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Displacement of Aphytis chrysomphali by Aphytis melinus, parasitoids of the California red scale, in the Iberian Peninsula

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Abstract

Parasitoids are the main natural enemies of the California red scale, Aonidiella aurantii (Maskell) and on occasion can regulate their populations. To increase their effectiveness, inoculative or augmentative releases of parasitoids are promoted. Previous to the implementation of any release strategy an important and necessary step is to acquire knowledge on the parasitoid fauna associated with this key phytophagous pest. Parasitoids were surveyed and quantified in Spanish citrus orchards between 2005 and 2009. Aphytis melinus DeBach (87.1%) resulted as the dominant species, followed by Aphytis chrysomphali (Mercet) (15.9%), Encarsia perniciosi (Tower) (2.4%) and Aphycus hederaceus (Westwood) (0.004%). Overall, higher levels of parasitism were recorded in fruit than in twigs. Scales in fruit were parasitized at similar levels by the different parasitoid species whereas E. perniciosi was more active in twigs. Data eventually reveal the recent displacement of A. chrysomphali by A. melinus. The implications of these results on the biological control of A. aurantii are discussed and this information will be useful in the decision of IPM strategies for this pest.

Additional key words: biological control; Aonidiella aurantii; climatic conditions; Encarsia perniciosi.

Introduction

Hymenopteran parasitoids of the genus Aphelinidae are the main natural enemies of armoured scale insects, particularly the ectoparasitoids of the genus Aphytis (Rosen, 1994) and the endoparasitoids of the genus Encarsia (Viggiani, 1990). Several species of these genera have been widely used in the regulation of California red scale (CRS), Aonidiella aurantii (Maskell) (Hemiptera: Diaspididae) populations (Viggiani, 1990; Rosen, 1994). This phytophagous pest, CRS, can cause the commercial depreciation of fruit and due to its unsatisfactory control is considered key pest in almost all citrus-growing areas worldwide (Jacas & Urbaneja, 2010).

Natural occurring CRS parasitoid guilds are insufficient to keep pest populations under economic injury levels (Viggiani, 1994). Hence, to increase the benefits provided by these natural enemies, biological control (BC) programs, based on inoculative or augmentative releases of CRS parasitoids as well as conservation measures addressed to maintain naturally occurring parasitoid guilds, are being promoted (Jacas & Urbaneja, 2010; Tena et al., 2013b; Vanaclocha et al., 2013a; Urbaneja et al., 2014). Previous to the implementation of any of these measures an important and necessary step is to acquire knowledge on the parasitoid fauna associated with this phytophage, such as guild composition, ecology and activity in the areas where they are going to be implemented. Previous studies have been conducted on A. aurantii parasitoids in Spanish citrus orchards (Rodrigo et al., 1996; Pina et al., 2003; Sorribas et al., 2008). Unfortunately, all of them were performed in the same citrus growing area, the Valencia region. In these studies, the parasitoids associated with A. aurantii were the ectoparasitoids Aphytis chrysomphali (Mercet), Aphytis melinus De-Bach, Aphytis lingnanensis Compere, Aphytis hispanicus (Mercet) and the endoparasitoid Encarsia perniciosi Tower. Aphytis chrysomphali and A. hispanicus are considered native species in Spain. Aphytis chrysomphali was found to be the prevalent parasitoid in earlier studies (Rodrigo et al., 1996; Pina et al.,

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2003), but at present, *A. melinus* has displaced the native one becoming the most abundant species associated with CRS (Vanaclocha *et al.*, 2009; Sorribas *et al.*, 2010; Tena *et al.*, 2013a). *Aphytis melinus* was first introduced in the Valencia region in 1976 (Meliá & Blasco, 1980). *Aphytis lingnanensis* and *E. perniciosi* were also introduced in this region, but their establishment was not successful (Rodrigo *et al.*, 1996; Pina *et al.*, 2003; Sorribas *et al.*, 2010).

Parasitoids of CRS are usually found as species guilds that may vary in their composition at different sites and different substrates of the plant due to different environmental condition requirements as well as host stage preferences (Yu et al., 1990; Rodrigo et al., 1996). For their ecological success, parasitoids must adapt to a range of environmental conditions similar to the ones required by their host. However, when two or more species compete in the same ecological niche, small variations on any environmental variable may change the dominance hierarchy within the guild (Chapin et al., 2000; Hance et al., 2007). As a result, a reduction in the relative abundance of some species or even their extinction could result (Hance et al., 2007). The effect of environmental variables on parasitoid guilds can be followed through geographical or temporal studies. Areas under different climatic conditions may present different guild compositions. Temporal series in which these variables change may explain the ecological succession from a past to a present guild. Thus, climate change is recognised as an important factor affecting the distribution of a wide range of organisms (Pimm, 2001; Parmesan, 2006; Hance et al., 2007). Meta-analysis estimates an average displacement per decade of northern and altitudinal species distribution boundaries of 6.1 km and 6.1 m northward and upward respectively (Parmesan & Yohe, 2003). In addition to the more predictable effects of abiotic factors on single species, species idiosyncratic phenological responses to changes in environmental variables are expected to trigger a cascade of changes on species interactions at all ecological levels with unpredictable consequences (Davis et al., 1998; Chapin et al., 2000; Van Nouhuys & Lei, 2004). In the present work, a comprehensive study describing CRS parasitoid assemblages in the main Spanish citrus growing areas has been conducted. The species composition and their relative abundance have been determined for each assemblage and correlated to the CRS parasitism ratios found. Results obtained were compared with those of a previous study done on a more local scale

(Sorribas *et al.*, 2010). The information compiled provides a better understanding of the mechanisms determining the CRS parasitoid hierarchy under most of the climatic conditions found on the Iberian Peninsula, and help to predict potential changes in the hierarchy with regard to a global warming scenario. The information herein provided may be used in the design of Integrated Pest Management (IPM) strategies adapted to more local conditions.

Material and methods

Study sites and sampling methods

Aonidiella aurantii-infested fruit and twigs of citrus trees (sweet orange and clementine mandarin) were sampled between 2005 and 2009, from August to November, in 21 orchards located throughout the main citrus-growing areas of Spain (Table 1 and Fig. 1). During this period, twig and fruit samples were randomly collected from each plot at least once per month and taken to the laboratory, where leaves were removed. The first generation of the scale (May-June) was not sampled since fruit was not available for parasitism comparison with twigs. Twigs of approximately 20 cm were examined under a binocular microscope. From each sample, both twig and fruit, irrespective of the number of scales present, up to ten individuals were checked to standardise the sampling. One hundred susceptible scales of A. aurantii per sampling site and date for parasitism (second nymphal stage, young females and prepupa and pupa males), were checked. Parasitoid species were determined according to their pupa pigmentation pattern and, when necessary, pupae were transferred into gelatine capsules for further development. Adults emerged from pupae were determined to a species level.

Data analysis

Active parasitism rate, given as an average \pm standard error, was calculated as the number of live parasitized CRS over the total number of scales susceptible to being parasitized. According to the parasitoids obtained, percentages of active parasitism were classified in four different assemblage combinations found in the sampled orchards: 1) orchards where *A. melinus* was predominant (> 95% of the parasitoids found)

Table 1. Relative abundance of parasitoid species observed in 21 citrus groves for the sampled period 2005-2009. The different citrus groves sampled were located through the Spain citrus area. AL: Almería; CA: Cádiz; CS: Castellón; IB: Islas Baleares; HU: Huelva; MA: Málaga; MU: Murcia; VL: Valencia

Code	Location	Citrus species and cultivar	Year	Parasitoids (n)	A. melinus (%)	A. chyrsomphali (%)	E. perniciosi (%)	A. hederaceus (%)
1	Almenara (CS)	Citrus clementina Hort. Ex Tan. cv. Oronules	2007	1,884	81.2	18.8		
2	Benifairó de la Vall- dingna (VL)	Citrus sinensis (L.) Osb. cv. Valencia Late	2008 2009	426 270	96.9 100.0	3.1		
3	Llíria (VL)	Citrus clementina Hort. ex Tan. cv. Clemenules	2008 2009	199 50	87.4 100.0	12.6		
4	Bétera (VL)	Citrus sinensis (L.) Osb. cv. Navelina	2005 2007 2008 2009	171 364 131 29	64.9 36.5 96.2 72.4	35.1 63.5 3.8 27.6		
5	Moncada (VL)	Citrus sinensis (L.) Osb. cv. Lane Late	2005	118	32.2	67.8		
6	La Pobla de Vallbona (VL)	Citrus clementina Hort. ex Tan. cv. Esbal	2007 2008 2009	1,192 300 616	93.7 98.7 99.7	6.3 1.3 0.3		
7	Inca (PM)	Citrus sinensis (L.) Osb. cv. Navelina	2008	34	73.5	26.5		
8	Bellreguard (VL)	Citrus sinensis (L.) Osb. cv. Valencia Late	2005	229	41.9	58.1		
9	Castellonet (VL)	Citrus clementina Hort. ex Tan. cv. Orogrande	2005	177	39.5	60.5		
10	L'Alcúdia de Crespins (VL)	Citrus sinensis (L.) Osb. cv. Valencia Late	2009	26	84.6	15.4		
11	El Mirador (MU)	Citrus sinensis (L.) Osb. cv. Lane Late	2006	89	83.1	16.9		
12	Mondujar (AL)	Citrus sinensis (L.) Osb. cv. Castellana	2005 2006 2007	7 41 23	100.0 95.1 91.3		4.9 8.7	
13	Mojonera (AL)	Citrus sinensis (L.) Osb. cv. Valencia Late	2005 2006 2007	18 58 23	72.2 98.3 100.0		27.8 1.7	
14	Churriana (MA)	Citrus sinensis (L.) Osb. cv. Valencia Late	2005 2006 2007	6 11 31	100.0 100.0 90.3		9.7	
15	Alhaurín de la Torre (MA)	Citrus sinensis (L.) Osb. cv. Valencia Late	2005 2006 2007	11 65 21	45.5 92.3 57.1		54.5 7.7 42.9	
16	Pizarra (MA)	Citrus sinensis (L.) Osb. cv. Valencia Late	2005 2006 2007	14 44 13	85.7 95.5 76.9		14.3 4.5 23.1	
17	Casarabo- nela (MA)	Citrus sinensis (L.) Osb. cv. Valencia Late	2005 2006 2007	7 30 10	100.0 100.0 40.0		60.0	

Table 1 (cont.). Relative abundance of parasitoid species observed in 21 citrus groves for the sampled period 2005-2009. The different citrus groves sampled were located through the Spain citrus area, AL: Almería; CA: Cádiz; CS: Castellón; IB: Islas Baleares; HU: Huelva; MA: Málaga; MU: Murcia; VL: Valencia

Code	Location	Citrus species and cultivar	Year	Parasitoids (n)	A. melinus (%)	A. chyrsomphali (%)	E. perniciosi (%)	A. hederaceus (%)
18	San Enrique (CA)	Citrus sinensis (L.) Osb. cv. Newhall	2005	21	33.3		52.4	14.3
			2006	102	31.4		68.6	
			2007	48	43.8		56.3	
19	Moguer	Citrus clementina Hort.	2005	6	100.0			
	(HU)	ex Tan. cv. Clemenpons	2006	11	100.0			
		•	2007	11	72.7		27.3	
20	Cartaya	Citrus sinensis (L.) Osb.	2005	17	100.0			
	(HU)	cv. Valencia Late	2006	18	88.9		11.1	
			2007	14	92.9		7.1	
21	Nerva (HU)	Citrus sinensis (L.) Osb.	2005	39	97.4		2.6	
	` ,	cv. Valencia Late	2006	45	100.0			
			2007	22	68.2		31.8	
	Sub-total		2005	841	51.5	45.2	3.0	0.4
			2006	514	81.1	2.9	15.9	_
			2007	3,656	80.3	18.1	1.7	_
			2008	1,090	94.8	5.2	_	_
			2009	991	98.6	1.4	_	_
	Total			7,092	81.7	15.9	2.4 (6.91)	0.0004

¹ % in localities with presence of *Encarsia perniciosi*.

(Am); 2) orchards where E. perniciosi was present (<95% of the parasitoids were A. melinus and 5-100% were E. perniciosi) (Am + Ep); 3) coexistence of A. melinus and A. chrysomphali, but predominance of A. melinus (51-95% A. melinus and 5-49% A. chrysomphali) (Am + Ac) and 4) coexistence of A. melinus and A. chrysomphali but predominance of A. chrysomphali ($\le 50\%$ A. melinus and $\ge 50\%$ A. chrysomphali) (Ac + Am). Parasitism percentages were subjected to a two-way analysis of variance (factors: substrate and parasitoid assemblage) and the Bonferroni-test was used for mean separation at p < 0.05. Data underwent angular transformation before the analysis to meet the assumption of normality and homogeneity of variance.

Results

Four species of parasitoids were found associated with A. aurantii: Aphytis melinus, A. chrysomphali, E. perniciosi and Aphycus hederaceus (Westwood) (Encyrtidae).

Aphytis melinus was the most widespread and the predominant species in the majority of the study locations accounting for 81.7% of A. aurantii parasitism.

Aphytis chrysomphali and E. perniciosi accounted for 15.9% and 2.4% of CRS parasitism (Table 1). The incidence of A. hederaceus was negligible, with only three specimens found in the south of Spain in 2005. Aphytis melinus was present throughout all the study area, while A. chrysomphali and E. perniciosi had more restricted and rather allopatric distributions. Aphytis chrysomphali spread in the eastern citrus areas situated at around 37° 50' N latitude (locations 1 to 11 in Table 1 and Fig. 1); conversely, E. perniciosi was distributed south of that latitude, in the Andalusia region (locations 12 to 21 in Table 1 and Fig. 1). In the sympatric area encompassing A. melinus and A. chrysomphali (Valencia region and Balearic Islands), the incidence of the latter dropped from 55.5% in 2005 to 8.7% in 2009. In its distribution area, E. perniciosi reached on average, a relative abundance of 6.9%. No association between parasitoid relative abundances and citrus species was observed (Table 1).

Parasitism of scales was significantly higher in fruit than in twigs (fruit: $21.2 \pm 8.3\%$; twigs: $15.7 \pm 8.7\%$) ($F_{1,140} = 13.2$; p = 0.0004) (Fig. 2). Different parasitism levels were recorded for the different species assemblages in twigs, and were statistically higher where E. perniciosi was present ($F_{3,77} = 6.5$; p = 0.0006) (Fig. 2a).

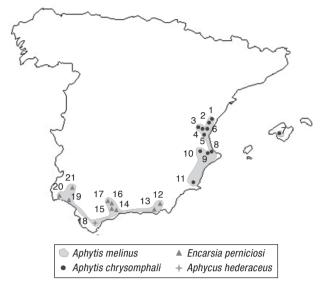
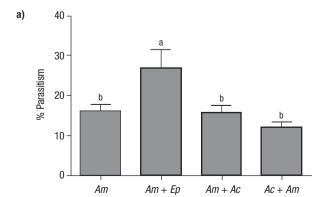


Figure 1. Sampling locations and distribution of species of parasitoids founded associated to *Aonidiella aurantii* along the main citrus growing areas in Spain. Each number indicates the location. 1, Almenara (CS); 2, Benifairó de la Valldigna (VL); 3, Llíria (VL); 4, Bétera (VL); 5, Moncada (VL); 6, La Pobla de Vallbona (VL); 7, Inca (PM); 8, Bellreguard (VL); 9, Castellonet (VL); 10, L'Alcúdia de Crespins (VL); 11, El Mirador (MU); 12, Mondujar (AL); 13, Mojonera (AL); 14, Churriana (MA); 15, Alhaurín de la Torre (MA); 16, Pizarra (MA); 17, Casarabonela (MA); 18, San Enrique (CA); 19, Moguer (HU); 20, Cartaya (HU); 21, Nerva (HU). AL: Almería; CA: Cádiz; CS: Castellón; PM: Palma de Mallorca; HU: Huelva; MA: Málaga; MU: Murcia; VL: Valencia.

However, no significant differences between assemblages were found in fruit ($F_{3,62} = 1.0$; p = 0.39) (Fig. 2b). *Aphytis melinus* and *A. chrysomphali* were found in similar percentages in both twigs and fruit whereas 96% of *E. perniciosi* parasitoids were found in twigs.



Discussion

Aphytis melinus and A. chrysomphali are widespread biological agents of CRS worldwide (Dahms & Smith, 1994; Viggiani, 1994; Noves, 2003). In Spain A. melinus was first released in the Valencia region in 1976 as part of a BC program; later it was also released in Andalusia in 1987 (Pina & Verdú, 2007a). According to our results, A. melinus has been established very successfully in all citrus-growing areas of Spain. Aphytis chrysomphali is considered an indigenous species. It was first found in Spain on Chrysomphalus dictvospermi (Morgan) (Hemiptera: Diaspididae) in Andalusia, the Valencia region and the Balearic Islands by García-Mercet (1912). Later, this ectoparasitoid was found on both C. dictyospermi and CRS in Andalusia (García-Mercet, 1930) and the Valencia region (Limón et al., 1976; Rodrigo et al., 1996). The results here obtained demonstrate that A. melinus has become the dominant CRS parasitoid species in Spanish citrus and seems to be progressively displacing A. chrysomphali, at least in the hottest and driest locations as suggested by Sorribas et al. (2010) in Valencian citrus. This displacement has also been observed in other Mediterranean regions (Siscaro et al., 1999).

The endoparasitoid *E. perniciosi* is a common CRS parasitoid widely distributed around the world (Furness *et al.*, 1983; Heraty *et al.*, 2007). To our knowledge, this species has only been previously recorded in eastern Spain (Pina & Verdú, 2007b; Sorribas *et al.*, 2008) where some inoculative releases were carried out (Pina & Verdú, 2007b). *Aphicus hederaceus* is widespread across Europe (Noyes, 2003), and has already been recorded in Spain (García-Mercet, 1921). In

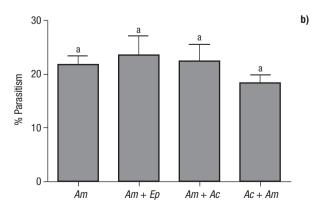


Figure 2. Parasitism percentages in twigs (a) and in fruit (b) for the different parasitoid assemblages. Am : > 95% Aphytis melinus; Am + Ep : < 95% Aphytis melinus and 5-100% Encarsia perniciosi; Am + Ac : 51-95% Aphytis melinus and 5-49% Aphytis chrysomphali; $Ac + Am : \le 50\%$ Aphytis melinus and $\ge 50\%$ Aphytis chrysomphali.

the past, authors considered *A. hederaceus* as a parasitoid exclusive of soft scales, but García-Mercet (1921) revealed that this species also parasitizes the armoured scale *Aspidiotus hederae* (Vallot).

Aphytis chrysomphali was the dominant species parasitizing CRS in Spain before A. melinus releases started. In Andalusia, there is no data about the time in which the displacement by A. melinus occurred, but it probably happened after the releases accomplished in 1987 (Pina & Verdú, 2007a). In our study, no A. chrysomphali specimens were found in that citrus growing area during the three seasons when samples were collected. In relation to the Valencia region, in the 90's, Rodrigo et al. (1996) found that A. chrysomphali accounted for 98% of CRS parasitisim. At the end of that decade the relative abundance of this parasitoid declined to 77.7% (Pina et al., 2003), and later, it dropped to 50% (Sorribas et al., 2008). The data presented in this work confirms this tendency showing a decline from 55.5% in 2005 to 8.7% in 2009. A similar parasitoid displacement of A. chrysomphali by A. lingnanensis (DeBach & Sisojevic, 1960) and later of A. lingnanensis by A. melinus (DeBach et al., 1969) occurred in California; in Greece, a displacement of A. chrysomphali by A. melinus also took place (De-Bach & Argyriou, 1967).

The very recent displacement of A. chrysomphali by A. melinus in Spain caused either by climatic conditions, persistent use of pesticides, competition with A. melinus or possibly a combination of all these factors, implies a change in the performance of A. chrysomphali in controlling CRS. The average high temperatures of the Spanish Mediterranean climate is favourable to the presence of A. melinus (Samways, 1985) while, A. chrysomphali and E. perniciosi are better adapted to more moderate climatic conditions (Abdelrahman, 1974; Pina & Verdú, 2007b). Indeed, the rapid displacement which occurred in Andalusia was probably partly due to the significantly warmer temperatures of this area in comparison to the east coast (Fig. 1). Sorribas et al. (2010) observed this phenomenon on a smaller scale in the Valencia region, where A. melinus was super-dominant in those assemblages found in warmer areas whereas A. chrysomphali was able to coexist via temporal niche partitioning in those areas with colder springs and winters.

As yet, we cannot explain why, according to the current data, *A. chrysomphali* and *E. perniciosi* spread in allopatric ranges, the former in northern regions and the latter in southern ones.

The higher percentages of parasitism in fruit (21.2%) than in twigs (15.7%) found in this work are similar to those found in other related studies (Atkinson, 1977; Asplanato & García-Marí, 2002). We have shown that A. melinus and E. perniciosi are sympatric in groves of southern Spain, and that the latter shows a preference for twigs. This coincides with the situation on the California coasts described by Yu et al. (1990), who stated that this resource partitioning could explain the coexistence of A. melinus and E. perniciosi. In this work CRS sampling was not conducted during the first CRS generation, May-June, when fruit is not present yet and therefore, A. melinus and E. perniciosi are forced to compete for the same resource. Future studies which focus on this period of year will help to discern the role played by E. perniciosi on CRS suppression and the importance of competition between these two parasitoid species in warmer areas.

Several authors established that parasitism rates around 20% could control CRS (DeBach et al., 1969). This percentage was reached in some of the orchards sampled in our study; however, unfortunately, parasitoids were unable to keep CRS populations under economic injury levels. The insufficient natural BC provided by CRS natural enemies, and especially by parasitoids, places CRS in the category of key pest in Spain (Jacas & Urbaneja, 2010; Urbaneja et al., 2014). In recent years, emphasis has been placed on implementing environmentally safe measures to control A. aurantii in Spain, rather than using traditional chemical insecticides (Vacas et al., 2012; Vanaclocha et al., 2012, 2013b). For this purpose, augmentative releases of CRS parasitoids should be one of the alternatives considered for controlling this pest. According to our results, the recommended species to use in such augmentative programs is A. melinus because it is clearly well-adapted to Spanish citrus growing conditions. Furthermore, because *E. perniciosi* plays a role in controlling CRS in twigs, releases in those areas where it can become established should be continued.

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