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5 **Does host quality dictate the outcome of interference competition**
6 **between sympatric parasitoids? Effects on their coexistence**

7 **Ruth Cebolla, Pablo Bru, Alberto Urbaneja, Alejandro Tena**

8 Instituto Valenciano de Investigaciones Agrarias, IVIA. Centro de Protección Vegetal. Unidad Asociada
9 de Entomología UJI-IVIA. Carretera Moncada-Náquera km 4.5, 46113 Moncada (Valencia, Spain)

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11

12

13

14

Corresponding author: Alejandro Tena

15

Centro Protección Vegetal y Biotecnología.

16

Instituto Valenciano de Investigaciones Agrarias (IVIA)

17

Carretera Moncada-Náquera km 4.5.

18

46113 Moncada (Valencia, Spain)

19

Phone: (+34) 96 342 41 51

20

Fax: (+34) 96 342 40 01

21

Email: atena@ivia.es

22

23

24 **ABSTRACT**

25 Herbivore suitability and quality for hymenopteran parasitoids is dynamic, varying with host
26 development. Generally, within the same host species, large hosts (i.e. older instars) are
27 considered of higher quality for progeny development. Studies of interspecific competition
28 between parasitoids have considered the effect of host instar on indirect competition but its
29 effect on interference competition remains unknown. Here, we report the first results on whether
30 host instars (of different quality for immature development) might dictate the outcome of
31 interference competition between sympatric parasitoids of the genus *Aphytis* Howard
32 (Hymenoptera: Aphelinidae) when they attack low (second) and high quality (third) instars of
33 their common host *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae). Oviposition
34 behavior (host acceptance and clutch size) in low and high quality host instars was similar for
35 both *Aphytis* species in the absence of competition. When they found heterospecific parasitized
36 hosts of high quality, *Aphytis melinus* DeBach laid more eggs and accepted significantly more
37 hosts than *A. chrysomphali* (Mercet), whereas there were no significant differences in the low
38 quality instar. This result suggests that interference competition is mediated by host quality.
39 However, the progeny proportion of both parasitoids in multiparasitized hosts (outcome of
40 competition) was independent of host quality and *A. melinus* always emerged at higher rates.
41 Therefore, the final result of interference competition between these sympatric parasitoids was
42 not affected by host quality and this competition will contribute to the displacement of the
43 native *A. chrysomphali* by the introduced *A. melinus*, as it has been observed in some areas of
44 the Mediterranean basin.

45

46 **Keywords:** interspecific competition, intrinsic competition, competitive exclusion, size-
47 mediated interactions, infanticide, *Aphytis*, *Aonidiella aurantii*, armored scales.

48 **1. INTRODUCTION**

49 Interspecific competition drives community structure and function (Morin, 2011). In extreme
50 cases, stronger competitors can drive weaker competitors to extinction by monopolizing
51 resources (Chesson, 2000). Herbivorous insects are frequently attacked by several
52 hymenopteran parasitoid species whose larvae engage in interspecific competition (Godfray,
53 1994). Herbivore suitability and quality vary during development and can dictate the outcome
54 of competition among developing parasitoids sharing a host (Harvey, Poelman, & Tanaka,
55 2013; Price, 1972). Generally, parasitoid species that find and parasitize younger hosts have an
56 exploitative advantage over their antagonists because they can use their host earlier in the
57 season and, also because they have a head start in intrinsic competition. The former type of
58 competition among free-living adult parasitoids searching for and using hosts of different
59 sizes/instars is a type of interference competition and has been documented in the field (Bográn,
60 Heinz, & Ciomperlik, 2002; Luck & Podoler, 1985) as well as in theoretical studies (Briggs,
61 1993; Harvey et al., 2013; Murdoch, Briggs, & Nisbet, 1996). However, the effect of the host
62 instar/stage on interference competition has never been tested, and we hypothesize that the host
63 instar might facilitate the coexistence of ecological homologue parasitoids when the outcome of
64 competition depends on the parasitized instar/stage.

65 Several mechanisms related to the behaviour of the mother and/or competition between larvae
66 might explain the apparent instar-related attenuation of superiority (Collier, Hunter, & Kelly,
67 2007; Cusumano, Peri, & Colazza, 2016; Harvey et al., 2013). First, the mother can provide an
68 advantage to its own progeny by killing immature individuals of the competing species or by
69 laying a larger clutch size (Cusumano et al., 2016; Tena, Kapranas, Garcia-Marí, & Luck,
70 2008). We expect this behaviour to vary depending on host suitability and quality (instar)
71 (Hopper, Prager, & Heimpel, 2013). We thus hypothesize that a mother will be less willing to
72 expend energy and time killing progeny of a competitor species in a heterospecific-parasitized
73 host of low quality (i.e., small in size or young instar). Second, competition between immature
74 parasitoids through either physical contests or a scramble for host resources may also depend on

75 the host instar. For example, parasitoid species with long embryonic-development times might
76 have a higher probability of surviving in adult hosts than in young/small hosts in which
77 resources are scarcer. Finally, the outcome might depend on a combination of maternal
78 behaviour and offspring competition.

79 Here, we study whether the host instar/stage dictates the outcome of interference competition
80 between parasitoids of the genus *Aphytis* Howard (Hymenoptera: Aphelinidae) and facilitates
81 their coexistence in sympatry in Mediterranean citrus (Pekas, Tena, Harvey, Garcia-Marí, &
82 Frago, 2016; Sorribas, Rodríguez, & Garcia-Marí, 2010). The introduced species *Aphytis*
83 *melinus* DeBach is a superior competitor compared to the native *Aphytis chrysomphali* (Mercet)
84 as a parasitoid of *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae). Their coexistence has
85 been attributed to fluctuating environmental conditions, seasonal variation in parasitoid
86 abundance (Boyero et al., 2014; Pina, 2006; Sorribas et al., 2010) and, more recently, to the
87 plasticity of *A. chrysomphali* in exploiting different host instars depending on the *A. melinus*
88 density (Pekas et al., 2016). That latter field study shows that *A. chrysomphali* are recovered in
89 greatest numbers from second-instar host, which are poorer quality hosts, when the *A. melinus*
90 density is high and exploits the third-instar (a higher quality host) (Pekas et al., 2016). However,
91 we hypothesize that this conditional patch partitioning might reflect the fact that *A. melinus* is a
92 superior competitor when both parasitoids parasitize third-instar hosts (high quality), but is less
93 advantageous in second-instar hosts (low quality).

94 To test our hypothesis and the mechanisms underlying it, we first observed female parasitoids to
95 directly investigate whether females can provide an advantage to their own progeny by laying a
96 larger clutch or killing the progeny of the competitor, depending on the host instar. Then, we
97 analyzed the intrinsic competition between parasitoid species to test whether the outcome
98 depends on host instar and/or order of attack (generally, the offspring of the first female has an
99 advantage). Finally, we provide an explanation for the coexistence of *A. melinus* and *A.*
100 *chrysomphali* in terms of the results obtained here and in a field study (Pekas et al., 2016).

101

102 2. MATERIAL AND METHODS

103 2.1 System

104 Parasitoids of the genus *Aphytis* are the most successful and widespread biological control
105 agents of *A. aurantii* in citrus (DeBach & Rosen, 1991; Forster & Luck, 1996; Murdoch, Briggs,
106 & Swarbrick, 2005). These specialist parasitoids can reduce their shared host to levels nearly
107 200 times lower than the average density observed in their absence (DeBach, Rosen, & Kennett,
108 1971), suggesting strong resource competition between parasitoid species (Borer, Murdoch, &
109 Swarbrick, 2004). In fact, species of the genus *Aphytis* represent one of the best-known cases of
110 competitive displacement in insects (Luck et al., 1982; Luck and Podoler, 1985; Luck and
111 Nunney, 1999; Sorribas et al., 2010; Pekas et al., 2016). *Aphytis melinus* displaced *Aphytis*
112 *lingnanensis* Compere (Hare & Luck, 1991) in interior California (Luck & Podoler, 1985; Luck
113 et al., 1982; Podoler, 1981) because the former uses smaller host sizes for production of female
114 progeny such that it exploits its hosts before they reach a size suitable for the production of
115 female *A. lingnanensis* (Hudak, 2003; Luck & Nunney, 1999; Luck & Podoler, 1985). Thus,
116 host size represents a resource that is available for the developing parasitoid and is probably the
117 most reliable cue of host quality for *Aphytis* (Hare & Luck, 1991; Pekas, Aguilar, Tena, &
118 Garcia-Marí, 2010). In fact, larger adults of *A. melinus* and *A. chrysomphali* emerge from third
119 than from the second-instar, they prefer the third-instar when both instars are available, and the
120 immature mortality is slightly lower in the third than in the second-instar (Hare & Luck, 1991;
121 Pina, 2006; Pekas, Aguilar, Tena, & Garcia-Marí, 2010; Pekas et al., 2016; Table 2). In the
122 Mediterranean basin, *A. melinus* has displaced *A. chrysomphali* in some areas, whereas the
123 species coexist in other areas (Sorribas et al., 2010). Although *A. chrysomphali* reproduces
124 parthenogenetically and produces only females when it is infested with the bacterium
125 *Wolbachia* (Pina, 2006), *A. melinus* is considered to be a superior competitor in the field
126 because it has a higher capacity for dispersion (McLaren, 1976) and is better adapted to climates
127 where citrus is cultivated (Abdelrahman, 1974; Rosen & DeBach, 1979).

128 2.2 Insects.

129 The host herbivore *Aonidiella aurantii* was reared on lemons (*Citrus limon* (L.) from a
130 laboratory colony at the Instituto Valenciano de Investigaciones Agrarias, IVIA (Moncada,
131 Valencia, Spain). This colony was initiated in 1999 from scales collected from citrus fields in
132 Alzira (Valencia, Spain) and renewed every two to three years with field-collected scales (Tena,
133 Llácer, & Urbaneja, 2013). Approximately two-thirds of the surface of each lemon was covered
134 with red paraffin around the mid-section to retard its desiccation. The red paraffin was prepared
135 with a mixture of 1 kg of paraffin pearls (Parafina USP Perlas; Guinama S.L., Alboraya, Spain)
136 and 1 g of red pigment (Sudan III; Panreac Química S.A., Castellar del Vallés, Spain). The
137 remaining surface (approx. 24-cm² area) of the lemons was infested by exposure to gravid
138 female scales for 48 h on the *A. aurantii* colony. Once they were infested, lemons were
139 maintained at 27 ± 1 °C at $70 \pm 5\%$ RH and darkness until female scales reached the second (9-
140 11 days) and third nymphal instars (19-22 days), both of which were used in these assays.

141 *A. melinus* and *A. chrysomphali* were obtained by exposing third-instar *A. aurantii* on lemons to
142 parasitism by insectary-reared adult wasps maintained in the laboratory at 26 ± 1 °C, $60 \pm 5\%$
143 RH and LD 16:8 h. The colonies of *A. melinus* and *A. chrysomphali* were initiated in 2008 and
144 2013, respectively, from *A. aurantii* scales collected in citrus fields located in the Valencia
145 region (Valencia, Spain). Both colonies are renewed yearly with field-collected parasitoids.

146 Between five and ten late-stage pupae of both parasitoids were removed from parasitized scales
147 and held separately in crystal vials that were 8 mm in diameter and 35 mm long. At emergence,
148 parasitoids were sexed and held in these vials for one day to obtain mated females of *A. melinus*.
149 One day after their emergence, females were isolated in the same vials described above, and one
150 *A. aurantii* female body was introduced daily to allow them to feed on a host until they were
151 used two to three days later (Heimpel, Rosenheim, & Kattari, 1997). Since *Aphytis* do not obtain
152 sugars from host feeding (Tena et al., 2013) and adults die within three days without a
153 carbohydrate source (Heimpel, Rosenheim, & Kattari, 1997), a drop of honey was added on the

154 inside wall of each vial , which were stoppered with a cotton plug. The vials were stored in a
155 climatic chamber (SANYO MLR- 350; Sanyo, Japan) at $25 \pm 1^\circ\text{C}$, 50-70% RH and LD 14:10 h.

156 **2.3 Experimental arena.**

157 We conducted behavioural observations on a lemon from the colony, where we measured and
158 selected a second- (0.5-0.7 mm²) or third-instar scale (0.8-1 mm²) (Luck & Podoler, 1985; Opp
159 & Luck, 1986; Pekas et al., 2010). To measure the surface of each scale, we used a dissecting
160 microscope with a Leica EC 3 3.1 megapixel digital color camera (Leica Microsystems GmbH,
161 Spain). Images were processed with Leica LAS EX imaging software for Windows (Leica
162 Microsystems GmbH, Spain) and the area of the scales (mm²) were measured with ImageJ, a
163 public-domain Java Image-processing program (Rasband, 2016). The selected scale was
164 mapped, and the remaining scales were removed using an entomological needle and a paper
165 moistened with water.

166 **2.4 Adult female behaviour.**

167 Female parasitoids were introduced individually into a glass petri dish (diameter 4 cm, height
168 1.5 cm), and the petri dish was placed over an individual scale on a lemon. The behaviours of *A.*
169 *melinus* and *A. chrysomphali* were observed and recorded under four different conditions for
170 each *A. aurantii* nymphal instar (second- or third-instar) (see different treatments in Table 1). In
171 “control treatments”, parasitoids were introduced individually into the experimental arenas
172 described above. In the “competition treatments”, *A. melinus* and *A. chrysomphali* were
173 introduced sequentially in both possible orders; the first female was observed until she
174 oviposited in the host and was then removed, the female of the other species was introduced 2 h
175 later and observed. Each treatment was replicated 30 times (Table 1).

176 In each replicate, we continuously observed the behaviour of the second female using a
177 dissecting microscope at 10x to 50x magnification and a cool fiber light to illuminate the arena.
178 We initiated behavioural recording when the female recognized the host, i.e., she drummed the
179 scale by positioning herself on the scale cover and moved from the centre to the edge while

180 tapping the cover with her antennae and sometimes her mouthparts (van Lenteren, 1994). Each
181 observation terminated when the female left the scale or rested for more than two min. Three
182 separate behavioural components were identified, timed and recorded: (1) rejection, (2)
183 oviposition and (3) host-feeding. After drumming the scale with her antennae, the female might
184 investigate the host by probing, a behaviour set that includes using her ovipositor to drill
185 through the scale cover, explore the cavity between the scale body and cover, and pierce the
186 body and explore the haemocoel (Casas, Swarbrick, & Murdoch, 2004). The parasitoid may
187 leave the host at any time during this process (rejection), may accept the host (oviposition) or
188 may consume the scale's body fluids (host-feeding) (Casas et al., 2004). The time spent probing
189 the host was recorded (not including probes that ended in oviposition). We identified
190 oviposition as occurring when female abdominal vibrations occurred while probing with
191 exudation of a viscous substance from the ovipositor tip. The ovipositor was then withdrawn,
192 and the female either left the scale or retract their ovipositor to puncture the scale cover again to
193 lay another egg (Pina, 2006). We recorded additional behavioural data during the observations:
194 (i) the position and number of ovipositions per scale and (ii) the position and number of probes.
195 We used the acceptance rates and clutch size to test whether *A. melinus* females accept and lay
196 more eggs than *A. chrysomphali* in the third but not the second-instar when both instars were
197 parasitized by the competitor.
198 After the observation period ended, the parasitoid was removed and each lemon was kept in a
199 plastic container (14 x 14 x 8 cm) covered with a piece of muslin. The containers were kept in
200 the same climatic chamber described above ($25 \pm 1^\circ\text{C}$, 50-70% RH and LD 14:10 h) to
201 determine the outcomes of these encounters.

202 **2.4.1 Possible heterospecific ovidicide**

203 To determine whether *A. melinus* and *A. chrysomphali* are able to detect and kill the eggs of the
204 competing female (heterospecific ovidicide), we first determined and compared the duration of
205 probing in healthy and parasitized hosts and then determined whether the second female probed
206 the scale in the direction of the clutch laid by the first female (Netting & Hunter, 2000). Later,

207 we checked whether these second probes were lethal. We considered only ovicidal behaviour in
208 third-instar hosts because it was difficult to determine the outcome in the second-instar. Both
209 parasitoid species probed close to the eggs because of the small size of this instar.

210 **2.5 Outcome of competition**

211 To determine the outcome of competition between *A. melinus* and *A. chrysomphali*, we
212 observed parasitized and multiparasitized scales between 10 and 12 days after the original
213 observations (time needed to reach the pupal stage under our conditions). The covers of the
214 scales were removed carefully with an entomological needle under a binocular lens. Then, we
215 identified the species and recorded the sex and number of parasitoid pupae. To differentiate
216 between species, we check the conspicuous longitudinal black line that is present in the
217 mesosternum of *A. chrysomphali* pupae but not in *A. melinus* (Rosen & DeBach, 1979). These
218 data were used to determine and compare the outcome of competition measured as the immature
219 mortality, brood size, sex ratio and proportion of emergence, depending on the host instar. We
220 expected that the proportion of emergent *A. melinus* would be higher than that of *A.*
221 *chrysomphali* in the third instar, but not in the second, independent of the order of attack.

222 **2.6 Statistical analysis.**

223 We compared the probing duration across treatments using ANOVA. The normality assumption
224 was assessed using Shapiro's test, and homoscedasticity was assessed by the Levene test.
225 Proportional and count data were analyzed with generalized linear models (GLMs). Initially, we
226 assumed a binomial error variance for proportional data (host acceptance, probing in the
227 direction of the first clutch, progeny proportion, sex ratio) and a Poisson error variance for count
228 data (clutch and brood size). We assessed the assumed error structures using a heterogeneity
229 factor equal to the residual deviance divided by the residual degrees of freedom. If we detected
230 over- or underdispersion, we re-evaluated the significance of the explanatory variables using an
231 *F* test after rescaling the statistical model by a Pearson's chi-square divided by the residual
232 degrees of freedom (Crawley, 2007). We present the means of the untransformed proportion and

233 count data (in preference to less intuitive statistics, such as the back-transformed means of logit-
234 transformed data). All statistical analyses were performed with R studio (Version 0.98.501 – ©
235 2009-2013 RStudio, Inc).

236 **3. RESULTS**

237 **3.1 Behaviour of adult females**

238 3.1.1 Host Acceptance

239 In the absence of competition, *A. melinus* and *A. chrysomphali* displayed similar rates of
240 acceptance for second-instar hosts (acceptance of healthy host by *A. melinus*: 0.67 ± 0.09 and *A.*
241 *chrysomphali*: 0.53 ± 0.09 ; $F_{1,58} = 1.08$; $P = 0.30$) and third-instar hosts (*A. melinus*: 0.77 ± 0.08
242 and *A. chrysomphali*: 0.67 ± 0.09 ; $F_{1,58} = 1.23$; $P = 0.27$) (Fig. 1). When the scales had been
243 previously parasitized by their competitor, *A. melinus* accepted heterospecific-parasitized third-
244 instar *A. aurantii* more frequently (0.77 ± 0.08) than did *A. chrysomphali* (0.43 ± 0.09) ($F_{1,58} =$
245 6.87 ; $P = 0.011$). However, the two parasitoids accepted second-instar hosts at similar rates (*A.*
246 *melinus*: 0.43 ± 0.09 and *A. chrysomphali*: 0.40 ± 0.09 ; $F_{1,58} = 0.07$; $P = 0.8$), as predicted by
247 our hypothesis (superiority diminishes in the second-instar).

248 3.1.2 Clutch size

249 In the absence of competition, *A. melinus* and *A. chrysomphali* laid similar-sized clutches of
250 eggs in second-instar hosts (eggs laid in healthy hosts by *A. melinus*: 1.05 ± 0.05 and *A.*
251 *chrysomphali*: 1.06 ± 0.06 ; $F_{1,34} = 0.03$; $P = 0.88$) and third-instar hosts (*A. melinus*: 1.3 ± 0.12
252 and *A. chrysomphali*: 1.32 ± 0.11 ; $F_{1,37} = 0.04$; $P = 0.84$) (Fig. 2).

253 When the scales had been previously parasitized by their competitor, *A. melinus* laid a
254 significantly larger clutch size (1.39 ± 0.14) than did *A. chrysomphali* (1) on third-instar hosts
255 ($F_{1,34} = 5.25$; $P = 0.028$). However, the two parasitoids laid a similar numbers of eggs in the
256 second-instar (*A. melinus*: 1.08 ± 0.08 and *A. chrysomphali*: 1 ; $F_{1,23} = 0.95$; $P = 0.34$), as
257 predicted by our hypothesis (the advantage diminishes in the second-instar).

259 **3.2 Probing time, site and potential heterospecific ovicide**

260 When the scales had been previously parasitized by their competitor, the time spent probing the
 261 second-instar was similar for both parasitoid species (*A. melinus* = 44.2 ± 16.1 sec; *A.*
 262 *chrysomphali* = 57 ± 11.4 sec; $F_{1,23} = 0.41$; $P = 0.53$). The time spent probing the third instar
 263 increased, but there were no significant differences between parasitoid species (*A. melinus* =
 264 190.7 ± 12.7 sec; *A. chrysomphali* = 171.5 ± 21.2 sec; $F_{1,34} = 1.13$; $P = 0.72$). However, *A.*
 265 *melinus* tended to probe the scale in the direction of the first clutch (ratio: 0.65 ± 0.10) more
 266 frequently than did *A. chrysomphali* (ratio: 0.33 ± 0.13) ($\chi^2 = 42.7$; $P = 0.049$; $n = 36$) when they
 267 encountered heterospecific-parasitized third-instar hosts. Finally, no *A. chrysomphali* emerged
 268 from the nine hosts in which *A. melinus* had probed in the direction of the first clutch.

269 **3.3 Outcome of competition**

270 Given the acceptance ratios, clutch size laid, ovicide and immature competition (next section),
 271 *A. melinus* produced a greater proportion of progeny (~ 0.7) than did *A. chrysomphali* (~ 0.3),
 272 independent of the host instar ($F_{1,57} = 0.085$; $P = 0.77$) and order of exposure ($F_{1,57} = 0.02$; $P =$
 273 0.89 (Fig. 3)). The interaction between the host instar and order of exposure was not significant
 274 ($F_{1,56} = 0.01$; $P = 0.91$). According to these results, and contrary to our hypothesis, *A. melinus*
 275 was a superior competitor in both instars.

276 3.3.1 Immature mortality

277 In the absence of competition, the immature mortality rates of *A. melinus* and *A. chrysomphali*
 278 were similar in second-instar ($F_{1,34} = 2.44$; $P = 0.13$) and third-instar hosts ($F_{1,41} = 0.09$; $P =$
 279 0.77) (Table 2). In multiparasitized hosts, the immature mortalities of *A. melinus* and *A.*
 280 *chrysomphali* were similar in third-instar hosts ($F_{1,34} = 0.56$; $P = 0.46$), but mortality was
 281 significantly higher for *A. chrysomphali* than for *A. melinus* in the second instar ($F_{1,23} = 5.89$; $P =$
 282 0.02) (Table 2).

283 3.3.2 Brood size

284 In the absence of competition, the brood sizes of *A. melinus* and *A. chrysomphali* were similar
285 when they emerged from second-instar (always emerged one parasitoid) and third-instar hosts
286 ($F_{1,37} = 0.43$; $P = 0.52$) (Table 2). Similarly, in multiparasitized hosts, the brood sizes of *A.*
287 *melinus* and *A. chrysomphali* were similar when they emerged from second-instar (always one
288 parasitoid) and third-instar hosts ($F_{1,13} = 0.37$; $P = 0.55$) (Table 2).

289 3.3.3 Sex ratio

290 In the absence of competition, the secondary sex ratio of *A. melinus* was male-biased in second-
291 instar hosts and became female-biased in the third instar (Table 2). *Aphytis melinus* sex ratio
292 followed the same pattern in multiparasitized hosts. As expected, all emerging *A. chrysomphali*
293 were females and, therefore, we could not compare the sex ratios between parasitoid species.

294 4. DISCUSSION

295 Effect of host instar on interference competition

296 Our results contradict our initial hypothesis that *A. melinus* is a superior competitor when both
297 parasitoids parasitize the third-instar (high quality) but that this advantage diminishes in the
298 second-instar. Given adult female behaviour and immature competition, the introduced
299 parasitoid *A. melinus* was a superior competitor compared to the native *A. chrysomphali*,
300 independent of the host instar (low vs. high quality hosts) and sequence of attack. The
301 superiority of *A. melinus* is explained by the higher aggressiveness of the mother on
302 encountering third-instar hosts (accepting more hosts, laying more eggs, and high rates of
303 ovide) and the higher mortality of *A. chrysomphali* larvae when they develop in
304 multiparasitized second-instar hosts. The higher mortality of *A. chrysomphali* larvae compared
305 to *A. melinus* larvae might be the result of physical and physiological mechanisms employed by
306 dominant parasitoids to suppress their competitors (Harvey et al., 2013). These mechanisms
307 have never been studied in *Aphytis*, but their larvae have mandibles (Eliraz & Rosen, 1978;
308 Rosen & DeBach, 1979) that can be used to kill the eggs or larvae of competitors in different

309 larval instars, as occurs in other parasitoid species (Tena et al., 2008). It has been generally
310 assumed that species with shorter developmental times are at an advantage because they can
311 ingest resources earlier. However, the developmental time of *A. chrysomphali* is shorter than
312 that of *A. melinus* at 25°C (the conditions of the experiment) (Abdelrahman, 1974).

313 **Effect of interference competition on the coexistence of *A. melinus* and *A. chrysomphali***

314 Theoretical studies have highlighted the role of interspecific competition in structuring
315 parasitoid communities (Borer, Briggs, Murdoch, & Swarbrick, 2003; Borer, 2002; May &
316 Hassell, 1981; Murdoch et al., 1996). At present, however, there is relatively little information
317 explaining how parasitoids with broadly overlapping host niches coexist in nature (but see
318 (Bográn et al., 2002; Borer et al., 2004; Snyder, Borer, & Chesson, 2005; Tschardtke, 1992). In
319 some areas of the Mediterranean basin, *A. melinus* and *A. chrysomphali* coexist as parasitoids of
320 *A. aurantii*. Pekas et al. (2016) showed that *A. chrysomphali* is recovered mostly from second-
321 instar hosts of poorer quality when the density of the superior competitor *A. melinus* is high in
322 areas where they coexist. We hypothesized that this conditional patch partitioning might reflect
323 the fact that *A. melinus* is a superior competitor when both parasitoids parasitize the third-instar
324 (high quality), but that this advantage diminishes in the second-instar. Contrary to our initial
325 hypothesis, our data indicate that *A. melinus* is a superior competitor compared to native *A.*
326 *chrysomphali* when they compete for the same individual host, independent of host instar.
327 Therefore, the superior biological traits of *A. melinus* described herein, together with its higher
328 capacities for dispersion (McLaren, 1976) and parasitism (Pekas et al., 2010), contribute to the
329 displacement of *A. chrysomphali*, as has occurred in southeastern Spain (Boyero et al., 2014;
330 Sorribas et al., 2010).

331 On the other hand, other factors may affect the intrinsic competition between these parasitoids,
332 favouring their coexistence. For example, the facultative symbiont *Hamiltonella defensa* can
333 reverse the outcome of competition between two parasitoids of the pea aphid *Acyrtosiphon*
334 *pisum* (Harris) (Hemiptera: Aphididae) (McLean & Godfray, 2016). In the case of *Aphyits*, both
335 parasitoids are infected with the bacterium *Wolbachia* and it is unknown whether its absence

336 might modify the competition. Another factor that can modify the outcome of competition and
337 their coexistence is the presence of alternative hosts. The outcome of the intrinsic competition
338 between the parasitoids *Hyposoter ebeninus* (Gravenhorst) (Hymenoptera: Ichneumonidae) and
339 *Cotesia glomerata* L. (Hymenoptera: Braconidae) depends on both plant and herbivore host
340 species (Poelman et al., 2014). It is well known that *A. melinus* and *A. chrysomphali* can find
341 and develop in alternative hosts in the Mediterranean basin ([http://www.nhm.ac.uk/our-](http://www.nhm.ac.uk/our-science/data/chalcidoids/database/)
342 [science/data/chalcidoids/database/](http://www.nhm.ac.uk/our-science/data/chalcidoids/database/)). The presence of these alternative plants and hosts might
343 also facilitate their coexistence as suggested by Pekas et al. (2016).

344 Finally, the results obtained herein, together with those of Pekas et al. (2016), suggest that *A.*
345 *chrysomphali* is able to evaluate the density of its competitor, *A. melinus*, and alter the use of
346 the host instar (quality) depending on the density of the former. This ability provides *A.*
347 *chrysomphali* with competition-free host resources and, together with favorable climatic
348 conditions (Sorribas et al., 2010), permits sympatry with the dominant *A. melinus* in
349 northeastern Spain, which is not possible elsewhere in this range. Further research is necessary
350 to corroborate this hypothesis.

351 **Heterospecific ovicide in the genus *Aphytis***

352 *Aphytis melinus* females tended to probe the scale in the direction of the eggs laid by *A.*
353 *chrysomphali* and likely killed them with their ovipositor, eliminating competitors for their
354 offspring. Heterospecific ovicide has been documented in several species of ectoparasitoids
355 (Infante, Mumford, Baker, Barrera, & Fowler, 2001; Pérez-Lachaud, Batchelor, & Hardy,
356 2004). Ectoparasitoids find heterospecific eggs outside the host cuticle and either eat them or
357 stab them with their ovipositor (Collier et al., 2007). Nonetheless, ovicide might be less feasible
358 for specialized ectoparasitoids of diaspine hosts, such as *Aphytis*, because the eggs are located
359 under the scale cuticle and sometimes even under the host body (Luck et al., 1982). Therefore,
360 *Aphytis* females must pierce one or two barriers with their ovipositor to reach the first female's
361 eggs, behaving as an endoparasitoid. With all this, it was impossible to determine whether
362 females probed eggs through the dark scale cuticle of *A. aurantii*. Rather than observe the

363 probes, we observed how females moved their ovipositors towards the first female's eggs. The
364 fact that none of these supposedly probed eggs survived the attack suggests ovicide. This
365 hypothesis was supported by two other observations. First, *A. chrysomphali* survived ($37.5\% \pm$
366 18.3) when it shared the scale with *A. melinus* whose mother had not tried to probe the eggs of
367 the competitor. Second, *A. melinus* females always allocated a clutch of eggs after probing in
368 the direction of the first female's eggs. *Aphytis melinus* also commits ovicide when females
369 sting the eggs of competitors while trying to host-feed on scales parasitized by conspecifics
370 (Collier per. observation cited in Collier & Hunter, 2001) and *Encarsia perniciosi* Tower (Yu,
371 Luck, & Murdoch, 1990).

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503

504 **Table 1.** Number of replicates used per treatment and per variable in the experiment.

505

Second-instar <i>A. aurantii</i>				
	Control		Multiparasitism	
	<i>A. melinus</i>	<i>A. chrysomphali</i>	<i>A. melinus</i>	<i>A. chrysomphali</i>
Behavioural observations	30	30	30	30
Clutch size	20	16	13	12
Immature mortality	20	16	13	12
Brood size	18	11	10	3
Sex ratio	18	*	10	*

Third-instar <i>A. aurantii</i>				
	Control		Multiparasitism	
	<i>A. melinus</i>	<i>A. chrysomphali</i>	<i>A. melinus</i>	<i>A. chrysomphali</i>
Behavioural observations	30	30	30	30
Clutch size	23	19	23	13
Immature mortality	23	19	23	13
Brood size	21	19	11	4
Sex ratio	20	*	10	*

506

507

508 **Table 2.** Mean immature mortality, secondary brood size (emerged adults) and sex ratio (%
 509 males) of *A. melinus* and *A. chrysomphali* that had accepted healthy (control) and
 510 heterospecific-parasitized second- and third-instars of *A. aurantii*.

		<i>A. melinus</i>		<i>A. chrysomphali</i>	
		Control	Multiparasitism	Control	Multiparasitism
Immature mortality	N2	0.1 ± 0.07	0.27 ± 0.12	0.31 ± 0.12	0.75 ± 0.13
	N3	0.15 ± 0.07	0.52 ± 0.1	0.13 ± 0.06	0.69 ± 0.13
Brood size	N2	1	1	1	1
	N3	1.1 ± 0.07	1.18 ± 0.18	1.16 ± 0.08	1
Sex Ratio	N2	0.59 ± 0.12	0.7 ± 0.02	0	0
	N3	0.13 ± 0.07	0.2 ± 0.09	0	0

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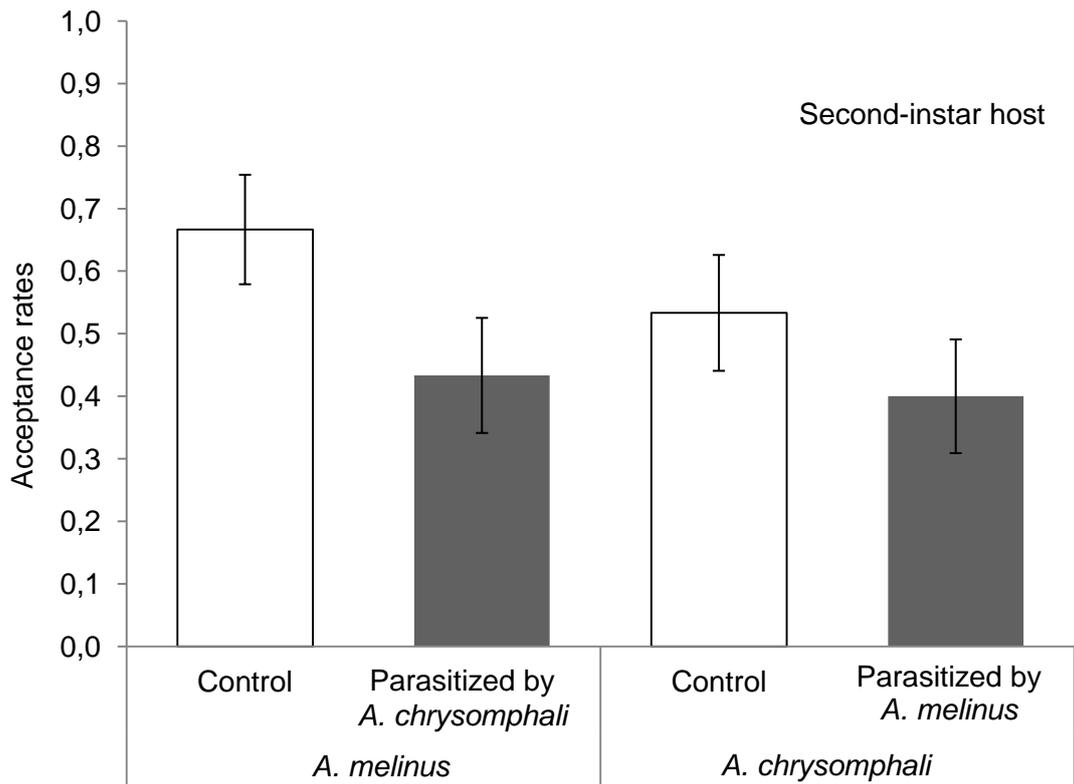
513 **Figure legends.**

514 **Fig. 1.** Acceptance rates (mean \pm SE) of healthy (control) and heterospecific-parasitized
515 *Aonidiella aurantii* by *Aphytis melinus* and *A. chrysomphali* when they encountered (A) second-
516 and (B) third-instar hosts. Different letters above columns denote significant differences
517 between parasitoids at $P < 0.05$.

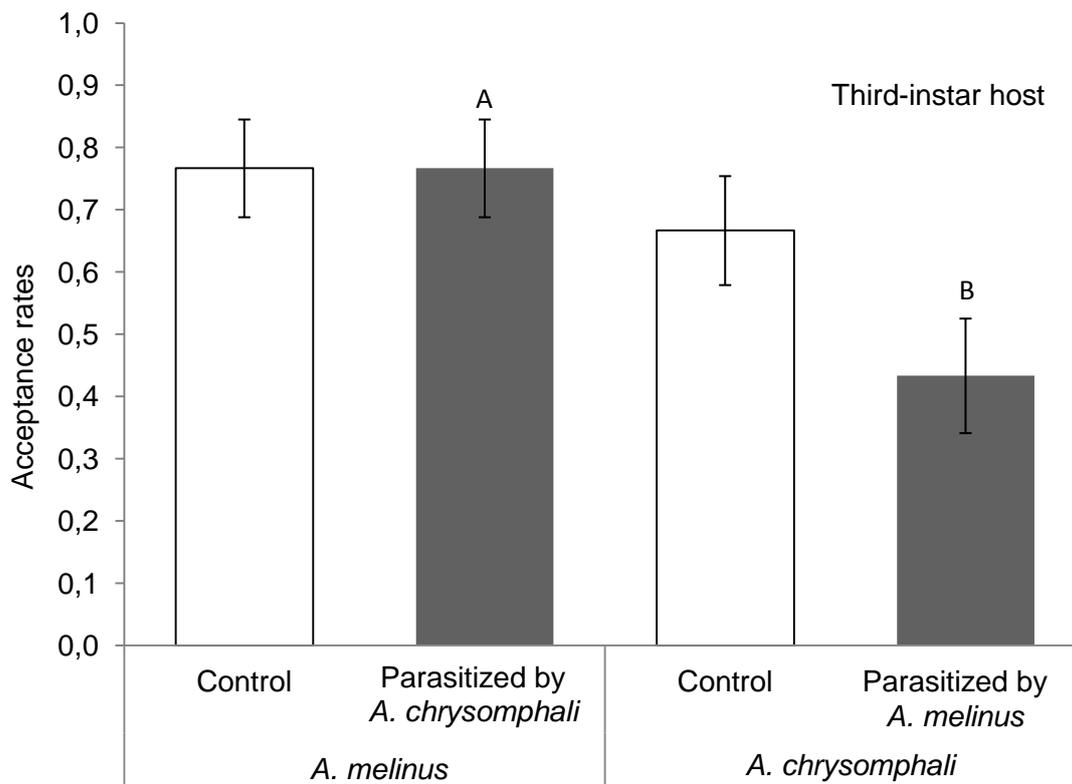
518 **Fig. 2.** Clutch size (mean number of eggs laid per host \pm SE) of *Aphytis melinus* and *A.*
519 *chrysomphali* when they accepted healthy (control) and heterospecific-parasitized second- and
520 third-instars of *Aurantii aurantii*. Different uppercase letters above columns denote significant
521 differences between parasitoid species at $P < 0.05$. Different lowercase letters above columns
522 denote significant differences between treatments (healthy vs. heterospecific-parasitized) within
523 a parasitoid species at $P < 0.05$.

524 **Fig. 3.** Effect of host instar (second- and third-instar) and order of attack on the outcome of
525 competition between *Aphytis melinus* and *A. chrysomphali* in multiparasitized *Aonidiella*
526 *aurantii*. Represented as the proportion of total progeny (mean \pm SE) produced by each species.
527

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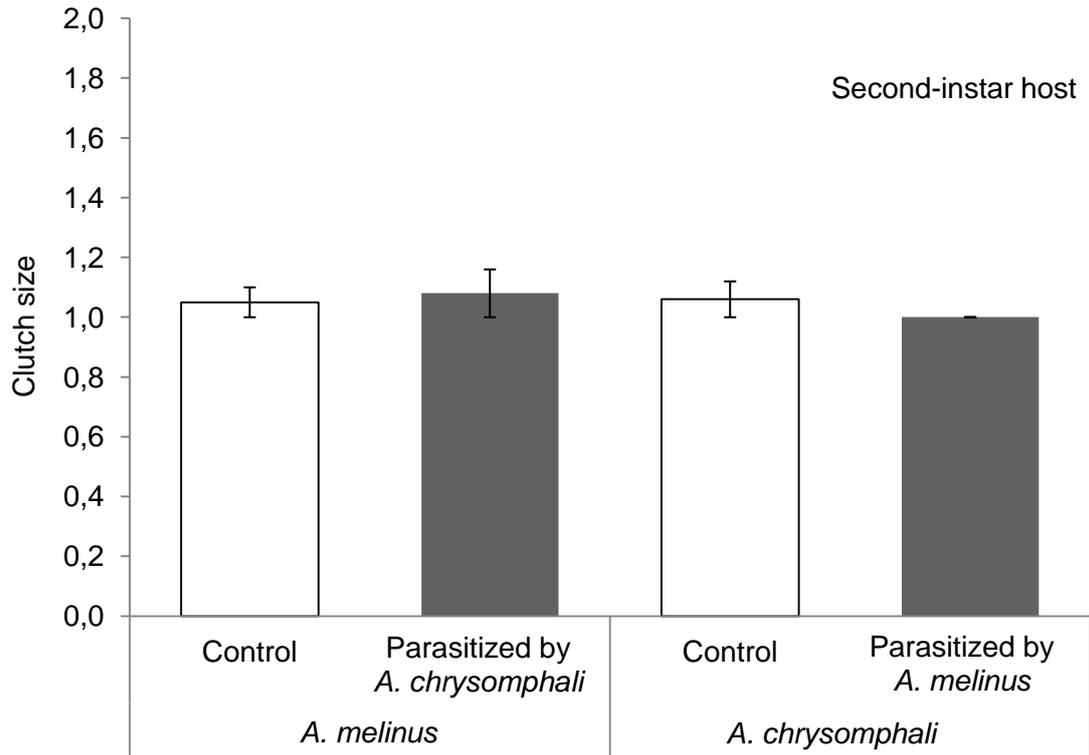


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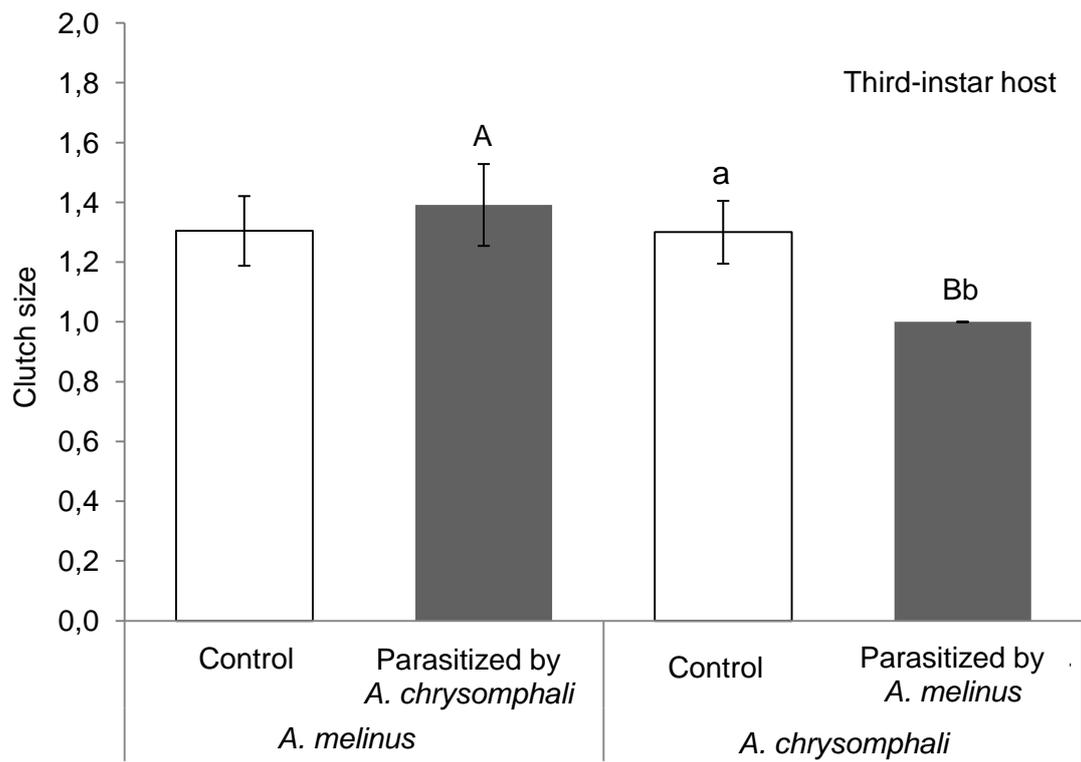
531 **Fig. 1.**

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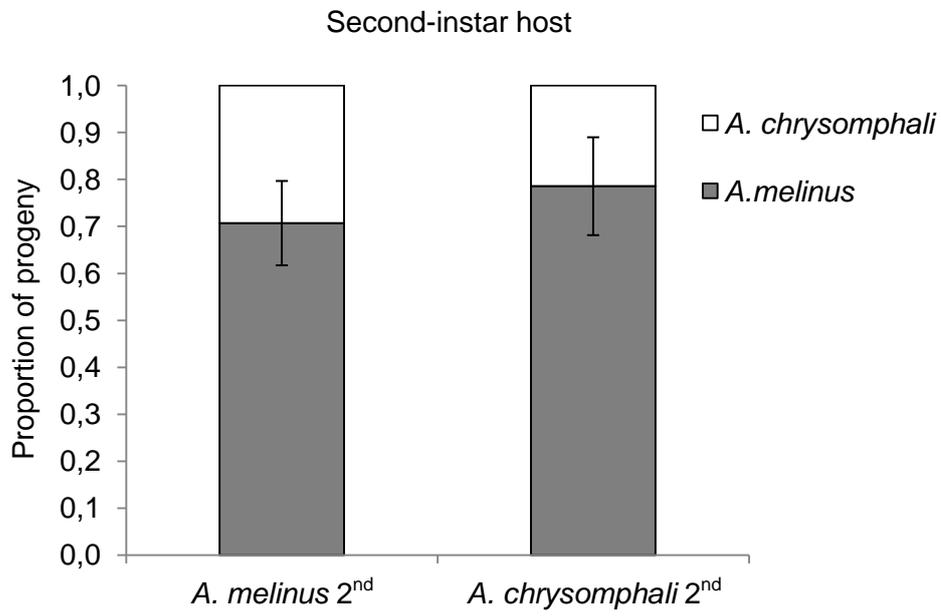


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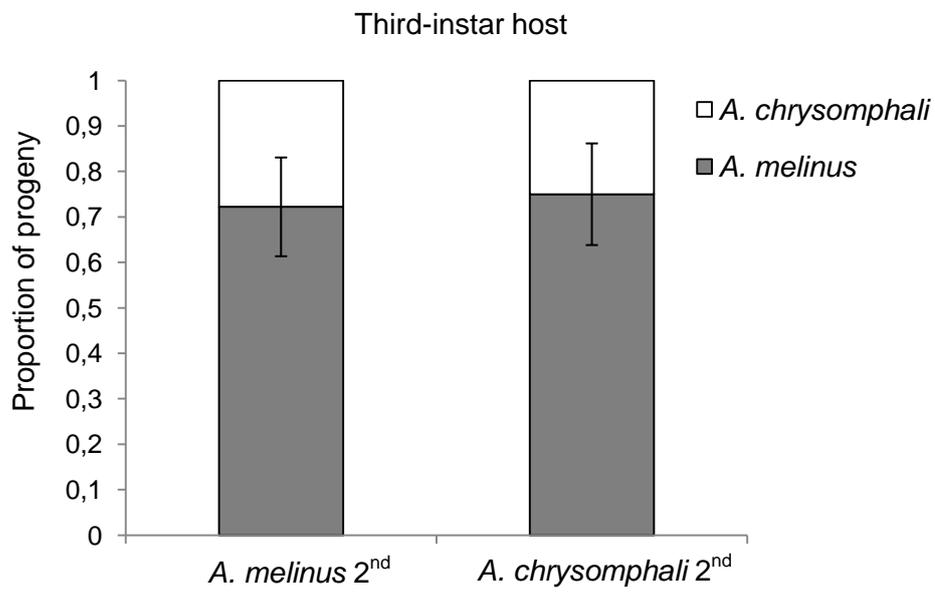
Fig. 2.

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541 **Fig. 3.**

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