and Michelakis 1975). In 1976, *M. helvolus* was successfully introduced into eastern Spain (Carrero 1981); however, ten years later, Ripollés (1990) reported that it was not well established in this region.

Metaphycus lounsburyi is a gregarious endoparasitoid that attacks 3rd instars, mature, and ovipositing female black scale (Barzman and Daane 2001). In Israel, *M. lounsburyi* is considered the principal parasitoid responsible for suppressing black scale (Argov and Rössler 1993). This species was introduced into eastern Spain in 1979 (Meliá and Blasco 1981) but its establishment and efficacy are unknown. A recent local study of black scale parasitoids in olive groves in eastern Spain by Noguera et al. (2003) found *M. lounsburyi*, but not *M. helvolus*.

A second genus of parasitoids associated with soft scales is *Coccophagus* (Hymenoptera: Aphelinidae). These are heteronomous hyperparasitoids in which the females are primary endoparasitoids of soft scales whereas the males are hyperparasitoids of their females (Walter 1983) or of other primary parasitoids including species of *Metaphycus* (Bernal et al. 2001). *Coccophagus lycimnia* has been collected from black scale infesting citrus and olives in Valencia (Carrero et al. 1977; Noguera et al. 2003). If *C. lycimnia* is abundant, it may be hyperparasitizing soft scales previously parasitized by *Metaphycus* and thereby reducing the potential of *Metaphycus* to suppress these soft scales (Bernal et al. 2001).

Scutellista caerulea is a well known cosmopolitan parasitoid of black scale, which larvae usually develop as an egg-predator feeding on the eggs beneath the scale body (Ehler 1989). However, it seldom consumes all the eggs and some often survive. The percentage of surviving eggs depends on the total number of eggs laid by the host female (Mendel et al. 1984). Although *S. caerulea* is relatively well-known, its incidence on black scale populations appears unclear because it varies depending on areas and crops. Thus, on olives in southern Spain, Montiel and Santaella (1995) detected *S. caerulea* in 5.9% of the black scale females, whereas on citrus in Israel, Mendel et al. (1984) found it in as many as 80% of the scales they assessed.

Despite being the most serious soft scale pest of citrus and olives in the Mediterranean region, no long-term, area-wide studies of the parasitoid complex of black scale have been conducted recently. Comparing the parasitoid complex on both crops would be especially interesting due to their coexistence all around the Mediterranean Basin (Carvalho et al. 2003). Moreover, after the introduction of *M. helvolus* and *M. lounsburyi* more than 30 years ago, their establishments and impact on native parasitoids remain unclear. Thus, we initiated a study of the parasitoid complex of black scale to determine the main species present in citrus and olives, their geographical distribution, their seasonal abundance to ensure correct timing for possible augmentative releases, and their incidence on the host population, as a first step in improving the biological control of black scale in Spain.

Materials and methods

Groves

We selected ten citrus and four olive groves in eastern Spain with sparse to dense black scale populations and sampled them between March 2003 and December 2005 (citrus) and May 2004–December 2005 (olives). Groves were included in an area 200 Km long (north-south) and 50 km wide (east-west) and they were classified according to their infestation levels in May, the period of the year when adult females are present, as high (H: more than five adult scales per twig), medium (M: between two and five adult scales per twig) or low (L: one or two adult scales per twig). The groves were sampled twice a month during periods of rapid scale growth (April–October) and monthly during the cooler, winter months. Each grove was sampled for different periods of time, ranging from 8 to 18 months. Groves were not treated with insecticides over the sampling period. Samples were collected from 2 to 4 citrus and olive groves on each date. The citrus groves were located in Albal (Medium infested, 14 months sampled), Alcora (H, 10), Altura (H, 10), Castellón (M, 10), Moncófar (M, 18), Museros (L, 8), Onda (H, 15), Real de Montroy (M, 12), Ribarroja (L, 12) and Xilxes (H, 18); and the olive groves in Altura (L, 15), Castellón (M, 10), Planes (L, 18), and Villar del Arzobispo (M, 18).

Black scale phenology and parasitoid incidence

Sixteen, 15-cm long twigs with green-wood and leaves were collected from a minimum of four trees. We only selected trees and twigs within trees that were infested with black scale because we wanted to determine whether parasitoids were present in these groves. The infested twigs were placed in plastic bags and transported to the laboratory for processing. Plastic bags were closed during transportation and opened and placed in a chamber at 10°C and 50% R.H. once in the laboratory. Samples were processed in the next 48 h. We processed each twig and four leaves (both sides) from each twig using stereomicroscope. We sought to determine the phenology of the black scale population and the identity and incidence of its parasitoids. To determine the age structure of black scale that was present on a sample date, we counted the total number of live scales and categorized them into the three immature stages (i.e., see Morillo 1977) and into three adult female stages: young females, females with eggs, and females with crawlers (or eggs and crawlers). Simultaneously, we examined immature black scales for signs of parasitism *by Metaphycus* spp. and *Coccophagus* spp. (i.e., parasitoid larvae or pupae). Parasitism rates for *M. flavus* and *M. helvolus* was a combined estimation because we could not differentiate the larval stages of these two species. We referred to this parasitism as *Metaphycus* spp. Similarly, we also combined the parasitism by *C. lycimnia* and *C. semicircularis* (Förster) (= *C. scutellaris* (Dalman)) and referred to as parasitism by *Coccophagus* spp. All adult females were slightly turned over, carefully examined for signs of parasitism by *Metaphycus lounsburyi* (the ventral part of the females appeared grey clear and deformed when had been parasitized by this parasitoid) and/or for presence of *Scutellista caerulea* larvae or pupae under the scale body, and returned to their original position to allow parasitoid development. *Scutellista caerulea* is usually referred as a parasitoid, even though its larvae are predators of black scale eggs beneath the scale's body. These assessments allowed us to calculate parasitism rates as the number of scales parasitized (with parasitism signs or presence of *S. caerulea*) / number of live and parasitized scales. Parasitism rates were calculated for each black scale stage when the number of live and parasitized scales were $\geq$20. These parasitism rates are presented in Table 2 and Fig. 4.

The twigs and leaves previously observed were placed in a ventilated transparent 25 × 8 cm plastic cage for parasitoid emergence. Other species of soft scales were removed from the plant material. The cages were held at 23–27°C, 16:8 (L:D) and 60–80% RH for 25–30 days and then held at −20°C for at least one day to kill surviving parasitoids. Finally, dried twigs, leaves and debris were brushed, sieved, and examined under stereomicroscope to count and identify the adult parasitoids that had emerged. This sampling method is referred in the text as ''emergence cages'' and its results are presented in Table 1.

Flight period of parasitoids

On each sampling date, adult parasitoids were collected from the tree canopy with a portable, engine-powered, suction device. The device was constructed by modifying a commercial vacuum-blower (McCulloch, model Mac 320 BV, Tucson, AZ) and adapting it to collect insects from the foliage. We modified it by adding a cylindrical plastic tube 30-cm long with a 30-cm diameter opening. The sampling was standardized by placing the opening of the cylindrical tube a total 70 times on the foliage of citrus or olive trees per date. We ensured that the samples were obtained around the tree canopy and up to 2 m high, on eight trees (8–10 suctions per tree), different from those trees used to collect the twigs and leaves and selected for their high levels of black scale infestation. The collected material from each grove was bagged and transported to the laboratory, where it was held at −20°C for one day to kill the insects. The black scale parasitoids were then counted

Table 1 Relative abundance of the main *Saissetia oleae* natural enemies, observed using three sampling methods in citrus (March 2003–December 2005) and olive groves (May 2004–December 2005) in eastern Spain

Species	Citrus			Olives			Combined crops	
	% Abundance[a]		Grove presence	% Abundance[a]		Grove presence	Total %	Grove presence
	Emerging parasitoids	Suction-sampled		Emerging parasitoids	Suction-sampled			
Aphelinidae								
Coccophagus lycimnia	4.0	6.2	7/10	10.8	13.4	3/4	6.5	10/14
C. semicircularis	0.8	0.7	3/10	0.3	0.4	1/4	0.7	4/14
Encyrtidae								
Metaphycus flavus	22.3	69.7	10/10	2.6	26.1	4/4	44.5	14/14
M. helvolus	6.1	3.2	5/10	5.6	8.2	3/4	4.7	8/14
M. lounsburyi	19.1	7.1	9/10	51.0	28.5	4/4	17.4	13/14
Pteromalidae								
Scutellista caerulea	47.7	13.2	10/10	29.7	23.3	4/4	26.6	14/14
Total number	2174	3460	10	694	536	4	6864	14

[a] Parasitoid species percentage in each sampling method

under a stereomicroscope and labeled by date and grove. These data are referred in the text as ''suction samples of parasitoids''.

We used the keys of Guerrieri and Noyes (2000) and Malipatil et al. (2000) to identify the Hymenoptera in the collected material and our identifications were confirmed by M. J. Verdú (Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain). The parasitic mite was identified by A. Baker and a cecidomyiid egg predator found at low levels was indentified by N. Wyatt (Natural History Museum, London, UK).

Statistical analysis

We compared the percent parasitism between years, host plants (citrus vs. olive), host stages, and parasitoid species using a multifactorial analysis of variance (ANOVA) (Statgraphics 1994). We arcsine-square-root transformed (z = arcsine $(x^{0.5})$) percentage parasitism to approximate a normal distribution before subjecting the data to analyses. Means were compared using an LSD test at a 5% significance level.

Results

Parasitoids species

During the three year study, 6,864 parasitoid specimens were obtained using two sampling methods. These parasitoids belonged to nine species, with the genus *Metaphycus*, *Scutellista* and *Coccophagus* comprising 99% of the parasitoid fauna. The most abundant and widely distributed parasitoids in both olive and citrus groves were *Metaphycus flavus* (Howard) (44.5% of 6,864), *Scutellista caerulea* (26.6%), *M. lounsburyi* (17.4%) and *Coccophagus lycimnia* (6.5%) (Table 1). Their abundance depended on the crop and the sampling methodology used.

Among the parasitoids of the immature scales, *M. flavus* was the most abundant (44.5%) and widely distributed, both on citrus and olive as well as by geographic sampling location. It was present in all the groves sampled, whereas *M. helvolus* was much less abundant (4.7%) and was present only in eight of the 14 groves sampled. Parasitoids of genus *Coccophagus* were more abundant on olives than on citrus. On olives, *Coccophagus lycimnia* was the most abundant parasitoid in the emergence cages (10.8%), where parasitoids of genus *Metaphycus* (*M. helvolus* (5.6%), *M. flavus* (2.6%)) were less abundant.

The female parasitoids *S. caerulea* and *M. lounsburyi* were widely distributed, because they were collected in almost all the citrus and olive groves sampled. The pteromalid *S. caerulea* was the most abundant if considering both crops, though the gregarious endoparasitoid *M. lounsburyi* was more abundant on olives than the pteromalid. The mite *Pyemotes herfsi* (Oudemans) (Prostigmata: Pyemotidae) was observed feeding on the larvae, pupae and adults of *Scutellista caerulea*. *Pyemotes herfsi* was found in three citrus and all four olive groves.

Two specimens of a male Pteromalinae (Pteromalidae: Hymenoptera), three female specimens of *Mycroteris nietneri* (Motschulsky) (Hymenoptera: Encyrtidae) and one specimen of the hyperparasitoid *Marietta picta* (André) (Aphelinidae: Hymenoptera) were also obtained in the emergence cages.

During the observations to calculate the parasitism rates, 23 predacious cecidomyiid larvae were observed feeding on black scale eggs under the female's body. It was found only in the citrus groves. The cecidomyiid was identified as *Lestodiplosis* sp. or another closely related genus.

Black scale phenology and seasonal trend of its parasitoids

Three years of data have been combined to compare the total number black scale and its age structure along with the total number of parasitoids recovered from the emergence cages and the parasitoid's relative abundance per crop and date (Figs. 1 and 2). The number of parasitoids recovered from the emergence cages peaked in both crops at the beginning of summer (June–July), just at the end of the female black scale's development. The main species recovered during these dates were the female parasitoids *M. lounsburyi* and *S. caerulea*. This peak did not occur during the summer of 2004 in citrus groves, due to the sparse number of black scale and parasitoids present that year.

When black scale occurred as 2nd and 3rd instars (between September and May each year) the number of parasitoids collected was lower (Figs. 1 and 2). *Metaphycus flavus* was the commonest species recovered in citrus, although other parasitoids of immature scales as *M. helvolus*, *C. lycimnia* and *C. semicircularis* were also present. Similarly, low numbers of *C. lycimnia*, *M. helvolus* and *M. flavus* were also collected in the olive groves during this period.

The suction samples of the adult parasitoids in the groves confirmed the seasonal trend observed in the emergence cages (Fig. 3). Adult specimens of *Metaphycus lounsburyi* and *S. caerulea* were captured in the groves in summer (June–September), after female black scales were abundant. These parasitoids were low or absent during the rest of the year. In contrast, the parasitoid *M. flavus* was present throughout the year. *Metaphycus flavus* numbers peaked at the end of the spring (June) and during autumn (October–November), during or shortly after 2nd and 3rd instar black scale occurred in the groves. These instars are the preferred stages for oviposition by *M. flavus*. Interestingly, a second *M. flavus* peak occurred in summer when suitable black scale stages for oviposition by *M. flavus* were absent. *Coccophagus lycimnia* also parasitizes the immature stages of black scale and was usually abundant during the spring (May–June). It was also abundant in one olive grove in autumn 2005.

Parasitism rates

Parasitism of black scale by *Metaphycus* spp. (*M. flavus* and *M. helvolus*) was greater than by *Coccophagus* spp. (*C. lycimnia* and *C. semicircularis*) (ANOVA: $F = 32.52$; $df = 1, 33$; $P < 0.0001$) (Table 2). *Metaphycus* spp. were found parasitizing all immature stages, although 1st instar was rarely parasitized. Third instar black scale suffered higher rates of parasitism than 2nd instar (ANOVA: $F = 9.95$; $df = 1, 16$; $P = 0.0036$). Moreover, *Metaphycus* spp. parasitized significantly more black scale in citrus than in olives groves (ANOVA: $F = 7.41$; $df = 1, 17$; $P = 0.0107$). Finally, *Metaphycus* spp. parasitized significantly more immature black scale in 2003 (8.96 ± 1.01%) than in 2004 (3.03 ± 0.63%) or 2005 (2.04 ± 0.57%) (ANOVA: $F = 17.2$; $df = 2, 13$; $P < 0.0001$).

Coccophagus spp. parasitized both 2nd and 3rd instar black scale but it parasitized a significantly higher percentage of the 3rd instars (ANOVA: $F = 6.2$; $df = 1, 16$;

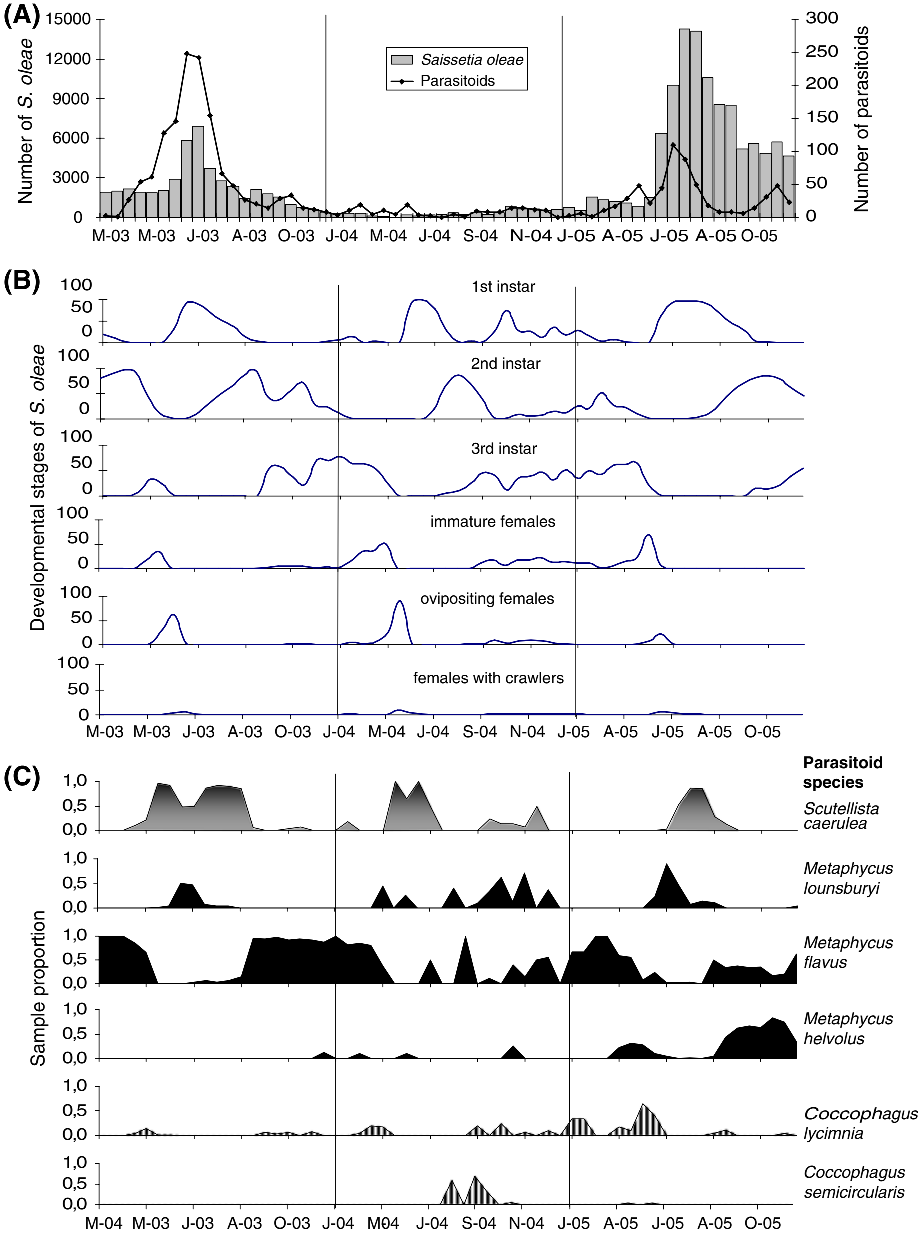

Fig. 1 *Saissetia oleae* phenology and the relative abundance of its main parasitoids on citrus in eastern Spain from March 2003 to December 2005. (**A**) Seasonal abundance of *Saissetia oleae* and total number of parasitoids recovered in the ''emerging cages''. (**B**) Relative abundance of *S. oleae* developmental stages. (**C**) Relative abundance of the main parasitoids recovered in the ''emerging cages''

P = 0.0186). Parasitism rates by this parasitoid were similar in citrus and olive groves (ANOVA: F = 0.98; df = 1, 17; P = 0.3311) and in different years (ANOVA: F = 0.49; df = 2, 13; P = 0.6181).

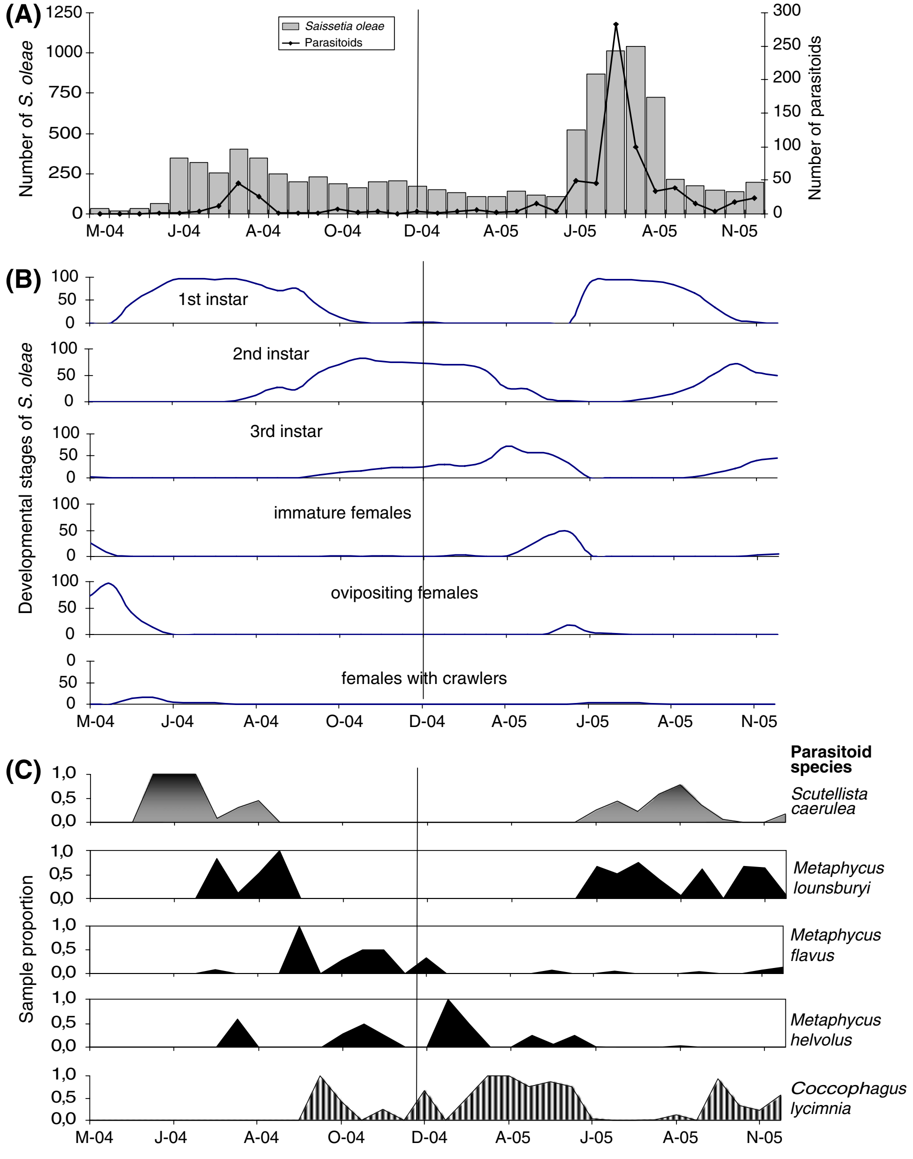

Fig. 2 *Saissetia oleae* phenology and the relative abundance of its main parasitoids on olives in eastern Spain from May 2004 to December 2005. (**A**) Seasonal abundance of *Saissetia oleae* and total number of parasitoids recovered in the ''emerging cages''. (**B**) Relative abundance of *S. oleae* developmental stages. (**C**) Relative abundance of the main parasitoids recovered in the ''emerging cages''

As expected, *S. caerulea* larvae were always found developing as egg predators. On citrus, its larvae and pupae were found beneath 35.4 ± 7.5% of the female black scale with eggs, or with eggs and crawlers, whereas on olives its larvae or pupae were found beneath 22.4 ± 3.5% of the females with eggs, or with eggs and crawlers. These represent the highest percentages of parasitism encountered in this study. Parasitism rates were similar

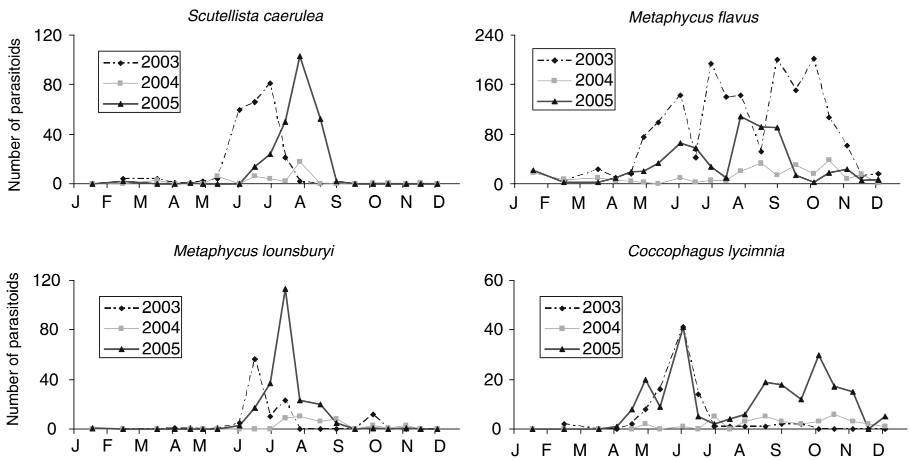

Fig. 3 Seasonal abundance of the main parasitoids of *Saissetia oleae* collected with a suction engine-powered device on citrus and olive groves in eastern Spain from February 2003 to December 2005

between crops (ANOVA: $F = 1.42$; $df = 1, 7$; $P = 0.2559$) and years (ANOVA: $F = 2.69$; $df = 2, 5$; $P = 0.1080$). The parasitism rates of *S. caerulea* reached high values (>80%) at the end of the development of the black scale females (end of June-beginning of July) (Fig. 3), being lower during the maximum of black scale females (May-beginning of June). Similarly, the incidence of *Pyemotes herfsi*, a parasite of *S. caerulea* larvae, pupae and adults, increased when the pteromalid was most abundant beneath the female scale body. In late July almost 100% of *S. caerulea* were found attacked by *P. herfsi*.

The percentage of gravid females parasitized by *M. lounsburyi* was much lower than by *S. caerulea* in both citrus and olive groves (Table 2). Parasitism by *M. lounsburyi* was similar in the citrus and olive groves (ANOVA: $F = 0.01$; $df = 1, 7$; $P = 0.9632$) and in both years (ANOVA: $F = 0.05$; $df = 2, 5$; $P = 0.9497$). *Metaphycus lounsburyi* rarely parasitized the 3rd instars of the black scale.

Table 2 Mean parasitism rates by the natural enemies of *Saissetia oleae*, in citrus (March 2003–December 2005) and olive groves (May 2004–December 2005) in eastern Spain

Natural enemies	Host	Citrus % ± SE (*n**)	Olives % ± SE (*n**)
Metaphycus spp.	L2	3.6 ± 1.2 (10)	0.8 ± 0.6 (4)
	L3	7.6 ± 1.6 (9)	1.3 ± 0.4 (4)
Coccophagus spp.	L2	0.1 ± 0.1 (10)	0.1 ± 0.0 (4)
	L3	0.4 ± 0.2 (10)	1.1 ± 0.7 (4)
Metaphycus lounsburyi	♀ with eggs	3.0 ± 1.4 (9)	4.8 ± 3.6 (4)
Scutellista caerulea	♀ with eggs	35.4 ± 7.5 (10)	22.4 ± 3.5 (4)
*Pyemotes herfsi***	*S.caerulea*	15.7 ± 7.1 (7)	2.5 ± 1.4 (3)

* Number of groves

** Parasite of any postembrionic *Scutellista caerulea* stage present beneath the body of *Saissetia oleae*

Discussion

Six species predominate in the parasitoid complex associated with black scale on citrus and olives in eastern Spain. According to their preferred host stage, they can be divided into those associated with immature stages and those associated with the female stage. The parasitoids of immature scales were *C. lycimnia*, *C. semicircularis*, *M. flavus* and *M. helvolus*; while the female parasitoids were *M. lounsburyi* and *S. caerulea*. All of them had already been reported in eastern Spain (Limón et al. 1976; Carrero et al. 1977; Ripollés 1986; Noguera et al. 2003), but their relative abundance, geographical and crop distribution, flight period and incidence were not previously assessed.

Metaphycus flavus was the most abundant and widely distributed parasitoid of immature scales in citrus groves and, consequently, it has not been displaced by the introduced parasitoid *M. helvolus* as it happened in Crete, Greece (Argyriou and Michelakis 1975). *Metaphycus flavus* parasitized black scale mainly in spring and fall, according to data obtained from emergence cages, but it was present all along the year in the groves, even when its black scale preferred stages were not present (Fig. 3). During that time, *M. flavus* might have emerged from alternate hosts present in Spanish citrus groves, as brown soft scale *Coccus hesperidum* L. (Hemiptera: Coccidae) (Llorens 1984). The availability of using alternate host species could explain, at least in part, the superiority of *M. flavus* over *M. helvolus* observed in our study, because *M. helvolus* suffers high encapsulation rates when developing in brown soft scale (Blumberg 1977). The encapsulation rates of *M. helvolus* when developing in *C. hesperidum* decrease at low temperatures (Blumberg and DeBach 1981), and in our observations this parasitoid was found at high levels just in the most interior and, consequently, the most continental citrus grove sampled, being scarce or absent in other nine groves.

On olives, where immature black scales appeared poorly parasitized, *C. lycimnia* predominate in the complex of parasitoids of immature scales. *Coccophagus lycimnia* is a facultative autoparasitoid (Walter 1983), with females acting as primary endoparasitoids and males as hyperparasitoids of females of other parasitoid species. In this study, *C. lycimnia* reached the highest population levels coinciding with the maximum of *M. flavus* in May–June. This result suggests that *C. lycimnia* could reduce the potential of *M. flavus* and *M. helvolus* to suppress soft scales in spring, as observed by Bernal et al. (2001). More studies are needed to clarify the role of *C. lycimnia* in the parasitoid complex of black scale.

The parasitoids of black scale females, *S. caerulea* and *M. lounsburyi*, play apparently a significant role in the biological control of black scale because they were encountered abundantly in almost all citrus and olive groves. *Scutellista caerulea* presented the highest parasitism rates in both crops, reaching almost 80% at the end of the scale development in 2003 and 2005 (Fig. 4). However, an important part of the eggs laid by black scale females usually escape predation by *S. caerulea* larvae (Mendel et al. 1984). We found *S. caerulea* beneath scale's body when most of the eggs had already hatched and crawlers had gone away. Thus, the real impact of *S. caerulea* on black scale populations is lower than our parasitism rates suggest. Although *Pyemotes herfsi* had not been previously cited in Spain as a parasite of *S. caerulea*, we have found that it is a common natural enemy regulating the pteromalid populations and, consequently, decreasing the efficacy of *S. caerulea* as a biological control agent. The parasitism levels reached by *P. herfsi* might be overestimated in our study, because *S. caerulea* parasitized by *P. herfsi* remain for longer beneath black scale body than the unparasitized, which emerge and migrate.

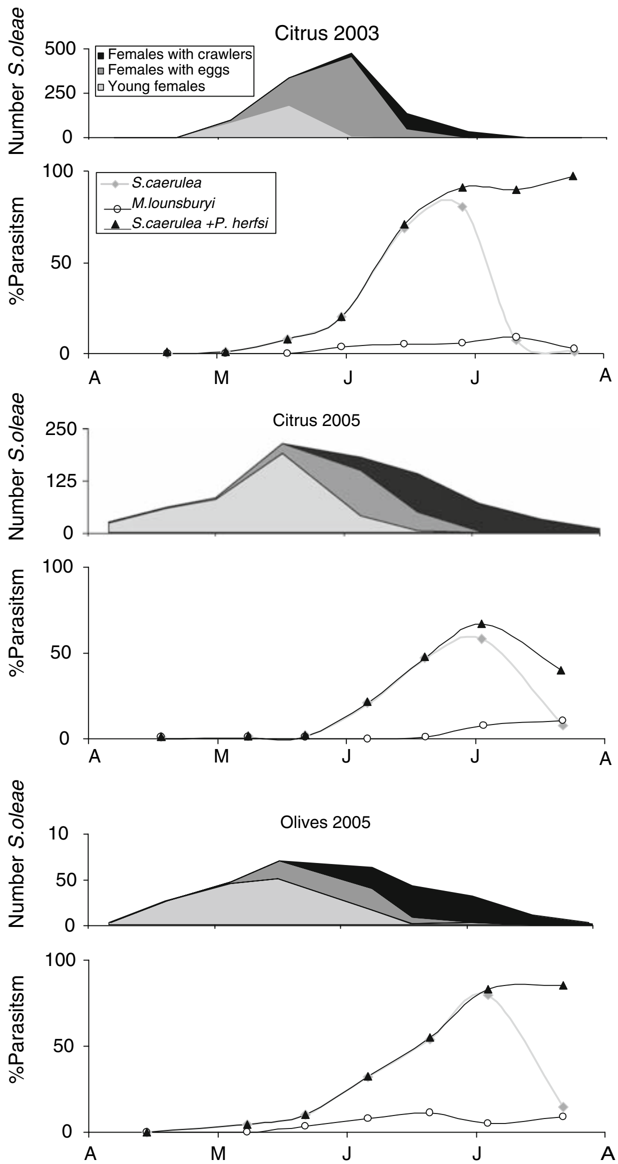

Fig. 4 Changes in parasitism rates by *Metaphycus lounsburyi* and *Scutellista caerulea* (either alone or with its parasite *Pyemotes herfsi*) related with the phenology of their host, *Saissetia oleae*. Each crop and year includes data collected from four different groves

Metaphycus lounsburyi appeared as one of the most abundant parasitoids, like in other studies in which the emergence cages have been used as sampling method (Lampson and Morse 1992; Mendel et al. 1984). However, the incidence of *M. lounsburyi* on black scale populations may be overestimated using this method because it is a gregarious parasitoid (Barzman and Daane 2001), emerging up to 12 adults per parasitized scale (personal observations). Thus, the incidence of *M. lounsburyi* on black scale populations is considerably lower when considering its parasitism rates (3 and 4.8% on citrus and olives respectively). Further, *M. lounsburyi* might build up the populations too late to prevent scale outbreaks, because the host scale is univoltine and no alternate hosts were present. Only one flight peak of *M. lounsburyi* was observed along the year from our suction samples.

The real impact of the parasitoids on black scale populations is difficult to assess, because its populations are affected not only by biotic but also by abiotic factors, mainly the climate (Bodenheimer 1951; Panis 1977; Montiel and Santaella 1995). During spring and autumn, *M. flavus* in the coast and *M. helvolus* in the interior may be responsible, at least in part, for the second and third instar mortality observed in citrus groves. Development of black scale females appears strongly synchronized during a short summer-time period, being highly attacked by *S. caerulea* and *M. lounsburyi* when the scales have already laid the eggs and many crawlers have hatched. Consequently, the effectiveness of *S. caerulea* and *M. lounsburyi* seems to be limited as biological control agents of black scale when the scale is univoltine, unless they were augmentatively released just before the female reach the optimum stage to be parasitized.

Overall, our results show that the most abundant and widely distributed parasitoids of black scale in citrus and olive crops in eastern Spain are *S. caerulea, M. flavus* and *M. lounsburyi*. These parasitoids should be considered when determining the side-effects of pesticides on beneficials, as an important component of Integrated Pest Management strategies. We also recommend the rearing and augmentative release of *M. flavus* instead of *M. helvolus* for black scale outbreaks in citrus, because the native parasitoid appears to be better adapted and, moreover, mass-production of *M. flavus* is less costly than that of *M. helvolus* (Scheweizer et al. 2003). Finally, more studies should be carried out to determine: (i) the effectiveness of augmentative release of *M. flavus*; (ii) the scarce distribution of *M. helvolus* in eastern Spain, especially in coastal areas; and (iii) the relationship between *C. lycimnia* and the abundance of *Metaphycus* females.

Acknowledgements We wish to thank Dr. Robert F. Luck and two anonymous referees for their advices and careful review of our manuscript. We are grateful to Alejandro Alicart, Andrés Alonso, Ana Cano, Salut Cuñat, Miriam García, Francisco Girona, José Miguel Martinez, Cristina Mases, Juan Carlos Meliá, Vicente Mestre, Santiago Mompó, José Enrique Sanz, M[a] Carmen Torralba and Manuel Viciedo for assistance in locating suitable groves for sampling. We also thank to the following staff at Entomology Department of the Universidad Politecnica de Valencia for their technical and taxonomic assistance: Carmen Marzal, Paco Ferragut, Lupita Alvis, Marta Martinez, Miguel Angel Martinez, Laura Bargues and Cristina Navarro. This study was supported by Ministerio de Ciencia y Tecnología into the Project AGL2002–00725.

References

Argyriou LC, Michelakis S (1975) *Metaphycus lounsburyi* Howard (Hym. Encyrtidae), parasite nouveau de *Saissetia oleae* Bern. in Crète, Grèce. Fruits 30:251–254

Argov Y, Rössler Y (1993) Biological control of the Mediterranean black scale, *Saissetia oleae* (Hom: Coccidae) in Israel. Entomophaga 38:89–100

Barzman MS, Daane MK (2001) Host-handling behaviours in parasitoids of the black scale: a case for ant-mediated evolution. Jour Anim Ecol 70:237

Bernal JS, Luck RL, Morse JG, Drury MS (2001) Seasonal and scale size relationships between Citricola Scale (Homoptera: Coccidae) and its parasitoid complex (Hymenoptera: Chalcidoidea) on San Joaquin Valley Citrus. Biol Control 20:210–221

Blumberg D (1977) Encapsulation of parasitoid eggs in soft scales (Homoptera: Coccidae). Ecol Entomol 2:185–192

Blumberg D, DeBach P (1981) Effects of temperature and host age upon the encapsulation of *Metaphycus stanleyi* and *Metaphycus helvolus* eggs by brown soft scale *Coccus hesperidum*. Jour Invert Pathology 37:73–79

Bodenheimer F (1951) Citrus Entomology in the Middle East. Dr. W. Hunk, The Hague, Netherlands

Carrero JM (1981) Etat actuel de la lutte biologique contre les cochenilles des agrumes a Valence. IOBC/WPRS Bulletin 42:25–31

Carrero JM, Limon F, Panis A (1977) Note biologique sur quelques insectes entomophages vivant sur olivier et sur agrumes en Espagne. Fruits 32:548–551

Daane KM, Barzman MS, Caltagirone LE (1991) Augmentative release of *Metaphycus helvolus* for control of black scale, *Saissetia oleae*, in olives. KAC Plant Protection Quarterly 2:7–9

Daane KM, Barzman MS, Kennet CE, Caltagirone LE (1991) Parasitoids of black scale in California: establishment of *Prococcophagus probus* Annecke & Mynhardt and *Coccophagus rusti* Compere (Hymenoptera: Aphelinidae) in olive orchards. Pan-Pac Entomol 67:99–106

Ehler LE (1989) Observations on *Scutellista cynea* Motsch. (Hymenoptera: Pteromalidae). Pan-Pac Entomol 65:151–155

Graebner L, Moreno DS, Baritelle JL (1984) The Fillmore citrus protective district: a success story in integrated pest management. Bull Entomol Soc Amer 30:27–33

Guerrieri E, Noyes JS (2000) Revision of European species of genus *Metaphycus* Mercet (Hymenoptera: Chalcidoidea: Encyrtidae), parasitoids of scale insects (Homoptera: Coccoidea). Syst Entomol 25:147–222

Kennett CE (1986) A survey of the parasitoid complex attacking black scale, *Saissetia oleae* (Olivier) in central and northern California (Hymenoptera: Chalcidoidea; Homoptera: Coccidae). Pan-Pac Entomol 62:363–369

Lampson LJ, Morse JG (1992) A survey of Black Scale, *Saissetia oleae* (Homoptera: Coccidae) Parasitoids (Hym.: Chalcidoidea) in Southern California. Entomophaga 37:373–390

Lampson LJ, Morse JG, Luck RF (1996) Host selection, sex allocation, and host feeding by *Metaphycus helvolus* (Hymenoptera: Encyrtidae) on *Saissetia oleae* (Homoptera: Coccidae) and its effect on parasitoid size, sex and quality. Environ Entomol 25:283–294

Limón F, Meliá A, Blasco J, Moner P (1976) Estudio de la distribución, nivel de ataque, parásitos y predadores de las cochinillas lecaninas (*Saissetia oleae* Bern y *Ceroplastes sinensis* Del Guercio) en los cítricos de la provincia de Castellón. Bol Serv Plagas 2:263–276

Llorens JM (1984) Las cochinillas de los agrios. Consellería de Agricultura, Pesca y Alimentación. Valencia, España

Malipatil MB, Dunn KL, Smith D (2000) An illustrated guide to the parasitic wasps associated with citrus scale insects and mealbugs in Australia. Knoxfield, Victoria

Meliá A, Blasco J (1981) Cochenilles nuisibles aux citrus de la région de Castellon et leurs parasites. IOBC/WPRS Bulletin 4:5–11

Mendel Z, Podoler H, Rosen D (1984) Population dynamics of the Mediterranean black scale, *Saissetia oleae* (Olivier), on the citrus in Israel. 4. The natural enemies. J Ent Soc Sth Afr 47:1–21

Montiel A, Santaella S (1995) Evolución de la población de *Saissetia oleae* Oliv. en condiciones naturales. Periodos susceptibles de control biológico Bol San Veg Plagas 21:445–455

Morillo C (1977) Morfología y biología de *Saissetia oleae* (Homoptera Coccidae). Bol R Soc Española Hist Nat (Biol) 75:87–108

Noguera V, Verdú MJ, Gómez-Cadenas A, Jacas JA (2003) Ciclo biológico, dinámica poblacional y enemigos naturales de *Saissetia oleae* Olivier (Homoptera: Coccidae), en olivares del Alto Palencia (Castellón). Bol San Veg Plagas 29:495–504

Panis A (1977) Contribución al conocimiento de la biología de la ''cochinilla negra de los agrios'' (*Saissetia oleae* Olivier). Bol San Veg Plagas 3:199–205

Passos de Carvalho J, Torres LM, Pereira JA, Bento AA (2003) A cochonilha-negra *Saissetia oleae* (Olivier, 1791) (Homoptera - Coccidae). Instituto Nacional de Investigaçao Agraria, Universidade de Tras-os-Montes e alto Douro, Escola Superior Agraria de Bragança

Pereira JAC (2004) Bioecologia da cochonilha negra *Saissetia oleae* (Olivier), na oliveira, em Trás-os-Montes. PhD disertation. Universidade de Trás-os-Montes e alto Douro, Vila Real

Ripollés JL (1986) Integrated Pest Management in citrus. Symposium Parasitis, Geneva, 9–13 December

Ripollés JL (1990) Las cochinillas de los agrios. Levante Agrícola 297:37–45

Schweizer H, Morse JG, Luck RF (2003) Evaluation of *Metaphycus* spp. for suppression of black scale (Homoptera: Coccidae) on southern California citrus. Biol Control 32:377–386

Statgraphics (1994) Version 4.0 Plus. Statistical graphics system by Statistical Graphics Corporation. Manugistics, Rockville MD

Walter GH (1983) Divergent male ontogenies in Aphelinidae (Hymenoptera: Chalcidoidea): a simplified classification and a suggested evolutionary sequence. Biol Jour Linnean Soc 19:63–82
Waterhouse DF, Sands DPA (2001) Classical biological control of arthropods in Australia. CSIRO Entomology, Camberra