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Effect of the olive fruit size on the parasitism rates of *Bactocera oleae* (Diptera: Tephritidae) by the figitid wasp *Aganaspis daci* (Hymenoptera: Figitidae), and first field releases of adult parasitoids in olive grove

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Summary The olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is the major pest of olives worldwide. The figitid wasp, *Aganaspis daci* (Hymenoptera: Figitidae), is a larval-prepupal endoparasitoid of fruit fly species, and it was found to successfully parasitize medfly larvae in field-infested figs in Greece. To assess the potential of *A. daci* as a biological control agent against *B. oleae*, we studied the effect of olive fruit size on parasitism rates of *A. daci* on 2nd and 3rd instar larvae of *B. oleae*, by using fruit of different size (cultivar 'Chalkidikis') and wild olive fruit. In addition, we conducted releases of *A. daci* females in a pilot olive grove in Volos, Magnesia. From July to October, we released 200 *A. daci* females/0.1 ha/week, followed by olive fruit sampling to estimate olive fruit infestation levels and the parasitism rates of *A. daci*. Laboratory trials revealed that fruit size and larvae instar were predictors of parasitism success of *A. daci*, with parasitism rates higher for small-size fruit of the cultivar "Chalkidikis" and the 3rd instar larvae of *B. oleae*. In field trials, no *A. daci* adults emerged from the olive fly infested fruit.

Additional keywords: biological control, larval instar, parasitism, olive fruit fly

Introduction

The olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is a monophagous species, of sub-Saharan African origin, that attacks fruits of African and Asian wild olive, *O. europaea* ssp. *cuspidate,* and the commercial olives *O. europaea* ssp. *europaea* (including wild olive fruit or naturalized 'oleasters') (Hoelmer *et al.*, 2011, Tzanakakis, 2006). Its presence has been well documented in the Mediterranean Basin, South and Central Africa, Middle East and India, and it has recently invaded California and northwestern Mexico (Nardi *et al.*, 2010). The olive fruit fly usually lays one or more eggs just below the surface of the fruit, and larvae form galleries while feeding on the pulp of the fruit. Besides secondary infestation from both bacteria and fungi, olive fruit fly infestation causes premature fruit drop and reduces fruit and olive oil quantity and quality (Manousis and Moore, 1987). Current management strategies for olive fruit fly populations rely primarily on insecticide application either through cover sprays or bait sprays that target adult fly. Resistance of *B. oleae* to insecticides has been documented both for olive fly populations from Greece (Kampouraki *et al.*, 2018) and California (Kakani *et al.*, 2010). Thus, *B. oleae* poses a serious threat to the olive industry worldwide and its control is challenging (Daane and Johnson, 2010).

Over nearly 100 years, considerable efforts have been made to manage the olive fruit fly in southern Europe by introducing primarily North African populations of *Psyttalia* (*Opius*) *concolor* (Szepligeti) (Hymenoptera: Braconidae), a koinobiont endoparasitoid of the second and third larval instar of the Mediterranean fruit fly (medfly), *Ceratitis capitata,* and the olive fly (Daane and Johnson, 2010). In Greece, initial trials in the island of Chalki gave promising results during the first year of release (18-37% parasitism rates) but parasitism rates declined

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(1-2%) the following years (Stavraki-Paulopoulou, 1966). In the island of Corfu, releases of *P. concolor* (from Tunisia) against heavily infested olive trees of the Lanolia variety during the spring revealed high parasitism rates (30-50%) even from the 1st week after the release (Kapatos *et al.*, 1977). The harvest period of olive fruit in Corfu (collected from the ground in the spring) differs from that of other regions in Greece (harvested in autumn). Apparently, unharvested fruit support an additional, spring generation of the olive fruit fly that gives adults late in May and/or June (Kapatos *et al.*, 1977). However, *P. concolor* rarely contributes to control of the olive fruit fly populations in spring without considering large inundative wasp releases (Liaropoulos *et al.*, 1977; Kapatos *et al.*, 1977).

In Spain, *P. concolor* was initially introduced in late 1970s, but failed to get established in several olive oil production areas (Jiménez *et al.*, 1998). More recently, high parasitism levels (>20%) of *B. oleae* pupae by *P. concolor* were recorded during August – September in organically-managed orchards in Majorca (Balearic Islands) (Miranda *et al.*, 2008). Apart from the Balearic Islands, *P. concolor* is also assumed to be established in southern Italy (Raspi and Loni, 1994). Moreover, field releases of the generalist, egg parasitoid of fruit flies, *Fopius arisanus* (Hymenoptera: Braconidae), revealed parasitism rates >20% during autumn when humidity levels are high in Italy (Moretti *et al.*, 2007).

In California (USA), numerous braconid parasitoids were screened from 2002 onwards as potential biological control agents of *B. oleae* and two larval endoparasitoids, *Psyttalia humilis* (from Namibia) and *P. lounsburyi* (from Kenya) have been released in olive groves of coastal and inland countries (Yokoyama *et al.*, 2011; Daane *et al.*, 2015). Recovery results revealed that the parasitoids have been established in coastal regions and, more importantly, *P. lounsburyi* can successfully overwinter and survive even at low *B. oleae* densities (Daane *et al.*, 2015).

The parasitoid *Aganaspis daci* (Hymenoptera: Figitidae, Eucoilinae) is another solitary, primary endoparasitoid that attacks the larval-prepupal stage of numerous fruit fly species in South-East Asia and Australia (Clausen *et al.*, 1965). It was first detected on parasitized larvae of *B. dorsalis* (Hendel) in Malaysia and Taiwan (Weld, 1951). In the Mediterranean basin, *A. daci* was first recovered from medfly puparia of field-infested figs in the Greek island of Chios in 1999, with the parasitism rate reaching 45% (Papadopoulos and Katsoyannos, 2003). Up to now, *A. daci* has been retrieved from medfly and *Rhagoletis cerasi* (Diptera: Tephritidae) pupae collected from field-infested citrus and cherry fruits in Thessaly and Attiki area, Greece (N.T. Papadopoulos and D. Papachristos, personal observations). In 2009, *A. daci* was recorded in Valencia, Spain, attacking medfly in fig and citrus fruits (Sabater-Muñoz *et al.*, 2012). Recently, *A. daci* was reported to be the predominant parasitoid of *C. capitata* pupae from field-infested loquat, grapefruit, peach and guava orchards in the coastal area of Tartous in Syria, with parasitism levels of 1.68%, 30.76%, 18.28% and 16.15%, respectively (Ali *et al.*, 2015; 2016).

In addition, *A. daci* has been introduced to many countries as a biological control agent of specific fruit fly species. Initially, it was introduced to Hawaii in 1948 for the biological control of *B. dorsalis,* where it was also successfully mass reared on medfly larvae/pupae (Clausen *et al.*, 1965). Releases for the control of the Caribbean fruit fly, *Anastrepha suspensa*, in Florida (USA) (introduced via Hawaii) resulted in establishment after three years (Baranowski *et al.*, 1993). In contrast, the parasitoid failed to become established in Mexico and Costa Rica (introduced via Hawaii) after being released against medfly and the Mexican fruit fly, *Anastrepha ludens* (Wharton *et al.*, 1998). In 2008, *A.daci* was introduced to Egypt (via Hawaii) for the biocontrol of *B. zonata*, with the initial parasitism rate ranging from 1.6 to 8% in a guava orchard and, interestingly, five years later, the parasitoid was detected in the wild in fruit fly infested citrus and guava fruits approximately 320km away from the initial release point (El-Heneidy *et al.*, 2019).

Even though *A. daci* is a native parasitoid

of *Bactrocera* spp. and previous releases for the control of *B. dorsalis* and *B. zonata* have been attempted with some success (Clausen *et al.*, 1965; El-Heneidy *et al.*, 2019), biological control efforts, using this parasitoid, against the olive fruit fly have yet to be assessed. Preliminary results revealed that *A. daci* can successfully parasitize *B. oleae* larvae under laboratory conditions (N.T. Papadopoulos and Ch. S. Ioannou, unpublished data), but its response to infested olive fruit both in laboratory and field conditions remained unknown. It is well documented that the olive fruit size is a significant predictor of the parasitic success of the African parasitoids *P. ponerophaga* (Sime et al., 2007), *P. concolor* (Wang *et al.*, 2009a) and *P. lounsburyi* (Wang *et al.*, 2009b) on *B. oleae* host. To this end, we assessed in the laboratory performance of *A. daci* on infested olive fruit of different size. In addition, we included fruit of wild olive trees that can also support *B. oleae* populations in Europe (Bigler and Delucchi, 1981). Last, releases of *A. daci* were carried out in an olive grove in Greece for assessing its potential as a biological control agent against *B. oleae* host in field conditions.

Material and Methods

Insects

Laboratory colonies of *B. oleae* and *A. daci* were maintained in an insect room under controlled conditions (25 ± 1°C, 60 ± 10% R.H, and a photoperiod of L14: D10) in the laboratory of Entomology and Agricultural Zoology at the University of Thessaly, Volos, Greece.

Aganaspis daci specimens were obtained from a laboratory colony housed at the Valencian Institute of Agrarian Research [Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia, Spain]. This colony was established in 2010 with specimens obtained from medfly-infested figs in Bétera, Spain. Since then, a laboratory colony was maintained on the host *C. capitata* (de Pedro *et al.*, 2016). After transferring to Volos, *A. daci* was reared on mature (L_3) medfly larvae, which were reared in the laboratory on an artificial diet as it is described by Boller (1985).

Olive flies obtained from infested olive fruit of the "Pelion" cultivar collected from organic olive groves in Volos in June 2016. Flies were reared for 2 generations on olive fruit that had been collected at an appropriate ripening stage (full size, green color) and stored at low temperatures (6-7°C) until being used. All adults were maintained in wooden framed, nylon-mesh-screened holding cages (30 x 30 x 30 cm) and had free access to food (mixture of yeast hydrolysate and sugar in a ratio of 1:4) and water. Olive fruit were offered to females for oviposition, and mature larvae dropped from the fruit into a container placed below. Pupae were collected and kept under the same conditions.

Effects of olive fruit size on parasitism success

To assess the effect of fruit size on the parasitism rate of *A. daci* (the number of *A. daci* adults recovered per olive fly pupae), we used i) olives of the cultivar "Chalkidikis", and ii) wild olive fruit (wild growing olive fruit of *O. europaea* ssp. *europaea*). The olive fruit size (length and width) and olive fruit thickness were measured for both "Chalkidikis" olives (small-sized and big-sized) and for wild olive fruit using a sample of 50 fruit per category. Pulp thickness (i.e., distance from the epidermis to pit) was averaged from three needle-probe measurements per fruit. Measurements were always taken from the mid-point along the longitudinal axis of a fruit. Fruit from "Chalikidikis" olives were mechanically categorized as small (length < 3.1cm) and big (length ≥ 3.1cm). All olive fruit were offered to mature, mated females of *B. oleae* for oviposition, allowing 1 - 2 oviposition stings per fruit. Larvae were left to develop inside fruit until reach 2nd or 3rd instar. Monitoring of larvae development was based on samples of dissected fruit. Olive fruit with 2nd and 3rd instar larvae were offered to *A. daci* females for parasitism. Five *A. daci* females without previous oviposition experience (5-10 days old) were trans-

ferred to plexiglass cages (15x15x15cm) with ten olive fruits infested with 2nd or 3rd instar *B. oleae* larvae. *Aganaspis daci* females were allowed to oviposit to olive fruits for 24h having free access to food (sugar and honey) and water. Then, fruit were individually transferred to ventilated petri dishes for collecting parasitized olive fruit fly pupae. Petri dishes with pupae were kept in the insect room and checked daily until adult olive fly and parasitoid emergence ceased. For each fruit size and larvae instar (2nd or 3rd instar) treatment, we run ten replicates (10 infested fruits with 5 *A. daci* female/replicate). Five replicates of ten infested fruits remained in a parasitoid unit without parasitoid for 24h as control treatment to assess the natural olive fly larval/pupae mortality.

Induced mortality was evaluated by comparing % mortality in the treatment with % mortality in its control, using the Schneider-Orelli formula (Püntener, 1981) as follows:

Induced mortality (%) = [(treatment mortality - control mortality)/ (100 - control mortality)] x 100%.

Induced mortality refers to mortality of host pupae attributed to parasitoids, from which adults do not emerge.

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

Field releases of *A. daci*

One rainfed, olive grove located in Nea Ionia Volos (Magnesia, Greece) (39°37'N; 22°93'E, 16m) was selected for *A. daci* field releases. The size of the olive grove was estimated at 0.5ha, and it was mainly planted with the cultivar "Pelion". The experimental field was surrounded by olive groves and wild growing olive trees and has been certified (according to EC 834/2007) as an organic farm for at least the last five year. To monitor the olive fly population, 10 McPhail traps loaded with ammonium sulphate 2% as attractant were deployed on 10th July, 2018. Adult captures recording and replacement of the attractant solution was conducted every week until the end of the experiment. Apart from male and female captures per trap per week, the percentage of fertile females caught was determined following dissection under a binocular microscope (Fletcher *et al.*, 1978). *Aganaspis daci* releases took place one week after the first capture of mature *B. oleae* females (bearing mature oocytes in ovaries) in the traps. Ten 10 parasitoid females (5-10 days old) per fertile olive fruit fly female captured were released, with a maximum release of 200 female parasitoids per 0.1 ha/week or 1000 female *A. daci*/week due to rearing constraints. One week after the first release, a total of 300 olive fruit were randomly collected, and kept under laboratory conditions until the emergence of *B. oleae* pupae. Then, collected pupae were placed in Petri dishes until the emergence of olive fruit flies and/or *A. daci* adults. The experiment was completed on 23rd October, 2018 because of the premature drop of almost all olive fruit caused by the heavy *B. oleae* infestation. Climatic data (temperature and humidity) during the pilot trials (from 10th July, 2018 to 23rd October, 2018) were obtained from the closest meteorological station located at Nea Anchialos Airport (39°21'N; 22°08'E, 25m a.s.l.) 17.8 km south from the pilot field site (Fig. 1).

Statistical analysis

The normality of data was assessed with the Kolmogorov–Smirnov test. Kruskal-Wallis test was used to compare olive fruit size metrics (length, width, pulp thickness). Kruskall–Wallis and Mann–Whitney tests were used to determine whether olive fruit size and larval instar (2nd and 3rd) had an effect on a) parasitism rate of *A. daci*, b) induced mortality and c) population reduction of olive fly, respectively. All statistical analyses were performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA).

Results

Effect of olive fruit size on parasitism success

The length of small and big categories of "Chalkidikis" fruit averaged 2.7 ± 0.3 cm and

3.6 ± 0.3 cm, respectively; fruit width averaged 1.8 ± 0.1 cm and 2.5 ± 0.1 cm, respectively. Mean length and width of wild olive fruit were estimated to be 2.0 ± 0.2 cm and 1.6 ± 0.1 cm, respectively. Hence wild olive fruit were smaller than the small size "Chalkidikis" fruit. The average pulp thickness of "Chalkidikis" olive fruit ranged from 0.57 ± 0.11 cm (small fruit) to 0.85 ± 0.01 cm (big fruit), while pulp thickness of wild olive fruit was estimated at 0.62 ± 0.11 cm. Kruskal-Wallis tests revealed that olive fruit differ in length (χ^2=133.197, df=2 P<0.001), width (χ^2=132.104, df=2, P<0.001) and fruit thickness (χ^2=99.189, df=2, P<0.001).

Parasitism rates of *A. daci* on 2nd and 3rd instar larvae of the olive fruit fly was lower than 11.4%, regardless of the size and cultivar. *Bactrocera oleae* population reduction was lower than 30% for 2nd and 3rd instar larvae in all olive sizes, except for L_3 on small "Chalkidikis" fruit (≈53%) (Table 1). Olive fly larvae developing in small olive fruit of the "Chalkidikis" cultivar seem to be more "vulnerable" to *A. daci* than those of the big fruit regardless of the larvae instar. No *A. daci* adults emerged from olive fly on wild olive fruit, irrespective of the larval instar. Olive fruit size was a significant predictor of parasitism rates (Kruskal-Wallis test, χ^2=14.885, df=2, P=0.001), as opposed to induced mortality (Kruskal-Wallis test, χ^2=0.687, df=2, P=0.709) and population reduction of olive fly (Kruskal-Wallis test, χ^2=1.749, df=2, P=0.417). Moreover, Mann-Whitney tests revealed that larval size (developmental stage) significantly affected parasitism rates (U=193.000, P=0.003) and population reduction (U=293.000, P=0.020), while it was marginally not significant predictor for the induced mortality caused by *A. daci* on olive fly populations (U=318.000, P=0.051).

Field releases of *A. daci*

Olive fruit fly adult captures were recorded from the first week of sampling. Captures peaked (300-500 adults per week) from 11 to 18 September, 2018 (Fig. 2). Fruit infestation was low (<15%) until the 11th of September and significantly increased after the 25th September, 2018 (>100% olive infestation) and remained high until the end of the survey. Percentage of mature females were higher than 85% during the first week of sampling and remained high (85-97%) until the end of the experiment. In total, 13,220 *A. daci* females were released from 24th July, 2018 to 23rd October, 2018. Fruit sampling revealed that 1,845 *B. oleae* adults emerged from 2,454 olive fly pupae; however, no *A. daci* adults emerged from the infested olives (Table 2). It is noted that no olive fruit

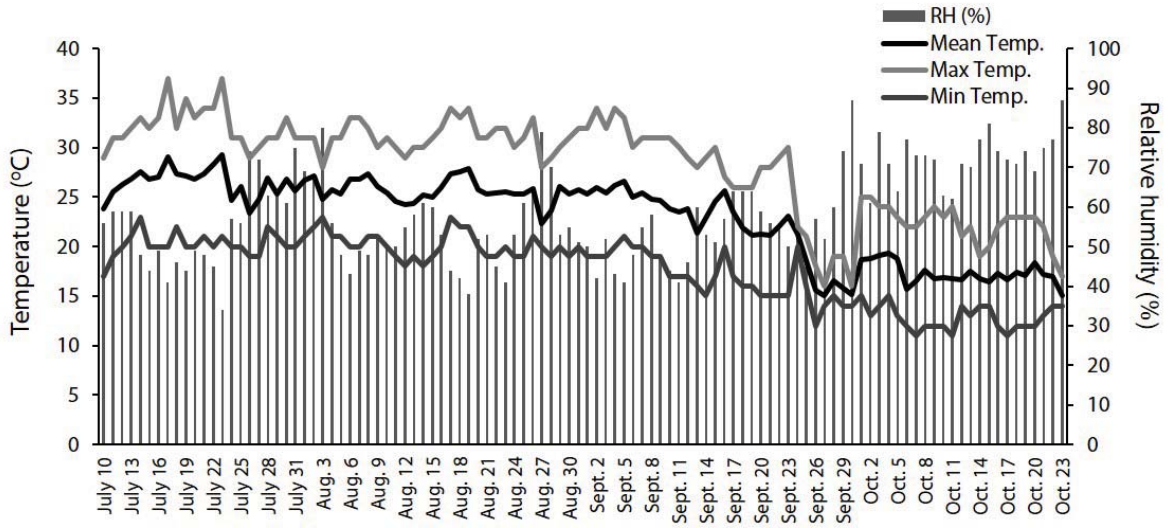

Figure 1. Climatic data from the pilot olive grove during the period of field releases. Data were obtained from the closest meteo station located at the airoport of Anchialos, 17.8 km away from the pilot field.

Table 1. Parasitism rates of *Aganaspis daci*, induced mortality and population reduction of olive fly on L_2, and L_3 larvae.

Larval instar	Olive fruit	*A. daci* parasitism (%) (mean ± SE)	Induced mortality (%)* (mean ± SE)	Population reduction (%)** (mean ± SE)
L_3	Wild olive fruit	0	24.4 ± 5.8	24.4 ± 5.8
	Chalkidiki (small)	11.4 ± 2.8	41.3 ± 10.7	52.7 ± 10.8
	Chalkidiki (big)	5.5 ± 1.9	22.9 ± 7.9	28.4 ± 7.2
L_2	Wild olive fruit	0	19.4 ± 5.4	19.4 ± 5.4
	Chalkidiki (small)	3 ± 1.6	15.4 ± 3.9	18.4 ± 3.7
	Chalkidiki (big)	0	18.3 ± 11.9	18.3 ± 11.9

*Induced mortality (%) = [(treatment mortality - control mortality)/ (100 - control mortality)] x 100%.
**Population reduction is the sum of induced mortality and percentage parasitism.

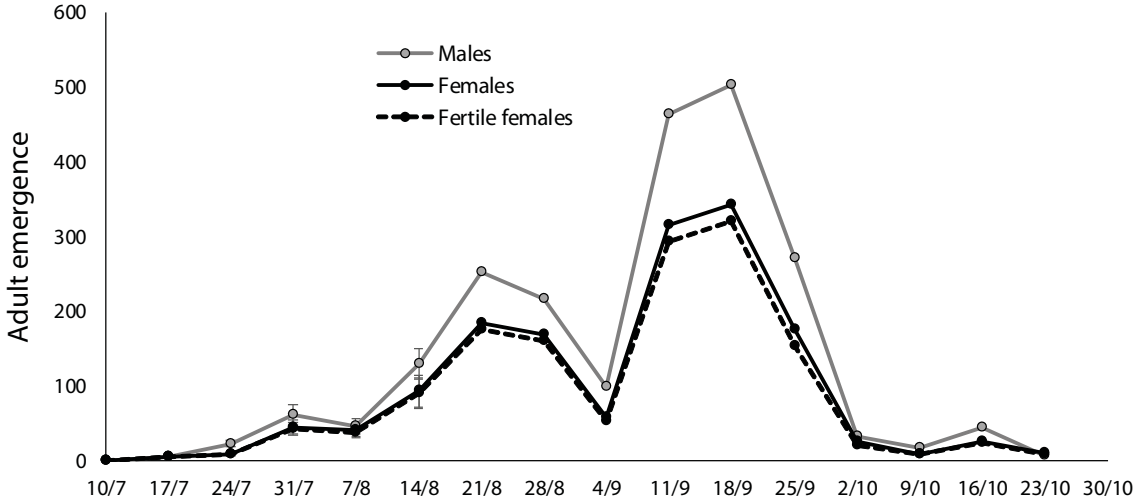

Figure 2. Mean captures (numbers ± SE) of olive fruit flies on McPhail traps per week during monitoring period. Males, females and fertile females are shown. Traps installed on 10th July, 2018.

were available for sampling following the last *A. daci* release conducted on 23rd October, 2018 due to the premature fruit drop caused by the heavy olive fly infestation.

Discussion

Our results suggest that parasitism success of *A. daci* on *B. oleae* larvae feeding on olive fruit was both affected by olive fruit size and larval instar under laboratory conditions. Specifically, the parasitism rates of the infested olive fruit of the "Chalkidikis" cultivar ranged from 11.4% to 3% for small and big fruit, respectively. Interestingly, *B. oleae* larvae were fully protected in wild olive fruit since no *A. daci* emergence was recorded. It is noted that wild olive fruit were smaller than those from the small "Chalkidikis" fruit; however, there was no difference in pulp thickness between wild olive and small "Chalkidikis" fruit. Moreover, both parasitism rates of *A. daci* and olive fly population reduction were higher for 3rd instar compared to 2nd instar larvae. In contrast,

Table 2. Results of olive fruit infestation rates and parasitism rates of *Aganaspis daci* on olive fruit fly after releases during the period of 24th July – 23rd October, 2018.

A. daci release		Olive infestation levels		Number of emerged individuals (n)		% parasitism of *B. oleae*	% *B. oleae* infested olives*	% *B. oleae* adult emergence (conside-ring formed pupae)
Release date	*A. daci* females	Fruit collection date	*B. oleae* pupae	*B. oleae*	*A. daci*			
24/7/2018	560	7/8/2018	25	17	0	0	8.3	68.0
31/7/2018	840							
7/8/2018	1000	14/8/2018	42	34	0	0	14	81.0
14/8/2018	1000	21/8/2018	34	21	0	0	11.3	61.8
21/8/2018	1000	28/8/2018	33	29	0	0	11	87.9
28/8/2018	1000	4/9/2018	36	26	0	0	12	72.2
4/9/2018	1000	11/9/2018	23	16	0	0	7.7	69.6
11/9/2018	1000	18/9/2018	41	30	0	0	13.7	73.2
18/9/2018	1000	25/9/2018	209	194	0	0	69.7	92.8
25/9/2018	1000	2/10/2018	431	325	0	0	143.7	75.4
2/10/2018	1000	9/10/2018	652	408	0	0	217.3	62.6
9/10/2018	1000	16/10/2018	448	352	0	0	149.3	78.6
16/10/2018	820	23/10/2018	480	393	0	0	160	81.9
23/10/2018	1000							
Total	13220		2454	1845	0			

*Sample of 300 olive fruit per sampling date

olive fruit size and larvae instar have no impact on induced mortality. Our field trials revealed that olive fruit fly adult captures in traps were high from the middle to the end September, resulting in high infestation rates of olive fruit from the end of September to the end of October, when our trials ceased due to premature olive fruit drop. Zero parasitoid recovery rates reveal that *A. daci* failed to get established and parasitize the olive fruit fly in the pilot orchard under the prevailing conditions.

Effect of olive fruit size on parasitism success

Our laboratory experiments support that *A. daci* adults are not able to emerge from infested wild olive fruit, being in line with a previous field study that revealed that wild olive is not an appropriate host of olive fly parasitoids in Crete (Neuenschwander *et al.*, 1983). However, olive fruit size and larval instar have been found to be significant predictors of the parasitism rates of *A. daci* on *B. oleae* larvae. For *Psyttalia* species, fruit size of the domesticated olives is known to have a negative impact on their parasitism success on *B. oleae* larvae (Sime *et al.*, 2007; Wang *et al.*, 2009a; 2009b). Given that *Psyttalia* species follow "a drill and sting" strategy for parasitizing their host (2nd and 3rd instar larvae), their ovipositor length is expected to be a significant predictor of parasitism success, in line with Latiere's hypothesis (Latiere, 1917). This is particularly true for the 2nd instar of *B. oleae* larvae that tend to tunnel deeper in large, fleshier fruit of European cultivars (Wang *et al.*, 2009a). On the other hand, *A. daci* females follow "an ingress and sting" strategy, which means that they enter the fruit in order to parasitize the host larvae favored by the smooth, compressed body shape. In this context, the relative ovipositor length of *A. daci* females seems to be

less important for the parasitism success of *B. oleae* on different-sized fruit, compared to the opiines' parasitoid. Indeed, pulp thickness of all three study olive fruit (5.7 – 8.5mm) is significant higher than the ovipositor length of *A. daci* (NT Papadopoulos, unpublished data) but still *A. daci* is capable of parasitizing *B. oleae* larvae. As a result, variability in *A. daci* parasitism efficiency in olive fruit cannot be explained by the ovipositor length. In addition, the 3rd instar *B. oleae* larvae were found to be more vulnerable to parasitism by *A. daci.* This is partly attributed to the fact that the L_3 (positioned closer to surface because on their way to either pupate (summer generation] or exit the fruit and pupate in soil (autumn winter generation) are more exposed to *A. daci* attacks than the L_2 larvae (Tzanakakis, 2006; Wang *et al.*, 2009b).

Field releases of *A. daci*

Although *A. daci* can successfully parasitize infested olive fruit in laboratory conditions, our results support no activity of *A. daci* in field conditions, despite of the prevailing optimal conditions for the field released wasps in terms of host availability. In general, field studies have so far revealed very low fertility rates and high immature mortality of *A. daci*, driven mainly by low (<20°C) or high (>30-35°C) temperatures (de Pedro *et al.*, 2016; 2017), even though exceptions exist (Papadopoulos and Katsoyannos, 2003; Ali *et al.*, 2016). Nevertheless, parasitism rates under low temperatures are expected to be high only under low humidity levels, while warm and wet conditions favor parasitism rates of *A. daci* during summer, suggesting that both temperature and relative humidity affect parasitism rates of *A. daci* in the Mediterranean basin (de Pedro *et al.*, 2017). For instance, in the coastal area of Syria, field parasitism of *A. daci* was recorded from May to September with a peak during June and August, when temperatures ranged from 22°C to 26°C (Ali *et al.*, 2016). In our study, high temperatures and low relative humidity prevailing in the area during July and August may resulted in high mortality rates of the released parasitoids. In addition, low temperatures (<20°C) during the last month of the field trial are likely to extend the duration of the immature stages of the parasitoid and decrease survival rates of *A. daci*, despite of the high olive fly infestation that offered an increased host availability (Tormos *et al.*, 2013).

Given that olive cultivar has an effect on parasitism rates of *A. daci*, it is possible to assume that the parasitism failure in pilot trials is attributed to cultivar type, although there is no data regarding *A. daci* response to olive fly larvae feeding on the "Pelion" cultivar.. In this sense, further studies are needed for assessing the parasitism rates of *A. daci* on olive fly infested fruit of the "Pelion" cultivar under both laboratory and field conditions following more controlled experimental approaches. Moreover, taking into account the oviposition strategy and the searching behavior of *A. daci*, it is expected that the figitid wasp will predominantly parasitize larvae in fallen fruits due to the easy entry through cracks of wounded fallen fruit, as previous studies have documented for the fruit fly parasitoids *A. pelleranoi* and *Odontosema anastrephae* (Hymenoptera: Figitidae) (Aluja *et al.*, 2009). In our study, high *B. oleae* infestation (particularly during the last month of the field trial) resulted in large numbers of fallen olive fruit but parasitism of olive fly larvae hosted in these fruit was not evaluated. Moreover, it is important to note that samples of *B. oleae* pupae that gave no adults (*B. oleae* or *A. daci*) were not examined for immature stages of *A. daci* that did not manage to reach adult stage. Hence, the possibility that parasitism exists on fallen fruit or pupae in the soil cannot be excluded.

Associative learning is well documented, particularly for the opiine parasitoid species (Giunti *et al.*, 2015). Laboratory experiments revealed that early adult learning affects host preferences (*B. oleae vs C. capitata*) in the medfly mass-reared *P. concolor* (Canale and Benelli, 2012; Giunti *et al.*, 2016). Even though parasitoid behavioral studies in the field are still scarce (Randlkofer *et al.*, 2010; Kostenko *et al.*, 2015), host plant appears to

be another important source of information for parasitoids' foraging capacity (Giunti *et al.*, 2015; Segura *et al.*, 2012; 2016). Recently, it was proved that host-fruit odor learning influences foraging capacity of the fruit fly parasitoid, *Diachasmimorpha krausii* (Hymenoptera: Braconidae), against the tephritid host fly *Bactrocera tryoni* in nectarines and tomatoes (Masry *et al.*, 2019). The role of olive trees and olive fruits on the performance of *A. daci* and other olive fruit fly parasitoids remain totally unexplored, despite of the long term efforts for the biological control of olive fruit fly (Daane and Johnson, 2010). In this sense, it is plausible to suggest that parasitism failure of *A. daci* against the heavily infested olive fruit may be partly attributed to olive trees/fruit recognition issues. Further studies are necessary for assessing the factors that affect foraging capacity of *A. daci* in olive groves.

Conclusions

To summarize, our study aimed at evaluating the potential of the figitid wasp, *A. daci*, as a biological control agent of the olive pest, *B. oleae*. To this end, we assessed the effects of olive fruit size and *B. oleae* larval instar on its parasitism success under laboratory conditions, and then we examined *A. daci* performance on *B. oleae* larvae in a pilot olive grove in Greece. Laboratory studies revealed that small-sized olives infested with 3rd instar larvae of olive fruit fly are most vulnerable to *A. daci* attacks. However, infested wild olive fruit found not to be an appropriate host for *A. daci*. Our first attempt of field control of olive fruit fly raised the well documented inherent difficulties of classical biological control of *B. oleae*, resulting in no parasitism. Overall, further studies are needed, both in laboratory and field conditions, for getting a better insight of *A. daci* potential in olive fly biological control programs.

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Literature Cited

Ali, A.Y., Ahmad, A.M. and Amar, J.A. 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, A.Y., Ahmad, A.M., Amar, J.A., Darwish, R.Y., Izzo, A.M. and Al-Ahmad, S.A. 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.

Aluja, M., Ovruski, S.M., Guillén, L., Oroño, L.E. and Sivinski, J. 2009. Comparison of the host searching and oviposition behaviors of the tephritid (Diptera) parasitoids *Aganaspis pelleranoi* and *Odontosema anastrephae* (Hymenoptera: Figitidae, Eucoilinae). *Journal of Insect Behavior*, 22: 423– 451.

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

Bigler, F. and Delucchi, V. 1981. The main mortality factors during the perpupal development of the olive fly, *Dacus oleae* Gmel. (Dipt., Tephritidae) on oleasters and cultivated olives in Western Crete, Greece. *Journal of Applied Entomology*, 92: 343–363.

Boller, E.F. 1985. *Rhagoletis cerasi* and *Ceratitis capitata*. In: P. Sing and R.F. Moore (eds). *Handbook of Insect Rearing*. Amsterdam, the Netherlands: Elsevier, pp. 135–144.

Canale, A. and Benelli, G. 2012. Impact of mass-rearing on the host seeking behavior and parasitism by the fruit fly parasitoid *Psyttalia concolor* (Szépligeti) (Hymenoptera: Braconidae). *Journal of Pest Science*, 85: 65–74.

Clausen, C.P., Clancy, D.W. and Chock, Q.C. 1965. Biological control of the Oriental fruit fly (*Dacus dorsalis* Hendel) and other fruit flies in Hawaii. *Bulletin of the U.S. Department of Agriculture*, 1322: 1-102.

Daane, K. M. and Johnson, M. W. 2010. Olive Fruit Fly: Managing an Ancient Pest in Modern Times. *Annual Review of Entomology*, 55: 151-169.

Daane, K. M., Wang, X-G., Nieto, D. J., Pickett, C. H., Hoelmer, K. A., Blanchet, A. and Johnson, M. W.

2015. Classic biological control of olive fruit fly in California: release and recovery of introduced parasitoids. *BioControl*, 60: 317-330.

El-Heneidy, A.M., Hosni, M.E. and Ramadan, M.M. 2019. Identity and biology of *Aganaspis daci* (Weld) (Hymenoptera: Figitidae), recently introduced to Egypt for biological control of *Bactrocera zonata* (Diptera: Tephritidae). *Entomologist's Monthly Magazine*, 155: 17–37.

Fletcher, B.S., Pappas, S. and Kapatos, E. 1978. Changes in the ovaries of olive flies (*Dacus oleae* (Gmelin)) during the summer, and their relationship to temperature, humidity and fruit availability. *Ecological Entomology*, 3: 99-107.

Giunti, G., Canale, A., Messing, R.H., Donati, E., Stefanini, C., Michaud, J.P. and Benelli, G. 2015. Parasitoid learning: current knowledge and implications for biological control. *Biological Control*, 90: 208–219.

Giunti, G., Benelli, G., Messing, R.H. and Canale, A. 2016. Early adult learning affects host preferences in the tephritid parasitoid *Psyttalia concolor* (Hymenoptera: Braconidae). *Journal of Pest Science*, 89: 529-537.

Hoelmer, K.A., Kirk, A.A., Pickett, C.H., Daane, K.M. and Johnson, M.W. 2011. Prospects for improving biological control of olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), with introduced parasitoids (Hymenoptera). *Biocontrol Science and Technology*, 21: 1005-1025.

Jiménez, A., Castillo, E., Beitia, F.J. and Esteban, J.R. 1998. Estimación del parasitismo de *Opius concolor* Szepligeti en *Bactrocera oleae* (=*Dacus oleae*) Gmelin, en condiciones de laboratorio. *Boletin de Sanidad Vegetal Plagas*, 24: 889–896.

Kakani, E.G., Zygouridis, N.E., Tsoumani, K.T., Seraphides, N., Zalom, F.G. and Mathiopoulos, K.D. 2010. Spinosad resistance development in wild olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae) populations in California. *Pest Management Science*, 66: 447–453.

Kampouraki, A., Stavrakaki, M., Karataraki, A., Katsikogiannis, G., Pitika, E., Varikou, K., Vlachaki, A., Chrysargyris, A., Malandraki, E., Sidiropoulos, N., Paraskevopoulos, A., Gkilpathi, D., Roditakis, E. and Vontas, J. 2018. Recent evolution and operational impact of insecticide resistance in olive fruit fly *Bactrocera oleae* populations from Greece. *Journal of Pest Science*, 91: 1429–1439.

Kapatos, E., Fletcher, B.S., Pappas, S. and Laudeho, Y. 1977. Release of *Opius concolor* and *Opius concolor* var. *siculus* (Hym.: Braconidae) against spring generation of *Dacus oleae* (Dipt.: Trypetidae) on Corfu. *Entomophaga,* 22: 265–270.

Kostenko, O., Lammers, M., Grootemaat, S., Kroon, T., Harvey, J.A., van Geem, M. and Bezemer, T.M. 2015. Effects of plant diversity and structural complexity on parasitoid behaviour in a field experiment. *Ecological Entomology*, 40: 748–758.

Latiere, H., 1917. La Lutte contre les Maladies des Plantes en Italie. *Annales du Service des épiphyties*, 4: 76–114.

Liaropoulos, C., Louskas, C., Canard, M. and Laudeho, Y. 1977. Release of *Opius concolor* (Hym.: Braconidae) on spring populations of *Dacus oleae* (Dipt.: Trypetidae): 1. Experiments in continental Greece. *Entomophaga*, 22: 259-264.

Manousis, T, and Moore, N. 1987. Control of *Dacus oleae*, a major pest of olives. *Insect Science and Its Application*, 8:1–9.

Masry, A., Cunningham, J.P. and Clarke, A.R. 2019. From laboratory to the field: consistent effects of experience on host location by the fruit fly parasitoid *Diachasmimorpha kraussii* (Hymenoptera: Braconidae). *Insect Science*, 26: 863–872.

Miranda, M.A., Miquel, M., Terrassa, J., Melis, N. and Monerris, M. 2008. Parasitism of *Bactrocera oleae* (Diptera, Tephritidae) by *Psyttalia concolor* (Hymenoptera: Braconidae) in the Balearic Islands (Spain). J. Appl. Entomol. 132: 798-805.

Moretti, R., Lampazzi, E., Reina, P. and Calvitti M. 2007. On the use of the exotic oo-pupal parasitoid Fopius arisanus for the biological control of *Bactrocera oleae* in Italy. *Integrated Protection of Olive Crops IOBC/wprs Bulletin*, 30: 49-60.

Nardi, F., Carapelli, A., Boore, J.L., Roderick, G.K., Dallai, R. and Frati, F. 2010. Domestication of olive fly through a multi-regional host shift to cultivated olives: comparative dating using complete mitochondrial genomes. *Molecular Phylogenetics and Evolution*, 57:678–686.

Neuenschwander, P., Bigler, F., Delucchi, V. and Michelakis, S. 1983. Natural enemies of preimaginal stages of *Dacus oleae* Gmel. (Dipt., Tephritidae) in western Crete. I. Bionomics and phenologies. *Bollettino del Laboratorio di Entomologia Agraria 'Filippo Silvestri'*, 40: 3-32.

Papadopoulos, N.T. and Katsoyannos, B.I. 2003. Field parasitism of *C. capitata* larvae by *Aganaspis daci* in Chios, Greece. *Biocontrol*, 48, 191-195.

de Pedro, L., Beitia, F., Sabater-Muñoz, B., Asís, J.D. and 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., Sabater-Muñoz, B., Harbi, A., Ferrara, F., Polidori, C., Asís, J.D. and Tormos, J. 2017. Parasitism of *Aganaspis daci* against *Ceratitis capitata* under Mediterranean climate conditions. *Entomologia Experimentalis et Applicata*, 163: 287–295.

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

Raspi, A. and Loni, A. 1994. Alcune note sull'allevamento massale di *Opius concolor* Szépligeti (Hym.: Bra-

conidae) e su recnti tentative d'introduzione della specie in Toscana e Liguria. *Frustula Entomologia,* 30: 135–145.

Randlkofer, B., Obermaier, E., Casas, J. and Meiners, T. 2010. Connectivity counts: disentangling effects of vegetation structure elements on the searching movement of a parasitoid. *Ecological Entomology*, 35: 446–455.

Sabater-Muñoz, B., Falcó, J.V., de Pedro, L., Tormos, J., Asís, J.D., Papadopoulos, N.T., Verdú, M.J. and Beitia, F. 2012. First record, surveillance and biological parameters of *Aganaspis daci* (Hymenoptera: Figitidae), as parasitoid of *Ceratitis capitata* (Diptera: Tephritidae) in Spain. In: *Second International Symposium of TEAM (Tephritid Workers of Europe Africa and the Middle East)*, Kolymbari, Crete (Greece), 2-6 July 2012, p.117.

Segura, D.F., Viscarret, M.M., Ovruski, S.M. and Cladera, J.L. 2012. Response of the fruit fly parasitoid *Diachasmimorpha longicaudata* to host and host-habitat volatile cues. *Entomologia Experimentalis et Applicata*, 143: 164– 176.

Segura, D.F., Nussenbaum, A.L., Viscarret, M.M., Devescovi, F., Bachmann, G.E., Corley, J.C., Ovruski, S.M. and Cladera, J.L. 2016**.** Innate host habitat preference in the parasitoid *Diachasmimorpha longicaudata*: functional significance and modifications through learning. *PLoS ONE*, 11: e0152222.

Sime, K.R., Daane, K.M., Kirk, A., Andrews, J.W., Johnson, M.W. and Messing, R.H. 2007. *Psyttalia ponerophaga* (Hymenoptera: Braconidae) as a potential biological control agent for the olive fruit fly in California. *Bulletin of Entomological Research*, 97: 233–242.

Stavraki-Paulopoulou, H.G., 1966. Contribution a l'étude de la capacite reproductrice et de la fecondite reelle d'*Opius consolor* Szepl. (Hymenoptera- Braconidae). *Ann. Epiphyties*, 17: 391 435.

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

Tzanakakis, M.E. 2006. Insects and mites feeding on olive: distribution, importance habits seasonal development and dormancy. Brill Academic Publication, Leiden.

Yokoyama, V.Y., Rendón, P.A., Wang, X.G., Opp, S.B., Johnson, M.W. and Daane, K.M. 2011. Response of *Psyttalia humilis* (Hymenoptera: Braconidae) to olive fruit fly (Diptera: Tephritidae) and conditions in California olive orchards. *Environmental Entomology*, 40: 315–323.

Wang, X.G., Johnson, M.W., Daane, K.M. and Yokoyama, V.Y. 2009a**.** Larger olive fruit size reduces the efficiency of *Psyttalia concolor*, as a parasitoid of the olive fruit fly. *Biological Control*, 49: 45–51.

Wang, X.G., Nadel, H., Johnson, M.W., Daane, K.M., Hoelmer, K., Walton, V.M., Pickett, C.H. and Sime, K.R. 2009b. Crop domestication relaxes both top-down and bottom-up effects on a specialist herbivore. *Basic and Applied Ecology*, 10: 216–227.

Weld, L.H., 1951. A new species of Trybliographa (Hymenoptera: Cynipidae). *Proceedings of the Hawaiian Entomological Society*, 14: 331-332.

Wharton, R.A., Ovruski, S.M. and Gilstrap, F.E. 1998. Neotropical Eucoilidae (Cynipoidea) associated with fruit infesting Tephritidae, with new records from Argentina, Bolivia and Costa Rica. *Journal of Hymenoptera Research*, 7: 102–115.

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Επίδραση του μεγέθους του ελαιοκάρπου στον παρασιτισμό του *Bactocera oleae* (Diptera: Tephritidae) από το παρασιτοειδές *Aganaspis daci* (Hymenoptera: Figitidae) και πιλοτική εξαπόλυση του παρασιτοειδούς σε ελαιώνα

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Περίληψη Ο δάκος της ελιάς, *Bactocera oleae* (Diptera: Tephritidae), αποτελεί τον σημαντικότερο εχθρό της ελαιοκαλλιέργειας παγκοσμίως, του οποίου η βιολογική αντιμετώπιση παραμένει μάλλον ανεπιτυχής στον αγρό. Το *Aganaspis daci* (Hymenoptera: Figitidae) είναι ένα ενδοπαρασιτοειδές που προσβάλλει μεγάλης ηλικίας προνύμφες των ειδών της οικογένειας Tephritidae και βρέθηκε να παρασιτεί επιτυχώς προνύμφες της μύγας της Μεσογείου σε προσβεβλημένα στον αγρό σύκα

στην Ελλάδα. Προκειμένου να εξεταστεί η δυνατότητα αξιοποίησης του *A. daci* ως παράγοντα βιολογικής καταπολέμησης του δάκου της ελιάς, μελετήθηκε η επίδραση του μεγέθους του ελαιοκάρπου στον παρασιτισμό προνυμφών 2ης και 3ης ηλικίας του δάκου της ελιάς, χρησιμοποιώντας διαφορετικού μεγέθους καρπούς της ποικιλίας «Χαλκιδικής» και καρπούς αγριελιάς. Επιπρόσθετα, πραγματοποιήθηκαν εξαπολύσεις του *A. daci* σε πιλοτικό ελαιώνα στην ευρύτερη περιοχή του Βόλου, Μαγνησίας. Κατά την περίοδο Ιουλίου – Οκτωβρίου 2018, πραγματοποιήθηκαν σε εβδομαδιαία βάση εξαπολύσεις 200 θηλυκών ατόμων/στρέμμα με παράλληλες συλλογές ελαιοκάρπων (300 ελαιόκαρποι/εβδομάδα) για έλεγχο του ποσοστού προσβολής των καρπών από τον δάκο της ελιάς και του ποσοστού παρασιτισμού από το *A. daci*. Από τα αποτελέσματα προκύπτει ότι τόσο το μέγεθος του ελαιοκάρπου όσο και το στάδιο ανάπτυξης της προνύμφης του δάκου της ελιάς είναι καθοριστικοί παράγοντες για τον επιτυχή παρασιτισμό από το *A. daci*. Υψηλά ποσοστά παρασιτισμού καταγράφηκαν στους μικρού μεγέθους καρπούς της ποικιλίας «Χαλκιδικής» και στις προνύμφες 3ης ηλικίας του δάκου της ελιάς σε εργαστηριακές συνθήκες. Αντίθετα, δεν προέκυψαν ενήλικα του *A. daci* από προσβεβλημένους ελαιόκαρπους κατά τη διάρκεια των πιλοτικών δοκιμών στον αγρό.

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