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Pekas, A.; Navarro-Llopis, V.; Garcia Marí, F.; Primo Millo, J.; Vacas González, S. (2015). Effect of the California red scale *Aonidiella aurantii* sex pheromone on the natural parasitism by *Aphytis* spp. in Mediterranean citrus. *Biological Control*. 90:61-66.  
doi:10.1016/j.biocontrol.2015.05.016.



The final publication is available at

<http://dx.doi.org/10.1016/j.biocontrol.2015.05.016>

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Additional Information

1 **Title:** Effect of the California red scale *Aonidiella aurantii* sex pheromone on  
2 the natural parasitism by *Aphytis* spp. in Mediterranean citrus

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15

16 **Abstract**

17 Mating disruption has proved successful against California red scale (CRS), *Aonidiella*  
18 *aurantii* Maskell (Hemiptera: Diaspididae) in Mediterranean citrus. Although mating  
19 disruption does not affect negatively the parasitism by *Aphytis melinus* DeBach  
20 (Hymenoptera: Aphelinidae), a CRS parasitoid introduced to the Mediterranean, there is no  
21 information regarding its potential effect on the native *Aphytis* species. In the present  
22 study, the effect of CRS mating disruption on the field parasitism inflicted by *Aphytis* spp.  
23 has been assessed and compared to a mineral oil and a control treatment. In order to  
24 confirm the effectiveness of the mating disruption we also evaluated its effect on the  
25 captures of the CRS males and on fruit infestation. Moreover, the potential role of the CRS  
26 sex pheromone as kairomone for the *Aphytis* species was also evaluated by comparing  
27 captures of parasitoids on sticky traps with or without pheromone. Significantly lower CRS  
28 male captures and fruit damage were registered in the mating disruption respect to the  
29 control or oil treatments indicating that mating disruption was effective. In September,  
30 when compared to the control, parasitism by *Aphytis* spp. was significantly lower in the  
31 mating disruption and mineral oil treatments and crucially no *A. chrysomphali* were  
32 registered in the mating disruption treatment. Finally, while the captures of both *A. melinus*  
33 and *A. lepidosaphes* (Mercet) were not significantly different between traps with or  
34 without pheromone, *A. chrysomphali* Marcet captures were significantly higher in traps  
35 baited with CRS pheromone. These results suggest a possible kairomonal effect of the CRS  
36 pheromone on *A. chrysomphali*.

37

38 Keywords: *Aphytis melinus*, *Aphytis lepidoshapes*, *Aphytis chrysomphali*, kairomone,  
39 mating disruption, host recognition

40

## 41 **1. Introduction**

42 California red scale (CRS), *Aonidiella aurantii* Maskell) (Hemiptera: Diaspididae), is a  
43 major pest of citrus worldwide. Although only heavy infestations are able to kill the trees,  
44 the sole presence of scales on fruits considerably reduces their market value causing huge  
45 economic losses (Jacas et al. 2010). Currently, integrated pest management including  
46 applications of pesticides, mineral oil sprays, biological control and methods based on  
47 semiochemicals is employed to control CRS infestations in citrus orchards. The CRS sex  
48 pheromone was used exclusively for monitoring purposes, however, recently mating  
49 disruption (MD) against this pest was employed successfully in Mediterranean citrus  
50 proven at least as effective as conventional mineral oil sprays (Vacas et al., 2009, 2010). In  
51 fact, CRS presents the first case of successful mating disruption for a diaspidid scale insect.  
52 The use of mating disruption has been found not only to be effective against CRS but was  
53 also innocuous for the parasitism caused by *Aphytis melinus* DeBach a CRS parasitoid  
54 introduced to the Mediterranean (Vacas et al., 2011, Vanaclocha et al., 2012).  
55 Nevertheless, there is no information regarding the potential impact of mating disruption  
56 on the CRS parasitism inflicted by the *Aphytis* species native to the Mediterranean.

57         Alternative pest management methods have to ensure sustainability from both the  
58 socioeconomic and the environmental perspectives, which involves the conservation of  
59 beneficial insects. In general, parasitoids exploit a range of stimuli for host location which  
60 can derive from the microhabitat or the plant, from the presence of the host (i.e. frass,  
61 honeydew) or from the host itself (Godfray, 1994). In the latter case, sex or aggregation  
62 pheromones, which are deliberately emitted by the host for its own purposes, can be  
63 exploited by the parasitoids. For example, sex pheromones have been described to serve as  
64 chemical cues for host location for egg parasitoids such as *Trichogramma* spp., *Telenomus*  
65 spp. (Powell 1998) and aphid parasitoids (Powell, 1998; Birkett and Pickett, 2003; Powell

66 and Pickett, 2003). In the concrete case of entomophagous arthropods of scale insects, the  
67 predator *Elatophilus hebraicus* Pericart (Hemiptera: Anthocoridae) is reported to be  
68 attracted to the racemic mixture of the female sex pheromone of *Matsucoccus josephi*  
69 Bodenheimer et Harpaz (Mendel et al., 1995). Similarly, the sex pheromone of  
70 *Planococcus ficus* (Signoret) acts as a kairomone for the parasitoid *Anagyrus pseudococci*  
71 (Girault) (Hymenoptera: Encyrtidae) (Franco et al., 2008). There is also evidence that  
72 aphelinid parasitoids are attracted to the sex pheromone of their scale hosts. *Encarsia*  
73 *perniciosa* Tower (Hymenoptera: Aphelinidae) significantly responds to synthetic  
74 pheromone and virgin females of the San Jose scale, *Diaspidiotus perniciosus* (Comstock)  
75 (Rice and Jones, 1982, McClain et al., 1990, Bayoumy et al., 2011).

76         The principal natural enemies of CRS in the Mediterranean basin are the  
77 ectoparasitoids *Aphytis chrysomphali* Mercet (Hymenoptera: Aphelinidae) and *A. melinus*  
78 (Rodrigo et al., 1996, Pina, 2007, Pekas et al., 2010) and to a lesser extent some  
79 endoparasitoids and generalist predators (Vanaclocha et al., 2009, Pina, 2007). Sternlicht  
80 (1973) reported attraction of *A. melinus* and *A. coheni* DeBach to CRS female sex  
81 pheromone. This was confirmed by other studies concluding that *A. melinus* females are  
82 attracted to airborne cues from hosts, i.e. CRS virgin females (Bernal and Luck, 2007;  
83 Zappalà et al., 2012). Nevertheless, various experiments proved that the recognition and  
84 acceptance of *A. aurantii* as host by *A. melinus* is mainly based on a contact, non-volatile  
85 kairomone (Hare et al., 1993; Morgan and Hare, 1998). In laboratory experiments, Vacas  
86 et al. (2011) demonstrated that *A. melinus* mating behavior and parasitism were not  
87 affected when parasitoids were exposed inside cages to CRS pheromone concentrations  
88 even higher than in orchards where mating disruption was applied. Most crucially, Vacas et  
89 al. (2011) demonstrated the compatibility of mating disruption with augmentative releases

90 of *A. melinus* in extensive field trials, where CRS mortality caused by the released  
91 parasitoids was not affected in the orchards with mating disruption treatments.

92         Although the effect of the mating disruption treatment on the parasitism inflicted by  
93 the introduced *A. melinus* appears to be not significant, the effect on other *Aphytis* species,  
94 especially the native to the Mediterranean *A. chrysomphali*, remains unknown. Likewise,  
95 there is no information regarding the impact of the commercially available pheromone  
96 employed routinely on sticky traps for CRS monitoring purposes on *Aphytis spp.*  
97 parasitoids. Thus, in the present study we asked the following questions: i) is CRS mating  
98 disruption having an effect on the field parasitism inflicted by the *Aphytis* species native to  
99 the Mediterranean? and ii) can the CRS sex pheromone act as a kairomone for the *Aphytis*  
100 species? The first question was addressed by assessing the parasitism rate of the *Aphytis*  
101 species in citrus orchards in plots treated with mating disruption dispensers, plots receiving  
102 mineral oil sprays and untreated (control) plots. In order to test the effectiveness of the  
103 mating disruption treatment and corroborate its potential impact on the parasitoids we also  
104 evaluated its effect on the flight of the CRS males and on the fruit infestation. To answer  
105 the second question, we compared the captures of *Aphytis* species on sticky traps baited  
106 with the female CRS sex pheromone.

107

## 108 **2. Material and methods**

### 109 2.1. Mating disruption field trials

110 The trials were conducted in a 5-ha sweet orange (*Citrus sinensis* Osbeck, var. Lane late)  
111 orchard located in Denia (Alicante, Spain; UTM: X243500 Y4303900). The California red  
112 scale sex pheromone was released in the field by installing the mesoporous pheromone  
113 dispensers described by Vacas et al. (2009, 2010). The dispensers were developed by  
114 Universitat Politècnica de València and Ecología y Protección Agrícola (Valencia, Spain)

115 and are now registered and commercialized in Spain under the name Dardo<sup>®</sup> (Syngenta  
116 Agro SA, Madrid, Spain). Each dispenser consisted in a cylindrical tablet, containing 70  
117 mg of the diastereomeric mixture (3S,6R and 3S,6S) of the 3-methyl-6-isopropenyl-9-  
118 decen-1-yl acetate.

119 Mating disruption dispensers were deployed, one per tree, in three 0.5 ha plot on 25  
120 March 2009, before the first CRS males' flight, and in three 0.5 ha plots on 11 May 2009,  
121 before the second CRS males' flight. The trees were spaced 6×4 m apart (~420  
122 dispensers/ha) and dispensers were placed on the internal tree branches at a height of 1.5–  
123 2.0 m. Three 0.4 ha plots received conventional mineral oil applications which were timed  
124 for the presence of crawlers. Finally, three 0.25 ha plots were left without treatment as an  
125 untreated reference (control).

126

## 127 2.2.Mating disruption efficacy

128 The efficacy of the pheromone treatment was evaluated according to the CRS male  
129 flight disruption and the fruit infestation assessment. One commercial white sticky  
130 pheromone trap (Pherocon<sup>®</sup> V Trap; Trécé Inc., Adair, OK) was placed in each plot to  
131 compare male captures between the different control strategies every 7 or 15 days, from  
132 March to November 2009. The inhibition of male captures that occurs in pheromone-  
133 treated plots is the first indicator for male disorientation. Flight Inhibition Index (FII) was  
134 calculated according to the formula  $FII = (1-(x/y)) \times 100$ , where  $x$  is the number of males  
135 captured in MD plot, and  $y$  is the number of captures in the untreated plot. Finally, fruit  
136 infestation was evaluated on 10 November 2009, by counting the number of scales present  
137 on 40 fruits per tree (10 fruits per orientation) of the 4 central trees in each plot. The  
138 percentage of fruit with more than 5 scales was recorded as it is a common damage  
139 threshold employed for marketable fruit.

140 The pheromone release profile of the mating disruption dispensers was studied during  
141 the trial to determine the mean release rate and their life-span. Additional dispensers were  
142 aged under field conditions in a nearby area, in order to extract and quantify by gas  
143 chromatography (GC-FID) their residual pheromone content at different days of ageing.

144

### 145 2.3. Influence of mating disruption on CRS parasitism

146 Parasitism rate was evaluated on 9 September and 10 November 2009. On each sampling  
147 date, we collected 40 branches (less than 10 mm in diameter and bearing at least ten  
148 leaves), and 40 fruits (10 per orientation), infested by CRS, from at least ten different trees  
149 per treatment. Samples were transferred to the laboratory and were processed using a  
150 stereomicroscope. Parasitized CRS scales were identified by the presence of parasitoid  
151 eggs, larvae, prepupae or pupae. For every parasitized scale, parasitoid species was  
152 identified based on the pupae coloration (Rosen and DeBach, 1979). Eggs, larvae and  
153 prepupae were transferred to glass vials (3.0 by 0.8 cm) and maintained at 22–25 °C, 60–  
154 70% RH and 16:8 L:D photoperiod for development to pupa and identification.

155

### 156 2.4. Attraction of parasitoids and CRS males to pheromone baited traps

157 The trial was conducted in a nearby 3-ha mandarin (*Citrus reticulata* Blanco; var.  
158 Ortanique) orchard without mating disruption treatment. The possible kairomonal response  
159 of *Aphytis* sp. to the sex pheromone of CRS was tested by evaluating the attraction to traps  
160 baited with Pherocon<sup>®</sup> rubber monitoring lures (Trécé Inc., Adair, OK), loaded with 250  
161 µg CRS female sex pheromone. The effect of trap color on captures was also tested by  
162 including commercial white sticky Pherocon<sup>®</sup> V traps and transparent traps, made from  
163 transparent PVC sheets with Tangle-Trap<sup>™</sup> sticky coating (Biagro SL, Valencia, Spain).  
164 Thus, traps included in the trial were: (1) white with monitoring pheromone lure, (2)



165 transparent with monitoring pheromone lure, (3) white without pheromone, (4) transparent  
166 without pheromone. A fifth (5) white trap was included, baited with a mating disruption  
167 dispenser, to check for the effect of higher pheromone loads on parasitoid attraction. Three  
168 blocks with these five traps were installed on 12 August 2009. Traps were attached to tree  
169 branches at 1.5-2.0 m from the ground. Distance between traps was 20 m and blocks were  
170 located at least 50 m apart. The number of *Aphytis* sp individuals and *A. aurantii* males  
171 captured on the traps were recorded on 9 September, 8 October, 23 October and 5  
172 November 2009. On each sampling date, the position of traps was rotated within each  
173 block and sticky boards were replaced by new ones. The collected boards were transferred  
174 to the laboratory and were processed using a stereomicroscope. The *Aphytis* captured on  
175 the traps were extracted, mounted, and identified under a microscope according to Rosen  
176 and DeBach (1979).

177

## 178 2.5. Statistical analysis

179 Simple regression was used to study the evolution of the GC-FID quantified residual  
180 pheromone load (mg) versus time (days) and calculate mean emission rate for the mating  
181 disruption dispensers employed. Regarding mating disruption efficacy assessment, the  
182 number of males captured per trap and day (MTD) was transformed by  $\log(n+1)$  in order to  
183 homogenize variance and normalize the distributions before analysis of variance  
184 (ANOVA). Tukey HSD test ( $P < 0.05$ ) was performed to assess the effect of treatment on  
185 the CRS male flight activity. In the same trial, a one-way ANOVA model was employed  
186 with  $\arcsin(\sqrt{n})$  transformed data of percentage of infested fruits to compare the  
187 level of infestation among treatments (Tukey HSD test at  $P < 0.05$ ). The Statgraphics  
188 Centurion XVI (v. 16.1.11) package was used for these statistical analyses (Statpoint  
189 Technologies Inc., 2010).

190 Using generalized linear model techniques, two different models (one for each assessment  
191 date), assuming binomial error variance, were constructed to compare the rate of  
192 parasitized individuals of CRS in the different treatments. Likewise, we used generalized  
193 lineal model techniques assuming Poisson error variance to compare the number of *Aphytis*  
194 spp. parasitoids or CRS males captured per trap. Given the highly male-biased sex ratio of  
195 the captured parasitoids (see below) only the female parasitoids were considered for the  
196 analyses. For each species, we constructed different models with the number of individuals  
197 captured per trap as the dependent variable and trap type, sampling date and block and  
198 their interaction as the explanatory variables.

199 In all the models the significance of the explanatory variables was assessed by backward  
200 elimination of the non-significant terms from the model and subsequent comparison of the  
201 two models using the F test statistic. When significant effects were found the *glht* function  
202 in the *multcomp* package (Hothorn et al., 2008) was used to perform TukeyHSD tests for  
203 post-hoc pairwise comparisons. These statistical analyses were conducted with R (R  
204 Development Core Team, 2012).

205

### 206 **3. Results**

#### 207 3.1. Mating disruption efficacy

208 *Aonidiella aurantii* MD dispensers had a useful life of approximately 110 days, providing a  
209 mean release rate of approximately 402 µg/day during 15 weeks, which was consistent  
210 with the emission rates required to obtain enough pheromone concentration in the orchard  
211 to disrupt CRS male flights (Vacas et al., 2009, 2010). Indeed, the mean number of males  
212 per trap and day (MTD) captured in the monitoring traps was significantly influenced by  
213 the treatment applied in each plot ( $F = 82.17$ ;  $df = 3,168$ ;  $P < 0.0001$ ). Neither block ( $F =$   
214  $2.44$ ;  $df = 2,168$ ;  $P = 0.09$ ) nor the interaction block x treatment ( $F = 1.86$ ;  $df = 6,168$ ;  $P =$

215 0.09) were significant. Both mating disruption treatments, employed either in March or  
216 May, obtained significantly lower CRS male captures respect to control and oil plots  
217 (Table 1). MD treatments inhibited male captures by > 90%, indicating that the mating  
218 disruption environment managed to disorientate the CRS males. It is important to mention  
219 that *Aphytis* individuals were observed in the monitoring traps in all the plots.  
220 Fruit infestation was also significantly affected by the different control measures applied  
221 ( $F = 12.23$ ;  $df = 3,61$ ;  $P < 0.0001$ ). Both mating disruption treatments reduced the  
222 percentage of fruit with more than 5 scales compared to the control but MD-May achieved  
223 significantly lower infestations compared to oil treatment (Table 1).

224

### 225 3.2. Influence of mating disruption on the CRS parasitism

226 In September we registered 188 scales parasitized by *A. melinus* (80% of the total) and *A.*  
227 *chrysomphali* (20%) in all treatments. Treatment had a significant effect on the CRS  
228 parasitism ( $F = 6.26$ ,  $df = 3, 584$ ,  $P = 0.0003$ ) (Fig. 1). Compared to the control treatment,  
229 the CRS parasitism rate was significantly lower in the mineral oil ( $P < 0.001$ ) and in the  
230 mating disruption-March treatments ( $P = 0.01$ ) (Tukey test; adjusted P values with single  
231 step method). It is important to highlight that in September, no *A. chrysomphali* was  
232 registered in the mating disruption treatments whereas we did find it in the mineral oil and  
233 control treatments.

234 Given that oil treatments were performed only in June, no significant differences were  
235 expected between the parasitism rate in control and oil treated plots. Thus in November,  
236 the oil treatment was not sampled. We registered 108 scales parasitized by *A. melinus*  
237 (77%) and *A. chrysomphali* (23%). The CRS parasitism rate was similar between  
238 treatments ( $F = 0.0025$ ;  $df = 1, 412$ ;  $P = 0.96$ ) and *A. chrysomphali* was found in all  
239 treatments including the mating disruption (55% of the total *A. chrysomphali* registered).

240

### 241 3.3.Attraction of parasitoids and CRS males to pheromone baited traps

242 The number of CRS males captured per trap was significantly influenced by trap type ( $F =$   
243  $26.86$ ;  $df = 4, 75$ ;  $P < 0.0001$ ) (Fig. 2). The effect of trap type was independent of sampling  
244 date (interaction trap x sampling date:  $F = 1.09$ ;  $df = 12, 55$ ;  $P = 0.38$ ) or block (trap type x  
245 block:  $F = 1.93$ ;  $df = 4, 67$ ;  $P = 0.12$ ). Overall, the number of CRS males captured was  
246 significantly higher on the traps with pheromone either white ( $166.06 \pm 34.80$ ) or  
247 transparent ( $120.5 \pm 30.49$ ) (Tukey test; adjusted P values with single step method).

248 The most abundant parasitoid species captured on the traps was *A. melinus* (1165  
249 individuals; 55 females, 1110 males), followed by *A. lepidosaphes* (1145 individuals; 369  
250 females, 776 males) and *A. chrysomphali* (84 individuals; 81 females, 3 males). The highly  
251 male-biased sex ratio of the captured parasitoids indicates a possible “calling effect” of the  
252 females captured on the traps, therefore, only the female parasitoids were considered for  
253 the analyses. Moreover, only females can inflict mortality to the host through host feeding  
254 and parasitism and in that sense they are more relevant for assessing any effects of our  
255 treatments on the biocontrol services provided by the parasitoids.

256 Specifically, the trap type significantly affected the number of *A. chrysomphali* captured ( $F$   
257  $= 13.86$ ;  $df = 4, 75$ ;  $P < 0.0001$ ) (Fig. 3). The effect of the trap did not vary among  
258 sampling dates (interaction trap type x sampling date:  $F = 1.12$ ;  $df = 12, 55$ ;  $P = 0.37$ ) or  
259 block (trap type x block:  $F = 2.23$ ;  $df = 4, 67$ ;  $P = 0.08$ ). Overall, the number of *A.*  
260 *chrysomphali* captured was significantly higher in the white ( $2.35 \pm 0.91$  parasitoids per  
261 trap) and transparent traps ( $1.37 \pm 0.49$ ) both loaded with the CRS pheromone.

262 The effect of trap type on the number of *A. lepidosaphes* captured was marginally non-  
263 significant ( $F = 2.46$ ;  $df = 4, 75$ ;  $P = 0.06$ ) (Fig. 4). The trap effect was independent of  
264 sampling date (interaction trap x sampling date:  $F = 0.39$ ;  $df = 12, 55$ ;  $P = 0.95$ ) or block

265 (trap x block:  $F = 1.14$ ;  $df = 4, 67$ ;  $P = 0.35$ ). Overall, the highest number of *A.*  
266 *lepidosaphes* was captured on the white traps with or without pheromone suggesting a  
267 possible role of the trap color in the attraction of this species.  
268 Finally, the number of *A. melinus* captured was not affected by trap type ( $F = 1.86$ ;  $df = 4,$   
269  $75$ ;  $P = 0.13$ ) (Fig. 5).

270

#### 271 **4. Discussion**

272 Mating disruption again has proven to be efficient in reducing CRS infestations in citrus,  
273 by inhibiting the male flight and reducing fruit infestation. When the pheromone  
274 dispensers were employed in March, before the first CRS male flight, fruit infestation was  
275 significantly reduced compared to the control, at a level similar to that in the oil spray  
276 treatment. However, mating disruption employed in May gave significantly better results,  
277 allowing for a more rational pheromone use. In this way, using the same pheromone dose  
278 dispensers' life span will last long enough to cover the most important CRS male flights, as  
279 reported by Vacas et al. 2015. Moreover, we found that the mating disruption method,  
280 especially the one employed in May, has the additional benefit of not affecting the  
281 parasitism inflicted by *Aphytis* spp. in the orchards where these treatments were applied.  
282 Therefore, the deployment of the dispensers in May is the optimal option in terms of  
283 reducing fruit infestation, selectivity towards natural *Aphytis* parasitism and also from an  
284 economic point of view.

285 Our results show diverse responses of the *Aphytis* spp. present in the study area to  
286 various trap types tested. Specifically, *A. melinus* captures were not affected by the white  
287 trap color. This is in agreement with the previously reported results by Moreno et al.  
288 (1984) according to which *A. melinus* did not distinguish opaque from transparent  
289 rectangles and, moreover, it responded less to white compared to green or yellow trap

290 color. In general, *Aphytis* spp. are attracted to the yellow-green frequencies of the  
291 electromagnetic spectrum (Rosen and DeBach, 1979). Likewise, *A. melinus* captures were  
292 not affected by the presence of CRS pheromone in the traps. These results are in agreement  
293 with previous laboratory trials reporting that the CRS sex pheromone does not act as a  
294 kairomone for *A. melinus* (Morgan and Hare, 1998). Similarly, the fact that the parasitism  
295 inflicted by *A. melinus* was unaffected by the MD environment (Vacas et al, 2011,  
296 Vanaclocha et al., 2012) provides strong evidence that the CRS sex pheromone,  
297 independently of concentration or formulation, has no effect on the host location or the  
298 parasitism behavior of this parasitoid. This is of special relevance for biological control  
299 given that *A. melinus* is the most abundant parasitoid attacking CRS in the Mediterranean  
300 (Pekas et al., 2010). Finally, our results show that loading sticky traps with CRS sex  
301 pheromone for studies monitoring *A. melinus* abundance in the field is not necessary.

302         Regarding *A. lepidosaphes*, captures seemed to be more affected by the color rather  
303 than the presence of CRS pheromone on the traps although the captures between white and  
304 transparent traps were not statistically significant. *A. lepidosaphes* parasitizes the armored  
305 scale *Lepidosaphes beckii* (Newman) and it has not been reported attacking the CRS.  
306 Therefore, it seems quite consistent not be attracted by the CRS sex pheromone.

307         On the other hand, *A. chrysomphali* captures were significantly higher in traps  
308 baited with the CRS sex pheromone. In contrast to *A. melinus*, no previous studies have  
309 examined the effect of CRS sex pheromone on *A. chrysomphali*. Our results suggest that *A.*  
310 *chrysomphali* may be employing the CRS sex pheromone as a kairomone for host location.  
311 Additional indirect evidence supporting this hypothesis may be provided by our MD trials  
312 where *A. chrysomphali* was not found in the MD treated plots in September. In this period,  
313 pheromone emission was still high enough to disrupt CRS flight and probably *A.*  
314 *chrysomphali* behavior. Conversely, in November the dispenser life span is near depletion

315 (Vacas et al. 2010) and consequently, airborne pheromone concentration in the field was  
316 lower, resulting in *A. chrysomphali* individuals captured also in the mating disruption  
317 treatment. We consider that these results are not due to the variation of the *Aphytis* spp.  
318 abundance along the year because both *A. melinus* and *A. chrysomphali* peak their  
319 abundances in the study area in the period between September and November (Sorribas et  
320 al., 2008). Moreover, and given that *A. melinus* is apparently unaffected by the CRS sex  
321 pheromone the reduction of the parasitism in the mating disruption treatments in  
322 September may be due to the reduced activity of *A. chrysomphali*. *A. chrysomphali* is the  
323 second most important parasitoid of CRS in the Mediterranean citrus and any possible  
324 effects of the CRS pheromone on its behavior and parasitism may have important  
325 implications for the biological control of the scale. However, more detailed laboratory  
326 studies are needed in order to draw definitive conclusions about this issue.

327         It was already reported that several *Aphytis* spp. employ a kairomone from the scale  
328 cover and body in making oviposition decisions. Luck and Uygun (1986) demonstrated  
329 that *A. melinus*, *A. lignanensis* and *A. coheni* responded to water and ethanol extracts of  
330 CRS covers. Later, Millar and Hare (1993) isolated and identified this kairomonal  
331 compound as *O*-caffeoyltyrosine. Response of *A. melinus* to this kairomone is considered  
332 as an innate cue which may arise from its co-evolutionary background. The evolutionary  
333 host of *A. melinus* is *Aonidiella orientalis* (Newstead), which is a congener of *A. aurantii*  
334 (Morgan and Hare, 1998). Likewise, innate responses to sex pheromones are likely to  
335 happen in the case of coevolution or when the cue is shared with the evolutionary host. In  
336 Mediterranean citrus *A. chrysomphali* has been found parasitizing *Chrysomphalus*  
337 *dictyospermi* (Morgan), which is a closely related species of *A. aurantii* (Garcia Marí,  
338 2012). However, no information on a sex pheromone produced by *C. dictyospermi* is  
339 available.

340 We conclude that mating disruption with mesoporous dispensers was confirmed  
341 once again as a solid alternative for the management of the CRS in citrus. The optimal  
342 period to place the dispensers in terms of reducing fruit infestation as well as in terms of  
343 selectivity towards the natural *Aphytis* sp. parasitism is May. The CRS sex pheromone  
344 used for monitoring and also the high concentration employed for the MD do not have an  
345 effect on *A. melinus*, however, we provide evidence for a possible effect on the sibling  
346 species *A. chrysomphali*.

347

### 348 **Acknowledgements**

349 We would like to thank Amparo Aguilar for her valuable help with the field sampling and  
350 in the laboratory. Authors also want to thank Fernando and Cristina Alfaro for providing  
351 the experimental orchards.

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447 **Fig. 1.** Mean ( $\pm$ SE) parasitism rate among treatments inflicted by *Aphytis* spp. on the  
448 California red scale *Aonidiella aurantii* in September 9 in a citrus orchard in Valencia  
449 Spain. Columns bearing different letters are significantly different at  $P < 0.05$ . (MD=  
450 mating disruption employed either in March or May).

451 **Fig. 2.** Mean ( $\pm$ SE) number of California red scale (CRS) *Aonidiella aurantii* males caught  
452 on different trap types. Columns bearing different letters are significantly different at  $P <$   
453 0.05.

454 **Fig. 3.** Mean ( $\pm$ SE) number of *Aphytis chrysomphali* parasitoids caught on different trap  
455 types (CRS= California red scale, *Aonidiella aurantii*). Columns bearing different letters  
456 are significantly different at  $P < 0.05$ .

457 **Fig. 4.** Mean ( $\pm$ SE) number of *Aphytis lepidosaphes* parasitoids caught on different trap  
458 types (CRS= California red scale, *Aonidiella aurantii*).

459 **Fig. 5.** Mean ( $\pm$ SE) number of *Aphytis melinus* parasitoids caught on different trap types  
460 (CRS= California red scale, *Aonidiella aurantii*).

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