Abstract: Longevity, developmental time and offspring survival of parasitoid wasps are decisive in their effective performance as biocontrol agents. Optimum temperature range determines parasitoid survival, development and reproduction. Thus, controlling this abiotic factor is a key to the success of pest management programs. Adult longevity, developmental time from egg to adult and survival of immatures of Aganaspis daci were assessed in the laboratory under different constant temperatures; adult longevity without hosts, but with the provision of water and honey, and developmental time and survival of immatures from host pupae, whose larvae had been exposed to parasitoids. Results showed that longevity depended on temperature decreasing in the range 15–20 °C (36–25 days), but was lower in the range 25–35 °C (10–7 days). Regarding developmental time from egg to adult and survival of immatures, our results showed that 20 and 25 °C are the most suitable temperatures. At 15 and 30 °C mortality of the immature stages was very high (> 90%) or developmental time to adult was very slow (> 3 months). Immatures did not survive at 35 °C. We found no significant differences in developmental time to adult or survival of immatures between 20 and 25 °C. The sex ratio of parasitoid progeny was female biased at 25 °C; the proportion of females increased at all cases with temperature. The $t_0$ and $K$ for total development were 8.5 °C and 500 DD, respectively. Our findings provide some guidance for future inundative or inoculative field releases of this parasitoid for the management of Ceratitis capitata in Spain.

Keywords: Medfly; Aganaspis daci; Longevity; Developmental time; Thermal requirements.
1. Introduction

*Aganaspis daci* (Weld) (Hymenoptera: Figitidae), a larval-pupal solitary primary endoparasitoid, is an efficient fruit-fly pest-control agent, which has been successfully used to control *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) (Baranowski et al., 1993). This species was first recorded in 1951 as a larval parasitoid of *Bactrocera dorsalis* (Hendel) (=*Dacus dorsalis*) (Diptera: Tephritidae) in Malaysia and Taiwan (Weld, 1951). Since then, it has been introduced into several countries to control tephritid species (Clausen, 1978; Wharton et al., 1981). In the Mediterranean basin, the first record of *A. daci* parasitizing larvae of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) was on the Greek Island of Chios in 2003 (Papadopoulos and Katsoyannos, 2003). Lately, it has been introduced in Israel and Egypt from a “colony” bred in USDA-ARS Hawaii (El-Heneidy and Ramadan, 2010). In Spain, only the species *Spalangia cameroni* Perkins and *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) were found as native pupal parasitoids of the medfly, with no records of *A. daci* during a four-year survey (2000−04). In 2003, an ongoing biocontrol project was started with several imported exotic egg-larval parasitoids: *Fopius arisanus* (Sonan), *Diachasmimorpha tryoni* (Cameron) and *D. longicaudata* (Ashmead) (Hymenoptera: Braconidae). Following the release trials of these species (2007−12), several specimens of *A. daci* were recovered from medfly larva collected from fig and citrus fruits in the summer of 2009. Phylogenetic analysis of cytochrome oxidase I (COI) and internal transcribed spacer (ITS) sequences from Spanish specimens confirmed their assignment to this species after comparison with individuals from Greece, Israel, Hawaii and Egypt, and to the closely related species *Aganaspis pelleranoi* (Brethes) and *Ganaspis xanthopoda* (Ashmead) (Hymenoptera: Figitidae) (Sabater-Muñoz et al., 2012). At present, the biology of this parasitoid on *C. capitata* is under study to assess its adaptability to mass rearing.

Despite the aforementioned research, to date little attention has been paid to those aspects of *A. daci* biology that may contribute to its use in biological monitoring projects. Among contributors to this field, we should highlight Clausen et al. (1965); Nuñez-Bueno (1982); Ovruski (1994); Andleeb et al. (2010); Hosni et al. (2011) and Tormos et al. (2013). Here we have investigated and reported on adult *A. daci* longevity without hosts, developmental time from egg to adult and survival of immatures in laboratory conditions (at several constant temperatures). Thermal constants are often used to create predictive models of pest development in various environments, for example: stored products, greenhouses and orchards, and the knowledge of thermal constants provide essential information to determine the development thresholds and the development rates of arthropod species (Malina and Praslička, 2008). Longevity and development rate usually play a key role in the effectiveness of parasitoid species.
as biocontrol agents. We have analysed these parameters of the biotic potential for an environmental variable, namely temperature, with the object to evaluate the potential of this parasitoid as a biological agent to control *C. capitata*.

2. Materials and methods

2.1. Study centre and insects

*A. daci* was obtained from a laboratory colony housed at the Valencian Institute of Agrarian Research [Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia, Spain]. This colony was established in 2010 with specimens obtained from medfly larvae taken from figs in a nearby Valencian village (Bétera, Spain). Since then, a laboratory rearing has been maintained on the host *C. capitata* (Martínez-Torres, 2011). The medfly has been reared at the IVIA for over 6 years, following the methodology of Pérez-Hinarejos and Beitia (2008). Experiments comply with Spanish law currently in force.

2.2. Experimental design

Two experiments were conducted to assess the effect of the temperature on the longevity and the developmental time from egg to adult and survival of immatures of *A. daci*. Both experiments were run in a climate cabinet (Sanyo MLR 350; Sartorius, Barcelona, Spain) under the following constant temperatures (with a variation (SD) of ± 0.2 °C): 15 °C, 20 °C, 25 °C, 30 °C and 35 °C, 65 ± 10% relative humidity and 16:8 h (light/darkness) photoperiod. L3 (third instar larvae) not exposed to parasitoids were used as control.

In experiment 1, we analysed longevity of males and females until adult death. In the assays we used translucent plastic ventilated cylinders (15 x 20 cm) with 10 individuals, from a newly emerged cohort (males or females), supplied with water and honey ad libitum. There were three replicates for each sex and each temperature. Cylinders were checked daily and mortality rates recorded.

Experiment 2 was designed to evaluate the developmental time and survival of immatures until adult emergence. In the assays we used translucent plastic boxes (20 x 15 x 10 cm), with 330 L3 of *C. capitata* that had been exposed in artificial diet previously to parasitism by a rearing cohort for 4 h. This cohort consisted of 100 males and 100 females, between 6 and 8 days old, kept separate from their hosts during 24 h in order to obtain a homogeneous clutch. There were three replicates for each temperature. Translucent plastic boxes were checked from the host pupation (2 or 3 days after exposing L3 to parasitoids) and emergence, sex ratio (males%) and mortality rates were recorded.
2.3. Statistical analysis

The effect of temperature on the longevity of adult parasitoids, as well as on developmental time from egg to adult emergence, was represented by Kaplan–Meier survivorship curves. Log-rank and Breslow tests were used with a survival model to compare longevity, and developmental time from egg to adult, between different constant temperatures. The relationships between the sex ratio and the temperature were analysed by Chi squared analysis ($\chi^2$). The $\chi^2$ was applied to a 2 x 2 contingency table with sex and temperature as the defining factors. Additionally, a linear regression was applied to show the effect of temperature on sex ratio as well as to compute the lower developmental thresholds of $A. \ dacii$, using developmental rate data ($1/D$, $D$ being the time in days required for total development from egg to adult at the different temperatures) as dependent variables ($y$-axis) and constant temperature treatments of 15–30 °C as independent variables ($x$-axis). The lower developmental threshold ($t_0$) was determined as $x$-intercept of the linear equation ($y = 0$). The thermal units (K, DD = degree-day) required for the time to become an adult is given by the inverse of the equation slope (Campbell et al., 1974). Values are reported as means ± SE. Analyses were performed using the IBM SPSS statistical software package (v20; critical $p$ value used 0.05).

3. Results

3.1. Experiment 1

The log-rank test revealed that the longevity of $A. \ dacii$ (Table 1) differed significantly with respect to temperature in both males [Global Log-rank test: $v_2 = 155.896$; df = 4; $P < 0.001$; Global Breslow (generalized Wilcoxon) test: $v_2 = 122.399$; df = 4; $P < 0.001$] and females [Global Log-rank test: $v_2 = 181.365$; df = 4; $P < 0.001$; Global Breslow (generalized Wilcoxon) test: $v_2 = 134.538$; df = 4; $P = 0.001$], except between 25 °C and 30 °C in males [Log-rank test: $v_2 = 0.610$; df = 1; $P = 0.435$; Global Breslow (generalized Wilcoxon) test: $v_2 = 0.076$; df = 1; $P = 0.782$] and 30 °C and 35 °C in females [Log-rank test: $v_2 = 0.707$; df = 1; $P = 0.400$; Global Breslow (generalized Wilcoxon) test: $v_2 = 0.635$; df = 4; $P = 0.426$], but did not differ for sex [Global Log-rank test: $v_2 = 1.008$; df = 1; $P = 0.315$; Global Breslow (generalized Wilcoxon) test: $v_2 = 0.125$; df = 1; $P = 0.723$], except at 15 °C [Global Log-rank test: $v_2 = 4.826$; df = 1; $P = 0.028$; Global Breslow (generalized Wilcoxon) test: $v_2 = 5.089$; df = 4; $P = 0.024$] and at 30 °C [Global Log-rank test: $v_2 = 10.814$; df = 1; $P = 0.001$; Global Breslow (generalized Wilcoxon) test: $v_2 = 11.462$; df = 4; $P = 0.001$].
3.2. Experiment 2

The log-rank and Breslow tests revealed that the developmental time together with survival of immatures until adult emergence of *A. daci* (Table 2) differed significantly with respect to temperature (both, pairwise temperatures as pooled) in both males [Fig. 1, Global Log-rank test: $\chi^2 = 1466.834$; df = 3; $P < 0.001$; Global Breslow test: $\chi^2 = 1479.727$; df = 3; $P < 0.001$], and females [Fig. 2, Global Log-rank test: $\chi^2 = 638.963$; df = 3; $P < 0.001$; Global Breslow test: $\chi^2 = 598.742$; df = 3; $P < 0.001$], and also differed with respect to sex [Fig. 3, Log-rank test: $\chi^2 = 133.180$; df = 1; $P < 0.001$; Global Breslow test: $\chi^2 = 138.603$; df = 1; $P < 0.001$], except at 15 ºC [Global Log-rank test: $\chi^2 = 1.463$; df = 1; $P = 0.226$; Global Breslow (generalized Wilcoxon) test: $\chi^2 = 1.510$; df = 1; $P = 0.219$).

There were significant differences in the sex ratio (Table 3) with temperature in the range studied: 15–25 ºC ($\chi^2 = 10.621$; df = 1; $P = 0.001$), 15–30 ºC ($\chi^2 = 5.098$; df = 1; $P = 0.034$), 20–25 ºC ($\chi^2 = 21.36$; df = 1; $P = 0.001$) and 20–30 ºC ($\chi^2 = 8.807$; df = 1; $P = 0.003$), except for 15–20 ºC and 25–30 ºC. The sex ratio at 20 ºC ($\chi^2 = 21.845$; df = 1; $P < 0.001$) and 25 ºC ($\chi^2 = 4.669$; df = 1; $P = 0.03$) was significantly biased toward males and females, respectively. At 15 ºC and 30 ºC, on the other hand, the sex ratio lightly biased toward males and females, respectively, though the difference was not statistically significant. A linear regression analysis (Fig. 4) revealed a significant relationship between the temperature rise and
the sex ratio (the proportion of females increases with temperature) ($F = 70.673; \text{df} = 1, 11; P < 0.001; R^2 = 0.876$).

A linear regression analysis comparing temperature treatment ($15–30$ °C) with parasitoid developmental rate resulted in the equation $y = 0.002x - 0.017$ ($R^2 = 0.961; F = 246.374; \text{df} = 1, 11; P < 0.001$). Therefore, the adult parasitoid required 500 DD above a lower developmental threshold of 8.5 °C.
Table 2. Parasitism and developmental time obtained at different temperature from exposure for 4 h of 330 L3 of *C. capitata* to cohorts of 200 parasitoids (100 ♂♂ and 100 ♀♀) of *A. daci* of ≈ 6–8 days of age. Exposure was carried out in translucent plastic boxes (20 x 15 x 10 cm) placed in a climatic chamber (Tº: 15 °C/20 °C/25 °C/30 °C/35 °C, RH: 65 ± 10%, photoperiod: 16L:8D). [n = 3 replicates exposures for each temperature].

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>L3 exposed to parasitoids</th>
<th>Parasitised a Censored b Control c Developmental time (days)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Total</td>
</tr>
<tr>
<td>15</td>
<td>990</td>
<td>4.81%</td>
<td>3.13%</td>
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<tr>
<td>20</td>
<td>990</td>
<td>34.11%</td>
<td>22.82%</td>
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<tr>
<td>25</td>
<td>990</td>
<td>35.95%</td>
<td>41.89%</td>
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<tr>
<td>30</td>
<td>990</td>
<td>2.88%</td>
<td>4.23%</td>
</tr>
<tr>
<td>35</td>
<td>990</td>
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</tbody>
</table>

*a* Parasitised pupae (parasitized pupae from which emerged parasitoids).

*b* Censored pupae (dead, aborted parasitoid…).

*c* Control (aborted pupae of *C. capitata* according the temperature).
Fig. 1. Survivorship curves from egg to adult emergence for *Aganaspis daci* males at different constant temperatures.

Fig. 2. Survivorship curves from egg to adult emergence for *Aganaspis daci* females at different constant temperatures.
Fig. 3. Survivorship curves from egg to adult emergence for *Aganaspis daci*, according to sex.

**Table 3.** Sex ratio (number males/number males + females) at different temperatures. Proportions of males with different capital letter differ significantly at 95% level. The * indicates a significant bias towards males or females.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Sex ratio (% male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>60 A</td>
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<tr>
<td>20</td>
<td>*59 A</td>
</tr>
<tr>
<td>25</td>
<td>*46 B</td>
</tr>
<tr>
<td>30</td>
<td>41 B</td>
</tr>
<tr>
<td>35</td>
<td>-</td>
</tr>
</tbody>
</table>
Regarding insect life-span, the duration of an insect adult life is usually referred to as longevity (Blackburn, 1991a, b). Many evolutionary biologists have explored longevity in insects (natural enemies) because it is a component of individual fitness (Rivero and West, 2002) and can be considered as an indicator of survival capacity. Likewise its relationship to female fecundity, prey death rate and predator growth rate are of interest from the point of view of population dynamics (Jervis, 2005).

Longevity is a highly variable species characteristic, influenced by both physical and biotic factors (Jervis, 2005; Tormos et al., 2012). Regarding physical factors little is known about the influence of photoperiod on longevity (Jervis, 2005). By contrast, many experimental studies reveal that natural enemies have optimum humidity and temperature ranges, outside which the survival is severely compromised (Wysoki et al., 1988; Herard et al., 1988; Krishnamoorthy, 1989; Jervis, 2005).

Fig. 4. Relationship between the temperature rise and the sex ratio* (the proportion of females increases with temperature). *number males/number males + females.

4. Discussion and conclusions

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The results obtained in this study on *A. daci* show that longevity decreases with increasing temperature within the effective range, usually in both males and females (McDougall and Mills, 1997; Liu and Tsai, 2002; Seal et al., 2002; Jervis, 2005). In this respect, the average summer temperatures in the Valencia region (Spain) [in the last ten years ranged between 22 and 27 °C (IVIA’s meteorological data, http://riegos.ivia.es)] indicate that *A. daci* can adapt well to the climate in this region. Garzón-Luque et al. (2008) reported similar results for another *C. capitata* parasitoid: *D. tryoni*. Further evidence is that *A. daci* was commonly recovered from field samples in 2003 with reports of high rates of parasitism (Papadopoulos and Katsoyannos, 2003; Karamouna et al., 2010) in Mediterranean areas with similar climate to the Valencian region. These data also support the hypothesis that this species can survive the winter in this area, further evidenced by the fact that it has been found living in the wild for three consecutive years in Valencia (de Pedro et al., 2013).

Regarding the other parameter of biotic potential studied here, developmental time to adult, the results obtained in this study show that temperature significantly influences bionomics of the parasitoid species. This is consistent with earlier findings for the same parasitoid species on other tephritid flies. For example, according to Andleeb et al. (2010) the duration of the life cycle of *A. daci* males and females was 16–18 days and 18–20 days, respectively, on *Bractrocera zonata* (Saunders) (Diptera: Tephritidae) at 24–26 °C, and 54–56% relative humidity. By contrast, Hosni et al. (2011) reported 25.3 days on the same host at 25 ± 2 °C, 54–65% relative humidity and 14:10 h (light/darkness) photoperiod. According to other authors (Fernandes-Da-Silva and Zucoloto, 1993; Duyck et al., 2004), these discrepancies could be due, in addition to environmental variables, to differences among pest strains in different geographical areas, or the type of food provided for the larvae. Additionally, our results indicated that none of the *A. daci* immatures survived to complete their development at 35 °C.

Low temperature thresholds for *C. capitata* (10 °C) (Szyniszewska and Tatem, 2014) and for *A. daci* reared on *C. capitata* (8.5 °C) confirm that these species are well adapted to a wide-ranging temperature climate and, therefore, to the Mediterranean area. The developmental threshold of the parasitoid *A. daci* is lower than that of the *C. capitata*, thus this parasitoid should be present in the field earlier than the host, and already be well established when host populations begin to grow. Nevertheless, it should be pointed out that both high temperatures (between 30 and 35 °C) and low temperatures (around 15 °C) lead to slow and long developmental time and poor offspring survival in *A. daci*. These results, together with the fact that sex ratio was male biased at 15 °C, suggest that this parasitoid may not reach sufficient levels to control adequately *C. capitata* in field conditions. Additionally, it is possible that this parasitoid could have better performance under fluctuating conditions. In this respect, data obtained in the Valencia region (Spain) (de Pedro et al., 2013) about *A. daci* field parasitism on
C. capitata show that, even though A. daci is able to parasitize the medfly at any time of year, Mediterranean climate variability has a negative impact on development, emergence and survival of parasitoid immature stages. The K obtained in the results for total development of A. daci (this species required 500 DD above a lower developmental threshold of 8.5 °C) is informative only as we are aware that an estimation of degree-days in the laboratory is not adequate for pest management, a comparison of heat units required in the laboratory and estimates in the field are necessary.

Unlike the results obtained by Hosni et al. (2011) on B. zonata at 25 °C (male/female = 1: 1), our results found a sex ratio significantly biased toward females at the same temperature and using C. capitata as host.

We conclude that A. daci could be recommended as a natural enemy in a sensu stricto biological control programme. Likewise, A. daci may also be useful in biocontrol projects through both inundative and inoculative releases, although primarily through inundative releases at appropriate times and even using the parasitoid in hotspot control. However, in both cases temperature should be taken into account as extreme hot and cold temperatures may hinder effective control. Despite its usefulness as a biological control agent, A. daci cannot be expected to achieve a total and natural biological control of C. capitata.

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