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**Negative effect of global warming on biological control is mitigated by  
direct competition between sympatric parasitoids**

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## ABSTRACT

Parasitoids are among the most important and successful groups of natural enemies used in the biological control of insect pests. In most systems, several parasitoid species can parasitize the same pest. The coexistence of parasitoids in agroecosystems and their efficacy as biological control agents may be disrupted by global warming. An increase of approximately 3°C is predicted by the end of the twenty-first century in the Mediterranean basin (IPCC, 2014). In this context, we compared the present and future performance of two sympatric parasitoids of the genus *Aphytis* (Hymenoptera: Aphelinidae), which control the armoured scale *Aonidiella aurantii* (Hemiptera: Diaspididae) in Mediterranean citrus, either alone or in combination. The net reproductive rate (R<sub>0</sub>) of the introduced *Aphytis melinus* DeBach was higher than that of its competitor, the native *Aphytis chrysomphali* (Mercet), under current conditions. The two parasitoids responded differently to higher temperature and competition. The R<sub>0</sub> of *A. chrysomphali* decreased by 50% when both parasitoids competed in the same patch, but was not affected by the temperature increase. The R<sub>0</sub> of *A. melinus* decreased approximately 40% with the increase in temperature because the proportion of females was reduced. However, the presence of *A. chrysomphali* competing in the same patch mitigated the negative effect of the increase in temperature on *A. melinus* (R<sub>0</sub> decreased by only 20%). Overall, our results suggest that global warming will have a negative effect on the biological control of *A. aurantii* and that this effect will be higher in areas, such as southern Spain, where *A. melinus* has displaced *A. chrysomphali*.

**Keywords:** *Aphytis*; *Aonidiella aurantii*; citrus; interspecific competition; displacement

## 1. INTRODUCTION

Due to global warming, average temperatures have risen by approximately 0.8°C since the early twentieth century, and a further increase of 3°C is predicted by the end of the twenty-first century for summer temperatures in the Mediterranean basin (IPCC, 2014). Because insects are ectotherms, their physiology, behaviour and fitness are directly affected by ambient temperature variation (Hance et al., 2007). The impact of global warming is likely to be less predictable in higher trophic levels than in lower trophic levels because the former also depend on the capacity of the lower trophic level to adapt to these changes (Hance et al., 2007; van Baaren et al., 2010). That impact is observed for parasitoids in which immature individuals feed and develop in (endoparasitoids) or on (ectoparasitoids) hosts. Parasitoids are the most important and successful group of natural enemies used in the biological control of insect pests (Godfray, 1994; Jervis, 2005), and their efficacy could be disrupted by changes in environmental conditions (van Baaren et al., 2010).

An increase in temperature can affect host-parasitoid relationships, mostly because they may have different thermal preferences (developmental or phenological), which can affect the temporal or geographical synchronization of the suitable instar/stage of the host with the adult parasitoids (Hance et al., 2007). For parasitoids, an increase in temperature can affect their morphology (body size, wing size, wing loading), fecundity, longevity, dispersal capacity, metabolism rate, trade-offs between life-history traits, capacity to locate and evaluate host quality and the capacity of the larvae to evade or overcome the host immune response (Hance et al., 2007; Moiroux et al., 2010; Vayssade et al., 2012; Vuarin et al., 2012; van Baaren et al., 2010). Indeed, an increase in temperature may induce a number of physiological changes, the cost of which may be expressed by a reduction in reproductive output, decrease in growth of immature stages and in lifespan and/or changes in mating behaviour (Angilletta, 2009; Hance et al., 2007; Omer et al., 1996; Řežucha et al., 2010). Understanding these characteristics is crucial for using parasitoids as biological control agents.

Because of interspecific differences in thermal responses among parasitoid species (Berg et al., 2010), global warming can also have a major influence on interspecific competition between species (Northfield and Ives, 2013). As such, global warming will have consequences for parasitoid

species distributions, community compositions, and ecosystem services, i.e., biological control (Bale et al., 2002; Hance et al., 2007; Northfield and Ives, 2013; Tougeron et al., 2017).

In this context, we investigated the influence of the expected temperature increase on the efficacy and competition of parasitoids of the genus *Aphytis* (Hymenoptera: Aphelinidae), which are the most successful and widespread biological control agents of *Aonidiella aurantii* (Hemiptera: Diaspididae) in citrus (DeBach and Rosen, 1991; Forster and Luck, 1996; Murdoch et al., 2005; Pekas et al., 2016).

These specialist parasitoids can reduce their shared host to levels nearly 200 times below the average density observed in their absence (DeBach et al., 1971), suggesting strong resource competition between parasitoid species because they parasitized the same host stages and niches (Borer et al., 2004; Pekas et al., 2016). Species of the genus *Aphytis* represent one of the best-known cases of competitive displacement in insects (Luck et al., 1982; Luck and Nunney, 1999; Luck and Podoler, 1985; Pekas et al., 2016; Sorribas et al., 2010). In the Mediterranean basin, *A. aurantii* became a key citrus pest at the end of the last century, and it was rapidly parasitized by the native parasitoid *Aphytis chrysomphali* (Mercet). Later, its coevolved parasitoid *Aphytis melinus* DeBach was introduced into a classical biological control programme, and it has displaced *A. chrysomphali* in some areas, whereas both species coexist in others (Boyero et al., 2014; Sorribas et al., 2010). Both parasitoids are present in the field from February to November showing higher parasitism levels between August and November (highest in September) and lower in February and March (Pekas et al., 2010). Although *A. chrysomphali* reproduces parthenogenetically and produces only females (Pina, 2007; Cebolla et al., 2017), *A. melinus* is considered to be a superior competitor in the field because it has a higher capacity for dispersion (McLaren, 1976) and is better adapted to dry and hot climates where citrus is cultivated (Abdelrahman, 1974a; Rosen and DeBach, 1979). Consequently, the relative proportion of *A. melinus* is higher during the warm months, and the abundance of *A. chrysomphali* increases from south to north, being higher in the cooler northern areas. This alteration in parasitoid dominance could be one of the reasons why the more efficient parasitoid *A. melinus* has not completely displaced *A. chrysomphali* in most Valencia citrus orchards (Sorribas et al., 2010). Therefore, we hypothesize that the superiority of *A. melinus* will be accentuated when the temperature increases due to global

warming, possibly leading to the extinction of the weaker competitor. To test this hypothesis, we performed laboratory experiments to evaluate how the increase in temperature in summer, when *A. chrysomphali* is a weaker competitor, will affect i) the fecundity and parasitism rate of both parasitoids when they exploit hosts alone or in competition and ii) the influence of temperature and competition on their efficacy as the biological control of *A. aurantii* by comparing their net reproductive rates ( $R_0$ ) and host-induced mortality.

## 2. MATERIALS AND METHODS

### 2.1 Abiotic conditions and treatments

The climate data used for rearing hosts and parasitoids and for experiments was  $26/20 \pm 1^\circ\text{C}$  (day/night) to mimic average summer temperatures in the last fifteen years in the Valencia Region (Moncada meteorological station: <http://riegos.ivia.es/>) and  $29/23 \pm 1^\circ\text{C}$  (day/night) to mimic warmer average summer temperatures predicted by the end of the twenty-first century (IPCC, 2014) at  $70 \pm 5\%$  RH and. Similarly, the photoperiod was selected to mimic the average number of light-darkness hours in summer months in the Valencia Region (Moncada meteorological station: <http://riegos.ivia.es/>), this is L:D 12:12.

The experiment consisted of four treatments in the two different abiotic conditions explained above for a total of eight combinations: 1) exploitation of 40 third-instar *A. aurantii* per patch by a single female of *A. melinus* ( $n = 42$ ); 2) exploitation of 40 third-instar *A. aurantii* per patch by a single female of *A. chrysomphali* ( $n = 46$ ); 3) exploitation of 40 third-instar *A. aurantii* per patch by *A. melinus* and *A. chrysomphali* simultaneously ( $n = 44$ ); and 4) 40 third-instar *A. aurantii* (number of replicates = 37).

### 2.2 Insects

The phytophagous host, *Aonidiella aurantii*, was reared on lemons from a laboratory colony at the Instituto Valenciano de Investigaciones Agrarias, IVIA (Moncada, Valencia, Spain). This colony was initiated in 1999 from scales collected in citrus fields in Alzira (Valencia, Spain) and renewed

every 2-3 years with field-collected scales. Approximately 2/3 of the surface of each lemon was covered with red paraffin around the mid-section to retard its desiccation. The red paraffin was prepared with a mixture of 1 kg of paraffin pearls (Parafina USP Perlas; Guinama S.L., Alboraya, Spain) and 1 g of red pigment (Sudan III; Panreac Química S.A., Castellar del Vallés, Spain). The remaining surface (aprox. 24-cm<sup>2</sup> area) of the lemons was infested by exposing them to gravid female scales of the *A. aurantii* colony for 48 h at 27 ± 1°C at 70 ± 5% RH and LD 14:10. Once infested, lemons were kept in climate chambers (SANYO MLR- 350; Sanyo, Japan) at the two temperature conditions described above until female scales reached the third nymphal instar (19-25 days), which was later used for rearing the parasitoids and for the experiments (Treatments 2,3 and 4) or until females become gravid (43-50 days) for the fecundity investigation (Treatment 1).

*Aphytis melinus* and *A. chrysomphali* are facultative gregarious ectoparasitoids (Rosen & DeBach, 1979). Females of both species mature eggs throughout their adult life (synovigenic) and lay between 2 and 6 eggs per day (Heimpel et al., 1997; Casas et al., 2000; Tena et al., 2015). These species are also idiobibionts (i.e., the host is paralyzed and arrests development once parasitized) and feed on the haemolymph of hosts which they do not use to lay eggs. Individuals of both species were obtained by exposing third-instar *A. aurantii* on lemons to parasitism by insectary-reared adult wasps. The colonies of *A. melinus* and *A. chrysomphali* were initiated in 2008 and 2013, respectively, from *A. aurantii* scales collected in citrus fields located in the Valencia region (Valencia, Spain). Both colonies are renewed yearly with field-collected parasitoids.

For this experiment, five adults of each species were transferred to rearing cages containing third-instar *A. aurantii* on lemons reared in the two abiotic conditions (described above) and were maintained in climatic chambers at these two abiotic conditions to obtain parasitoids. Between 10-12 days later, scales were observed under binoculars and late-stage pupae of both parasitoids were removed from parasitized scales. Pupae were held in crystal vials 8 mm in diameter and 35 mm long tapped with a cotton plug and with a drop of honey on the wall. At emergence, parasitoids were held in these vials for one day to obtain mated females of *A. melinus* [*A. chrysomphali* reproduces parthenogenetically (Gottlieb *et al.* 1998)]. Next, parasitoids were sexed, and females were isolated in

vials (same as above). One *A. aurantii* female was introduced daily to let them feed on the host until they were used 2-3 days later (Heimpel *et al.* 1997). Vials were stored in a climatic chamber at the two different abiotic conditions until they were used in the experiment (Treatments 2, 3 and 4).

### 2.3 Experimental microcosm and measures

For all treatments, the experimental microcosm was composed of a polystyrene plastic box (10 × 14×14 cm) with a lateral hole (4 × 9 cm) covered with muslin. One lemon infested with *A. aurantii* was introduced in the box. An acrylic cylinder (5 cm diameter) was used to hold the lemons. We used a dissecting microscope to select 40 scales from the lemon surface, and we removed the rest using an entomological pin and a paper towel moistened with water. The selected scales were  $0.85 \pm 0.05 \text{ mm}^2$ . To estimate scale sizes, photos of the scales were taken with a Leica EC 3 3.1 megapixel digital colour camera (Leica Microsystems GmbH, Spain), and the images were processed with Leica LAS EZ imaging software for Windows (Leica Microsystems GmbH, Spain).

### 2.4 Effect of competition and a temperature increase on parasitoid fitness and biological control potential

For treatments 1, 2 and 3, parasitoids were introduced in the box and remained in contact with hosts for 72 hours. *Aphytis* parasitoids parasitize between two to five hosts per day and per female (Heimpel *et al.*, 1997; Pina, 2007; Cebolla *et al.*, submitted). Therefore, microcosms contained hosts *ad libitum* in all the treatments. A drop of honey was added to the inside wall as a food source. Microcosms were held in climatic chambers at one of the two abiotic conditions. Seven days after parasitoid removal, scale covers were carefully removed with an entomological needle under a binocular lens. The numbers of unparasitized (turgent), parasitized and dead hosts were counted. We used these data to evaluate i) the number of parasitized and ii) the dead hosts per patch. The number of dead was the sum of parasitized and hosts dead by unknown reasons (caused by the parasitoids or natural death of hosts). *Aphytis* parasitoids cause the death of their host when they host-feed and when they probe the host with their ovipositor even when they reject the host (Cebolla *et al.*, 2017).

The parasitoid pupae of each parasitized host were measured and then transferred to crystal vials as described above. Between one and three pupae were obtained per host. To measure the effect



of temperature and parasitoid competition on parasitoid size (measured as the length of the pupae), only pupae of females that developed in solitary were considered because sex and brood size can affect pupae size (Salt, 1940; Abdelrahman, 1974b). At emergence, parasitoids were identified and sexed. *Aphytis chrysomphali* pupae are identified by the presence of a longitudinal black line on the mesosternum which is not present in *A. melinus* (Rosen & DeBach, 1979). Progeny production was calculated as the number of adults of each species that emerged per patch. Secondary sex ratio was calculated as the proportion of males of each species per host and patch.

## 2.5 Effect of an increase in temperature on host fecundity

To determine the effect of an increase in temperature on host fecundity (Treatment 4), we used the same methodology described by Vanaclocha et al. (2012). In detail, infested lemons were kept in climatic chambers at the two abiotic conditions. Before the female scales began to produce crawlers (~40 days), four scales per lemon were isolated with a double-sided sticky plastic ring (3M Scotch®; CergyPontoise Cedex, France) to trap the crawlers. Sticky plastic rings were replaced twice a week and the crawlers were counted under binoculars. The total number of progeny was calculated as the sum of each ring per female.

## 2.6 Effect of an increase in temperature on parasitoid net reproductive rate (R0)

The net reproductive rate (R0) during 72 hours was compared between the two parasitoid species in treatments 1, 2 (without competition) and 3 (with competition). R0 was calculated as  $R_0 = \sum l_x \cdot m_x$ . R0 represents the mean number of female offspring produced by each female (Birch, 1948; Carey, 1993), where x is the age class,  $l_x$  is the probability of survival till class x, and  $m_x$  is the fecundity of class x. Values of  $R_0 < 1$  indicate a declining population,  $R_0 > 1$  an increasing population, and  $R_0 = 1$  a stable population (Carey, 1993). To facilitate comparison of the demographic parameters, we calculated the standard errors (SE) of the demographic parameters at each temperature using a jackknife algorithm described by Meyer et al. (1986). The jackknife analysis method removes one observation at a time from the original dataset and recalculates the statistic of interest from the truncated data. The values of R0 per female and per temperature were calculated in Microsoft Excel and its numerical solver. The method can estimate R0 values with their respective jackknife variances.

## 2.7 Statistical analyses

Proportional and count data were analysed with generalized linear models (GLMs). Initially, we assumed a Poisson error variance for count data (number of parasitized hosts and number of progeny per patch) and a binomial error variance for proportional data (sex ratio and hosts killed per patch). We assessed the assumed error structures by a heterogeneity factor equal to the residual deviance divided by the residual degrees of freedom. If we detected an over- or underdispersion, we re-evaluated the significance of the explanatory variables using an F test after rescaling the statistical model by a Pearson's chi-square divided by the residual degrees of freedom (Crawley, 2007). We present the means of untransformed proportion and count data (in preference to less intuitive statistics such as the back-transformed means of logit-transformed data).

We compared pupa size of the offspring, host fecundity and  $R_0$  using ANOVAs. The normality assumption was assessed using Shapiro's test, and the homoscedasticity assumption was assessed with the Levene test. All data analyses were performed with the R freeware statistical package (<http://www.R-project.org/>).

## 3. RESULTS

### 3.1 Effect of competition and an increase in temperature on parasitoids fitness and biological control potential

#### 3.1.1 Number of parasitized hosts

The number of hosts parasitized by *A. melinus* females was independent of the increase in temperature ( $F_{1, 83} = 0.34$ ;  $P = 0.56$ ) and the presence of their competitor, *A. chrysomphali* ( $F_{1, 83} = 0.31$ ;  $P = 0.58$ ) (Fig. 1). The interaction between temperature and competition was not significant ( $F_{1, 82} = 0.31$ ;  $P = 0.58$ ).

The number of hosts parasitized by *A. chrysomphali* females was independent of temperature ( $F_{1, 87} = 0.15$ ;  $P = 0.70$ ) but it decreased with the presence of *A. melinus* (interspecific competition) ( $F_{1, 87} = 13.92$ ;  $P < 0.001$ ) (Fig. 1). The interaction between temperature and competition was not significant ( $F_{1, 86} = 0.74$ ;  $P = 0.79$ ).

### 3.1.2 Progeny production

The amount of progeny emerging from the hosts parasitized by *A. melinus* was independent of temperature ( $F_{1,83} = 0.044$ ;  $P = 0.83$ ) and competition ( $F_{1,83} = 0.55$ ;  $P = 0.46$ ) (Fig. 2). The interaction between temperature and competition was not significant ( $F_{1,82} = 0.041$ ;  $P = 0.84$ ).

The amount of progeny emerging from the hosts parasitized by *A. chrysomphali* was independent of temperature ( $F_{1,87} = 0.012$ ;  $P = 0.91$ ) but it decreased in the presence of *A. melinus* (competition) ( $F_{1,87} = 13.86$ ;  $P < 0.001$ ) (Fig. 2). The interaction between temperature and competition was not significant ( $F_{1,86} = 0.33$ ;  $P = 0.56$ ).

### 3.1.3 Parasitoid size

The pupae size of *A. melinus* and *A. chrysomphali* offspring was not affected by the increase in temperature (*A. melinus*:  $F_{1,46} = 2.93$ ;  $P = 0.094$ ; *A. chrysomphali*:  $F_{1,41} = 0.26$ ;  $P = 0.61$ ) or competition (*A. melinus*:  $F_{1,46} = 0.31$ ;  $P = 0.58$ ; *A. chrysomphali*:  $F_{1,41} = 0.044$ ;  $P = 0.83$ ) (Fig. 3). The interaction between temperature and competition was not significant in either parasitoid species (*A. melinus*:  $F_{1,45} = 0.032$ ;  $P = 0.86$ ; *A. chrysomphali*:  $F_{1,40} = 1.54$ ;  $P = 0.22$ ).

### 3.1.4 Sex ratio

The secondary sex ratio (proportion of males out of the total emerging wasps) of *A. melinus* was affected by temperature ( $F_{1,65} = 5.37$ ;  $P = 0.024$ ). The proportion of males increased with temperature. However, sex ratio was independent of the presence of the competitor, *A. chrysomphali* ( $F_{1,65} = 1.83$ ;  $P = 0.18$ ) (Fig. 4). The interaction between temperature and competition was not significant ( $F_{1,64} = 0.049$ ;  $P = 0.83$ ). As expected, all emerging *A. chrysomphali* were females.

### 3.1.5 Host mortality

In the absence of competition, the number of *A. aurantii* hosts killed by *A. melinus* and *A. chrysomphali* was not affected by the increase in temperature (*A. melinus*:  $F_{1,40} = 0.24$ ;  $P = 0.63$ ; *A. chrysomphali*:  $F_{1,44} = 0.028$ ;  $P = 0.87$ ). The same phenomenon occurred when both parasitoids were competing ( $F_{1,86} = 0.18$ ;  $P = 0.68$ ) (Fig. 5).

### 3.2 Effect of an increase in temperature on host fecundity

*Aonidiella aurantii* fecundity, measured as the total number of crawlers, was not affected by an increase in temperature (20-26°C =  $224.82 \pm 11.99$  crawlers; 23-29°C =  $242.29 \pm 23.37$  crawlers;  $F_{1,35} = 0.48$ ;  $P = 0.49$ ).

### 3.3 Effect of an increase in temperature on parasitoid net reproductive rate (R0)

The net reproductive rate (R0) of *A. melinus* was affected by the interaction between temperature and competition ( $F_{1,88} = 279.73$ ;  $P < 0.0001$ ) (Table 1), indicating that R0 increased with competition when there is a high temperature but it decreased with competition at a low temperature. This finding showed that the negative effect of a temperature increase is reduced in the presence of an inferior competitor.

The net reproductive rate (R0) of *A. chrysomphali* was affected by the interaction between temperature and competition ( $F_{1,91} = 50.69$ ;  $P < 0.0001$ ) (Table 1), indicating that R0 decreased with the increase in temperature when there was no competition but it increased with the increase in temperature when there was competition. This showed that the negative effect of a temperature increase is absent in the presence of a superior competitor.

## 4. DISCUSSION

The presence of the introduced parasitoid *Aphytis melinus* reduced the efficacy of the native *Aphytis chrysomphali* as a biological control agent of the California red scale (*Aonidiella aurantii*) by approximately 50% in the present conditions. The potential of *Aphytis* parasitoids was measured using

the net reproductive rate ( $R_0$ ), which represents the mean number of female offspring produced by each female, considering also the probability of survival of the female. The reduction in net reproductive rate was mainly due to the reduction of scales successfully parasitized by *A. chrysomphali* females when they searched in the same patch as *A. melinus* females compared to patches without competition. Several biological traits described previously in both parasitoids can explain the reduction in the number of scales successfully parasitized by *A. chrysomphali* when it shared the patch with its competitor. *Aphytis melinus* females tend to kill *A. chrysomphali* eggs (i.e., ovicide) before laying their own egg when they are locating a host already parasitized by *A. chrysomphali* (Cebolla et al., 2017a, b). Moreover, *A. melinus* larvae seem to be more aggressive than those of *A. chrysomphali* (Cebolla et al., 2017a, b). Therefore, *A. melinus* might have killed some of the progeny of *A. chrysomphali*, reducing its  $R_0$ . Another non-mutually exclusive reason that may explain the reduction of *A. chrysomphali*  $R_0$  is that females of this species might avoid using patches where *A. melinus* are searching. However, this hypothesis needs to be tested. Overall, the 50% reduction of *A. chrysomphali*  $R_0$  when it searches in the same patch as *A. melinus* represents another result that explains the displacement of *A. chrysomphali* by *A. melinus* in southern Spain (Sorribas et al., 2010; Boyero et al. 2014). Other reasons are the lower searching capacity and tolerance to hot and dry climates of *A. chrysomphali* (Abdelrahman, 1974a, b; McLaren, 1976). Both parasitoids, however, coexist in eastern Spain where the proportion of *A. chrysomphali* increases with latitude and colder temperatures (Sorribas et al., 2010; Pekas et al., 2010, 2016). Under this scenario, we expected that the predicted increase in temperature under global warming, especially in the summer, would negatively affect the net reproductive rate of *A. chrysomphali*, accelerating its displacement by *A. melinus*. Our results, however, showed that the net reproductive rate of *A. chrysomphali* was not affected by the expected increase of temperature.

The expected increase in temperature in summer will hinder the efficacy of *A. melinus* as a biological control agent of *A. aurantii*, as the  $R_0$  of this parasitoid was reduced by half. The reduction of *A. melinus*  $R_0$  when temperature increased was mostly due to the reduction in the proportion of females (i.e., increase of the secondary sex ratio proportion). This result is in accordance with previous

studies of hymenopteran parasitoids, which suggest that the proportion of males increases with temperature (King, 1987). The biological reasons behind the change in sex ratio are less clear. Two hypotheses might explain the increase in *A. melinus* sex ratio with temperature. First, mothers might consider high temperatures unfavourable for the development of their offspring and thus may have intentionally allocated sons, as suggested by Force and Messenger (1964) and Moiroux et al. (2014). Second, the high temperatures used in this assay might also decrease the ability of *A. melinus* to mate, as was previously observed in parasitoids exposed to hot thermal stresses (Abram et al., 2016; Jørgensen et al., 2006; Krebs and Loeschke, 1994; Patton and Krebs, 2001; Rohmer et al., 2004; Roux et al., 2010; Sisodia and Singh, 2006; Wilkes, 1963). In our assay, adult parasitoids were paired in small vials under the two selected abiotic conditions before the experiments started. Two out of the 24 couples exposed to the temperatures expected for 2035 did not produce females, whereas all couples produced at least one female under the current summer temperatures. This result suggests that mating was negatively affected by the increase in temperature but can, together with other traits, explain the increase in sex ratio. Conversely, we do not expect that host size and/or the infection of *A. melinus* by *Wolbachia* affected the sex ratio of *A. melinus* because we used hosts of similar size in the experiment (see section 2.3 in Materials and Methods) and temperatures below 30°C do not completely cure *A. melinus* of the infection (Vasquez et al., 2011). The curation of females might have produced incompatible crosses and production of males by cured females (Vasquez et al., 2011).

The presence of *A. chrysomphali* did not reduce *A. melinus*  $R_0$  when females of both species shared the same patch. Additionally, the competition between both parasitoids mitigated the negative effect of the increase in temperature on *A. melinus*. Under the summer temperatures expected for the end of the twenty-first century, the introduced parasitoid had higher  $R_0$  when females competed with *A. chrysomphali* than when they searched alone in a patch. Generally, high temperatures affect the metabolic rate of insects and can diminish their locomotion speed and activity level (Vogt et al. 2003; Irlich et al. 2009), increasing, in the case of the hymenopteran parasitoids, the handling time per oviposition (Langer et al., 2004; Wu et al., 2011). The presence of a competitor could, however, stimulate *A. melinus* to complete oviposition faster as occurs in the solitary egg parasitoid *Enoggera*

*nassau* (Griault) (Hymenoptera: Pteromalidae) when it shares a patch with competitors (Mansfield, 2016). Another possibility is that the presence of a competitor provoked an aggressive behaviour in *A. melinus*. There are numerous studies linking female-female competition and aggressiveness in diverse animal taxa (Stockley and Campbell, 2013). This aggressiveness could be reflected in direct aggressive behaviours towards the competing parasitoid, which would explain the reduction of *A. chrysomphali*  $R_0$  when competing together but could also be reflected in an increase in parasitism that could compensate for the diminution of the  $R_0$  consequence of the temperature increase.

Overall, our study highlights the importance of considering competition to predict the consequences of global warming for biological control. Our results, however, have to be interpreted with care, since these are based on small microcosms (cages) and, therefore, the effect of global warming on the dispersion capacity of both parasitoids has not been considered. Further research could use larger cages or flying studies under different temperatures to include their dispersion capacity and to confirm the results. Nevertheless, our data strongly suggests that the expected increase in temperature in the Mediterranean basin will negatively affect the introduced parasitoid *A. melinus* and, consequently, the biological control of *A. aurantii*, one of the main citrus pests (Tena and Garcia-Mari, 2011). Interestingly, in southern Spain and other areas where *A. melinus* has already displaced the native *A. chrysomphali*, the effect of global warming will be higher than in eastern Spain and other areas where both parasitoids coexist. This is because *A. chrysomphali*  $R_0$  will not be affected by the increase in temperature and competition will mitigate the negative effect of the increase in temperature on *A. melinus*. Several non-excluding hypotheses have been proposed to explain their coexistence in eastern Spain. These hypotheses include the presence of alternative hosts and climate, conditional patch partitioning and local weather conditions (Pina, 2007; Sorribas et al., 2010; Pekas et al., 2016; Cebolla et al., 2017a). If the native is finally displaced in this area, a decline in the biological control of *A. aurantii* can also be expected.

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**Table 1.** Net reproductive rate (R0) of the parasitoids *Aphytis melinus* and *A. chrysomphali* at two temperatures, with and without interspecific competition.

Parasitoid species and competition	R0	
	20-26°C	23-29°C
<i>A. melinus</i>		
Without competition	8.53 ± 0.04 aA	4.64 ± 0.14 bB
Competition	7.02 ± 0.03 aB	5.93 ± 0.05 bA
<i>A. chrysomphali</i>		
Without competition	3.64 ± 0.04 aA	3.38 ± 0.03 bA
Competition	1.74 ± 0.02 bB	1.89 ± 0.02 aB

Data are presented as the mean ± SE, obtained by the jackknife method. Different lowercase letters denote significant differences between temperatures within a treatment (without competition and with competition) and parasitoid species at  $P < 0.05$ . Different uppercase letters denote significant differences between treatments (with and without competition) within a temperature and parasitoid species at  $P < 0.05$ .

**Figure legend**

**Fig. 1.** Effect of temperature (20-26°C or 23-29°C) and interspecific competition on the number of hosts parasitized by the parasitoids *Aphytis melinus* and *A. chrysomphali* during 72 hours. The different temperatures represent the mean temperature of summer from 2009 to 2014 in the Mediterranean basin and the temperature predicted by the end of the twenty-first century by the IPCC. Different letters above columns denote significant differences between treatments (with and without competition) within a parasitoid species and temperature at  $P < 0.05$ .

**Fig. 2.** Effect of temperature (20-26°C or 23-29°C) and interspecific competition on the number of progeny of the parasitoids *Aphytis melinus* and *A. chrysomphali* during 72 hours. The different temperatures represent the mean temperature of summer from 2009 to 2014 in the Mediterranean basin and the temperature predicted by the end of the twenty-first century by the IPCC. Different letters above columns denote significant differences between treatments (with and without competition) within a parasitoid species and temperature at  $P < 0.05$ .

**Fig. 3.** Effect of temperature (20-26°C or 23-29°C) and interspecific competition on the pupa size of the parasitoids *Aphytis melinus* and *A. chrysomphali*. The different temperatures represent the mean temperature of summer from 2009 to 2014 in the Mediterranean basin and the temperature predicted by the end of the twenty-first century by the IPCC).

**Fig. 4.** Effect of temperature (20-26°C or 23-29°C) and interspecific competition on the sex ratio of the parasitoid *Aphytis melinus*. Presented as the mean proportion of males of *A. melinus* ( $\pm$  SE). The different temperatures represent the mean temperature of summer from 2009 to 2014 in the Mediterranean basin and the temperature predicted by the end of the twenty-first century by the IPCC. Different letters above columns denote significant differences between temperatures within the same competition treatment at  $P < 0.05$ .

**Fig. 5.** Effect of parasitoid species (*Aphytis melinus* or *A. chrysomphali*), temperature (20-26°C or 23-29°C) and parasitoid competition on the mortality of their common host, *Aonidiella aurantii*. Presented as the mean number of *A. aurantii* killed ( $\pm$  SE) by the parasitoids during 72 hours. The different temperatures represent the mean temperature of summer from 2009 to 2014 in the Mediterranean basin and the temperature predicted by the end of the twenty-first century by the IPCC.



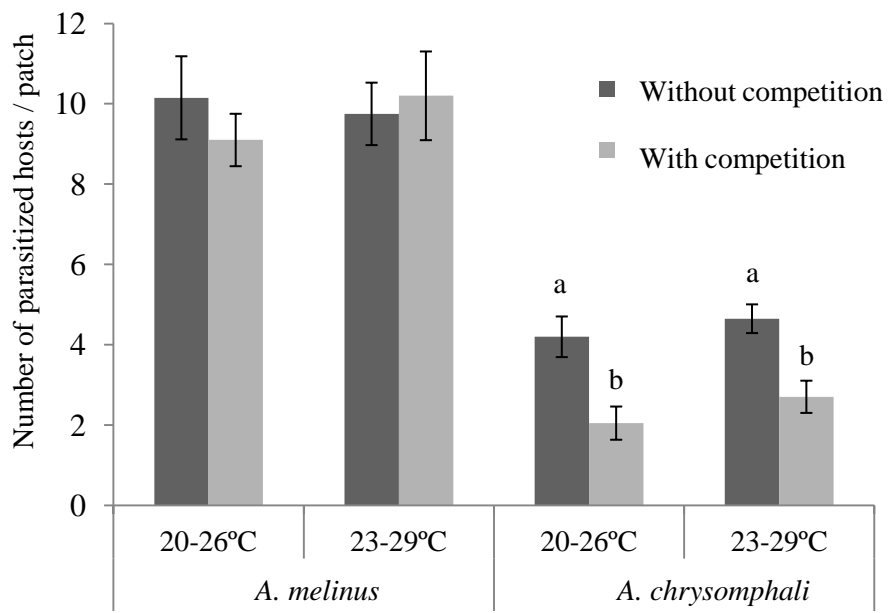


Fig. 1.

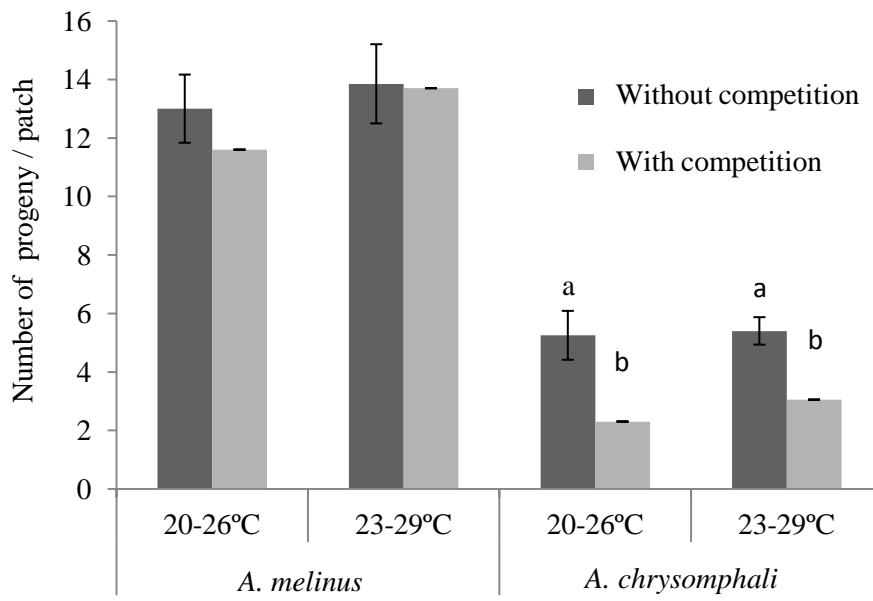
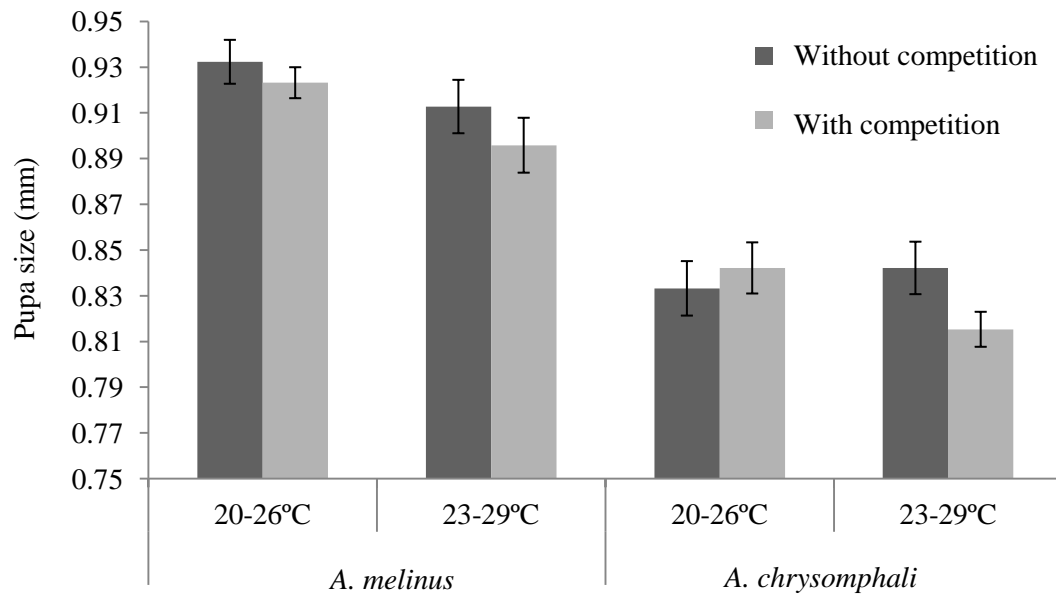
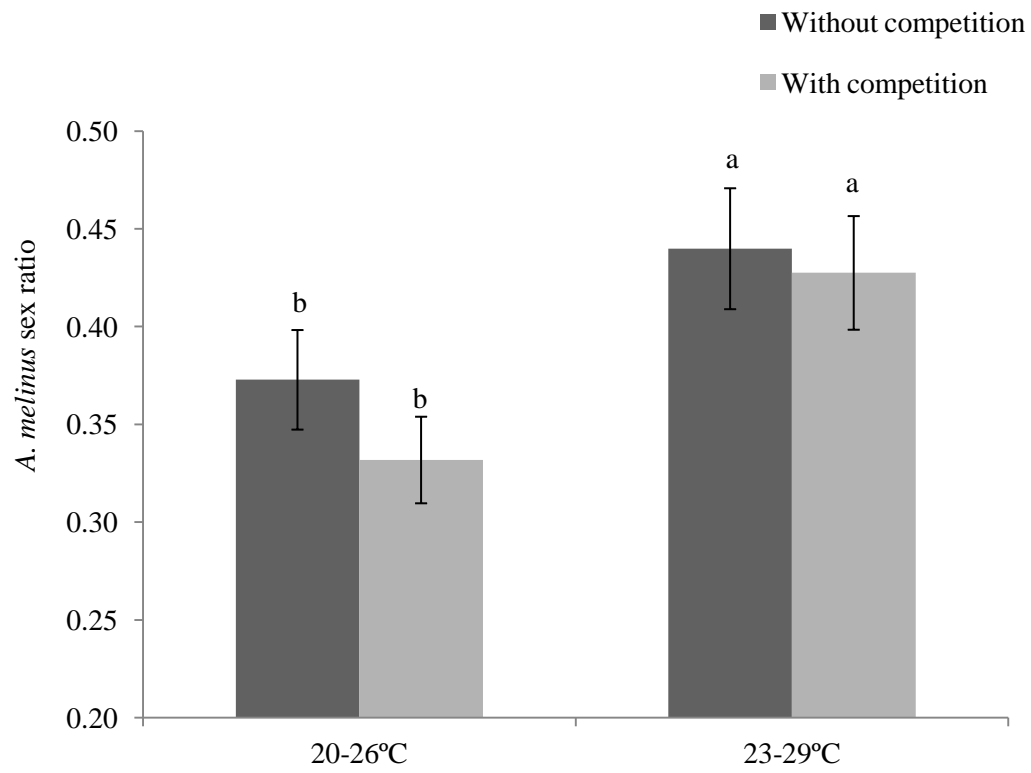
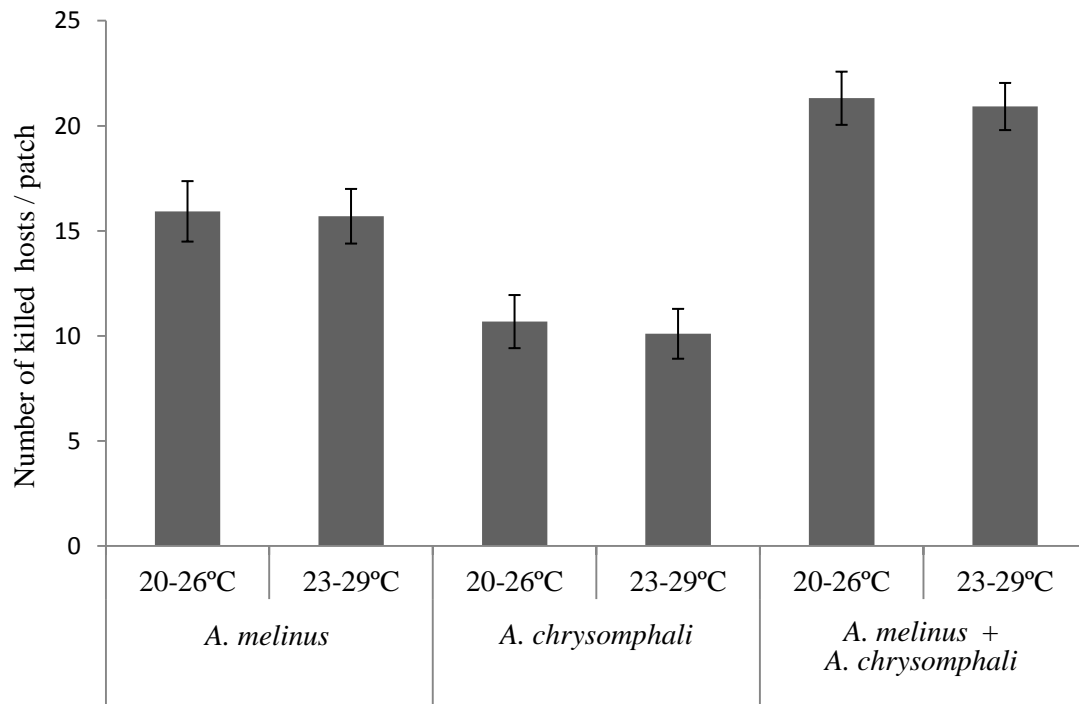


Fig. 2.

**Fig. 3.**

**Fig. 4.**

**Fig. 5.**

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**Highlights**

Control of *Aonidiella aurantii* by two parasitoids was measured under two temperature regimes

*Aphytis chrysomphali*  $R_0$  was decreased by 50% in competition, but was unaffected by higher temperature

*A. melinus*  $R_0$  decreased 40% at higher temperature because the proportion of females was reduced

*A. chrysomphali* presence partially mitigated the effect of higher temperature on *A. melinus*

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