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Effect of host density and location on the percentage parasitism, fertility and induced mortality of *Aganaspis daci* (Hymenoptera: Figitidae), a parasitoid of *Ceratitis capitata* (Diptera: Tephritidae)

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Abstract: The percentage parasitism, fertility and induced mortality (mortality of host pupae attributed to parasitoids, from which adults do not emerge) of the parasitoid *Aganaspis daci* (Weld) infesting larvae of *Ceratitis capitata* (Wiedemann) were studied under laboratory and greenhouse conditions for temperature, host location (larvae provided in artificial diet or inside fruit) and host density. For larvae in artificial diet in the laboratory, our findings revealed that 23–25 ˚C is the most suitable temperature range, leading to high rates of parasitism, fertility, induced mortality, population reduction (total mortality of *C. capitata* caused by the parasitoid, i.e., induced mortality + percentage parasitism), and female-biased sex ratio (♀♀/♀♀ + ♂♂ = 0.55). Regarding host density, parasitism and fertility corresponded to a Type III functional response with a female-biased sex ratio (0.54–0.61), while induced mortality decreased with an increase in host density, and population reduction presented the same type of response as parasitism and fertility. Regarding the larvae inside fruits in the laboratory, the analysis showed that parasitism and fertility, depending on host density, also showed a Type III functional response and female-biased sex ratio (0.51–0.62). For larvae provided inside fruits in the greenhouse, our results showed no significant differences regarding fruit position or host density on the percentage parasitism, induced mortality or population reduction, although there was a female-biased sex ratio (fruit position: 0.64–0.72; density: 0.66–0.76). Regarding host density, parasitism and fertility corresponded to a Type II functional response. The induced mortality decreased with increasing density and host population reduction had the same type of response as parasitism and fertility. The information reported here on the key demographic parameters and functional response of *A. daci* infesting *C. capitata* under various environments and situations, will assist to evaluate the potential use of this parasitoid as a biological control agent against this pest. Our results show *A. daci* displays an excellent ability to locate, capture, parasitise, or simply kill *C. capitata*, under laboratory and field conditions.

Keywords: Parasitism (%); Fertility; Induced mortality; Population reduction; Functional response; *A. daci*.

1. Introduction

Aganaspis daci (Weld) (Hymenoptera: Figitidae) is a larval-pupal, solitary, primary endoparasitoid that has been successfully used to control the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) (Baranowski et al., 1993). Therefore, nowadays, it is considered an efficient fruit fly pest control agent. This species was first recorded in 1951 as a larval parasitoid of the oriental fruit fly, *Bactrocera* (formerly *Dacus*) *dorsalis* (Hendel) (Diptera: Tephritidae) in Malaysia and Taiwan (Weld, 1951). Once its successful control over fruit fly pests in these countries was established, *A. daci* was introduced into several countries to control tephritid species (Clausen, 1978; Wharton et al., 1981). It was first introduced in Hawaii as a potential biological control agent against *B. dorsalis* (Clausen et al., 1965) and, later, it was successfully established in Florida, for biological control of *A. suspensa*, at lower population levels (Baranowski et al., 1993). Other countries where this species has been introduced are México (Jiménez-Jiménez, 1956), Costa Rica (Wharton et al., 1981) and Egypt (El-Heneidy and Ramadan, 2010).

In the Mediterranean Basin, *A. daci* was first recorded parasitising larvae of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) on fig fruits on the Greek Island of Chios in 2003 (Papadopoulos and Katsoyannos, 2003). In Spain, this species was not reported until 2009, when, following trialled release of the imported exotic egg-larval parasitoids *Fopius arisanus* (Sonan), *Diachasmimorpha tryoni* (Cameron) and *D. longicaudata* (Ashmead) (Hymenoptera: Braconidae), several *A. daci* specimens were recovered from medfly puparia collected from fig and citrus fruits (Verdú et al., 2011). Previously, only *Spalangia cameroni* Perkins and *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) had been found as native pupal parasitoids of the medfly (Falcó et al., 2006), with no records of *A. daci* during a 4-year survey from 2000–04. Phylogenetic analysis of cytochrome oxidase I (COI) and internal transcribed spacer (ITS) sequences from *A.daci* 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). Current studies on the biology, ecology and parasitic activity of this parasitoid on *C. capitata* aim to assess its adaptability to mass rearing and its potential as a biological control agent against medfly.

An advanced understanding of the interactions between parasitoids and their hosts can be achieved by studying how parasitoids respond to changes in host density. In this context, the term functional response, originally coined by Solomon (1949), describes the response shown

by individual natural enemies to varying host density. More precisely, the functional response is defined as the relationship between the number of prey or hosts attacked by a predator or parasitoid as a function of prey density. Generally, functional responses are classified intro three types, type I to III, according to the graphical shape of definition function (i.e. relationship between number of attacked hosts/prey versus host/prey density). In this respect, the type II functional response is the most common one for invertebrate parasitoids and predators (Fernández-Arhex and Corley, 2004; Vanaclocha et al., 2013); graphically, it is an asymptotic curve that decelerates constantly as prey numbers increase, due to the handling time (i.e., the time it takes the predator to manipulate its prey); the asymptote reflects the maximum attack rate. This type of response leads to inverse density-dependent predation or parasitism (Fernández-Arhex and Corley, 2003). However, a type III functional response could also be characteristic for invertebrate natural enemies (Vanaclocha et al., 2013). This type of response is represented by a sigmoid curve; in this case, as host density rises, the response initially accelerates due to the parasitoid or predator becoming increasingly efficient at finding hosts or prey (attack rate increases or handling time decreases). It then levels off under the influence of handling time or satiation (Fernández-Arhex and Corley, 2003). The type III is the only functional response which may lead to direct density dependence when prey densities are low and thus can potentially stabilize predator-prey interactions (Hassell, 1978).

The study of functional responses under different conditions is essential to describe the parasitic activity of a parasitoid (Oaten and Murdoch, 1975), understand host-parasitoid co-evolutionary relationships (Houck and Strauss, 1985), and evaluate their potential as biological control pest agents (Fernández-Arhex and Corley, 2003). Therefore, in the present study, fertility (as the number of descendants produced by parasitoids), the percentage parasitism (= parasitism rate, as the number of descendants per recovered pupae), induced mortality (as the mortality (i.e., pupae that remain closed) due to parasitoid activity) and population reduction (as the sum of induced mortality plus percentage parasitism) of *A. daci* on *C. capitata* were assessed with regard to the temperature, host location (larvae provided in an artificial diet versus larvae infesting fruits) and host density, under two climatic conditions (laboratory and greenhouse).

2. Material and methods

2.1. Study centre and insects

All experiments in this study were performed in compliance with current Spanish law. *A. daci* specimens were obtained from a laboratory colony housed at the Instituto Valenciano de Investigaciones Agrarias (IVIA, Valencia, Spain). The colony was established in 2010 with 40

specimens obtained from medfly larvae taken from figs in a nearby Valencian village (Bétera, Spain). Laboratory rearing has since been maintained on the host, *C. capitata* (Martínez-Torres, 2011) (rearing conditions: 24 ± 1 ºC, 65 ± 10% relative humidity (RH) and 16:8 (L:D) photoperiod). The medfly has been reared at the IVIA for over 6 years, using the method of Pérez-Hinarejos and Beitia (2008) (rearing conditions: 27 ± 1 ºC, 65 ± 10% RH and 16:8 (L:D) photoperiod).

2.2. Experimental design

Three experiments were conducted to assess the effect of temperature, host location and host density on parasitism rate, fertility and induced mortality of *A. daci* on *C. capitata*. These experiments differed not only in the conditions under which they were conducted but also in the way host larvae were offered to the parasitoid.

2.2.1. Experiment 1. Larvae provided in artificial diet under laboratory conditions

This experiment was performed in duplicate in a climatic chamber under laboratory conditions (23–28 ºC; 65 ± 10% RH; 16:8 (L:D) photoperiod). *C. capitata* larvae were offered to parasitoids in artificial medfly diet. Twenty-four translucent plastic boxes (20 x 15 x 10 cm) were used as experimental units or parasitism units. Each unit contained water, honey and sugar *ad libitum* as nutritional sources. The parasitism units were provided with side muslin windows for ventilation and an upper muslin window through which 3rd instar larvae of *C. capitata* in artificial diet were offered to parasitoids to assess their parasitic activity. Three 3–5-day-old *A. daci* mating couples were introduced into each parasitism unit. For 24 h over 3 consecutive days, *C. capitata* larvae were supplied to the parasitism units at 15, 60 and 120 larvae per parasitism unit. Therefore, eight parasitism units were used for each host-density treatment. Nine additional parasitism units, containing no parasitoids (three per host density treatment), were used as controls to assess the natural mortality of *C. capitata* larvae. Every day, those larvae that had been exposed the previous day were collected and placed inside a ventilated Petri dish (one per parasitism unit). Petri dishes containing collected larvae/pupae were kept in the abovementioned climatic chamber, beside parasitism units, until the emergence of parasitoid and medfly adults. Emergences and closed puparia were then counted. The effect of temperature on *A. daci* parasitic activity was also assessed in this experiment. Thus, within each replicate, two different temperature ranges, 23–25 ºC (bottom shelf of the chamber) and 26–28 ºC (top shelf), were assessed.

For each treatment, we analysed fertility; percentage parasitism; offspring sex ratio; induced mortality; and population reduction.

2.2.2. Experiment 2. Larvae provided in fruit under laboratory conditions

This experiment was conducted in duplicate under laboratory conditions, using the bottom shelf of the abovementioned climatic chamber (23–25 ºC, 65 ± 10% RH; 16:8 (L:D) photoperiod). In this instance, larvae were offered to parasitoids inside fruits, reproducing the way they can be found in the field. Fifteen plastic boxes (30 x 25 x 20 cm) were used as parasitism units, each containing water and sugar *ad libitum*. These parasitism units incorporated an upper muslin window for ventilation. Five 3–5-day-old *A. daci* mating couples were placed inside each parasitism unit. Thirty apples (var. Royal Gala) were artificially infested according to Martins et al. (2010), with 30 $2^{nd}/3^{rd}$ instar larvae of the medfly (ten holes per fruit and three larvae per hole). These apples were placed in the parasitism units at 30, 60 and 90 larvae per parasitism unit, establishing three different treatments in relation to host density. Thus, one, two or three apples were placed in five parasitism units in each instance. A layer of vermiculite was previously placed in each parasitism unit to provide the larvae with a suitable substratum to pupate. Apples/larvae were exposed for 4 days. Then, medfly pupae were recovered and put in ventilated Petri dishes (one per parasitism unit), kept in the abovementioned climatic chamber, beside the parasitism units, until the emergence of parasitoid and medfly adults. Emergences and closed puparia were then counted. For each treatment, we analysed: fertility; percentage parasitism; and offspring sex ratio.

2.2.3. Experiment 3. Larvae provided in fruit under greenhouse conditions

This experiment was conducted under semi-field conditions, inside a greenhouse located in the experimental field at the IVIA, between May–July of 2013 with five replicates. Medfly larvae were also provided inside apples. The greenhouse was equipped with four plastic-framed mesh cages (125 x 120 x 70 cm), each containing water and honey as nutritional sources. Fifty 3–5-day-old *A. daci* mating couples were placed inside three of the cages and 10 infested apples were introduced inside each cage. The number of medfly larvae per apple differed among cages, establishing three treatments for host density. In the first cage, each apple contained six larvae (two holes per fruit and three larvae per hole). In the second cage, each apple contained 30 larvae (ten holes per fruit and three larvae per hole), whilst in the third cage, each apple contained 60 larvae (twenty holes per fruit and three larvae per hole). In the fourth cage, without parasitoids, nine apples (three per host density treatment) were individually confined in plastic ventilated cylinders, as control treatments, to assess natural medfly larvae mortality. Furthermore, not only did we consider the effect of host density on *A. daci* parasitic activity, but also the effect of fruit position. Thus, in each cage containing parasitoids, half of the apples were placed on a plastic tray (with a layer of vermiculite for pupation) on the cage floor (soil position), whereas the other five apples were individually fitted inside a polyethylene

mesh (1 cm diameter holes) and hung from an orange tree placed inside the cage (tree position). Apples/larvae were exposed for 4 days and then medfly pupae were recovered. Pupae recovered from the plastic tray were considered from soil treatment, and those recovered from the cage floor (out of the tray) were considered from tree treatment. These recovered pupae were put into ventilated plastic boxes (20 x 15 x 10 cm; one per cage (host density) and position treatment) and kept under laboratory conditions, on the bottom shelf of the abovementioned climatic chamber, until the emergence of medfly/parasitoid adults. Emergences and closed puparia were then counted. For each treatment, we analysed: fertility; percentage parasitism; offspring sex ratio; induced mortality; and population reduction.

2.3. Statistical tools and analysis

Data were analysed using IBM SPSS 17.0 with a significance set at p = 0.05. Two-way ANOVA with posthoc Tukey´s test was performed to test the percentage parasitism, fertility, induced mortality and population reduction, depending on the factors host density and temperature. Induced mortality (i.e., corrected mortality) was calculated using the Schneider-Orelli formula (Püntener, 1981) as follows:

Corrected mortality (%) = ((Treatment mortality % – Control mortality %)/(100 – Control mortality %)) x 100

Here, population reduction is defined as the sum of induced mortality and percentage parasitism.

Prior to the analysis, data normality was checked and data were transformed, where necessary. Pearson´s chi-squared (χ^2) test was used to show any significant differences in the sex ratio (n= females/n= males + females), depending on the temperature and host density.

A generalised linear model was used to analyse the functional response, with the aim to discriminate between type II and type III responses. The data were fit to a binomial distribution with Logit link function, then fit to their corresponding functional response equation (Juliano, 2001; Fernández-Arhex and Corley, 2004; Vanaclocha et al., 2013). For the type III response, the attack rate coefficient, which varied with prey density, was obtained from the following equation (Hassell, 1978): $a' = b \times 'x'/(1 + c \times 'x')$, where x is the host density and b and c are constants of itself. Data were adjusted by nonlinear least-squares regression using the iterative estimation method of Levenber–Maarquardt (Monzó, 2010). From the adjustment of these equations, we estimated the parameters of the functional response, attack rate (a') and handling time (Tm).

3. Results

3.1. Experiment 1. Larvae provided in artificial diet under laboratory conditions

Three-way ANOVA [σ^2 (residual variance): 49.98, σ_B^2 (block variance): 0.14] showed that for the variable % parasitism, with respect to factors temperature and host density, the null hypothesis should be discarded (temperature: $F_{1, 282} = 30.10$, $P < 0.001$; host density: $F_{2, 282} = 7.933$, $P < 0.001$) (Table 1). The interaction among these factors was not significant ($F_{2, 282} = 2.249$, $P = 0.107$). A post-hoc Tukey´s test only highlighted significant differences ($P < 0.001$) for parasitism percentage at the low-level host densities. Similarly, three-way ANOVA [σ^2 (residual variance): 959.14, σ_B^2 (block variance): 4.2×10^{-5}] showed differences for fertility with regard to the factors temperature ($F_{1, 282} = 12.38$, $P = 0.001$) and host density ($F_{1, 282} = 446.22$, $P \leq 0.001$). The interaction of these factors was not significant ($F_{2, 282} = 5.29$, $P = 0.006$). A post-hoc Tukey´s test showed significant differences ($P < 0.001$) for fertility, for all host densities.

The estimated linear and quadratic coefficients were both positive (Table 2), indicating a type III functional response (Juliano, 2001; Vanaclocha et al., 2013). The estimated *b* and *c* parameters were 0.110 ± 0.116 and 0.145 ± 0.184, respectively. The estimated attack rate coefficients were 0.52 $days^{-1}$ for a density of 15 larvae, 0.68 $days^{-1}$ (60 larvae), and 0.72 $days^{-1}$ (120 larvae). The estimated handling time was 0.0029 ± 0.017 days (Table 3). The estimated maximum number of emergences per female was 115 specimens in 24 h (Fig. 1).

Significant differences in the sex ratio were obtained for both temperature ranges ($\chi^2 = 66.88$, d.f. = 1, $P \leq 0.001$). Additionally, for each range, we observed a biased sex ratio (range 23–25 ˚C, $\chi^2 = 33.83$, d.f. = 1, $P \leq 0.001$; range 26–28 ˚C, $\chi^2 = 175.88$, d.f. = 1, $P \leq 0.001$), female-biased in both cases. Regarding the sex ratio obtained for host density, the chi-squared test showed significant differences ($\chi^2 = 65.36$, d.f. = 2, $P \leq 0.001$). For each density, we observed a female-biased sex ratio (density 45 larvae (5 larvae per couple/day), $\chi^2 = 28.62$, d.f. = 1, $P \leq 0.001$; density 180 larvae (20 larvae per couple/day), $\chi^2 = 8.75$, d.f. = 1, $P = 0.003$; density 360 larvae (40 larvae per couple/day), $\chi^2 = 172.38$, d.f. = 1, $P \leq 0.001$). Regarding induced mortality and population reduction, a three-way ANOVA [induced mortality: σ^2 (residual variance): 39.43, σ_B^2 (block variance): 0.21; population reduction residual variance: 40.41, σ_B^2 (block variance): 0.23] showed differences with respect to the factors temperature (induced mortality: $F_{1, 224} = 39.64$, $P < 0.001$; population reduction: $F_{1, 224} = 40.57$, $P < 0.001$) and host density (induced mortality: $F_{2, 224} = 19.97$, $P < 0.001$; population reduction: $F_{2, 224} = 20.91$, $P < 0.001$). The interaction of these factors was not significant (induced mortality: $F_{2, 224} = 4.05$, $P = 0.401$; population reduction: $F_{2, 224} = 4.15$, $P = 0.431$). A post-hoc Tukey´s test only showed significant differences ($P < 0.001$) for lower host densities.

Table 1. Parasitism, fertility, induced mortality, population reduction and sex ratio displayed by *A. daci* – at different temperatures and host densities – on *C. capitata* larvae provided in artificial diet under laboratory conditions (asterisk indicates significant differences, $P \leq 0.05$).

	Temperature		Host density		
	23–25 ˚C	26–28 ˚C	15 larvae[a]	60 larvae[b]	120 larvae[c]
% parasitism (Range; Mean ± SE)	0–100; 67.93 ± 1.63*	0–100; 54.06 ± 2.02*	0–100; 54.02 ± 2.69*	0–94.5; 65.73 ± 2.08	1.6–91.4; 63.24 ± 2.08
Fertility (Range; Mean ± SE)	0–107; 41.88 ± 2.67*	0–108; 35.72 ± 2.34*	0–14; 7.64 ± 0.38*	0–52; 37.21 ± 1.18*	2–108; 71.55 ± 2.41*
Induced mortality (Range; Mean ± SE)	0–90; 17.58 ± 2.59*	0–23; 3.54 ± 0.82*	0–90; 10.56 ± 1.53*	0–52.8; 6.54 ± 1.04	0–46.6; 3.09 ± 0.34
Population reduction (Mean ± SE)	85.51 ± 3.2*	57.60 ± 1.01*	64.58 ± 2.12*	72.27 ± 1.26	66.33 ± 1.02
Sex ratio (♂♂ , ♀♀; ♀♀/♀♀ + ♂♂)	2679, 3315; 0.55*	1906, 3239; 0.62*	269, 429; 0.61*	1690,1943; 0.54*	2625, 4144; 0.61*

Induced mortality (= corrected mortality %).

Sex ratio (n = females/n = females + males).

[a]5 larvae per couple (female + male) and day x 3 couples (15 larvae per day) x 8 parasitism units x 3 repetitions (days) x 2 temperatures x 2 replicates.

[b]20 larvae per couple (female + male) and day x 3 couples (60 larvae per day) x 8 parasitism units x 3 repetitions (days) x 2 temperatures x 2 replicates.

[c]40 larvae per couple (female + male) and day x 3 couples (120 larvae per day) x 8 parasitism units x 3 repetitions (days) x 2 temperatures x 2 replicates.

Table 2. Maximum likelihood estimation parameters from the generalised linear model of the proportion of parasitised hosts as a function of initial host densities by fertilised *A. daci* females under laboratory and greenhouse conditions.

	Parameter	Estimate	SE	χ^2	df	P
Laboratory diet	Linear	0.018	0.0024	57.24	1	<0.0001
	Quadratic	0.00001	1.53E-0005	53.14	1	<0.0001
Laboratory fruit	Linear	0.037	0.0195	3.62	1	=0.05
	Quadratic	0.00001	0.002	3.58	1	=0.05
Greenhouse	Linear	-0.006	0.0019	11.514	1	=0.001
	Quadratic	2.34E-006	4.60E-006	25.917	1	<0.0001

Table 3. Functional response type (FR), attack rate (a) (days^{-1}) and estimated prey-handling time (T_h) (days) obtained from non-linear regression of the number of hosts by fertilised *A. daci* females under laboratory and greenhouse conditions.

	FR	a	SE	95% CI	T_h	SE	95% CI	R^2
Laboratory diet	Type III	b = 0.110	0.116	-0.118-0.337	0.0029	0.002	-0.002-0.006	0.745
		c = 0.145	0.184	-0.217-0.507				
Laboratory fruit	Type III	b = 0.039	0.098	-0.162-0.239	0.008	0.020	-0.032-0.049	0.822
		c = 0.112	0.374	-0.656-0.880				
Greenhouse	Type II	0.064	0.030	0.002-0.126	0.003	0.027	-0.052-0.057	0.607

R^2 = are the coefficients of determination from R^2 of each regression.

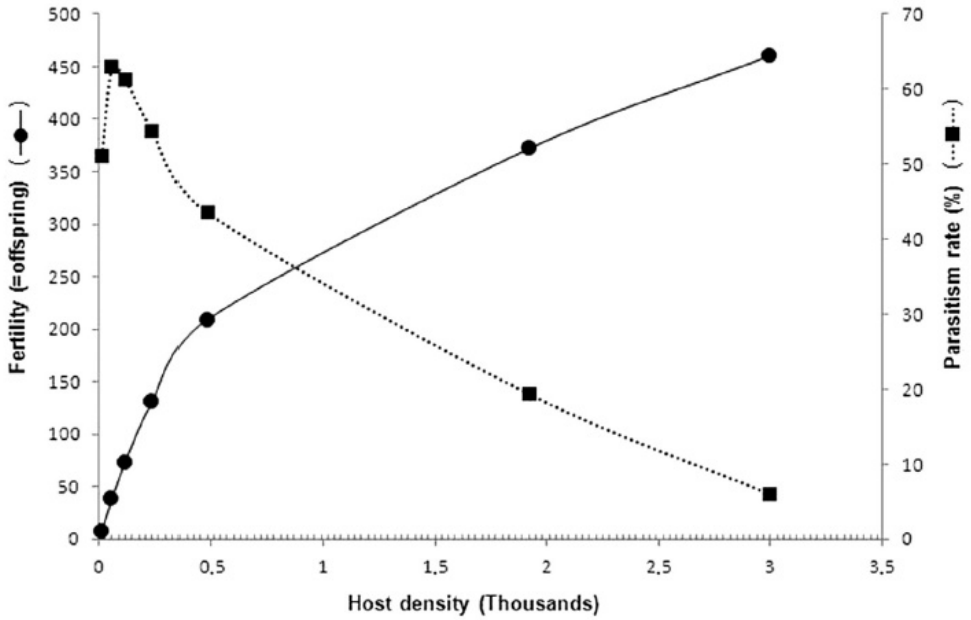

Fig. 1. Functional response curve fit by non-linear least-squares regression of fertilised *A. daci* females infesting larvae exposed in the laboratory in artificial diet[1] (Type III).

[1] 3 mating couples (host density: 5, 20 or 40 larvae per couple and day).

3.2. Experiment 2. Larvae provided in fruit under laboratory conditions

A two-way ANOVA [σ^2 (residual variance): 41.46, σ_B^2 (block variance): 0.20] showed that for the variable % parasitism with respect to the factor host density, the null hypothesis was acceptable ($F_{1, 27}$ = 0.626, P = 0.542) (Table 4). However, a two-way ANOVA [σ^2 (residual variance): 469.11, σ_B^2 (block variance): 5.1 x 10^{-5}] showed differences in fertility with respect to the host density factor ($F_{2, 27}$ = 62.36, P ≤ 0.001).

A generalised linear model for the proportion of parasitised hosts showed that the estimated values for the linear and quadratic coefficients were both positive (Table 2), indicating a type III functional response (Juliano, 2001; Vanaclocha et al., 2013). The estimated *b* and *c* parameters were 0.0039 ± 0.098 and 0.112 ± 0.374, respectively. The estimated attack rate coefficients were 0.27 $days^{-1}$ for a density of 30 larvae, 0.30 $days^{-1}$ for a density of 60 larvae, and 0.31 $days^{-1}$ for a density of 90 larvae. The estimated handling time was 0.008 ± 0.020 days (Table 3). The estimated maximum number of emergences per female was 25 specimens in 24 h (Fig. 2).

Similarly, regarding sex ratio obtained according to host density, the chi-squared test showed significant differences (χ^2 = 12.12, d.f. = 2, P ≤ 0.002). However, a biased sex ratio was only observed for higher densities (density of 60 larvae: χ^2 = 27.83, d.f. = 1, P ≤ 0.001; density of 90 larvae: χ^2 = 19.15, d.f. = 1, P ≤ 0.001). In both instances, the sex ratio was female-biased.

Table 4. Parasitism, fertility and sex ratio shown by *A. daci* – at different host densities – on *C. capitata* larvae provided inside fruit (apples) under laboratory conditions (asterisk indicates significant differences, $P \leq 0.05$).

	Host density		
	30 larvae[a]	60 larvae[b]	90 larvae[c]
% parasitism (Range; Mean ± SE)	50–100; 79.16 ± 4.74	81.1–91.5; 85.49 ± 1.07	38.9–93.3; 82.04 ± 4.94
Fertility (Range; Mean ± SE)	13–30; 22.50 ± 1.51*	37–54; 47.10 ± 1.62*	30–84; 68.70 ± 4.55*
Sex ratio (♂♂ , ♀♀; ♀♀/♀♀ + ♂♂)	224,226; 0.51*	217, 398; 0.65*	262, 425; 0.62*

Sex ratio (n = females/n = females + males).

[a]6 larvae per couple (female + male) (exposition 4 days) x 2 replicates.

[b]12 larvae per couple (female + male) (exposition 4 days) x 2 replicates.

[c]18 larvae per couple (female + male) (exposition 4 days) x 2 replicates.

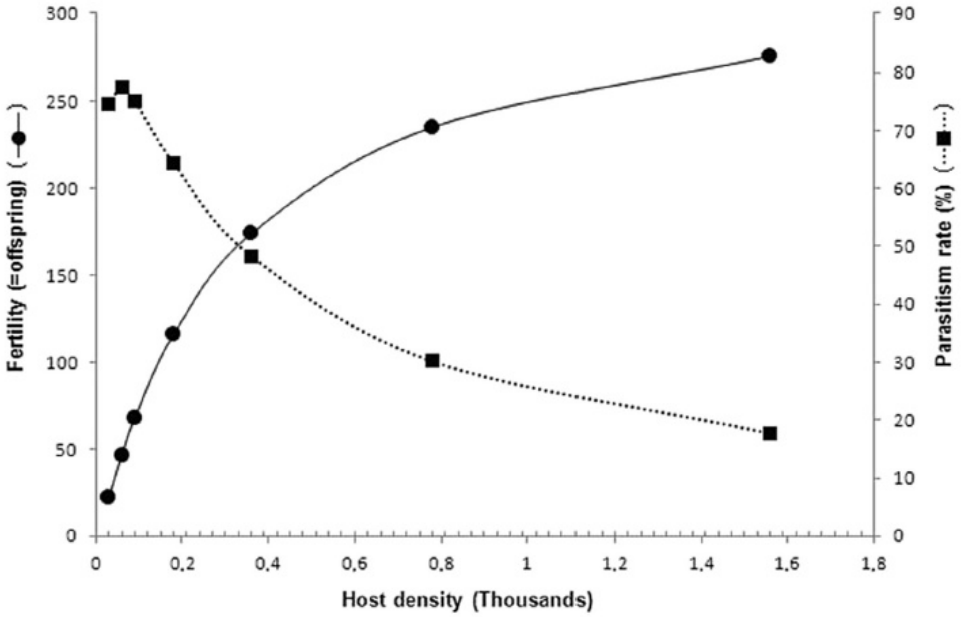

Fig. 2. Functional response curve fit by non-linear least-squares regression of fertilised *A. daci* females infesting larvae exposed in the laboratory inside fruit[2] (Type III).

[2] 5 mating couples (host density: 6, 12 or 18 larvae per couple and 4 exposure days).

3.3. Experiment 3. Larvae provided in fruit under greenhouse conditions

Three-way ANOVA [σ^2 (residual variance): 50.12, σ_B^2 (block variance): 4.6 x 10^{-5}] showed that for the variable % parasitism, with respect to the factors position and host density, the null hypothesis was acceptable (host position: $F_{1, 22} = 1.06$, $P = 0.313$; host density: $F_{3, 22} = 0.235$, $P = 0.871$) (Table 5). The interaction of these factors was not significant ($F_{3, 23} = 0.02$, $P = 0.996$). Likewise, the three-way ANOVA [σ^2 (residual variance): 1106.87, σ_B^2 (block variance): 0.62] did not show differences for fertility with respect to position ($F_{1, 22} = 0.56$, $P = 0.461$), although there were differences with respect to host density ($F_{3, 22} = 18.26$, $P \leq 0.001$). The interaction of these two factors, was not significant ($F_{3, 22} = 0.14$, $P = 0.933$). A post-hoc Tukey´s test showed significant differences ($P < 0.002$) for fertility, among the different host densities.

A generalised linear model of the proportion of parasitised hosts showed that the estimated value of the linear coefficient was negative, while the quadratic coefficient was positive (Table 2), indicating a type II functional response (Juliano, 2001; Vanaclocha et al., 2013). The estimated attack rate coefficient was 0.064 ± 0.030 $days^{-1}$ and the estimated prey-handling time was 0.003 ± 0.027 days (Table 3). The estimated maximum number of emergences per female was 7 specimens in 24 h (Fig. 3).

The chi-squared test showed that there were significant differences in the sex ratio obtained in the two positions ($\chi^2 = 65.36$, d.f. = 1, $P \leq 0.001$). For each position, we observed a biased sex ratio (tree position: $\chi^2 = 155.34$, d.f. = 1, $P \leq 0.001$; soil position: $\chi^2 = 41.67$, d.f. = 1, $P \leq 0.001$). In both instances the sex ratio was female-biased.

Likewise, regarding the sex ratio obtained according to host density, the chi-squared test showed significant differences ($\chi^2 = 15.15$, d.f. = 2, P = 0.001). Moreover, for each of the densities, a biased sex ratio was observed (density of 60 larvae: $\chi^2 = 80.04$, d.f. = 1, $P \leq 0.001$; density of 300 larvae: $\chi^2 = 87.96$, d.f. = 1, $P \leq 0.001$; density of 600 larvae, $\chi^2 = 28.89$, d.f. = 1, $P \leq 0.001$). In all three instances, the sex ratio was female-biased.

With respect to induced mortality and population reduction a three-way ANOVA [induced mortality: σ^2 (residual variance): 58.67, σ_B^2 (block variance): 0.32; population reduction: σ^2 (residual variance): 59.17, σ_B^2 (block variance): 0.34] did not show significant differences with respect to the factors position (induced mortality: $F_{1, 22} = 0.617$, P = 0.440; population reduction: $F_{1, 22} = 0.638$, P = 0.452) and host density (induced mortality: $F_{3, 22} = 1.12$, P = 0.362; population reduction: $F_{3, 22} = 1.27$, P = 0.392). The interaction of these factors was not significant (induced mortality: $F_{3, 22} = 0.12$, P = 0.946; population reduction: $F_{3, 22} = 0.11$, P = 0.926).

Table 5. Parasitism, fertility, induced mortality, population reduction and sex ratio displayed by *A. daci* – at different host densities – on *C. capitata* larvae inside fruit (apples) in different positions under greenhouse conditions (asterisk indicates significant differences, $P \leq 0.05$).

	Fruit position		Host density		
	Tree	Soil	60 larvae[a]	300 larvae[b]	600 larvae[c]
% parasitism (Range; Mean ± SE)	6.9–58.9; 28.84 ± 4.40	3.7–74.8; 38.15 ± 5.26	28–39.1; 33.56 ± 5.56	6.9–56; 31.35 ± 7.52	10.7–63.2; 30.77 ± 6.9
Fertility (Range; Mean ± SE)	2–154; 45.93 ± 12.11	0–108; 35.72 ± 2.34	0–14; 10.06 ± 0.38*	5–110; 47.1 ± 10.23*	30–181; 92.32 ± 15.86*
Induced mortality (Range; Mean ± SE)	18.4–92.5; 61.89 ± 6.5	3.1–96; 53.81 ± 7.01	58.2–73.3; 65.79 ± 7.53	36–92.5; 61.9 ± 8.79	3.1–88.9; 45.1 ± 8.98
Population reduction (Mean ± SE)	90.73 ± 3.26	91.96 ± 5.28	99.35 ± 5.18	93.25 ± 5.96	75.87 ± 5.29
Sex ratio (♂♂ , ♀♀; ♀♀/♀♀ + ♂♂)	332, 888; 0.72*	497, 919; 0.64*	535, 1028; 0.66*	248, 632; 0.72*	46, 147; 0.76*

Induced mortality (= corrected mortality %).

Sex ratio (n = females/n = females + males).

[a]30 larvae per 50 couple (female + male) and position (tree/soil) (exposition 4 days) x 5 replicates.

[b]150 larvae per 50 couple (female + male) and position (tree/soil) (exposition 4 days) x 5 replicates.

[c]300 larvae per 50 couple (female + male) and position (tree/soil) (exposition 4 days) x 5 replicates.

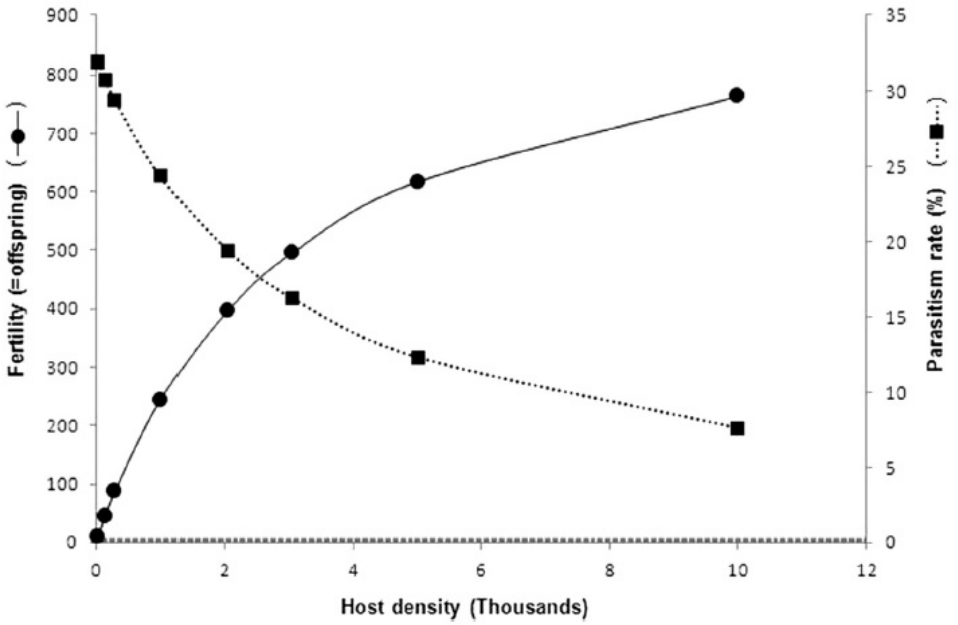

Fig. 3. Functional response curve fit by non-linear least-squares regression of fertilised *A. daci* females infesting larvae provided inside fruit in the greenhouse[3] (Type II).

[3] 50 mating couples (host density: 60/50, 6 or 12 larvae per couple and 4 exposure days).

4. Discussion

The results of this study indicate that the behaviour and population dynamics of *A. daci*, while infesting *C. capitata*, are not influenced by the particular biology of this tephritid species. In this respect, the results reported herein on the functional response of *A. daci* show that the exposition of this parasitoid under confined laboratory conditions, at high and low host densities, to both larvae provided in the diet and inside the fruit, correspond to a type III functional response. In this instance, we observed how the proportion of parasitised hosts per time unit accelerates with increasing host density. This could be associated with greater efficiency in the search by the parasitoid or a decrease in host-handling time due to learning behaviour (Fernández-Arhex and Corley, 2004), reaching a point where host-handling time limits parasitism rates. Under greenhouse conditions (more similar to natural conditions), with hosts inside fruits (suspended, simulating their position on the tree; or on the floor, simulating fallen fruit) and a host density per female rate more similar to that normally found in the field, the functional response corresponded to type II. This type of response, the most common found in parasitoids (Fernández-Arhex and Corley, 2004), is influenced at lower host densities by the longer time spent searching for hosts, and at higher densities by the greater time spent handling them. The limitation in the eggs that a female parasitoid can deposit can also represent a limiting effect (Hassell, 2000); although in our study *A. daci* females reached fertility rates of 40 offspring per day, meaning 120 descendants in 3 days.

As expected, induced mortality, due to factors including superparasitoidism (see Tormos et al., 2012, 2013), was higher at lower host densities both in the laboratory and the greenhouse. Additionally, the location of the host (exposed in artificial diet or inside the fruit), as well as slight variations in the environmental variables (much more pronounced under greenhouse than laboratory conditions) should result in fertility being significantly lower under greenhouse than the laboratory conditions. However, in both conditions, population reduction was similar and very high, given that lower fertility in the greenhouse was offset by higher induced mortality. This fact, among others, recently led de Pedro et al. (2016) to conclude that *A. daci* is a good candidate for use in biological control and, fundamentally, in inundative releases against *C. capitata*.

The female-biased sex ratio observed in all instances could be due to the temperature at which the trials were carried out. In this respect, previous studies about the effect of this environmental variable (de Pedro et al., 2016) on the developmental time, survival of immature stages and *A. daci* adult longevity, concluded that in the suitable temperature range for development, the proportion of females rises in parallel with increases in temperature.

In summary, this article contributes to increasing our knowledge of the demographic parameters and functional response of *A. daci* attacking *C. capitata* larvae in different environments and situations, adding new results to recent contributions on the effect of environmental variables on the biotic potential of this parasitoid (de Pedro et al., 2016). Collectively, this information leads us to conclude that although *A. daci* cannot be expected to achieve total biological control of *C. capitata* populations, it can certainly be useful in the fight against this pest, primarily through inundative releases made at appropriate times and even using the parasitoid in hotspot control campaigns.

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