Parasitism activity of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) and *Aganaspis daci* (Weld) (Hymenoptera: Figitidae) against *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) under Mediterranean climatic conditions

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Abstract

Background: Ceratitis capitata (Wied.), the Mediterranean fruit fly, is one of the key pest species affecting citrus production around the Mediterranean coasts of Spain, Morocco and Tunisia. During the past decade the IVIA (Valencian Institute for Agricultural Research) has imported several parasitoid species to enhance a Biological Control (BC) program against this pest. Soon after the introduction of *Diachasmimorpha longicaudata* (Ashmead) in 2009, a native parasitoid, the figitid *Aganaspis daci* (Weld), was identified in Bétera, a town in the province of Valencia. This work will contribute to highlight the importance of the two species within the BC program against *C. capitata* in the study areas.

Methods: To determine the influence of climatic factors on parasitism rate and Medfly mortality, apples artificially infested with Medfly larvae were individually exposed to five parasitoid couples for one week under natural conditions. Forty wood-framed mesh cages (twenty for each parasitism rate and immature development) of each parasitoid species were tested weekly over 10 weeks across one year.

Results: Under Mediterranean climatic conditions, *D. longicaudata* exerted a high parasitism rate compared with *A. daci*. Extreme winter and summer temperatures seem to affect the immature development of both species. A higher immature mortality was observed for *A. daci* throughout the year, compared with *D. longicaudata*. Adult parasitoid species were capable of parasitizing *C. capitata* L2/L3 larvae at the extreme temperatures tested.

Conclusions: This study suggests that *A. daci* shows a good performance as well as *D. longicaudata* against *C. capitata* under Mediterranean climatic conditions. Further studies are required to determine the influence of other climatic factors and whether the two parasitoid species act in synergism.

Keywords: infested fruits, field conditions, parasitism rate.

Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is a destructive pest of over 330 species of fruits and vegetables (Liquido et al., 1989; Beitia et al., 2003). It is a major pest of *Citrus* and also infests deciduous fruits, such as peach, pear, and apple (Thomas et al., 2010). It originated in Tropical Africa its origin from where it has spread to the Mediterranean area and to parts of Central and South America (Malacrida et al., 2007). It is one of the most important pests of tropical, subtropical and temperate regions (FAO/IAEA, 1993). It has a great ability to disperse, to use alternative hosts and presents a great developmental plasticity which allows its survival during all seasons. In addition to crop losses, it is responsible for the establishment of quarantine restrictions that prevent or hinder the development of agricultural exports wherever it occurs (Rendon et al., 2006). In most of the countries with these problems, the control programs are essentially based on chemical treatments, mass trapping, the sterile insect technique (SIT), chemosterilization and on biological control by the use of parasitoids, despite this is the least used method (Beitia et al., 2003; Castañera et al., 2003). To establish an Integrated Pest Management program involving environmentally safe control systems (like biological control) as requested/recommended in the European normative 2009/128/CE, Spain established cooperation programs with Morocco and Tunisia for the search of native parasitoids and to share the imported ones (Harbi et al., 2015).

Nowadays in the Valencian Community (Spain), biological control against *C. capitata* is being developed and the use of two promising species is proposed: *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) and *Aganaspis daci* (Weld) (Hymenoptera: Figitidae).

Diachasmimorpha longicaudata is an exotic parasitoid, introduced from Mexico to Spain by the Instituto Valenciano de Investigaciones Agrarias (IVIA, Valencian Institute for Agricultural Research) in 2009 (Sabater-Muñoz et al., 2009; Harbi et al., 2015). It is a larvo-pupal endoparasitoid of fruit flies, native to the Indo-Pacific region (Oroño & Ovruski, 2007; Carbajal-Paladino et al., 2010). This is the most important parasitoid species that is being used worldwide as part of integrated pest management programs against fruit flies of the genera *Bactrocera*, *Anastrepha* and *Ceratitis* (Carbajal-Paladino et al., 2010).

Aganaspis daci, also from Indo-Pacific region, was found in the Valencian Community in 2009 (Sabater-Muñoz et al., 2012) in *C. capitata* pupae from fig fruits. This parasitoid species was first found exclusively in the Greek island of Chios in 2003 (Papadopoulos & Katsoyannos, 2003). This species is also a larvo–pupal endoparasitoid (Tormos et al., 2013) and is used as an agent for biological control of many species of Tephritidae (Papadopoulos & Katsoyannos, 2003).

Despite both parasitoids species are being used in many American countries, studies including abiotic factors (climatic) concerning their use in citrus crops on the Mediterranean basin (west

Palearctic eco-region) are scarce. Both parasitoids are being tested in laboratory, greenhouse, semi-field and field conditions to assess its efficacy as biological control agents of the Medfly for its inclusion in IPM programms in citrus orchards. In this work, we show preliminary results on a semi-field experiment on the parasitism ability and induced medfly mortality of both species under natural Mediterranean conditions.

Material and Methods

Experimental area

All the trials were conducted in a 30-year old lemon (Citrus x lemon) plot, subjected to IPM, located in the experimental station of IVIA (Valencian Institute of Agricultural Research, Moncada, Valencia, Spain).

Insect rearing

Ceratitis capitata, *D. longicaudata* and *A. daci*, were reared in climatic chambers at the Entomology Unit of the IVIA. The *C. capitata* 'IVIA 2002' strain was reared in artificial diet, and was used to maintain *D. longicaudata* and *A. daci* colonies, since 2009 and 2010 respectively, as described in Harbi et al. (2015). Briefly, 4-8 hours-old medfly eggs (0.3ml) were collected on water recipients located below medfly rearing cages, placed in artificial larval diet (30% Miller's wheat bran, 7.5% sugar, 3.6% brewer's yeast, and 0.24% benzoic acid, 0.2% metil-paraben and 0.2% propil-paraben as preservatives) and allowed to develop till L3 at 25±2ºC, 65±10% relative humidity (RH) and in complete darkness. Medfly L3 larva were either allowed to develop into adult medflies or used as host for the parasitoids. The laboratory conditions for the medfly rearing were 27 ± 2ºC, 65 ± 10% RH and 16:8 h (L:D) photoperiod; whereas the parasitoids were reared at a lower temperature 23 ± 2ºC, keeping the same RH and photoperiod as for the Medfly (Harbi et al., 2015; De Pedro et al., 2013). Medfly adults were feed with a mixture of sugar: yeast extract (4:1), with *ad libitum* water and house-hold granulated sugar. Adults of both parasitoids species were fed with house-hold granulated sugar, *ad libitum* water, and with a daily supply of natural bee honey (honey of thousand flowers).

Parasitism Experimental Protocol

Parasitism ability was determined independently for *D. longicaudata* and *A. daci* throughout a period of twelve months (from July 2012 until May 2013) to cover all climatic seasons under Mediterranean conditions.

The experimental unit consisted in an apple (*Malus domestica* var. Royal Gala, from organic management fields) artificially infested with thirty 2nd/3rd-instar larvae of the medfly (ten holes per fruit and three larvae per hole). Apples were selected as medfly host for their availability throughout the year and by per previous results (unpublished PhD theses from A. Harbi and L. de Pedro). Each apple was confined in a ventilated plastic cylinder with five couples of the parasitoid species (3-5 days old). Each cylinder had a 50 ml water container, and parasitoids were provided with honey every two days. Cylinders were kept inside wood-framed mesh cages as protection against rain, and placed under one single lemon tree for protection against direct sunlight. After one week of exposure, cylinders were retrieved to the laboratory, pupae collected in 125 ml plastic cups, covered with vermiculite and kept at laboratory conditions (25 ± 2 ºC, 50-70% RH, 16:8 L:D) until adult emergence or up to 60 days from pick-up of pupae from the field (after this 60-days period, pupae not emerged were dissected to identify if possible, the parasitoid or medfly remains). Each trial/treatment consisted in 10 experimental units per parasitoid species, plus 10 experimental units without any parasitoid (control) to determine natural medfly mortality under each climatic condition. A total of 8 trials were conducted.

The parasitism rate was calculated for each replicate as the percentage of emerged adult parasitoids from recovered pupae (female realized fertility). The mortality of pupae (closed puparia) attributed to parasitoids was also evaluated by comparing the percentage of mortality in controls with those in treatments. This “corrected mortality” was calculated with Abbott’s formula (Abbott, 1925) as presented below.

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

Temperature and relative humidity were recorded by a datalogger (HD226-1, Delta Ohm, Padova, Italy) placed in one of the wooden cages with the cylinders. Other climatic conditions (wind direction, wind force, rain precipitation, UV radiation and daylight duration) were obtained from the weather station at the IVIA (located at less than 1 km from the lemon tree plot).

Results and Discussion

The parasitism rate of *D. longicaudata* and *A. daci* and climatic conditions of each trial are shown in Fig.1. As can be observed, both species were able to parasitize larvae of medfly throughout the year, despite the differences in temperature and RH for each trial (see the differences between continuous and dashed lines in Fig.1) were noticeable.

Moreover, each species show a differentiated parasitism rates in each season (see black continuous line for *D. longicaudata* and dashed black line for *A. daci*). Indeed, it seems that each parasitoid species has its own best period, when the parasitism rate is up for one species is low for the second. Further statistical analyses of these data are required to unveil any correlation between both species.

Diachasmimorpha longicaudata parasitism ability on C. capitata larvae under natural conditions.

Diachasmimorpha longicaudata parasitized *C. capitata* larvae during all the year and the parasitism rate reached its maximum (49%) in April. The best parasitism rates were recorded in summer, autumn and spring when average temperatures were between 18°C and 26°C. In the majority of cases, the lowest parasitism rates were recorded in the coldest periods (≤ 15°C), with one exception, September 2012, with an average temperature of 22.9°C, the parasitism rate achieved was lower (14%) than the general trend. This result could be explained by the influence of other climatic factors, like precipitation (high precipitations recorded during this period, data not shown) and daylight hours (autumn-winter in northern hemisphere at Valencia latitude has an average of 6.5 h of light) that should be further examined. Under laboratory conditions Liu et al. (2012), found that the best temperature range for the development and reproduction of *D. longicaudata* reared on *C. capitata* was between 24 and 27°C. Extreme low or high temperatures had an inhibitory effect on *D. longicaudata* development. The same temperature range (24-27°C) was recorded by Lawrence et al. (1976) as suitable for the development of *D. longicaudata* reared on *Anastrepha suspensa* (Loew). Results are also consistent with those reported by Appiah et al. (2013), who found that the most suitable temperature range for the optimum parasitism rate of *D. longicaudata* reared on *Bactrocera invadens* is between 20 and 25°C. Also Sime et al. (2006) showed that this moderate range of temperatures (22–25°C) is optimal for the development of both *D. longicaudata* and *D. kraussi* reared on *Bactrocera oleae* (Gmel.).

Aganaspis daci parasitism ability on C. capitata larvae under natural conditions.

For *A. daci*, the parasitism rates were relatively uniform along the study period, with a minimum value of 21% in July, when the average temperature was the highest. In contrast, the highest parasitism rates were achieved during the coldest period, with a maximum of 48.9% in December (9.2 °C) (Fig.1).

Comparatively, *A. daci* produced a higher parasitism rate in the coldest period, when *D. longicaudata* showed the lowest parasitism rate values. Given that no published data about the effect of the temperature on the development of *A. daci* is available, our results suggests that this figitid species may be adapted to lower temperatures compared to *D. longicaudata* (Fig.1). In the opposite hand, at average temperatures higher to 18°C, the parasitism rates of *D. longicaudata* were better than those of *A. daci* (Fig.1).

The results show the absence of a direct relationship between the temperature and the rate of parasitism of both species, suggesting that other climatic factors can play a major role on it, which deserve further analyses.

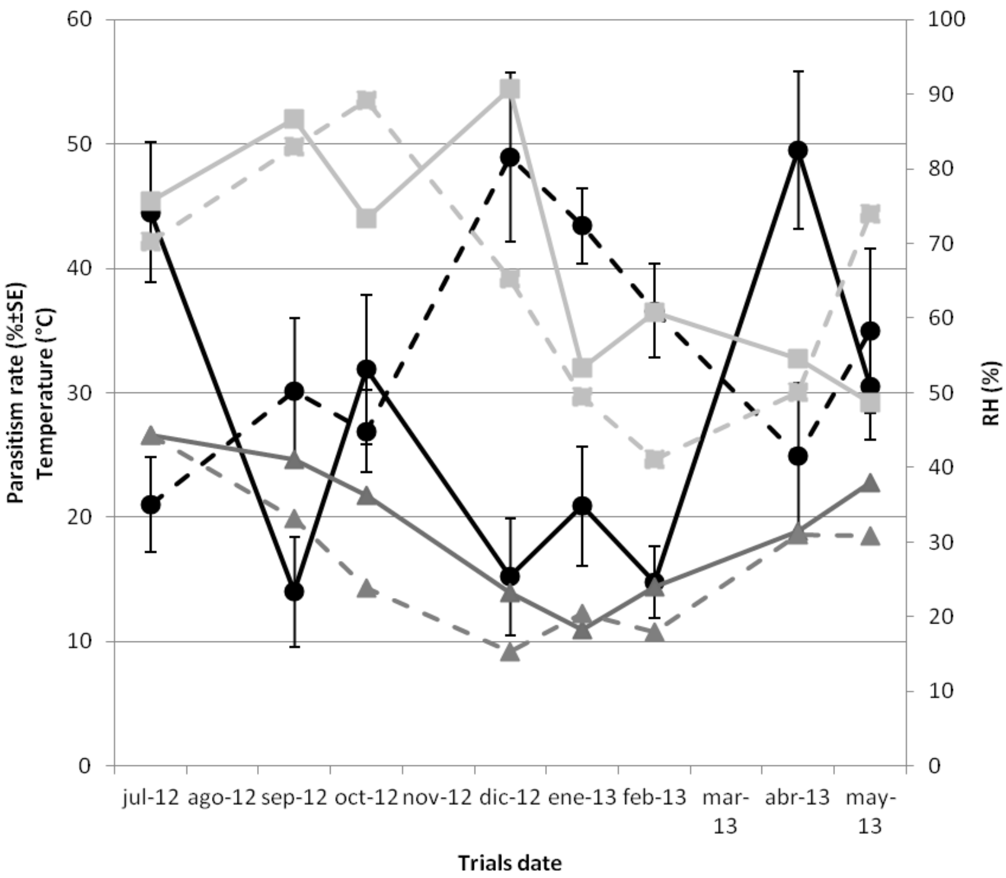

Fig. 1. Parasitism rate (% ± SE; in black), average temperature (Celsius degrees; in dark grey) and relative humidity (%; in light grey) obtained for each trial and parasitoid species. Continuous lines for *D. longicaudata*, and dashed ones for *A. daci*.

Parasitoid effect on C. capitata mortality

Corrected mortality, following Abbott's formula, puts into value the effect of each parasitoid, as includes the mortality of parasitoid immature stages (due to unfavorable climatic conditions) and medfly pupae killed by parasitoid females (by multiple oviposition events without the presence of parasitoid egg). Table 3 summarizes the results of mortality accounted in *C. capitata* pupae (denoted as pupa not emerged) exposed to *D. longicaudata* and *A. daci*, with the corresponding natural mortality of the controls.

In general, *C. capitata* mortality induced by *A. daci* was higher to that induced by *D. longicaudata* throughout the year (Table 1). The contribution of abiotic factors (climatic conditions) to this induced mortality should be further studied. However, as can be observed in Fig.2 and Fig.3, it seems exists a relationship between mortality, average temperature and RH, in opposite directions for each parasitoid species. As can be observed *A. daci* induces high mortality during hottest periods whereas *D. longicaudata* does it in coldest periods (Fig.2 and Fig.3 respectively).

Table 1. *Ceratitis capitata* immatures mortality (expressed as %) in treatments, corresponding controls, and the corrected mortality (Abbott's formula) due to the parasitoids *D. longicaudata* and *A. daci*.

Period	***D. longicaudata***			***A. daci***		
	Treatment	Control	Corrected	Treatment	Control	Corrected
July 2012	19.9	1.1	18.9	78.9	8.8	76.9
September 2012	15.8	12.1	4.2	64.3	6.1	62.0
October 2012	8.7	8.9	-0.2	67.2	9.3	63.8
December 2012	19.4	2.4	17.4	45.9	11.3	39.0
January 2013	37.4	9.7	30.7	52.9	13.0	45.9
February 2013	16.9	4.6	12.8	39.3	2.3	37.9
April 2013	13.6	1.1	12.6	72.0	9.4	69.1
May 2013	24.6	6.7	19.2	67.6	10.3	63.9

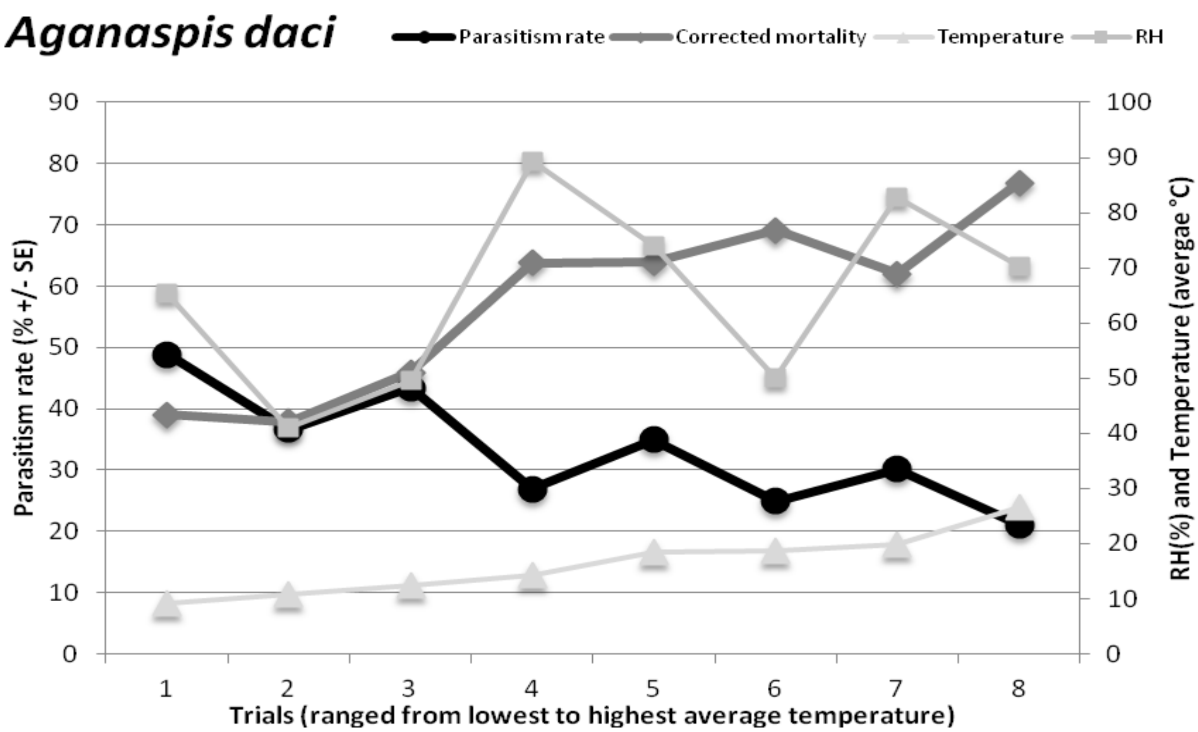

Fig. 2. Evolution of *A. daci* parasitism rate (%, black line) and corrected mortality (%, dark grey line) over *C. capitata* along the experiment, when trials are arranged in average ambience temperature (triangles in light grey) ascending order.

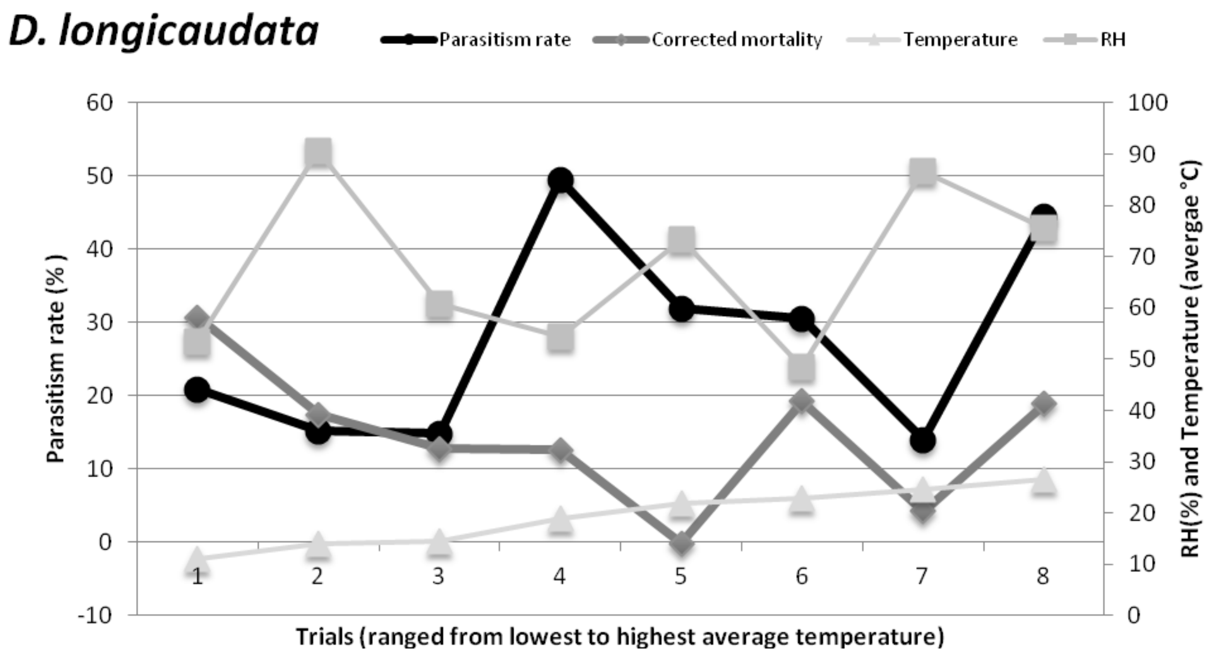

Fig. 3. Evolution of *D. longicaudata* parasitism rate (%, black line) and corrected mortality (%, dark grey line) over *C. capitata* along the experiment, when trials are arranged in average ambience temperature (triangles in light grey) ascending order.

It is important to note that, in general, the mortality induced for *A. daci* on pupae was higher compared to that induced by *D. longicaudata* throughout the year (Fig.2 line in dark grey vs Fig.3 in dark grey). It can be suggested that the corrected mortality reflects mainly the mortality of immature stages of the parasitoids: high rates of parasitism is related with low rates of mortality, so this fact reinforces the idea that low temperatures favor the parasitism of *A. daci* and in opposite side, *D. longicaudata* seems to prefer higher temperatures.

In summary, the results show that the reduction of medfly populations by the two parasitoid species can be affected not only by the parasitism activity but also by the mortality induced on pupae, and that both pest control factors are affected by climatic conditions (temperature and RH), but this last step deserves further research. In general, both species can be considered as potential biocontrol agents against the medfly in the Valencian Community, and that these results can be translated to Tunisia in the Mediterranean Region, which shows nearly similar climatic conditions. However, more work must be done to determine the way these parasitoids may interact in the field and along the time. Studies on the influence of the temperature and other climatic conditions (as the photoperiod, RH and the rainfall) on the parasitism rate of each of these two species, and also to know the effect of those climatic conditions on the development and mortality of the pre-imaginal stages of both parasitoid species in the field will surely provide valuable information.

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