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Parasitism of *Aganaspis daci* against *Ceratitis capitata* under Mediterranean climate conditions

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Abstract: The effect of environmental factors is essential to the success of parasitoids as biological control agents, as it determines their foraging activity, development, and survival. The larval-pupal parasitoid wasp *Aganaspis daci* (Weld) (Hymenoptera: Figitidae) is known to have a very low fertility (i.e., offspring production) in the field in certain Mediterranean areas, probably due to its inability to efficiently oviposit under such climatic conditions. In this study, the percentage of parasitism and induced mortality (mortality of host pupae attributed to parasitoids, from which adults do not emerge) caused by this wasp to the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), were assessed under field conditions across 1 year, using medfly-infested apples and parasitoid-confined release in a lemon orchard of southeastern Spain. As *A. daci* is known to have very few emergences in the field, fertility was assessed in the laboratory from parasitized pupae recovered from the field. We found average parasitism rates of 27% and high induced mortality rates of 66% under field conditions. Consequently, medfly population reduction (total mortality of *C. capitata* caused by *A. daci*, i.e., induced mortality + % parasitism) was, on average, 87%. Parasitism and induced mortality varied throughout the year, depending on the average temperature and relative humidity. The interaction of these factors resulted in the highest parasitism rates at low mean temperature and humidity values; likewise, the highest percentages of induced mortality were obtained with a combination of high mean temperature and low mean humidity values. In conclusion, *A. daci* may exert a strong impact on medfly populations, being a good candidate for inundative field releases for the management of *C. capitata* in the Mediterranean Basin.

Key words: Hymenoptera, Figitidae, parasitoid, Diptera, Tephritidae, medfly, biological control, induced mortality, population reduction, temperature, humidity.

1. Introduction

Currently, the Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the main global threats to fruit trees and citrus, with a range of more than 330 plant host species and known to be capable of adapting to a wide range of climates (Liquido et al., 1991; Papadopoulos, 2008). Control programmes against *C. capitata* are essential due to the negative economic effects of this species. The control strategies most used are environmentally friendly methods, such as sterile insect technique (SIT), mass trapping, chemosterilant traps, or biological control through the use of parasitoids (Delrio & Cocco, 2012).

Among the parasitoids of medfly, *Aganaspis daci* (Weld) (Hymenoptera: Figitidae), a larval-pupal solitary primary endoparasitoid, is one of the most promising species for biological control of medfly. This species was first recorded in 1951 as a larval parasitoid of *Bactrocera dorsalis* (Hendel) (= *Dacus dorsalis*) 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; El-Heneidy et al., 2014). However, although this wasp was proved to be effective against various species of fruit flies (Baranowski et al., 1993; Ovruski et al., 2000, 2007), its potential as control agent against *C. capitata* is still not fully clear, mostly because of scarcity of data from many areas and different medfly fruit hosts. *Aganaspis daci* was found to attack medfly in Greece in fig fruits (Papadopoulos & Katsoyannos, 2003), in Syria in loquat, guava, peach, and grapefruit (Ali et al., 2015, 2016), and in Spain in fig and citrus fruits (Verdú et al., 2011; Sabater-Muñoz et al., 2012). It also developed successfully from medfly-infested apples in controlled experiments in Spain (de Pedro et al., 2017).

Particularly unclear is whether *A. daci* can be effective in controlling medfly in the Mediterranean Basin. Indeed, despite high rates of parasitism (50–90%) recorded both in Greece and in Spain (Papadopoulos & Katsoyannos, 2003; Verdú et al., 2011; Sabater-Muñoz et al., 2012), this wasp is known to have a very low fertility (i.e., offspring production) in the field, compared with other parasitoids of *C. capitata* (Beitia et al., 2007; Garzón-Luque et al., 2008; Pérez-Hinarejos & Beitia, 2008; Stacconi et al., 2013; Harbi et al., 2014) in Mediterranean areas. The causes of this phenomenon are not clear, but one possibility is that *A. daci* is unable to efficiently oviposit under certain climatic conditions.

Here, we aimed to establish the influence of Mediterranean climate conditions on *A. daci* parasitic potential, by analysing the effects of these conditions on the percentage of parasitism (= parasitism rate, as the number of descendants produced by parasitoids per recovered pupa), induced mortality [as the mortality (i.e., pupae that remain closed) due to

parasitoid activity], and population reduction (as the sum of induced mortality + % parasitism) in this parasitoid on *C. capitata*.

2. Materials and methods

2.1. Experimental area and insect rearing

The assays were conducted in a lemon orchard located in the experimental field at the Instituto Valenciano de Investigaciones Agrarias (IVIA, Valencia, Spain) throughout a 1-year period (between 2012 and 2013). IVIA initiated in 2003 an Integrated Plan of Action against *C. capitata* in the Valencian Region (Comunidad Valenciana, Spain), which aims to include both exotic parasitoids (Beitia et al., 2003, 2011; Falcó et al., 2003) and native or naturalized parasitoids (Falcó et al., 2006). The latter group includes *A. daci,* for which IVIA established a rearing routine in 2010 in order to evaluate its efficacy as a biological control agent of medfly. This parasitoid was recovered for the first time in this area from medfly larvae recovered from fig and citrus fruits in the summer of 2009 in Valencia (Sabater-Muñoz et al., 2012). Since 2011 many experiments have been performed to increase our knowledge of the biology, ecology, and parasitic activity of this species (de Pedro et al., 2013a, 2016, 2017; Tormos et al., 2013).

Ceratitis capitata and its parasitoid *A. daci* were reared in separated climatic chambers at IVIA. Medfly was reared at 27 ± 1 ºC, 65 ± 10% r.h., and L16:D8 photoperiod; the parasitoid was reared at 24 ± 1 ºC, 65 ± 10% r.h., and L16:D8.

2.2. Experimental protocol

To evaluate the parasitism ability of *A. daci* against *C. capitata* under Mediterranean climate conditions, a total of 13 assays were conducted in 1 year, from May 2012 to May 2013. The assays covered the climatic conditions corresponding to the four seasons. Twenty-six apples (var. Royal Gala) were artificially infested according to Martins et al. (2010), with 30 second or third medfly instars in each assay – 10 holes (8 mm diameter, 2 cm deep) were dug in each fruit and three larvae were deposited in each hole. Apples were individually confined in a plastic ventilated cylinder (15 × 20 cm), with a total of 26 cylinders; five couples of parasitoids were introduced into 20 of them (this parasitoid/host ratio does not lead to high superparasitism rates, as observed by the authors), whereas the other six cylinders, without parasitoids, were considered as control treatments to assess the natural medfly larva mortality.

All cylinders, referred to as parasitism units, were introduced into wood-framed mesh cages and placed under lemon trees (to provide shelter from rain, avoiding the possible damages caused by rain). All cylinders were kept in place in the field for 1 week to obtain parasitism by female wasps. After that, medfly pupae were collected from the cylinders, in which a layer of

vermiculite had been placed previously in order to provide the larvae with a suitable substratum to pupate. Depending on the conditions for the development of immature stages, two treatments were considered, referred to as (1) laboratory and (2) field series.

Laboratory series: as *A. daci* is known to have a very low fertility (i.e., offspring production) in the field in some Mediterranean areas, pupae recovered from half of the cylinders (10 cylinders containing parasitoids + three controls) were put in translucent plastic boxes (20 × 15 × 10 cm) – one translucent plastic box per cylinder – and transferred to laboratory conditions (25 ± 2 ºC, 50-70% r.h., L16:D8 photoperiod) to develop under optimal conditions and to recover medfly and parasitoid adults. The adult parasitoids obtained enabled us to determine the climatic effects on the parasitic activity of *A. daci* under natural conditions. Medfly and parasitoid emergences and closed puparia were counted, and % parasitism and induced mortality were analysed. Induced mortality was evaluated by comparing % mortality in the treatment with % mortality in its control. Induced mortality (=corrected mortality) was calculated using the Schneider-Orelli formula (Püntener, 1981) as follows:

Induced mortality (%) = [(Treatment mortality – Control mortality) / (100 – Control mortality)] × 100%

Induced mortality is difficult to determine, but certainly includes oviposition (thus, related with parasitism) and other types of parasitoid behaviour unrelated with parasitism (e.g., host feeding).

Field series: from the other half of the cylinders (10 cylinders containing parasitoids + three controls), pupae were recovered and put in the same type of translucent plastic boxes, now placed inside another wooden-framed mesh cage, placed under a lemon tree, in the abovementioned lemon orchard. These plastic boxes were kept in the field until parasitoids emerged or alternatively until collection as closed pupae after a maximum of 4 months, to assess the effect of climatic conditions on *C. capitata* population reduction. Medfly and parasitoid emergences and closed puparia were counted to estimate % parasitism, induced mortality and, consequently, the within-year variation in medfly population reduction. In this case, induced mortality was evaluated by comparing % mortality with the laboratory control.

To establish the climatic conditions of each assay, six environmental variables were measured. Temperature and relative humidity were recorded once every hour of the day by a data logger placed in one of the wooden cages with the cylinders. The other environmental factors were number of sunshine hours, number and sum of cold hours [i.e., temperature below 8.5 °C, the minimum threshold for *A. daci*; the threshold for *C. capitata* is 10 ºC (Szyniszewska & Tatem, 2014; de Pedro et al., 2016)], solar radiation (MJ m^{-2}) and rainfall (mm). These were

obtained on a daily basis from the weather station at IVIA near the study plot [Model 2 of the agroclimatic information system for irrigated cultures (SiAR) of the Ministry of Agriculture, Food and Environment]. The mean values of all climatic variables across the assay periods (i.e., weeks) were used in the analysis. Each assay therefore corresponded to a particular climatic condition, as the environmental factors were different for each assay but identical across repetitions within assays (see Results).

2.3. Statistical analysis

ANOVA was performed to test the effect of the season of the year on the dependent variables % parasitism, % induced mortality, and % population reduction, followed by Tukey's honestly significant difference (HSD) tests for comparisons among seasons. Multiple linear regressions were used to analyse the possible effect of environmental factors and their interactions on dependent variables. Maximal models were simplified by manual stepwise backward selection ($\alpha = 0.05$). To avoid introducing correlated environmental factors in the regression model, we conducted an exploratory principal component analysis (McGarigal et al., 2000; Jackson, 2003) with the correlation matrix, calculated from the mean of the values obtained in each assay. When necessary the variables were normalized and the values (continuous variables) between 0 and 100 rescaled prior to analysis. In addition, the distributions of residuals were tested for normality. Analyses were performed with the SPSS v.20 statistical package (IBM SPSS, Armonk, NY, USA).

3. Results

Mean parasitism per assay across the 13 study periods ranged from 5 to 49%, on average (mean ± SE =) 27.4 ± 13.2% (Table 1). Season had a strong effect on % parasitism ($F_{3, 126} = 7.94$, $P < 0.001$). Parasitism in autumn and winter was higher than in spring, and in winter it was higher than in summer (Tukey's HSD test: $P<0.05$).

Mean induced mortality rate per assay across the 13 study periods ranged from 31 to 94%, on average 65.8 ± 18.4% (Table 1). Season had a strong effect on % induced mortality ($F_{3, 126} = 8.97$, $P < 0.001$). Induced mortality was lower in winter than in spring, summer, or autumn (Tukey's HSD test: $P<0.05$).

The multiple linear regression model indicated a significant effect of mean temperature (MT) and mean humidity (MH) (through its interaction with mean temperature) on % parasitism ($R^2_{aj} = 0.59$, AIC = -61.07, $F_{3,9} = 6.91$, $P = 0.011$) (Figure 1). These factors would influence the response of % parasitism according to the following equation:

$$\%\ parasitism = 1.37 - 0.0761 \times MT - 0.0116 \times MH + 0.000854 \times MT*MH$$

In particular, % parasitism of *A. daci* increased as MT increased but only with high levels of MH; with moderate and low levels of MH, increases in MT induced a reduction in % parasitism (Figure 1).

The multiple linear regression model for the effects of environmental factors on induced mortality also indicated a significant effect of both MT and MH, together with their interaction, on the dependent variable (R^2_{aj} = 0.77, AIC = 60.24, $F_{3,9}$ = 14.01, P = 0.001) (Figure 2). These factors would condition the response of the induced mortality according to the following equation:

*% induced mortality = –127.16 + 12.03 × MT + 2.23 × MH – 0.141 × MT*MH*

Thus, the induced mortality increased as MT increased for low and moderate values of MH, but decreased with temperature when MH was high (Figure 2).

Mean population reduction per assay across the 13 study periods ranged from 73 to 97%, on average (mean ± SE =) 86.7% ± 7.1% (Table 2). Although this variable was high throughout the study, between-season differences were apparent ($F_{3,\ 126}$ = 3.32, P = 0.02). Population reduction was lower in spring than in summer (Tukey's HSD test: P<0.05).

Table 1. Mean (± SE) climatic conditions of each assay in the laboratory series.

Assay[1]	Season	Period	Temperature (°C)		Humidity (%)		No. sunshine hours	No. hours of cold[3]	Solar radiation (MJ m^{-2})	Rainfall (mm)	% parasitism	% induced mortality[4]
			Mean ± SE (range)	CV[2]	Mean ± SE (range)	CV[2]						
1	Spring	18 May 2012	21.6 ± 8.4 (12.7–35.6)	18.0	55.8 ± 41.1 (13.5–99.4)	28.0	12.5	0	25.9	0.08	6 ± 2.13	89.2 ± 2.90
2		13 June	23.3 ± 9.0 (14.4–32.5)	20.1	70.1 ± 38.5 (30.7–100)	32.0	12.3	0	25.2	0.5	5 ± 2.21	93.6 ± 3.07
3	Summer	27 June	26.5 ± 9.9 (16.2–38.1)	21.0	70.3 ± 40.1 (28.8–99.8)	29.6	12.3	0	23.1	0	21 ± 4.06	76.8 ± 4.11
4		22 August	27.8 ± 8.9 (19.9–39.7)	17.0	81.7 ± 52.2 (21.3–100)	27.1	11.9	0	22.5	0	22 ± 5.37	76.7 ± 5.44
5		6 September	24.4 ± 7.8 (16.8–34.7)	24.9	87.3 ± 49.2 (35.4–100)	25.0	10.7	0	19.4	0	39 ± 7.14	58.0 ± 6.76
6	Autumn	27 September	19.9 ± 8.0 (13.1–31.1)	18.3	82.8 ± 53.1 (22.8–99.3)	10.7	8.3	0	11.4	4.8	30 ± 6.49	68.0 ± 6.24
7		10 October	20.3 ± 10.1 (10.3–34.5)	29.0	68.0 ± 48.1 (19.9–97.8)	37.2	9.1	0	13.4	0.8	19 ± 3.99	77.8 ± 4.96
8		25 October	14.3 ± 11.3 (3.0–27.6)	36.5	89.2 ± 59.6 (22.0–99.7)	17.8	8.3	3.2	11.1	0.5	27 ± 3.03	70.3 ± 3.66
9		28 November	9.2 ± 9.2 (0.1–20.6)	50.2	65.2 ± 36.0 (29.0–99.0)	30.5	7.8	8.4	9.5	0	49 ± 7.41	30.5 ± 6.19
10	Winter	23 January 2013	12.3 ± 11.8 (1.0–24.9)	42.4	49.4 ± 35.1 (16.5–98.9)	38.1	8.2	6.3	10.6	0.04	43 ± 3.16	49.1 ± 3.84
11		6 February	10.7 ± 7.6 (2.5–21.8)	34.9	41.1 ± 25.5 (18.2–73.9)	33.3	8.8	6.5	11.1	0.01	36 ± 4.48	37.8 ± 4.90
12	Spring	27 March	18.5 ± 10.1 (8.6–29.8)	22.2	50.0 ± 32.7 (18.3–99.7)	36.8	10.3	0.1	19.3	0.03	25 ± 6.50	72.2 ± 6.58
13		8 May	18.5 ± 10.6 (12.0–41.2)	32.0	73.9 ± 43.5 (22.8–99.9)	34.5	11.7	0	23.3	0.7	35 ± 7.33	60.6 ± 7.35

[1]Duration of each assay was 1 week.

[2]CV, coefficient of variation (= relative standard deviation, RSD).

[3]Hours with temperature below 8.5 ºC, the minimum threshold of *Aganaspis daci*.

[4]Corrected mortality {= [(treatment mortality – control mortality) / (100 – control mortality)] × 100%}.

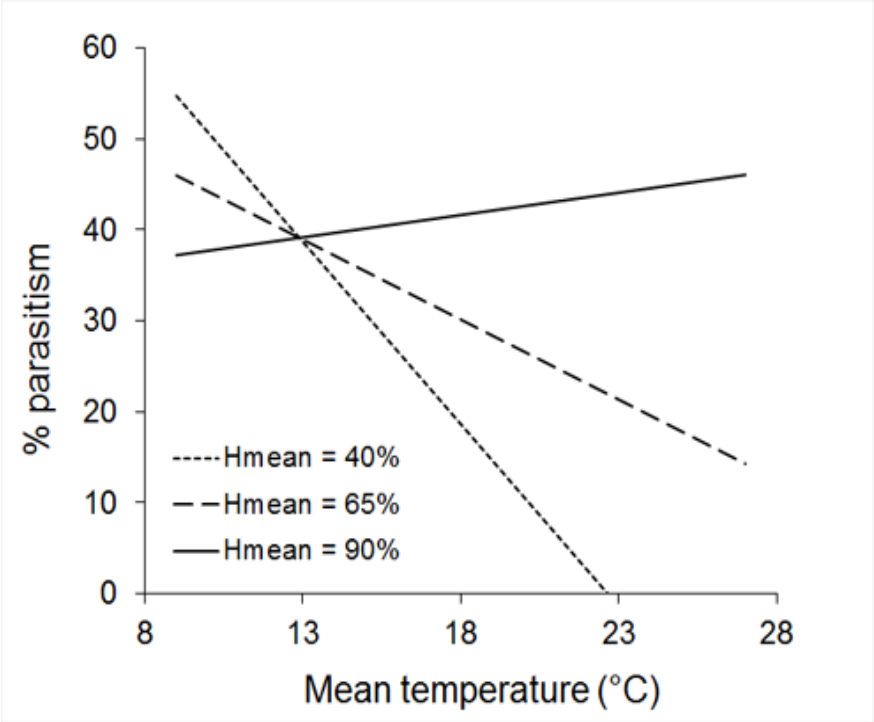

Figure 1. Relationship between parasitism (%) and mean temperature (°C), for high (90%), moderate (65%), and low (40%) mean humidity (Hmean). Notice how % parasitism of *Aganaspis daci* decreases as temperature increases when mean humidity is moderate or low; when mean humidity is high % parasitism increases as temperature increases.

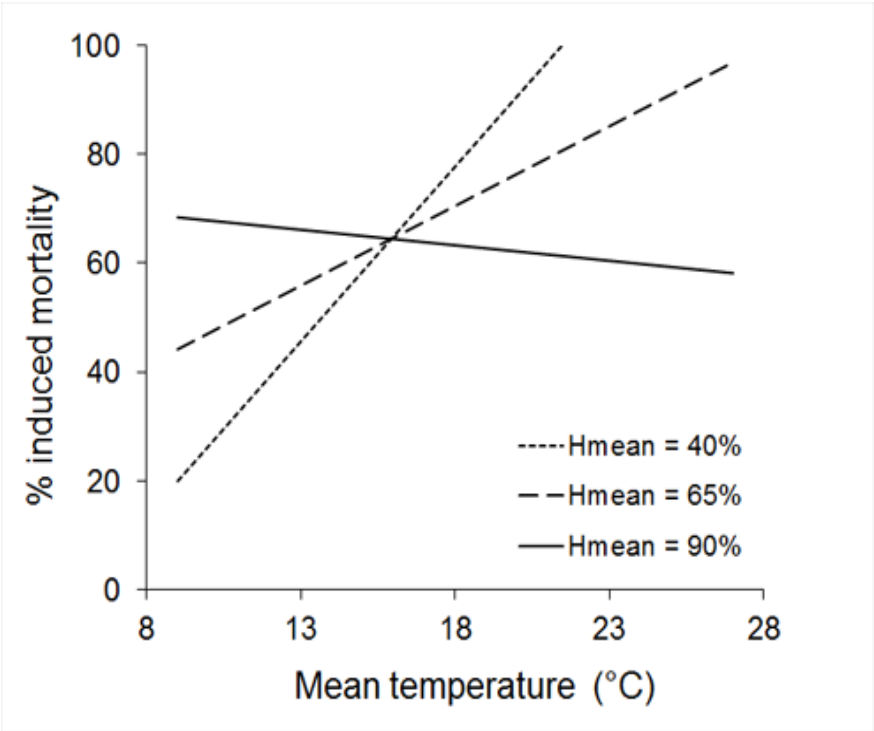

Figure 2. Relationship between induced mortality (%) and mean temperature (°C), for high (90%), moderate (65%), and low (40%) mean humidity (Hmean). Notice how induced mortality of *Aganaspis daci* increases with temperature when mean humidity is low or moderate, and how it decreases with temperature when mean humidity is high.

Table 2. Mean (± SE) climatic conditions of each assay in the field series.

Assay[1]	Season	Period	Temperature (°C)		Humidity (%)		No. sunshine hours	No. hours of cold[3]	Solar radiation (MJ m^{-2})	Rainfall (mm)	% population reduction
			Mean ± SE (range)	CV[2]	Mean ± SE (range)	CV[2]					
1	Spring	18 May 2012	25.3 ± 8.5 (17.1–34.5)	24.2	60.2 ± 27.5 (32.8–88.3)	37.5	13.0	0	28.2	0.1	80.1 ± 3.46
2		13 June	26.2 ± 7.1 (19.3–33.9)	20.1	68.6 ± 28.6 (36.3–96.1)	34.7	12.9	0	27.4	0.06	72.5 ± 0.00
3	Summer	27 June	27.2 ± 7.7 (19.6–35.5)	20.9	63.7 ± 28.4 (34.4–92.3)	35.9	13.1	0	27.8	0	90.5 ± 0.74
4		22 August	27.3 ± 7.1 (20.6–35.1)	71.8	71.8 ± 26.7 (39.9–97.1)	33.4	12.8	0	26.7	0	86.0 ± 3.94
5		6 September	22.2 ± 5.2 (17.6–29.4)	24.3	83.2 ± 20.5 (57.8–94.7)	25.9	9.6	0	14.3	1.8	96.5 ± 0.01
6	Autumn	27 September	19.7 ± 6.8 (13.8–28.6)	30.6	77.2 ± 29.1 (22.8–99.3)	26.4	8.9	0.4	13.2	1.0	93.0 ± 0.91
7		10 October	14.6 ± 4.7 (10.2–20.8)	35.6	79.5 ± 29.6 (54.7–96.1)	25.3	6.9	1.6	8.6	0.9	91.7 ± 0.01
8		25 October	12.7 ± 4.6 (8.7–18.8)	39.7	86.7 ± 22.1 (48.3–98.6)	49.9	6.9	3.4	7.3	2.1	90.2 ± 0.44
9		28 November	11.9 ± 4.4 (7.0–18.2)	40.6	60.2 ± 29.4 (35.0–83.9)	37.9	12.9	7.2	14.6	1.6	75.1 ± 4.45
10	Winter	23 January 2013	15.7 ± 9.0 (8.8–30.8)	45.1	65.1 ± 27.3 (24.1–92.9)	40.6	10.6	2.2	19.5	1.8	83.2 ± 2.60
11		6 February	20.9 ± 8.1 (12.4–30.3)	28.5	53.3 ± 29.1 (35.6–85.3)	33.1	12.1	0	26.9	0.2	88.4 ± 2.90
12	Spring	27 March	16.7 ± 5.2 (12.9–27.4)	32.6	78.2 ± 21.4 (57.9–94.9)	24.4	6.9	0.4	11.2	1.0	90.3 ± 0.34
13		8 May	22.2 ± 5.1 (17.6–29.4)	24.3	83.2 ± 17.8 (64.4–94.9)	25.9	9.6	0	14.4	1.9	89.7 ± 0.00

[1]Duration of each assay was until emergence of hosts and parasitoids, at least 1 month.

[2]CV, coefficient of variation (= relative standard deviation, RSD).

[3]Hours with temperature below 8.5 °C, the minimum threshold of *Aganaspis daci*.

4. Discussion

Temperature and humidity, and most likely their interaction, are known to affect parasitism parameters of parasitoids, such as developmental time, longevity, number of oocytes, fecundity, and parasitism rate (Kalyebi et al., 2006; Emana, 2007; Bruce et al., 2009; Quicke, 2015). Given their generally small size and, thus, their risk of water loss, humidity can also influence foraging activity of parasitoids and consequently parasitism rate (Moezipour et al., 2008; Quicke, 2015), particularly under hot conditions. *Aganaspis daci* appears no exception, although very few earlier studies addressed the effects of climatic conditions on its parasitism rate in the field – the strong dependence of its larval development upon environmental factors was already known (Andleeb et al., 2010; Hosni et al., 2011; de Pedro et al., 2016).

Under Mediterranean climate conditions, *A. daci* exhibited very low fertility compared to other parasitoids of *C. capitata*, such as the braconids *Diachasmimorpha longicaudata* (Ashmead) (Harbi et al., 2014) and *Diachasmimorpha tryoni* (Cameron) (Garzón-Luque et al., 2008), and the pteromalids *Spalangia cameroni* Perkins and *Pachycrepoideus vindemmiae* (Rondani) (Beitia et al., 2007; Pérez-Hinarejos & Beitia, 2008; Stacconi et al., 2013). According to these studies, although winter and summer temperatures seem to affect the immature development of all five species, *A. daci* presents higher immature mortality than the other four species throughout the year. Both high (30–35 °C) and low (ca. 15 ºC) temperatures lead to slow development and poor offspring survival in this parasitoid (de Pedro et al., 2016). One hypothesis is that the low fertility of *A. daci* is due to its inability to oviposit effectively under field climatic conditions. This contrasts with the results of Papadopoulos & Katsoyannos (2003) and the recent findings of Ali et al. (2016), which reported high levels of natural parasitism under Mediterranean climate conditions, but it concurs with their observation that natural populations are only present in the field during the summer, indicating only one generation per year, under the most favourable conditions.

Our experiments and assays indicated that *A. daci* induces mortality, parasitizes, and oviposits on *C. capitata* during all four seasons. Based on previous experiments on the optimal development of this parasitoid, such as suitable host fruits or parasitoid/host ratios (Andleeb et al., 2010; Hosni et al., 2011; de Pedro et al., 2013a,b, 2016, 2017), an experiment was designed to investigate the variation in parasitic activity of *A. daci* over time. We found that *A. daci* parasitic activity (and thus induced mortality) is correlated with temperature and humidity. Parasitism showed significant differences between seasons with greater climatic differences (spring and summer vs. autumn and winter), and increased as the optimal conditions required by the parasitoid were approached. De Pedro et al. (2013a,b) and Harbi et al. (2014) already found significant differences in induced mortality, mainly between summer and the other seasons. We

found that parasitism rates in winter (low temperature) are higher under low levels of humidity, and in summer (high temperature) increase under high humidity (opposite trends occurred for induced mortality). This indicates that during winter, humidity may have no beneficial effect on parasitoid activity, whereas during warmer periods of the year, when water loss risk is higher, *A. daci* benefits from higher humidity to keep its water content at an optimum level, allowing proper functioning of their metabolism. Similar results have been reported from other parasitoids of fruit flies, such as the braconid *Psyttalia concolor* (Szépligeti), which displays higher parasitism rates on *Bactrocera oleae* (Rossi) under warm and wet conditions that support growth and development of both parasitoids and hosts (Yokoyama et al., 2006, 2008; Yokoyama & Miller, 2007).

It can be concluded that *A. daci*, despite having a very low fertility under field conditions, exhibits acceptable parasitism rates. Together with its induced mortality, it results in a strong pest population reduction, which is the final aim of the integrated pest management programme against *C. capitata*. Therefore, *A. daci*, as previously suggested by de Pedro et al. (2013a,b) and Ali et al. (2016), is a good candidate to be used in inundative field releases for the management of *C. capitata* in the Mediterranean Basin.

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References

Ali AY, Ahmad AM & Amar JA (2015) Hymenopteran parasitoids (Figitidae and Pteromalidae) of *Ceratitis capitata* (Diptera: Tephritidae) on loquat and guava in Tartous, Syria. Biocontrol Science and Technology 25: 223–228.

Ali AY, Ahmad AM, Amar JA, Darwish RY, Izzo AM & Al-Ahmad SA (2016) Field parasitism levels of *Ceratitis capitata* larvae (Diptera: Tephritidae) by *Aganaspis daci* on different host fruit species in the coastal region of Tartous, Syria. Biocontrol Science and Technology 26: 1617–1625.

Andleeb S, Shahid MS & Mehmood R (2010) Biology of parasitoid *Aganaspis daci* (Weld) (Hymenoptera: Eucoilidae). Pakistan Journal of Scientific and Industrial Research 53: 201–204.

Baranowski R, Glenn H & Sivinski J (1993) Biological control of the Caribbean fruit fly (Diptera: Tephritidae). Florida Entomologist 76: 245–251.

Beitia F, Falcó JV, Pérez-Hinarejos M, Santiago S & Castañera P (2003) Importación de parasitoides exóticos para el control biológico de *Ceratitis capitata* en la Comunidad Valenciana. Comunitat Valenciana Agraria 24: 10–15.

Beitia F, Pérez-Hinarejos M, Garzón E, Santiago S, Tarazona I et al. (2007) Lucha biológica contra *Ceratitis capitata*: Himenópteros parasitoides autóctonos. Terralia 63: 34–44.

Beitia F, Sabater-Muñoz B & Malagón J (2011) Estrategias de manejo integrado de la mosca mediterránea de la fruta en la Comunidad Valenciana. Vida Rural 323: 40–46.

Bruce AY, Schulthess F & Mueke J (2009) Host acceptance, suitability, and effects of host deprivation on the West African egg parasitoid *Telenomus isis* (Hymenoptera: Scelionidae) reared on East African stemborers under varying temperature and relative humidity regimens. Environmental Entomology 38: 904–919.

Clausen CP (1978) Tephritidae (Trypetidae, Trupaneidae). Introduced Parasites and Predators of Arthropod Pests and Weeds: A World Review (ed. by CP Clausen), pp. 320–335. Agricultural Handbook No. 480. Washington, DC, USA, ARS-USDA.

Delrio G & Cocco A (2012) Tephritidae. Integrated Control of Citrus Pests in the Mediterranean Region (ed. by V Vacante & U Gerson), pp. 206–222. Bentham Science, Sharja, UAE.

El-Heneidy A, Hosny M & Ramadan M (2014) Potential of the parasitoid *Aganaspis daci* (Hymenoptera: Eucoilidae) for biological control of *Bactrocera zonata* (Diptera: Tephritidae) in Egypt. Ninth International Symposium on Fruit Flies of Economic Importance (ISFFEI), Book of Abstracts, Bangkok, Thailand, p. 220.

Emana GD (2007) Comparative studies of the influence of relative humidity and temperature on the longevity and fecundity of the parasitoid *Cotesia flavipes*. Journal of Insect Science 7: 19.

Falcó JV, Pérez-Hinarejos M, Santiago S, Navarro S & Beitia F (2003) Introducción de parasitoides exóticos para el control de insectos plaga: procedimientos de importación y cuarentena. Contribuciones al Conocimiento de las Especies Exóticas Invasoras, Vol. 1 (ed. by L Capdevila-Argüelles, B Zilletti & N Pérez Hidalgo), pp. 222-224. GEI Serie Técnica, León, Spain.

Falcó JV, Garzón-Luque E, Pérez-Hinarejos M, Tarazona I, Malagón J & Beitia F (2006) Two native pupal parasitoids of *Ceratitis capitata* (Diptera, Tephritidae) found in Spain. IOBC/WPRS Bulletin 29: 71–74.

Garzón-Luque E, Beitia F & Falcó JV (2008) Parasitism of *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) on the host *Ceratitis capitata* (Diptera: Tephritidae) under mediterranean temperatures. IOBC/WPRS Bulletin 38: 125–129.

Harbi A, de Pedro L, Ferrara FA, Tormos J, Beitia F & Sabater-Muñoz B (2014) Parasitism activity of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) and *Aganaspis daci* (Weld) (Hymenoptera: Figitidae) against *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) under Mediterranean Climatic Conditions. Ninth International Symposium on Fruit Flies of Economic Importance (ISFFEI), Book of Abstracts, Bangkok, Thailand, p. 214.

Hosni M, El-Husseini MM, El-Heneidy A & Atallah FA (2011) Biological aspects of the peach fruit fly, *Bactrocera zonata* (Saund.) (Diptera: Tephritidae) and its parasitoid species, *Aganaspis daci* Weld (Hymenoptera: Eucoilidae). Egyptian Journal of Biological Pest Control 21: 137–142.

Jackson JE (2003) A User's Guide to Principal Components. Wiley, Hoboken, NJ, USA.

Kalyebi A, Overholt WA, Schulthess F, Mueke J & Sithanantham S (2006) The effect of temperature and humidity on the bionomics of six African egg parasitoids (Hymenoptera, Trichogrammatidae). Bulletin of Entomological Research 96: 305–314.

Liquido NJ, Shindoa LA & Cunningham RT (1991) Host Plants of the Mediterranean Fruit Fly, *C. capitata* (Wiedemann) (Diptera: Tephritidae): An Annotated World Review. Miscellaneous Publication 77, Entomological Society of America, Lanham, MD, USA.

Martins DS, Skouri W, Chermiti B, Aboussaid H, El Messoussi S et al. (2010) Analysis of two larval-pupal parasitoids (Hymenoptera, Braconidae) in the biological control of *Ceratitis capitata* (Wiedemann) in Spanish Mediterranean areas. Proceedings of the 8th International Symposium on Fruit Flies of Economic Importance (ed. by B Sabater-Muñoz, V Navarro-Llopis & A Urbaneja), pp. 252–258. Polytechnic University of Valencia Editorial, Valencia, Spain.

McGarigal K, Cushman S & Stafford S (2000) Multivariate Statistics for Wildlife and Ecology Research. Springer, New York, NY, USA.

Moezipour M, Kafil M & Allahyari H (2008) Functional response of *Trichogramma brassicae* at different temperatures and relative humidities. Bulletin of Insectology 62: 245–250.

Ovruski SM, Aluja M, Sivinski J & Wharton RA (2000) Hymenopteran parasitoids on fruit-infesting Tephritidae (Diptera) in Latin America and the southern United States: diversity, distribution, taxonomic status and their use in fruit fly biological control. International Pest Management Reviews 5: 81–107.

Ovruski SM, Wharton RA, Rull J & Guillén L (2007) *Aganaspis alujai* (Hymenoptera: Figitidae: Eucoilinae), a new species attacking *Rhagoletis* (Diptera: Tephritidae) in the neotropical region. Florida Entomologist 90: 626–634.

Papadopoulos NT (2008) Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Encyclopedia of Entomology, Vol. 3 (ed. by JL Capinera), pp. 2318–2322. Springer, Heidelberg, Germany.

Papadopoulos NT & Katsoyannos BI (2003) Field parasitism of *Ceratitis capitata* larvae by *Aganaspis daci* in Chios, Greece. BioControl 48: 191–195.

de Pedro L, Harbi A, Ferrara FA, Tormos J, Verdú MJ et al. (2013a) Parasitismo generado por *Aganaspis daci* (Weld) (Hymenoptera: Figitidae), parasitoide de *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), en condiciones climáticas mediterráneas. VIII Congreso Anual de Entomología Aplicada, XIV Jornadas Científicas de la SEEA, Book of Abstracts, Mataró, Spain, p. 163.

de Pedro L, Martínez R, Harbi A, Ferrara FA, Tormos J et al. (2013b) Un nuevo enemigo natural de *Ceratitis capitata* (Diptera, Tephritidae) identificado en la Comunidad Valenciana: el parasitoide *Aganaspis daci* (Hymenoptera, Figitidae). Levante Agrícola 416: 153–157.

de Pedro L, Beitia F, Sabater-Muñoz B, Asís JD & Tormos J (2016) Effect of temperature on the developmental time, survival of immatures and adult longevity of *Aganaspis daci* (Hymenoptera: Figitidae), a natural enemy of *Ceratitis capitata* (Diptera: Tephritidae). Crop Protection 85: 17–22.

de Pedro L, Beitia F, Ferrara F, Asís JD, Sabater-Muñoz B & Tormos J (2017) 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). Crop Protection 92: 160–167.

Pérez-Hinarejos M & Beitia F (2008) Parasitism of *Spalangia cameroni* (Hymenoptera, Pteromalidae), an idiobiont parasitoid on pupae of *Ceratitis capitata* (Diptera, Tephritidae). IOBC/WPRS Bulletin 38: 130–133.

Püntener W (1981) Manual for Field Trials in Plant Protection, 2nd edn. Agricultural Division, Ciba Geigy, Basle, Switzerland.

Quicke DLJ (2015) Biology, Systematics, Evolution and Ecology of Braconid and Ichneumonid Parasitoid Wasps. Wiley Blackwell, Chichester, UK.

Sabater-Muñoz B, Falcó JV, de Pedro L, Tormos J, Asís JD et al. (2012) First record, surveillance and biological parameters of *Aganaspis daci* (Hymenoptera: Figitidae), as parasitoid of *Ceratitis capitata* (Diptera: Tephritidae) in Spain. Biological Invasions of Tephritidae: Ecological and Economic Impacts (ed. by NT Papadopoulos), p.117. Book of Abstracts, Kolymbari, Crete (Greece).

Stacconi MVR, Grassi A, Dalton T, Miller B, Ouantar M et al. (2013) First field records of *Pachycrepoideus vindemmiae* as a parasitoid of *Drosophila suzukii* in European and Oregon small fruit production areas. Entomologia 1: e3.

Szyniszewska AM & Tatem AJ (2014) Global assessment of seasonal potential distribution of mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). PLoS ONE 9: e111582.

Tormos J, de Pedro L, Beitia F, Sabater-Muñoz B, Asís JD & Polidori C (2013) Development, preimaginal phases and adult sensillar equipment in *Aganaspis* parasitoids (Hymenoptera: Figitidae) of fruit flies. Microscopy and Microanalysis 19: 1475–1489.

Verdú MJ, Falcó JV, Beitia F & Sabater-Muñoz B (2011) Identificación de un nuevo agente de control biológico de *Ceratitis capitata* en España, el himenóptero eucoilino *Aganaspis daci*. XXVIII Jornadas de la Asociación Española de Entomología (AeE). Book of Abstracts, Ponferrada, Spain, p. 25.

Weld LH (1951) A new species of *Trybliographa* (Hymenoptera: Cynipidae). Proceedings of the Hawaiian Entomological Society 14: 331–332.

Wharton RA, Gilstrap FE, Rhodei RH, Fischel MM & Hart WG (1981) Hymenopterous egg-pupal and larval-pupal parasitoids of *Ceratitis capitata* and *Anastrepha spp*. (Diptera, Tephritidae) in Costa Rica. Entomophaga 26: 285–290.

Yokoyama VY & Miller GT (2007) Olive fruit fly biology and cultural control practices in California. IOBC/WPRS Bulletin 30: 277–285.

Yokoyama VY, Miller GT, Stewart-Leslie J, Rice RE & Phillips PA (2006) Olive fruit fly (Diptera: Tephritidae) populations in relation to region, trap type, season, and availability of fruit. Journal of Economic Entomology 99: 2072–2079.

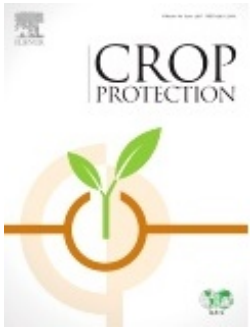
Yokoyama VY, Rendón PA & Sivinski J (2008) *Psyttalia* cf. *concolor* (Hymenoptera: Braconidae) for biological control of olive fruit fly (Diptera: Tephritidae) in California. Environmental Entomology 37: 764–773.

CAPíTVLO 6

"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)"

Efecto de la densidad y localización de hospedador sobre el porcentaje de parasitismo, la fertilidad y la mortalidad inducida de *Aganaspis daci* (Hymenoptera: Figitidae), parasitoide de *Ceratitis capitata* (Diptera: Tephritidae)

Luis de Pedro, Francisco Beitia, Fernando Ferrara, Josep Daniel Asís, Beatriz Sabater-Muñoz, José Tormos



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Resumen: El porcentaje de parasitismo, la fertilidad y la mortalidad inducida (mortalidad de pupas del hospedador, de las que no emergen adultos, atribuida a los parasitoides) del parasitoide *Aganaspis daci* (Weld) infestando larvas de *Ceratitis capitata* (Wiedemann) fueron estudiados en condiciones de laboratorio e invernadero en función de la temperatura y de la densidad y localización (larvas en dieta artificial o en el interior de frutos) del hospedador. Nuestros estudios mostraron que el rango de temperatura 23–25 ˚C es el más adecuado cuando se ofrecían larvas en dieta artificial y en el laboratorio, llevando a altas tasas de parasitismo, fertilidad, mortalidad inducida y reducción poblacional (mortalidad total de *C. capitata* causada por el parasitoide, es decir, mortalidad inducida + porcentaje de parasitismo) y a un sex ratio de la descendencia desplazado hacia las hembras (♀♀/♀♀ + ♂♂ = 0.55). Respecto a la densidad de hospedador, el parasitismo y la fertilidad mostraron una respuesta funcional de tipo III con un sex ratio desplazado hacia las hembras (0.54–0.61), mientras que la mortalidad inducida decrecía al incrementarse la densidad de hospedador, y la reducción poblacional mostraba el mismo tipo de respuesta que parasitismo y fertilidad. Cuando las larvas se ofrecían en frutos y en el laboratorio, el parasitismo y la fertilidad, en función de la densidad de hospedador, también mostraban una respuesta funcional de tipo III y un sex-ratio desplazado hacia las hembras (0.51–0.62). Para el caso de las larvas ofrecidas en frutos en el invernadero, nuestros resultados no mostraron diferencias significativas en porcentaje de parasitismo, mortalidad inducida o reducción poblacional respecto a la densidad de hospedador o a la posición del fruto (en suelo o en árbol), aunque el sex ratio sí aparecía desplazado hacia las hembras (posición del fruto: 0.64–0.72; densidad: 0.66–0.76). Respecto a la densidad de hospedador, parasitismo y fertilidad mostraban una respuesta funcional de tipo II. La mortalidad inducida decrecía al

aumentar la densidad, y la reducción poblacional respondía igual que el parasitismo y la fertilidad. Los datos aportados por este estudio acerca de los parámetros demográficos básicos y la respuesta funcional de *A. daci* sobre *C. capitata* bajo diversas condiciones y ambientes contribuirá a evaluar el uso potencial de este parasitoide como agente de control biológico contra esta plaga. Nuestros resultados muestran que *A. daci* presenta una gran habilidad para localizar, capturar, parasitar o, simplemente, matar a *C. capitata* en condiciones de laboratorio y campo.

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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