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Parasitism of *Psytallia concolor* (Hymenoptera: Braconidae) on *Bactrocera oleae* (Diptera: Tephritidae) infesting different olive varieties

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Abstract *Psytallia concolor* (Szépligeti) is a koinobiont endoparasitoid of many Tephritidae larvae, including *Bactrocera oleae* (Rossi), and has been used in Mediterranean areas for biological control of olive fruit fly by inundative release. The present study evaluates the influence of olive fruit variety (*Amfissis, Arbequina, Branquita de Elvas, Carolea, Kalamon, Koroneiki, Leccino, Manzanilla, Mastoidis, Moroccan Picholine* and *Picholine*) on *P. concolor* parasitism efficiency and performance in the field during two successive years. The results showed that the percentage of parasitism was significantly higher (>30%) in *Mastoidis* and *Koroneiki* (light-weight varieties <1.5 g) than *Leccino* which has a medium fruit weight, followed by *Amfissis*, *Moroccan Picholine*, *Picholine* and *Branquita de Elvas*. Only *Manzanilla* among large weight varieties, exhibited high percentage of parasitization (42.72%) during 2013. The mean weight of the pupae (>4.21 mg) as well as the length of the developed adult parasitoids (>3.5 cm) in *Mastoidis* and *Manzanilla* were significantly higher than these individuals developed from other varieties such as *Koroneiki* and *Kalamon*. Finally, the optimal host fruit for *P. concolor* development seems to be *Mastoidis* variety with great biological parameters and percentage of parasitism.

Keywords Olive fruit fly · *Psytallia concolor* · Olive varieties · Parasitism rate · Performance

Introduction

The olive tree (*Olea europaea* L. Lamiales: Oleaceae) is historic and classical feature of the Mediterranean landscape (Cherubini et al. 2013). It has been cultivated since the late prehistory, in the early Bronze Age, where it has been grown for its oil-rich fruit (Carrión et al. 2010). The olive tree is susceptible to pests and diseases, including the olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae). *B. oleae* is considered the major pest of olives around the world for more than 2000 years (Raspi and Viggiani 2008; Hepdurgun et al. 2009). Instead of many other Tephritidae which are polyphagous (White and Elson-Harris 1992; Liu et al. 2013), *B. oleae* is feeding exclusively on *Olea* species; olive fruit fly females lay an egg under the fruit surface thus the larvae develop inside olive fruits until they open an exit hole before pupate. On table olive groves the oviposition puncture leads to a serious reduction of crop value, while exit holes and pulp degradation can determine a quality and quantity loss of olive oil production and it can cause a significant quantitative and qualitative loss in the production of table olives and oil (Manousis and Moore 1987; Nardi et al. 2005). Recently Malacrinò et al. (2017) represented a further step to define the ecological role of the olive fruit fly, not only asdirect source of damage, but also as a major component of olive agroecosystem and as a vector of plant pathogenic

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fungi such as *Cladosporium* spp., *Alternaria* spp. and *Aureobasidium* spp., by plant pathogens like *Colletotrichum* spp. and *Pseudocercospora* spp., along with several other less abundant taxa whose ecology is unclear in most of the cases.

Over the last forty years, control of the olive fruit fly has relied mainly on chemical treatments, such as organophosphate insecticides (Kakani and Mathiopoulos 2008; Matallanas et al. 2013). However, their continue use led to the development of resistance in olive fruit fly populations (Kakani and Mathiopoulos 2008) and has ecological and toxicological side effects such as environmental pollution, contamination of olive oils (Amvrazi and Albanis 2009; Angioni et al. 2011) and serious effects on the environment and human health (Ruiz-Torres and Muñoz-Cobo 1997; Ruiz-Torres and Montiel-Bueno 2002). More recently, pyrethroid insecticides have been referred as a valuable alternative tool to control the olive fruit fly (Margaritopoulos et al. 2008; Youssef et al. 2004) as well as the macrocyclic lactone spinosad (Kakani et al. 2010). However, there is evidence that olive fruit fly populations are capable of developing resistance once pyrethroids started to be commonly used (Hawkes et al. 2005; Skouras et al. 2007; Margaritopoulos et al. 2008).

Alternative methods that do not involve insecticides were also developed and include: mass trapping (Broumas et al. 2002; Bento et al. 2003), attracting and killing (Bento et al. 1999; Mazomenos et al. 2002; Torres et al. 2002), the use of kaolin-based particle film (Saour and Makee 2004), sterile insect technique and biological control. From these control methods, biological control has long been investigated as a potential way to suppress olive fruit fly populations (Tzanakakis 2006; Wang et al. 2009a; Daane and Johnson 2010).

The focus of biological control has been on parasitic wasps. In fact, recent surveys suggest that a small group of braconids in the subfamilies Opiinae and Braconidae from Africa represent the primary natural enemies attacking olive fruit fly in its native range (Daane and Johnson 2010). Among them, *Psytallia concolor* (Szépligeti) (Hymenoptera: Braconidae) has been the most studied (Wang et al. 2011). It was first discovered in 1910 and distributed to several countries since then to control the olive fruit fly (Neuenschwander et al. 1983). Though no effort resulted in successful control of the olive fruit fly (Tzanakakis 2006). Various factors could have limited the success of these efforts, e.g. low winter temperatures, which affect parasitoid survival (Jiménez et al. 2002) or low quality of mass-reared parasitoids with low flight ability and abundance of fruit flies at the beginning of the summer (Delrio et al. 2005; Benelli and Canale 2012), oviposition experience influences the effectiveness of parasitoid release programs (Canale and Benelli 2012).

P. concolor is a larval–pupal koinobiont endoparasitoid that successfully attacks 14 Tephritidae species, including the olive fruit fly, *B. oleae* and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Fischer 1971; Wharton and Gilstrap 1983; Wharton 1997; Wharton et al. 2000; Sime et al. 2006; Wang et al. 2011; Benelli and Canale 2012). Parasitoid females naturally parasitized larvae inside fruits, usually preferring fully grown larvae (Canale and Benelli 2012) and emerge from the host puparium (Wharton 1997).

Female wasps are able to distinguish between infested and healthy fruit, preferring the first one, even if just olfactory cues are provided (Benelli et al. 2013). Giunti et al. (2016a) identified >70 volatile organic compounds (two of these were increased by *B. oleae* infestation, (*E*)-β-ocimene and 2-methyl-6-methylene-1,7-octadien-3-one, and four were decreased, α-pinene, β-pine ne, limonene, and β-elemene) emanating from uninfested and *B. oleae* infested olive fruits while mated *P. concolor* females were attracted only by (*E*)-β-ocimene. For olive fruit fly the process of host choice of a particular variety includes several mechanisms such as plant colour, shape, size, and particularly the volatiles emitted by the fruiting tree (Aluja and Mangan 2008) that may act as semiochemicals. Several authors (Dominici et al. 1986; Gümusay et al. 1990; Iannotta et al. 1999; Rizzo and Caleca 2006) showed that fruit morphometric indicators - fruit length, width, fresh weight were positively correlated with *B. oleae* infestation (Pucci and Ambrosi 1982; Neuenshwander et al. 1985; Rizzo et al. 2012; Garantonakis et al. 2016) Females may also adjust their choice according to the availability of olive varieties and their phenological stages, in order to optimize their reproductive success (Moreau et al. 2006).

Apart from the ability of a female *P. concolor* for oviposition on *B. oleae* larvae, which is related to olive fruit size, there are many other factors influencing the decision of the mother to oviposit its eggs in a specific host. The objective of the present study was to evaluate the influence of eleven *O. europaea* cultivars from the major European Mediterranean olive producing countries on parasitism efficiency and performance of *P. concolor*.

Materials and methods

Experimental orchard

The study was conducted on the island of Crete (southern part of Greece) in the experimental *O. europaea* grove of the Institute of Olive Tree, Subtropical Crops and Viticulture, located in Nerokourou village (1.1 ha, 35° 28′ 36. 76″ N- 24° 02′ 36. 44″ E-51 m) during 2012 and 2013. Fifty year-old, non-irrigated trees belonging to cultivars *Amfissis, Arbequina, Branquita de Elvas, Carolea, Kalamon, Koroneiki, Leccino, Manzanilla, Mastoidis, Moroccan Picholine* and *Picholine* were selected based on vegetative growth and fruit load uniformity. Eight trees of each cultivar were planted in a complete randomized block design within the orchard, spaced about 8 m apart and pruned regularly. The mean olive fruit production per tree and per field was estimated at ~70 and 50% of the normal yield (about 80–120 kg/tree) in 2012 and 2013, respectively. Chemical fertilizers were applied each winter according to soil and foliage analysis. Mean air temperature in this area was 18 °C, relative humidity (RH) was 64% and annual rainfall was 600–800 mm according to the records of the meteorological station of the Institute.

Olive fruit sampling

About 500 to 1000 of infested olive fruits (with oviposition sites and easily observed tunnels) were collected from each olive variety once during the last week of November 2012 and 2013 (end of the harvest period in Crete) in order to evaluate the parasitism rate of *B. oleae* larvae by the endoparasitoid *P. concolor* and its biological parameters. Fruits were collected at head height and along the four cardinal points of each tree and were immediately transferred to the laboratory for the measurements.

Except from the samples of infested fruits, 10 samples of 100 healthy fruits were also collected in order to measure weight per fruit and variety.

Parasitism rate and biological parameters of *P. concolor*

The infested fruits from each olive variety were enclosed into wooden cages (dimensions 30 × 30 × 30 cm). Pupae were daily collected from the cages, weighted and then each one was isolated in a small plastic cage and held until adult fly or parasitoid emerged. Every 10 plastic cages with pupae from the same olive variety were grouped in order to estimate *P. concolor* parasitism rate and sex ratio. Percentage parasitism was estimated based on the number of emerged wasps and flies. Parasitoids were anaesthetized with CO_2 and then the total body length: from top of head to tip of abdomen (of both sexes) as well as the sex ratio of the recorded adults (proportion of females out of total) were recorded under a stereomicroscope equipped with an ocular micrometer. All records were taken under controlled conditions of 22 ± 2 °C, 60 ± 5% RH and 12: 12 (L:D).

Data analysis

In order to compare differences among varieties, the data (fruit weight, weight of pupae, parasitism rate, parasitoids length, and parasitoids sex ratio) among varieties were analyzed by one-way analysis of variance (ANOVA) followed by the multiple comparison means by Tukey's honestly significant difference (HSD). Prior to analysis percentages of parasitism were normalized using the arcsine square root transformation. Means and standard errors based on the original data are presented in tables. The significant level for all analyses was 0.05. Data analysis was conducted using the statistical package JMP (SAS Institute 2008).

Results

Olive fruit weight

Olive fruit weight was significantly different among varieties in 2012 and 2013 (F = 47.33; df = 8, 81; p < 0.0001) and (F = 69.97; df = 8, 81; p < 0.0001) respectively.

During 2012, the highest fruit weight was observed in *Amfissis*, *Moroccan Picholine*, *Picholine*, *Branquita* while intermediate in *Kalamon* and *Leccino*. The lowest fruit weight was recorded in *Koroneiki* followed by *Mastoidis* and *Arbequina* (Table 1).

Table 1 Mean ± SE of olive fruit weight and parasitism levels of *P. concolor* on *B. oleae* among several olive varieties in 2012

Olive variety	Weight of 100 fruits (g)	% parasitism
Amfissis	402 ± 15 a	5.83 ± 2.03c
Arbequina	167 ± 10 d	7.33 ± 2.39bc
Branquita de Elvas	335 ± 18 ab	9.09 ± 3.02bc
Kalamon	296 ± 18bc	4.62 ± 2.07c
Koroneiki	96 ± 5e	29.66 ± 5.92ab
Leccino	258 ± 11c	0.00 ± 0.00c
Mastoidis	154 ± 12de	45.88 ± 5.77a
Moroccan Picholine	359 ± 15ab	1.82 ± 1.15c
Picholine	351 ± 19ab	5.71 ± 3.41bc

Means within a column followed by the same letter are not significantly different at $p = 0.05$

Next year, the highest fruit weight was observed in *Carolea* and *Amfissis* while intermediate in *Kalamon, Manzanilla*, *Picholine*, *Moroccan Picholine*, and *Leccino*. The lowest fruit weight was recorded in *Koroneiki* followed by *Mastoidis* (Table 2).

Parasitism rate

During 2012, parasitism of *B. oleae* larvae by *P. concolor* was significantly higher in *Mastoidis* (45.88%) compared to *Amfissis, Moroccan Picholine, Arbequina, Branquita, Leccino, Kalamon* and *Picholine* ($F = 9.84$; df = 8, 130; $p < 0.0001$) (Table 1).

During 2013, parasitism of *B. oleae* larvae by *P. concolor* was also significantly higher in *Mastoidis* (55.00%) compared to *Amfissis, Moroccan Picholine, Carolea, Leccino, Kalamon* and *Picholine* ($F = 8.98$; df = 8, 150; $p < 0.0001$) (Table 2).

Table 2 Mean ± SE of olive fruit weight and parasitism levels of *P. concolor* on *B. oleae* among several olive varieties in 2013

Olive variety	Weight of 100 fruits (g)	% parasitism
Amfissis	361 ± 15ab	1.11 ± 1.05c
Carolea	406 ± 9a	5.62 ± 2.16c
Kalamon	334 ± 9bc	14.44 ± 4.39bc
Koroneiki	93 ± 5f	43.93 ± 5.52a
Leccino	272 ± 7d	1.25 ± 1.15c
Manzanilla	329 ± 19bc	42.72 ± 8.12ab
Mastoidis	156 ± 10 e	55.00 ± 11.07a
Moroccan Picholine	307 ± 12 cd	12.22 ± 5.75bc
Picholine	312 ± 6bcd	10.76 ± 3.16bc

Means within a column followed by the same letter are not significantly different at $p = 0.05$

Biological parameters of *P. concolor*

Biological parameters of the *P. concolor,* emerged from olive varieties with very low low number of emerged parasitoids such as '*Kalamon'*, '*Manzanilla*', '*Picholine*, '*Leccino'*, '*Branquita de Elvas'*, '*Arbequina'* and '*Carolea'*, were not possible to be studied.

During 2012, pupal weight from *Koroneiki* (4.39 mg), was significantly lighter than pupal weight from *Mastoidis* ($F = 9.95$; df = 2, 177; $p < 0.0001$). Significantly larger size (adult length) of adult parasitoids was developed on *B. oleae* larvae feeding on olive fruits from *Mastoidis* (3.54 mm) compared to these from *Koroneiki* ($F = 7.57$; df = 2, 177; $p < 0.000$) (Table 3).

The percentage of female adults out of total, emerged from pupae ranged from 36.1% to 53.06% in *Mastoidis* and *Koroneiki* respectively, with no significant difference among varieties (Table 3).

Next year, the recorded pupal weight from *Kalamon* and *Koroneiki* (3.33 mg) and (3.96 mg) respectively, were significantly lighter than pupal weight from *Manzanilla* and *Mastoidis* ($F = 9.38$; df = 4, 404; $p < 0.0001$). Significantly larger size (adult length) was recorded in adult's parasitoids which were developed on *B. oleae* larvae feeding on olive fruits of *Manzanilla* and *Mastoidis* (3.63 mm) compared with these of *Kalamon* and *Koroneiki* ($F = 6.33$; df = 4, 404; $p < 0.000$). The percentage of females out of total, emerged from pupae ranged from 44.72% in *Koroneiki* to 59.17% in *Kalamon* with no significant difference among varieties (Table 4).

Discussion

Through our field measurements of *P. concolor* performance among several olive varieties under the same cultural and environmental conditions, we concluded that the optimal host fruit for *P. concolor* development seems to be *Mastoidis* variety with great biological parameters and percentage of parasitism. The female parasitoid chose for oviposition infested fruits of *Mastoidis* which has a relatively small weight, and gives to parasitoid's offspring a good fitness. Though there is an exception with the heavy-weight *Manzanilla*; female

Table 3 Mean ± SE of *P. concolor* biological parameters among several olive varieties in 2012

Olive variety	Weight of parasitized olive fruit fly pupae (mg)	Body length (mm)	% Sex ratio
Amfissis	5.14 ± 0.16ab	3.36 ± 0.09ab	36.70 ± 16.6a
Koroneiki	4.39 ± 0.17b	3.33 ± 0.04b	53.06 ± 6.04a
Mastoidis	5.42 ± 0.39a	3.54 ± 0.04a	36.10 ± 3.59a

Means within a column followed by the same letter are not significantly different at $p = 0.05$

parasitoid has choosen this variety for oviposition as a good resource for her offspring development.

Our study showed that parasitism of the olive fruit fly by *P. concolor* was not clearly determined by fruit weight. In both years of the study, the percentage of parasitism was significantly higher (>30%) in *Mastoidis* and *Koroneiki* (light-weight varieties <1.5 g) than *Leccino* which has a medium fruit weight, followed by *Amfissis*, *Moroccan Picholine*, *Picholine* and *Branquita*. There is an exception of a heavy-weight fruit *Manzanilla* (>3 g) with 42.72% parasitization during 2013. Varieties such as *Amfissis, Mastoidis, Manzanilla* as well as *Moroccan Picholine*, exhibited the greatest wasp performance as was indicated by the measurements performed as part of this study on *P. concolor*. Specifically in these olive varieties, the mean weight of the pupae (>4.21 mg) as well as the length of the developed adult parasitoids (>3.5 cm) were significantly higher than these individuals developed from other varieties such as *Koroneiki* and *Kalamon*. Finally our results indicated that olive variety did not affect the parasitoid's offspring sex ratio which ranged between 36.1 and 59.17% females. According Yokoyama et al. (2012) and Neuenschwander et al. (1983) the sex ratio of *Psytallia humilis* was similar to our study and ranged from 53 to 62% and 53%, respectively.

Several authors (Dominici et al. 1986; Gümusay et al. 1990; Iannotta et al. 1999; Rizzo and Caleca 2006) show that varieties with bigger fruits are heavily infested from *B. oleae* than smaller ones concluding also that within the same variety, the bigger ones can be more preferred than the smaller ones. Garantonakis et al. (2016) found that the percentage of alive infestation (live immature individuals of the olive fruit fly) was significantly influenced by the olive variety, with the lowest value recorded in *Mastoidis* (<2%%) compared to others. This can be probably linked with the significantly highest values of parasitization recorded at the same varieties, in this study.

B. oleae larvae prefer to feed deeper inside the fruit pulp with increasing fruit size and the reduction in parasitism levels by *P. concolor* in larger fruit is likely due to the parasitoid's relatively short ovipositor that limits them from reaching the maggots deeper within the fruit pulp (Wang et al. 2008). Thus, increased olive fruit size, which is associated with crop domestication, creates a better structural refuge for larval *B. oleae*. The success of parasitoid attack on more concealed pests depends on the match of the parasitoid's ovipositor with the depth at which their insect host feeds within plant organs (Feder 1995; Leyva et al. 1991; Lopez et al. 1999; Sivinski and Aluja 2003; Weis et al. 1985). The olive fruit fly parasitoid, *Psytallia lounsburyi* (Silvestri) (Hymenoptera: Braconidae), was less effective in attacking olive fruit fly larvae within larger than smaller olives in California, and this was attributed to its relatively short ovipositor in comparison to the depth of the pulp of mature cultivated olive fruit (Wang et al. 2009a).

Table 4 Mean ± SE of *P. concolor* biological parameters among several olive varieties in 2013

Olive variety	Weight of parasitized olive fruit fly pupae (mg)	Body length (mm)	% Sex ratio
Kalamon	3.33 ± 0.32b	3.39 ± 0.06b	59.17 ± 12.93a
Koroneiki	3.96 ± 0.21b	3.48 ± 0.02b	44.72 ± 3.42a
Manzanilla	4.98 ± 0.28a	3.63 ± 0.04a	53.43 ± 5.73a
Mastoidis	4.71 ± 0.12a	3.63 ± 0.04a	46.12 ± 7.29a
Moroccan Picholine	4.27 ± 0.19ab	3.57 ± 0.06ab	50.00 ± 11.78a

Means within a column followed by the same letter are not significantly different at $p = 0.05$

Sime et al. (2007) also reported that another larval olive fruit fly parasitoid, *P. ponerophaga* (Silvestri) (Hymenoptera: Braconidae) successfully produced more offspring from smaller than larger cultivated olives. Female flies allocate more offspring to large than to small fruit while at the same time specialist larval parasitoid, *P. lounsburyi*, more effectively parasitizes hosts in smaller than larger fruit (Wang et al. 2009a). In addition, olive fruit fly larvae can be attacked in large fruit when the larvae are exiting for pupation, or when the host density in each fruit is high (Wang et al. 2009b) such as in *Manzanilla* that has the highest recorded infestation among several varieties in field condition of Crete (Garantonakis et al. 2016).

Moreover the width of the mesocarp of a *Koroneiki* and *Mastoidis* olive fruit in which high values of parasitization were recorded is very close (ranged from 6 to 8 mm) while the length of the ovipositor of *P. concolor* was 1.93 mm (ranged from 1.4 to 2.4 mm) (Varikou unpublished data) meaning that it is easier to target the olive fly larva in such pulp thickness compared to larger ones; as flesh depth extends in larger sized and heavier drupes, second-instar hosts will be less accessible by the wasp because these tend to feed deeper within the fruit. An explanation of this is that the *Psytallia* species used in most of the early European field releases were probably originated from North Africa and collected from wild olives with small fruits (Rugman-Jones et al. 2009); the collected parasitoid is very well adapted in such small fruit Cretan varieties and favourable environmental conditions (>15 °C) for its development. It should be pointed also that Neuenschwander et al. (1983) was confused whether this parasitoid existed from Cretan orchards is indigenous or if it was introduced to island of Crete. There was an effort of introduction in Athens (1930) from Libya but due to lack of facilities its rearing and releasing was impossible. Anyway, the species was later, discovered in Ierapetra (Southeastern Crete) in 1963 (Stavraki 1967) and since then it exists in the island without new releases (Neuenschwander et al. 1983). The same researchers report that the parasitoid can probably develops on the olive fruit fly alone, survive and reproduce throughout the year on *B. oleae* and it has low population densities in summer and higher in winter.

Quality and quantity of host tissue are probably the most important factors influencing parasitoid size. Adult female weight may influence fitness by affecting the searching efficiency, longevity and fecundity of the wasp (Godfray 1994). Large hosts are expected to be more advantageous in terms of offspring fitness than small hosts because they contain a greater quantity of resources (Harvey et al. 1995). Other studies showed that the nutritional and endocrinological status could also impose constraints on the parasitoids' development (Harvey et al. 1995; Colinet et al. 2015).

Female parasitoid had the opportunity for selective oviposition determined by factors such as the quality of the larvae of the *B. oleae* as food for the larvae of the parasitoid, which is probably influenced by the olive variety. All these evidences point out that there are many other factors, beyond fruit size or weight, which influence host location and parasitism of *P. concolor*. Giunti et al. (2016b) referred that chemical cues produced by olive fruits under *B. oleae* attack, route the host location behavior of *P. concolor* females, acting as short-range 'kairomones'; 12 volatiles in infested *Arbequina* olives, 5 in *Frantoio* and 8 in *Leccino* ones.

Furthermore *P. concolor* can probably effectively parasitizes also other similar morphometrical varieties such as *Arbequina*; similar flesh weight (Garantonakis et al. 2016) and pulp thickness (according to the International Olive Council (IOC) on the basis of the Word Catalogue of Olive Varieties). Though, this topic needs further investigation.

Morphometrical characteristics differ among varieties, trees of a particular species and even within the canopies of individual trees, and these size patterns can underlie the distributions of fruit flies and their larval parasitoids. Structural refuges for insect herbivores exist even in natural systems (Dyer and Gentry 1999) and play an important part in sustaining multi-trophic interactions by preventing the overexploitation of hosts by their parasitoids (Hawkins et al. 1993). However, the large variability between the different tritrophic systems and the organisms involved requires thorough investigations and careful application of the gained knowledge.

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