Evaluating potential olive orchard sugar food sources for the olive fly parasitoid *Psyttalia concolor*

Abstract

Olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is a major olive pest in the Mediterranean basin where increasing insecticide resistance has enhanced damage and necessitates more reliance on other control strategies, such as biological control. Provision of floral resources has been reported to improve the effectiveness of natural enemies. Here, we tested the effect of six plant nectars and two honeydew sources on the survival of *Psyttalia concolor (*Szépligeti) (Hymenoptera: Braconidae), a parasitoid wasp used in the biological control of olive fruit fly. Our results showed a positive effect on survival associated with nectars of *Anchusa azurea* Mill., *Rosmarinus officinalis* L., *Lavatera cretica* L. and *Calamintha nepeta* (L.) Savi,, while honeydew proved to be a valuable alternative food source. When offering flowers directly to insects, *Anchusa azurea*, *Lavatera cretica*, and *Foeniculum vulgare* L. were found to be the most beneficial species, indicating also that *P. concolor* feed*s* predominantly on shallow corollas.

Keywords: *Hymenoptera: Braconidae*, nectar, honeydew, survival, conservation biological control

Introduction

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), is considered one of the most damaging olive pests in the Mediterranean basin (Tzanakakis 2003), and causes losses as high as 98% of a harvest, resulting into average losses exceeding one billion dollars per year (Bueno and Jones 2002). The fly has recently been introduced to Southern California from where it spread to almost the

entire state, becoming a serious threat to the olive industry of that region (Rice et al. 2003).

Control of olive fly has relied predominantly on application of chemical insecticides as sprays and in baits (Daane and Johnson 2010), however, growing concerns over effects of pesticides on environment and human health, the development of pesticide resistance (Kakani et al. 2014) and impending legislation aiming to reduce use of pesticide in Europe have induced a gradual shift towards more integrated pest control approaches. Accordingly, biological control measures will play a more significant role in the future, and will be complemented with other eco-friendly control methods such as the use of essential oils (Benelli et al. 2013a; Canale et al. 2013). Over the past 60 years, the main biological control agents used against *B. oleae* have been the Braconidae: Opiinae endoparasitoids *Psyttalia concolor* (Szépligeti), *Psyttalia humilis* (Silvestri) and *Psyttalia lounsburyi* (Silvestri) (Daane et al. 2011), which all belong to the *P. concolor* species complex (Rugman-Jones et al. 2009). *P. concolor* has been mass-reared in insectaries and repeatedly released in some Mediterranean regions but with limited success in controlling *B. oleae* (Delrio et al. 2005). Various factors could have limited the success of these trials, e.g. low winter temperatures, which affect survival (Jiménez et al. 2002), low quality of mass-reared parasitoids and abundance of fruit flies at the beginning of the summer (Delrio et al. 2005). It was also found that oviposition experience influences the effectiveness of parasitoid release programs (Canale and Benelli 2012) and that long periods of rearing *P. concolor* under laboratory conditions can affect behavioral traits (Benelli and Canale 2012) such as flight ability (Delrio et al. 2005). Exposure of insects to herbivore induced plant volatiles (Benelli et al. 2013c) or oviposition marking pheromones have been used to sensitize or train mass-reared parasitoids during the pre-release phase to improve post-release performance in

52 the field (Benelli et al. 2014). Habitat manipulation within or around orchards aimed at
53 increasing abundance of selected flowering plants and consequently abundance of
54 parasitoids within olive orchards, by providing nectar and honeydew as food resources
55 for parasitoids, has been reported to enhance effectiveness of olive fly control (Vattala
56 et al. 2006; Tompkins et al. 2010; Paredes et al. 2013a, 2013b). In fact, the survival of
57 parasitoid increases when they feed on sugar, enabling females to attack more hosts
58 over their lifetime (Idris and Grafius 1995; Lee et al. 2004), whilst ingested sugars may
59 also result in maturation of additional eggs in synovigenic species (Olson and Andow
60 1998) and can prevent parasitoids from resorbing eggs (Lee et al. 2004).
61 The visual or olfactory attractiveness of the flowers is a very important issue
62 because it influences insect foraging behavior, but nectar accessibility is not always
63 correlated with food sources attractiveness (Wäckers, 2004). The suitability of
64 flowering plants to provide nectar to a parasitoid depends ultimately on both the
65 parasitoid and the flower morphologies, as well as on the nectar quality and abundance
66 (Vattala et al. 2006). In addition to feeding on nectar, parasitoids may feed on
67 honeydew, a sugar-rich secretion produced by Sternorrhyncha (Lee et al. 2004). It is,
68 therefore, essential to know how floral and honeydew resources affect the life-cycle of
69 this group of insects to understand their management requirements and to propose
70 measures that could improve natural pest control by these parasitoids, at both landscape
71 and farm level. The aim of this research was to determine if average survival time of *P.*
72 *concolor* can be increased by feeding on floral nectar from six plant species commonly
73 found in or near Portuguese olive orchards, as well as on honeydew excreted by *Aphis*
74 *gossypii* Glover (Homoptera: Aphididae) and *Euphyllura olivina* Costa (Homoptera:
75 Psyllidae).
76

Material and methods

Psyttalia concolor rearing

Psytallia concolor wasps were reared on larvae of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), which are easier to maintain than *B. oleae*. Both insects were obtained from the Departmento de Producción Vegetal: Botánica y Protección Vegetal Unidad Protección de Cultivos E.T.S.I. Agrónomos UPM Madrid and reared at 23 ± 2 ºC, 40 ± 5% relative humidity (RH) with a photoperiod of 16 L: 8 D. Medfly adults were kept in methacrylate cages (30 x 40 x 30 cm) that contained around 3000 flies each, and fed with a 4:1 mixture of sucrose and enzymatic yeast hydrolysate (MP Biomedicals) (Albajes and Santiago-Alvarez 1980). About 2000 two to three days old eggs were collected and transferred to a plastic bowl (25 x 15x 4 cm) filled with 5 cm artificial culture medium. After 8-9 days the third instar larvae were collected and kept in small plastic containers to establish new medfly cages after adult emergence, while the remainder were parasitized.

About 500 *Psyttalia concolor* adults were kept in a plastic cage (30 x 40 x 30 cm) and fed a 4:1 mixture of ground sucrose and dried brewers yeast (Jacas and Viñuela 1994). About 500 *C. capitata* third stage larvae were placed in a nylon mesh bag directly on the *P. concolor* cage for 30 min. Parasitized larvae were transferred to a plastic cage (12x5 cm) and kept under the conditions described above. Cages were checked daily for newly emerged parasitoids, which were transferred either to rearing cages or to plastic containers for use in the bioassays.

Selected plants and nectar collecting

Anchusa azurea Mill. and *Echium plantagineum* L. (Boraginaceae), *Lavatera cretica* L. (Malvaceae), *Foeniculum vulgare* L. (Apiaceae), *Calamintha nepeta* (L.) Savi subsp. *nepeta* and *Rosmarinus officinalis* L. (Lamiaceae) were selected from a preliminary pool of 20 flowering plants common in olive orchards of South Portugal (Belo et al. 2009) according to their flowering period (to ensure nectar supply throughout the year – Table 1), theoretical accessibility (flower dimensions) and mean floral nectar production (Table 2). Flower dimensions were measured as upper width of corolla aperture, lower width around the nectaries, to make sure insects could fit into the corolla, and the length between these two points. Daily field production of nectar was quantified for each plant species by extracting nectar of 30 flowers with capillary micropipettes (Drummond Microcaps®). The volume was quantified under a binocular microscope. Flowers were covered with a gauze bag at noon 24 h before nectar collection to minimize nectar depletion by insects.

Insect measurements

To select flowering plants with suitable floral dimensions for the braconid *P. concolor*, insect head mean width and corolla mean width and depth were recorded from 30 wasps and 30 corollas per plant species. All measurements were recorded with an Olympus KL 1500 compact binocular microscope with an SC 30 digital camera and evaluated using the programs 'Analysis getit' and 'Measurit' (Olympus).

Floral nectar sugar composition and content

A total volume of 1 µl of nectar was collected from as many flowers as required using capillary micropipettes from all plant species, except *F. vulgare* – because of the high viscosity of its nectar. Samples were immediately frozen and dry weights obtained after freeze-drying. A 0.05 % (w/v) 2-Deoxy-D-glucose standard (98 %, Sigma-Aldrich) was used as the internal standard (IS) for quantification of soluble sugars. 100 µl of IS was added to nectar samples in Eppendorf® caps (5 replicates per species) and sugars extracted with 900 µl of ethanol/water (1:1 V/V) by sonicating for 5 min. The extraction was repeated twice using 1 ml of ethanol/water (1:1 ratio) and supernatants were pooled in 3 ml Eppendorf® caps. Extracts were analyzed by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD, ICS-3000, Dionex) using CarboPac PA-20 column (150 mm × 3 mm), with a CarboPac PA20 pre-column (Dionex) and isocratic elution with 10 mM NaOH solution containing 2 mM $Ba(OH)_2$. The eluent was kept under nitrogen to reduce carbonate build-up and biological contamination. The injection volume was 5 µl, the flow rate was 0.3 ml/min and the column temperature was maintained at 35 ºC during each run. The electrochemical detector consisted of an Au working electrode, Ag/AgCl reference electrode, and Ti counter electrode. The ED cell waveform was +0.1 V from 0.00 to 0.40 s, then −2.0 V from 0.41 to 0.42 s, and a ramp −2.0 to +0.6 V from 0.42 to 0.43 s, followed by −0.1 V from 0.44 to 0.50 s (end of cycle). The integration region was from 0.2 s to 0.4 s and the proportions of the three sugars (glucose, fructose, sucrose), in each sample, were determined by the integration of the correspondent chromatographic signals. The floral nectar sugar content was measured as a sucrose/hexose ratio, R= S/(F+G) (S=sucrose; F=fructose and G=glucose), and plant nectars categorized according to Baker and Baker (1983) as sucrose-dominant

151 (R>0.999) and sucrose-rich (0.999 < R < 0.500), hexose-rich (0.499 < R < 0.100) and
152 hexose-dominant (R<0.100).
153
154 Survival experiments
155
156 Two separate experiments were conducted to assess survival of *P. concolor*. In
157 the first, parasitoids were provided with a specified amount of manually collected nectar
158 and in the second the insects were provided with a specified number of flowers,
159 representing a similar amount of nectar. These experiments aimed to distinguish
160 theoretical and actual value of nectar as a food source to *P. concolor*, and to confirm the
161 adequacy of plant selection criteria with respect to accessibility of nectar by the insects.
162
163 *Experiments with collected nectar and honeydew*
164
165 Nectar was collected from *C. nepeta*, *R. officinalis*, *A. azurea*, *L. cretica* and *E.*
166 *plantagineum*, and stored frozen after collection at - 20ºC. Nectar from *F. vulgare* was
167 not collected due to its high viscosity. Sets of five newly emerged virgin wasps (less
168 than 24 h old) were placed in 90 mm diameter Petri dishes and subjected randomly to
169 the following treatments: (1) 0.25 µl of nectar/individual + humidified cotton, as a water
170 source (nectar-only hereafter), (2) humidified cotton only (negative control) and (3)
171 ~0.0004 g of artificial diet (ground sugar and dry yeast (4:1)) + humidified cotton
172 (positive control). All the assays were kept under the laboratory conditions described in
173 the section '*P. concolor* rearing'. The nectar volume had been determined in
174 preliminary experiments and found to be adequate for survival of *P. concolor*. Floral
175 nectar, artificial diet and water were renewed daily and wasp survival was checked

176 daily, up to 20 days. Tests were carried out in triplicate for each wasp sex and plant
177 species.

178 Drops of honeydew of *E. olivina* were collected in the laboratory with a needle
179 directly from infested flowering olive cuttings, and/or by shaking them a few times over
180 a sheet of paper, and tested only on female wasps due to its limited availability. Five
181 newly emerged female *P. concolor* were placed in each of three conical plastic cages
182 (11 cm Ø x 15 cm height) used per treatment and closed with netting. Treatments were:
183 (1) three drops of honeydew similar in size to *P. concolor* head, usually covered with a
184 very fine cover of *E. olivina* 'cotton'; (2) humidified cotton only (negative control) and
185 (3) 0.0004 g of artificial diet (positive control). Honeydew of *A. gossypii* was also tested
186 on newly emerged *P. concolor* females in the same set-up using: (1) three cuttings (5 to
187 8 cm) of *A. azurea* infested with *A. gossypii* and placed in a cylindrical plastic vial (5x3
188 cm) filled with water and sealed with parafilm to prevent wasps from drowning; (2)
189 three non-infested cuttings of *A. azurea* (negative control); (3) 0.0004 g of artificial diet
190 (positive control). A small portion of humidified cotton was provided as a source of
191 water for the insects in all assays and all flowers were removed from cuttings and
192 excision cuts sealed with parafilm to prevent wasps feed from plant sap. Cages were
193 arranged randomly and kept at 23 ± 2 ºC, 40 ± 5% RH with a photoperiod of 16 L: 8 D.
194 Survival was checked daily for 20 days, and *A. azurea* cuttings were replaced every two
195 days.

196

197 *Experiments with flowers*

198

199 Female and male *P. concolor* (five per cage, three cages per plant species) were
200 separately presented with flowers of *F. vulgare*, *R. officinalis*, *A. azurea*, *L. cretica*, *E.*

plantagineum and *C. nepeta* using the same set-up and procedure described above for newly emerged insects fed with honeydew of *A. gossypii*. Only flowers without aphid infestation or obvious damage were chosen and covered with a gauze bag at noon 24 h before each assay to minimize nectar depletion by insects. The number of flowers was determined according to their daily mean nectar production and required to provide an average of 0.25 μl nectar/wasp. Flowers were placed in the cages, inside small cylindrical water-filled plastic vials prior to the introduction of the wasps.

Statistical analysis

Data were evaluated for normality and homogeneity of variances with Kolmogorov–Smirnov and Levene tests, respectively, using the IBM SPSS statistical package v.20. One way analysis of variance (ANOVA) and two way analysis of covariance (ANCOVA) were used for evaluation of corolla size and daily mean floral nectar volume production, and for assessing wasp survival in relation to flowers, nectar-only and honeydew. Where statistical differences were found between categories Tukey HSD test was used for multiple comparison of means. Data on glucose, sucrose and fructose content of nectar were arcsine transformed for analysis because the distribution of percentages is binomial.

Results

Psyttalia concolor head measures

225 Mean head width of *Psyttalia concolor* males and females was very similar with
226 0.746 mm (±0.013 mm SE) for males and 0.791 mm (±0.023 mm SE) for females.
227
228 Corolla measures and nectar production
229
230 Flowers of *E. plantagineum* produced the highest mean daily nectar volume and
231 had the deepest corollas (Table 2). *C. nepeta* and *R. officinalis* also produced high daily
232 volumes of floral nectar but *R. officinalis* flowers had one of the smallest floral
233 dimensions (Table 2).
234
235 Floral nectar sugar composition and content
236
237 Percentages of sucrose, glucose and fructose and the sucrose/hexose ratio are
238 detailed in Table 3 and showed that *A. azurea*, *E. plantagineum* and *R. officinalis* have
239 sucrose-rich nectars and *L. cretica* and *C. nepeta* have hexose-rich nectars (Table 3).
240
241 Feeding experiments
242
243 Using three replicates with five wasps each appeared to be sufficient as no
244 statistically significant differences were detectable between replicates except for assays
245 with nectar of *R. officinalis* ($F = 4.245$, $df = 2, 14$, $P = 0.04$, S1 and S2, Supplementary
246 material). Overall, feeding wasps with nectar or honeydew of *A. gossypii* or *E. olivina*
247 showed a significant effect on their average survival time (Table 4). Female wasps
248 exhibited significantly higher survival time on all floral nectars and honeydews in
249 comparison to water-only treatment, on which wasps survived an average of 4.83 ± 0.24

250 days (Fig. 1a). Females survived longest when fed with nectar from *A. azurea* (20.0 ±
251 0.00 days), *R. officinalis* (17.80 ± 0.20 days), *L. cretica* (14.73 ± 2.66 days) and *C.*
252 *nepeta* (14.60 ± 2.16 days) (Fig. 1a). We should remark that females survival with *A.*
253 *azurea* (mean= 20.0 days; S.E.= 0.00) indicates that survival could be superior than 20
254 days if we had not ended the experiment, and thus might be underestimated. Survival
255 times associated with honeydew (*A. gossypii*: 14.27 ± 3.34 days; *E. olivina*: 13.67 ±
256 3.28 days) were similar to those associated with most of the floral nectars tested (Fig.
257 1a). Differences in survival were also observed when *P. concolor* fed directly on
258 flowers (Table 4). Female wasps feeding on *L. cretica* (18.53 ± 1.08 days), *A. azurea*
259 (17.54 ± 1.49 days) and *F. vulgare* (14.87 ± 1.38 days) showed the highest mean
260 survival time (Fig. 1b) which, however, did not differ significantly from survival of
261 wasps fed with artificial diet (positive control). By contrast, female wasps feeding on *R.*
262 *officinalis* (7.53 ± 1.77 days), *E. plantagineum* (4.67 ± 0.49 days) and *C. nepeta* (2.47 ±
263 0.36 days) flowers survived for significantly shorter times. In fact, the survival period
264 associated with *C. nepeta* (Fig. 1b) was significantly lower than that obtained with the
265 negative control.

266 Regarding *P. concolor* males, there were clear differences in mean survival
267 times between floral nectar treatments (Fig. 1c), with the highest mean survival time
268 associated to floral nectars from *A. azurea* (16.87 ± 1.24 days), *R. officinalis* (13.40 ±
269 1.66 days) and *C. nepeta* (13.27 ± 1.32 days). Males feeding on nectar from *E.*
270 *plantagineum* and *L. cretica* exhibited the lowest survival times and did not differ
271 significantly from the negative control (water; 4.87 ± 0.18 days). The effect of feeding
272 on flowers on male mean survival time was not as clear as observed with females. The
273 highest survival time observed in males feeding on *A. azurea* (13.07 ± 1.55 days) and *F.*
274 *vulgare* (10.40 ± 1.02) flowers was actually significantly lower than the mean survival

275 times associated with artificial diet (18.10 ± 0.70 days) (Fig. 1d). In summary, on
276 average, females lived longer than males (Fig. 1) and mean survival times differed
277 significantly between food provenance and wasp sex (Table 4).
278
279 **Discussion**
280
281 In our study the sucrose/hexose ratio does not seem to explain differences in
282 survival, a result similar with those found by Tompkins et al. (2010) which reported that
283 the sucrose/hexose ratio was not a significant factor to explain parasitoid survival of the
284 parasitoids *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) and
285 *Dolichogenidea tasmanica* (Hymenoptera: Braconidae). Even if sucrose-rich *A. azurea,*
286 as also found by Nepi et al. (2010), and sucrose-dominant *R. officinalis* nectars provided
287 survival times not different from the artificial diet, the nectar of the hexose-rich species
288 *C. nepeta* and *L. cretica* also resulted in similar survival periods of females, and males
289 (only with *C. nepeta* nectar). Also, survival times of both male and female wasps on *E.*
290 *plantagineum* were surprisingly low, considering that it also provides sucrose-rich
291 nectar which is more calorific then hexose-nectars (Nicolson 2007). Despite being a
292 known melittophilous species (Corbet and Delfosse 1984), nectar from *E. plantagineum*
293 contains pyrrolizidine alkaloids (Culvenor et al. 1981), which may have a deterrent
294 effect on *P. concolor* feeding behavior (Nicolson 2007). This fact could explain why
295 long survival periods as those observed with the other sucrose rich/dominat plants *A.*
296 *azurea* and *R. officinalis* weren't obtained with *E. plantagineum*, neither for females nor
297 males.
298

299 *P. concolor* feeding on flowers of *A. azurea*, *L. cretica* and *F. vulgare* exhibited
300 survival times similar to those when feeding on artificial diet. The findings justified the
301 selection of flowers based on corolla morphometry and head size. However, survival of
302 *P. concolor* feeding on *E. plantagineum* flowers was lower than when fed with nectar-
303 only. These findings suggest that *E. plantagineum* flower morphology or floral scent are
304 an additional constraint to pyrrolizidine alkaloids presence in nectar (Culvenor et al.
305 1981) and in itself affects survival. A similar effect was observed in *Episyrphus*
306 *balteatus* (Diptera: Syrphidae) feeding on *E. plantagineum* (Pinheiro et al. 2013). Even
307 though the corolla of *E. plantagineum* is broad enough for *P. concolor* to insert its head
308 but is also quite deep and it is uncertain if *P. concolor* can feed successfully on such a
309 relatively deep structure. Similarly, survival was much lower on flowers of *R. officinalis*
310 and *C. nepeta* than on their nectar. This finding suggests that the narrow width of the
311 corolla close to the nectaries (1.51 ± 0.425 and 1.61 ± 0.297 mm, respectively) in
312 combination with a comparably deep corolla prevents *P. concolor* from feeding
313 successfully. The results indicate clearly that laboratory observations on nectar feeding
314 may not always be transposed to field conditions, because floral morphology can
315 profoundly affect the foraging behavior of parasitoids and their ability to obtain nectar
316 (Patt et al. 1997; Wäckers and van Rijn 2012). Our results suggest that *P. concolor*, as
317 many hymenopteran parasitoids (Gilbert and Jervis 1998), feeds predominantly on
318 flowers with shallow corollas. Consequently, parasitoid head width and corolla depth
319 and width are important factors to consider in the choice of non-host food sources for
320 natural enemies of pests.

321 Feeding on honeydew resulted in survival times, which compared well to about
322 half of the floral nectars tested. It, therefore, represented another suitable food source
323 for *P. concolor*. A similar effect was reported by Beach et al. (2003) who found that

several honeydew sugars were readily accepted by the egg parasitoid *Anaphes iole* Girault (Hymenoptera: Mymaridae)d and by Idoine and Ferro (1988), who observed parasitoids failing to visit flowers but feeding easily on honeydew. The findings are contrasted by reports on several other hymenopteran parasitoids, in which honeydew was found to be an inferior food source (Idoine and Ferro 1988; Wäckers 2000, 2005; Wäckers et al. 2008). Honeydew as a food source could be very useful for some parasitoids since many crops lack nectar or provide it only during short periods of time (Wäckers 2005), whereas honeydew is often more readily available, making it the predominant sugar source in agro-ecosystems. However, honeydew is often highly viscous (Wäckers 2005) and because of its content of melezitose and raffinose, which crystallise easier than sucrose, sometimes only scattered as crystallized deposits across leaf surfaces, which are difficult to feed on for parasitoids (Wäckers 2000). *P. concolor* in particular has been observed to feed on liquid and even viscous honeydew but never on crystallised deposits (F. Rei personal observation). Because *P. concolor* has short mouthparts, which restrict feeding to more exposed floral nectars, the availability of other easily accessible sugar sources, such as honeydew, can be an important factor for their survival. In olive groves, honeydew provided by *E. olivina*, a common secondary olive pest, could potentially provide vital resources for *P. concolor*, especially when floral nectar is not available in sufficient quantity.

In conclusion, our results showed that nectar from all tested plants and honeydew from *A. gossypii* and *E. olivina* provide nutritional resources for *P. concolor* females during the active *B. oleae* periods, that is, in late spring and late summer/autumn. *Anchusa azurea*, *Lavatera cretica* and *Foeniculum vulgare* were the most beneficial species to *P. concolor* survival and could also be suitable for other parasitoids of *B. oleae*, especially those related to the *P. concolor* complex, but also for

many predatory arthropods (Coll and Guershon 2002). However, since the trials were conducted for only 20 days, this could have capped the longevity, resulting in underestimation of the survival time provided by some of the plants, mainly by *A. azurea*, which allowed survival of all individuals for 20 days. Other species under evaluation that provided high mean longevity and low SE might also have been underestimated. We therefore consider that sugar impacts and the differences between treatments could be better defined with longer experimental periods.

Maintenance of an herbaceous cover in inter-rows is a very useful measure for improving soil stability and fertility of the orchard, and should also include an adequate number of flowering species suitable as food sources for parasitoids to enhance their abundance and survival. Our results indicate that inclusion of *A. azurea*, *L. cretica* and *F. vulgare* in the inter-rows or in the olive orchard border would be a useful measure because the plants are a suitable food source for the olive fly parasitoid, *P. concolor*. Honeydew from *E. olivina* also constitutes a suitable food source for the parasitoid, and this should be considered in the management of this secondary pest, especially as it does not represent a significant risk for the adult olive orchard. Both measures together could enhance the effectiveness of biological control programs, making pest control less disruptive and improving sustainability of olive orchards. Future research should address effects of these food resources on the reproduction of *P. concolor*, to understand their effect on the entire life cycle of the wasp. For example, mating interactions are costly for both sexes of *P. concolor* (Benelli et al. 2013b) and may well reduce survival compared to that of virgin males and females used in this study.

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496 **Table 1** Flowering periods of selected plant species and botanical families ([a])

Species (Families)	Flowering Period											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Anchusa azurea (Boraginaceae)			■	■	■	■	■					
Calamintha nepeta (Lamiaceae)			■	■	■	■			■	■	■	■
Echium plantagineum (Boraginaceae)		■	■	■	■	■						
Foeniculum vulgare (Apiaceae)						■	■	■	■	■		
Lavatera cretica (Malvaceae)			■	■	■	■	■					
Rosmarinus officinalis (Lamiaceae)	■	■	■	■	■					■	■	■

497 [a]According to Coutinho (1939)

Table 2 Corolla size and daily mean floral nectar volume production (mean ± S.E.) of six plant species tested as potential food source for the olive-fly parasitoid *P. concolor*.

	Corolla size/measures (mm)				Nectar volume
Species (Families)	Depth (*F*=1274.78, *df*=5,179 *P*<0.001)	Upper width (*F*=2421.95, *df*=5,179 *P*<0.001)	Lower width (*F*=194.87, *df*=5.179 *P*<0.001)	Length (*F*=1348.32, *df*=*5,179* *P*=0.007)	(µl/flower/day) (*F*=18.916, *df*=5,179 *P*<0.001)
Anchusa azurea (Boraginaceae)	8.48 ± 0.435 b	2.87 ± 0.301 a	2.87 ± 0.302 b	12.07 ± 0.582 a	0.35 ± 0.345 ab
Calamintha nepeta (Lamiaceae)	13.60 ± 0.82 d	7.85 ± 1.260 b	1.61 ± 0.297 a	14.22 ± 1.036 b	0.94 ± 0.424 c
Echium plantagineum (Boraginaceae)	17.19 ± 1.773 e	21.05 ± 2.103 c	3.09 ± 0.332 b	17.19 ± 1.773 c	1.48 ± 1.298 d
Foeniculum vulgare (Apiaceae)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.09 ± 0.052 a
Lavatera cretica (Malvaceae)	8.36 ± 0.915 c	33.82 ± 2.270 d	4.97 ± 0.686 c	19.03 ± 2.371 d	0.45 ± 0.282 ab
Rosmarinus officinalis (Lamiaceae)	2.54 ± 0.590 a	1.84 ± 0.236 a	1.51 ± 0.425 a	11.09 ± 0.584 a	0.73 ± 0.528 bc

For each measure, means with the same letters are not significantly different (Tukey' HSD test).

502 **Table 3** Glucose, sucrose and fructose (%) content (mean ± S.E.) of floral nectar and
503 sucrose/hexose ratio (Baker and Baker 1983) from five plant species common on olive
504 orchards from South Portugal

Plant species	% Glucose (F=4.793, df=4,24, P=0.007)				% Sucrose (F=4.813, df=4,24, P=0.001)				% Frutose (F=3.705, df=4,24, P=0.021)				Sugar Ratio (R)			
Anchusa azurea	28.27	±	8.16	ab	35.11	±	5.31	a	20.67	±	6.79	a	0.58	±	0.12	Sucrose
Calamintha nepeta	31.15	±	9.17	a	23.46	±	3.74	a	30.48	±	7.68	ab	0.33	±	0.06	Hexose
Echium plantagineum	28.20	±	2.32	ab	32.54	±	7.89	a	39,26	±	5.80	ab	0.57	±	0.19	Sucrose
Lavatera cretica	32.21	±	3.43	a	26.04	±	9.31	a	41.74	±	6.63	b	0.45	±	0.19	Hexose
Rosmarinus officinalis	10.43	±	1.38	b	67.40	±	2.99	b	22.17	±	1.73	ab	2.19	±	0.33	Sucrose

505 For each sugar, means with the same letters are not significantly different (Tukey' HSD test).

506 *Sucrose-dominant (R>0.999); sucrose-rich (0.999 < R < 0.500); hexose-rich (0.499 < R < 0.100);

507 hexose-dominant (R<0.100)

508

509 **Table 4** Results of two-way ANCOVA of survival of *P. concolor* provided with flowers

510 and nectar from six plant species and honeydew

Survival	**df**	***F***	***P***
Associated to nectar and honeydew			
Treatment	8	14.030	<0.001
Sex*	1	15.713	<0.001
Rep**	1	0.796	0.379
Treatment x sex*	6	0.954	0.472
Error	33		
Total	47		
Associated to flowers			
Treatment	7	77.309	<0.001
Sex	1	30.158	<0.001
Rep**	1	0.153	0.698
Treatment x Sex	7	7.065	<0.001
Error	31		
Total	47		

511 *Performed only with nectar data, since honeydew data was not available for males.

512 ** Repetitions were considered in the analysis as covariates.

513

514 **Fig. 1** Survival (mean ± S.E.) of *Psytallia concolor* females (♀) fed for 20 days with **a)**
515 nectar-only of *Lavatera cretica*, *Anchusa azurea*, *Rosmarinus officinalis*, *Echium*
516 *plantagineum*, *Calamintha nepeta*, and honeydew from *Euphyllura olivina* and *Aphis*
517 *gossypii*, and with **b)** flowers of *L. cretica*, *A. azurea*, *Foeniculum vulgare*, *R.*
518 *officinalis*, *E. plantagineum*, *C. nepeta.* and males (♂) fed for 20 days with **c)** nectar-
519 only of *Lavatera cretica*, *Anchusa azurea*, *Rosmarinus officinalis*, *Echium*
520 *plantagineum*, *Calamintha nepeta*, and with **d)** flowers of *L. cretica*, *A. azurea*,
521 *Foeniculum vulgare*, *R. officinalis*, *E. plantagineum*, *C. nepeta.* In all cases, water-only
522 was the negative control and artificial diet was the positive control. Bars regarding
523 treatments with different letters are significantly different at $P < 0.05$ (Tukey' HSD
524 test). nt - not tested
525

526 **Fig. 1**

527

528

529