

# Wild flower resources and insect honeydew are potential food items for *Elasmus flabellatus*

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**Abstract** Adult parasitoids need non-host food such as nectar or honeydew for survival and reproduction. In a conservation biological control strategy, the knowledge about non-host feeding of parasitoid species is a key factor to successfully increase their action. The nutritional behavior of *Elasmus flabellatus* (Fonscolombe) (Hymenoptera: Eulophidae), a major parasitoid of the olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae), is completely unknown. Survival experiments were performed on two secondary olive pest honeydews and eight common flowering plant species in order to analyze their suitability as potential food sources for *E. flabellatus* females. Abdomen and gut dissections were carried out to verify the pollen consumption and the egg production. Floral architecture and insect morphology were described. Cox's proportional hazard regression models were used to analyze the differences between parasitoid survivals. Honeydews secreted by *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) and *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae) resulted in the best performance followed by the flowers of *Malva sylvestris* L. (Malvaceae), *Daucus carota* L. (Apiaceae), and the Cichorioideae *Tolpis barbata* (L.) and *Andryala integrifolia* L. Theoretical flower resources accessibility were assessed and related with the survival results.

*E. flabellatus* females did not consume pollen and did not produce eggs, suggesting that the species is synovigenic and requires additional foods for egg production. In sustainable pest control programs, this novel knowledge is a promising opportunity for improving suitable food resources of *E. flabellatus* in the field.

**Keywords** Nutritional ecology · Survival analysis · Cox proportional hazard model · Parasitoids · *Prays oleae* · Olive orchard

## 1 Introduction

Insect feeding is determined by several aspects such as availability, detectability, accessibility, and nutritional suitability of foods (Wäckers 2005). Adult parasitoid wasps need energy for maintenance, locomotion, and reproduction that is provided by non-host foods (Jervis et al. 2008). Several studies have been conducted to determine the influence of non-host resources (such as nectar, insect honeydews, and sugar solutions) on various parasitoid species. Different traits related to survival and reproduction were evaluated under laboratory and field conditions (e.g., Balzan and Wäckers 2013; Lavandero et al. 2006; Wäckers et al. 2008). In all these studies, parasitoids fed on sugary liquids. Pollen feeding by parasitoids has been studied less frequently than sugar feeding (Lundgren 2009 and references therein), and according to Jervis et al. (2008), only few groups feed on pollen (e.g., Mutillidae, Scoliidae, and some Bombyliidae). However, Patt et al. (1997) found that when crawling on the disc flowers, the Eulophidae *Edovum puttleri* Grissell and *Pediobius foveolatus* Crawford accumulated pollen on their bodies and they fed on that pollen. In other cases, parasitoids such as *Trichogramma brassicae* Bezdenko (Trichogrammatidae)

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benefited from pollen feeding, which increased its longevity and fecundity when fed on corn pollen (Zhang et al. 2004). Gut dissection can be used to analyze pollen consumption by insects, although few studies have been performed with parasitoid wasps (Jervis et al. 1993; Lundgren 2009).

The olive tree (*Olea europaea* L.) is among the oldest and most widespread crops in the Mediterranean region. Nowadays, it is cultivated in several regions of the world with climatic conditions that allow its establishment. The olive moth *Prays oleae* (Bernard) (Lepidoptera: Praydidae) is one of the most important pests in the olive grove agroecosystem. It has three generations a year, the phyllophagous (feeding on leaves), the anthophagous (feeding on flowers), and the carpophagous generation (feeding on fruit). This pest is parasitized by several hymenopteran species (Villa et al. 2016b); however, the information about the type of food resources exploited by them is scarce.

Species of the genus *Elasmus* Westwood occur in all the zoogeographical regions. *Elasmus flabellatus* (Fonscolombe) (Hymenoptera: Eulophidae) is an ectophagous and gregarious parasitoid, which attacks larvae and pupae of Lepidoptera order (e.g., Gelechiidae, Tortricidae, Noctuidae, Yponomeutidae, Heliozelidae, Plutellidae, Psychidae, and Pyralidae families) and larvae of Hymenoptera (e.g., Cephidae, Bethylidae, Braconidae, and Ichneumonidae families) (Yefremova and Strakhova 2010 and references therein). This is an idiobiont parasitoid, i.e., females stop the development of *P. oleae* last instar larva, lay their eggs, and develop outside of it (Bento et al. 2007). To our knowledge, there is no information about the type of ovigeny of this species. It acts as a facultative hyperparasitoid of other *P. oleae* parasitoids, and the levels of parasitism on this pest were found to vary from 10% for the phyllophagous generation (Bento et al. 2007 and reference therein) to 15.2% for the anthophagous generation (Villa et al. 2016b). Despite its hyperparasitoid behavior and parasitism rates, in some conditions, *E. flabellatus* can be considered an important *P. oleae* biocontrol agent. For instance, Villa et al. (2016b) found that in olive groves with herbicide application, after an extremely dry year and extraordinary low pest levels, *E. flabellatus* reached around the half of the overall parasitism with 10% of parasitism levels.

Usually, *E. flabellatus* attacks the phyllophagous and the anthophagous generations but not the carpophagous (e.g., Bento et al. 2007 and references therein). Increasing numbers of *E. flabellatus* in the agroecosystem might lead to higher parasitism of *P. oleae* larvae from the phyllophagous and the anthophagous generations and consequently to the diminution of the carpophagous generation and, more importantly, to the reduction of fruit damage. However, from the end of the anthophagous generation (at the end of the spring) to the beginning of the phyllophagous generation (at the end of the winter of the next year), *E. flabellatus* needs to survive. The adult feeding of *E. flabellatus* emerging from larva of the

anthophagous generation is therefore crucial to its success and additional studies are needed in order to elucidate which food resources could contribute to enhance *E. flabellatus* in the olive grove during that period.

Moreover, honeydews produced by some olive tree secondary pests, such as the black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) and the olive psyllid *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae), are mainly present during the anthophagous generation (Pereira 2004; Tzanakakis 2003 and references therein) and could have a final positive effect by nourishing parasitoids and improving their performance.

In this context, this work aims at (1) studying the survival of *E. flabellatus* females fed on honeydews secreted by *S. oleae* and *E. olivina* and eight spontaneous plant species: the Apiaceae *Daucus carota* L., the Asteraceae *Anthemis arvensis* L. and *Coleostephus myconis* (L.) Rchb.f. (subfamily Asteroideae), *Andryala integrifolia* L. and *Tolpis barbata* (L.) (subfamily Cichorioideae), the Boraginaceae *Echium plantagineum* L., the Hypericaceae *Hypericum perforatum* L., and the Malvaceae *Malva sylvestris* L., (2) analyzing the parasitoid accessibility to flower resources and the pollen consumption, and (3) determining its type of ovigeny.

## 2 Materials and methods

### 2.1 Parasitoids

*E. flabellatus* adults were obtained from parasitized olive moth larvae of the anthophagous generation collected in olive orchards from the Trás-os-Montes region (northeastern Portugal) at the end of the spring of 2013. In the laboratory, *P. oleae* larvae were transferred individually into tubes (1.7 cm in diameter and 12 cm length), closed with Parafilm®, and placed in a climate chamber at  $21 \pm 2$  °C,  $70 \pm 5\%$  RH, and a photoperiod of 16:8 h (L/D) until the emergence of *E. flabellatus* adults used in experiments described in Sections 2.3 and 2.5.

### 2.2 Selected foods

Selected plants were *A. arvensis*, *A. integrifolia*, *C. myconis*, *D. carota*, *E. plantagineum*, *H. perforatum*, *M. sylvestris*, and *T. barbata* flowering at the end of the spring, simultaneously with the flight period of *E. flabellatus* that emerged from the anthophagous generation of *P. oleae* and common in several agroecosystems. The plant selection was based on (1) the existence of records for flowering specimens in the Trás-os-Montes region (northeastern Portugal) in Flora-on (2015), which systematizes the geographical and ecological information of the Portuguese flora and is coordinated by the Botanical Society of Portugal, (2) their occurrence in olive

groves during the period of study (Villa, personal observation), and (3) their abundance (enough to perform the experiments and potentially feed insects in the field). Flowers were collected in the campus of the Polytechnic Institute of Bragança (in order to minimize the period between flower collection and experiment assemblage) between 9:00 and 10:00 AM, and only flowers without aphid infestation or damages were chosen. Small drops (of about 1–2  $\mu\text{l}$  of volume) of honeydews produced by *S. oleae* and *E. olivina* were collected overnight by placing a Parafilm® strip (4  $\text{cm}^2$ ) under infested leaves of olive trees grown in a climatic chamber [ $24 \pm 2^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and a photoperiod of 16:8 h (L/D)]. Strips with more than ten honeydew drops were used in the experiments in order to guarantee that *E. flabellatus* fed ad libitum.

### 2.3 Survival experiments

Newly emerged (<24 h) adult females of *E. flabellatus* were transferred into cages (2.7 cm in diameter and 12 cm length) and provided with water and one of the food sources. The different treatments were carried out simultaneously, all the individuals emerged during a period of 10 days, and all the plants were flowering during the period of the experiment. Between 18 and 34 females were tested: *A. arvensis*—20, *A. integrifolia*—18, *C. myconis*—24, *T. barbata*—22, *E. plantagineum*—26, *H. perforatum*—22, *M. sylvestris*—27, *D. carota*—32, *S. oleae* honeydew—31, and *E. olivina* honeydew—25. A negative control (water) and a positive control (honey solution 10%, w/v) were assembled (26 and 34 females, respectively). To ensure the repeatability of the experiment, a similar floral area was provided by treatment: two inflorescences of *A. arvensis*, three of *A. integrifolia*, one of *C. myconis*, three of *T. barbata*, two flowers of *E. plantagineum*, three of *H. perforatum*, one of *M. sylvestris*, and five umbellets of *D. carota*. The cut stems of the flowers were submerged in water in 1.5-ml microcentrifuge tubes and closed with Parafilm®. This procedure is considered adequate since previous studies did not find differences between observed lifespans with cut and non-cut flowers (Wade and Wratten 2007). In the negative and positive control treatments, water and honey 10% were provided by filling 1.5-ml microcentrifuge tubes with a strip of filter paper and closed with Parafilm®. The microcentrifuge tube was fixed inside the cage with a piece of adhesive putty. Food items were replaced three times a week, according to the durability of the flowers. Cages for survival analysis were kept in a climate chamber at  $21 \pm 2^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and a photoperiod of 16:8 h (L/D), and daily mortality was recorded until all insects were dead (Fig. 1).

### 2.4 Theoretical flower resource accessibility

Flower resource (pollen and nectar) accessibility was analyzed by determining the compatibility between flower architecture and the insect morphology. For that, the following parameters were measured in 20 specimens of each plant species: (1) in compound flowers (all Asteraceae and *D. carota*) the inflorescence opening (as the largest diameter of the inflorescence), (2) in open flowers (*H. perforatum* and *D. carota*) the flower opening (as the largest diameter of the flower), (3) in tubular flowers (*E. plantagineum* and disc flowers in the Asteroideae *A. arvensis* and *C. myconis*) the corolla opening (as the diameter of the narrowest part of the tube) and the depth of the flower (from the most proximal point of the corolla to the location of the nectar), and (4) in ligulate flowers (in the Cichorioideae *T. barbata* and *A. integrifolia*) the corolla length (from the most proximal point of the corolla to the location of the nectar). A caliper rule was used for structures larger than 5 mm. Leica Application Suite X version 3.8 software (Leica Microsystems, Germany) connected to a stereomicroscope was used to measure structures smaller than 5 mm. The head and thorax widths of 20 *E. flabellatus* females were measured.

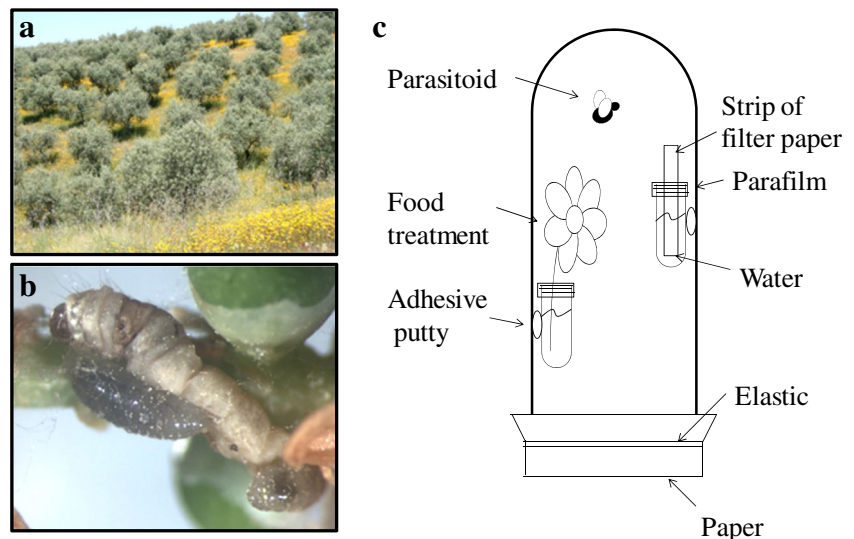
### 2.5 Pollen consumption and ovigeny

Ten newly emerged (<24 h) adult females of *E. flabellatus* were transferred into cages (2.7 cm in diameter and 12 cm length) and provided with water and one of the treatments. After 3 days, the parasitoids were frozen and then the pollen consumption and egg load were analyzed. In the case of the flower treatments, each parasitoid was washed in 96% alcohol to eliminate the external pollen. The abdomen was removed from the body and opened with the help of the scalpel, needles, and fine forceps using a stereomicroscope. In all the specimens, the abdominal content was released onto a glass slide. After that, two drops of glycerin jelly/water (1:1) were added, and a coverslip (22  $\times$  22 mm) was applied. To prevent pollen contamination between the samples, the scalpel, needles, and forceps were washed after each dissection. The consumption of pollen grains was evaluated using an optical microscope.

### 2.6 Data analysis

Firstly, 18 observations from each treatment were randomly selected using the *sample* function in R (R core team 2014) in order to ensure the balance of the sample size between treatments. Lifespan means and standard errors were calculated. Survival curves were represented for each treatment with Kaplan–Meier estimates using the *Surv* and *survfit* functions from the “survival” package (Therneau 2014). Death hazard

**Fig. 1** Olive grove in Trás-os-Montes, northeastern Portugal (a); larvae of *Elasmus flabellatus* feeding on a *Prays oleae* larva (b); setting up of the survival experiments (c)



differences between treatments were checked using Cox's proportional hazard regression model (Cox PHM) (Eq. (1))

$$h(t, x) = h_0(t) \exp \left( \sum_{i=1}^p \beta_i x_i \right) \quad (1)$$

where  $h(t, x)$  is the hazard function,  $t$  is the time,  $x \in \{c_0, c_1, \dots, c_k - 1\}$  represent the different treatments,  $h_0(t)$  is the baseline,  $\exp(\beta_i x_i)$  is the death hazard ratio for each treatment, and  $\beta$  is the slope (Cox and Oakes 2001). This is a semiparametric model that estimates the death hazard ratio between two treatments, with the advantage of indicating the dimension of the hazard ratio variation between two treatments. A death hazard ratio of 1 indicates that no differences exist among two treatments. A death hazard ratio of  $<1$  indicates lower death risk for the comparison group. A death hazard ratio of  $>1$  means higher death risk for the comparison group.

The Cox PHM was estimated through likelihood ratio test and using *coxph* function of the *survival* package (Therneau 2014) and with “food treatment” as explanatory variable. Efron's partial likelihood was used to estimate  $\exp(\beta_i)$  of the Cox PHM. The proportional hazard assumption of the Cox PHM was assessed by testing the no correlation between the Schoenfeld's residuals and the survival time using the *cox.zph* function of the same package and plotting the smoothed Schoenfeld's residuals against the different levels of the categorical variable. The Martingale residuals were plotted against the different levels of “food resources” to detect potential nonlinearity of the model. The occurrence of influential observations was evaluated by estimating changes in the regression coefficients upon deleting each observation in turn and diving by the standard errors using *type=dfbetas* in *residuals* function in R (Fox and Weisberg 2011). An observation was considered influential if *dfbetas*  $>1$  (Bollen and Jackman 1990).

### 3 Results and discussion

Food resources analyzed in this work are spatially and temporally coincident with *E. flabellatus* adults emerging from larvae of the anthophagous generation of *P. oleae* at the end of the spring (Flora-on 2015; Pereira 2004; Tzanakakis 2003). This synchrony enables parasitoids feeding.

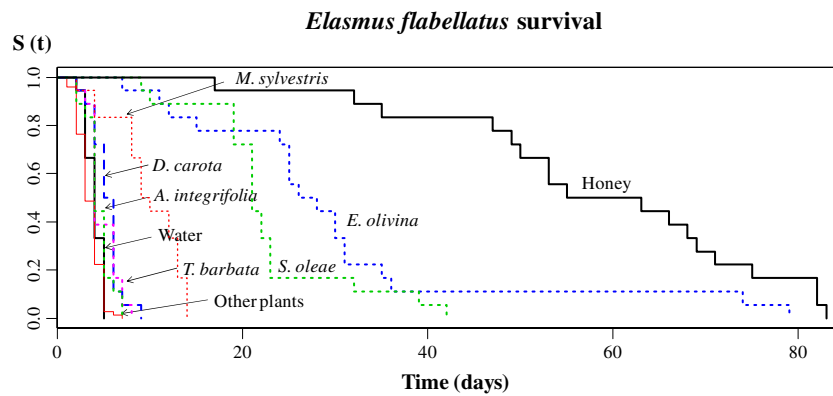
Figure 2 represents the survival curves obtained by Kaplan–Meier estimates for *E. flabellatus* females fed on different resources. The best survival was reached with honey followed by both honeydews and the flowers of *M. sylvestris*, *D. carota*, *T. barbata*, and *A. integrifolia*.

In the Cox PHM fitted using “food resource” the proportional hazard assumption was accepted and nonlinearity was not detected. No influential observations were detected (all *dfbetas*  $<1$ ).

The Cox PHM showed that female death hazards were significantly different among diet treatments ( $\chi^2 = 331$ , *df* = 11,  $p < 0.001$ ). The highest lifespan for females was observed on the honey solution ( $58.33 \pm 4.34$  days) (Fig. 3). The Cox PHM showed that honey reduced the risk of death when compared with *E. olivina* (69.60% reduction,  $p = 0.011$ ) and *S. oleae* honeydews (88.10% reduction,  $p < 0.001$ ) and with all the flowers and the negative control ( $>99\%$  reduction,  $p < 0.001$ ). These results suggest that honey contains components that can benefit *E. flabellatus* females. Higher quality and quantity of sugars and other minor components could be responsible for these results.

Food nutritional suitability depends on its composition and the ability of the insect to absorb, metabolize, and assimilate or store the food components (Wäckers 2005). Hemipteran honeydews contain oligosaccharides that are thought to be involved in osmoregulation functions (Byrne et al. 2003), but also in evasive strategies to avoid honeydew consumption by parasitoids and non-mutualistic predators (Wäckers 2005 and references therein). Sugar composition of honeydews





**Fig. 2** Survival curves obtained by Kaplan–Meier estimates for adult females *Elasmus flabellatus* fed on different resources. The “other plants” curve correspond to *A. arvensis*, *E. plantagineum*, *C. myconis*, and *H. perforatum* treatments that were gathered in a survival curve to

facilitate the reading of the graphic once the survival curves of these treatments were overlapped and not significantly different in the Cox’s proportional hazard regression model (Cox PHM)

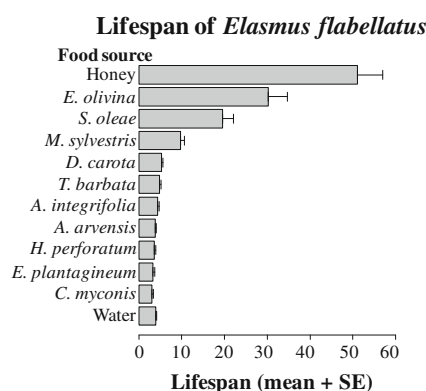
depends on both the sucking insect and the plant species (Hendrix et al. 1992). The composition of *S. oleae* honeydew was described for this pest growing on *Citrus sinensis* L., and fructose, sucrose, and glucose were the only carbohydrates identified (Byrne et al. 2003). As far as we know, no data are available about the composition of *S. oleae* and *E. olivina* honeydews growing on olive trees. In this study, the natural food resources that resulted in better reductions of death hazard of *E. flabellatus* females were *S. oleae* ( $22.61 \pm 1.923$  days) and *E. olivina* ( $30.22 \pm 4.42$  days) honeydews (Fig. 3). Both honeydews presented lower death risk than the rest of the treatments when compared with the flowers and only water (>92% reduction,  $p < 0.001$ ) and, therefore, seemed to be nutritionally suitable for females of *E. flabellatus*. Aphid honeydews have been observed to contain higher amounts of amino acids than phloem saps and the composition can be more balanced (Douglas 2006). A potential extra source of amino acids in *S. oleae* and *E. olivina* honeydews could be responsible for the increase of the survival of *E. flabellatus* fed on these foods. Parasitoid feeding has been observed to be negatively influenced by the viscosity

of sugary liquid food resources (Winkler et al. 2009). In this study, females fed on *E. olivina* reduced their risk of death by 60.60% when compared to females fed on *S. oleae* ( $p = 0.011$ ). The viscosity of *E. olivina* honeydew, which forms bigger and thicker droplets, is visibly higher than that secreted by *S. oleae* (Villa, personal observation). The death hazards were significantly lower for females fed on *E. olivina* honeydew, although its viscosity is higher than that of *S. oleae*, which suggests that *E. flabellatus* can feed on viscous sugary liquids.

The highest survival of females fed on flowers was observed on *M. sylvestris* ( $9.78 \pm 0.87$  days) which reduced the risk of death when compared with all the flowers and only water (>72.60% reduction,  $p < 0.001$ ). Following *M. sylvestris*, the best performance was showed by females fed on *D. carota* ( $5.28 \pm 0.37$  days) and *T. barbata* ( $4.83 \pm 0.37$  days), which reduced the risk of death when compared with all the other flowers and only water (*D. carota*, >65.10% reduction,  $p < 0.003$ ; *T. barbata*, >54.90% reduction,  $p = 0.02$ ), with the exception of *A. integrifolia* ( $4.44 \pm 0.33$  days) that did not show significant differences with *D. carota* ( $p = 0.09$ ) and *T. barbata* ( $p = 0.368$ ). Also, *D. carota* and *T. barbata* did not show significant differences among them ( $p = 0.445$ ). *A. integrifolia* ( $3.06 \pm 0.29$  days) only reduced the risk of death when compared with *C. myconis* (64.00% reduction,  $p < 0.001$ ) and *E. plantagineum* (49.10% reduction,  $p = 0.046$ ) (Fig. 3).

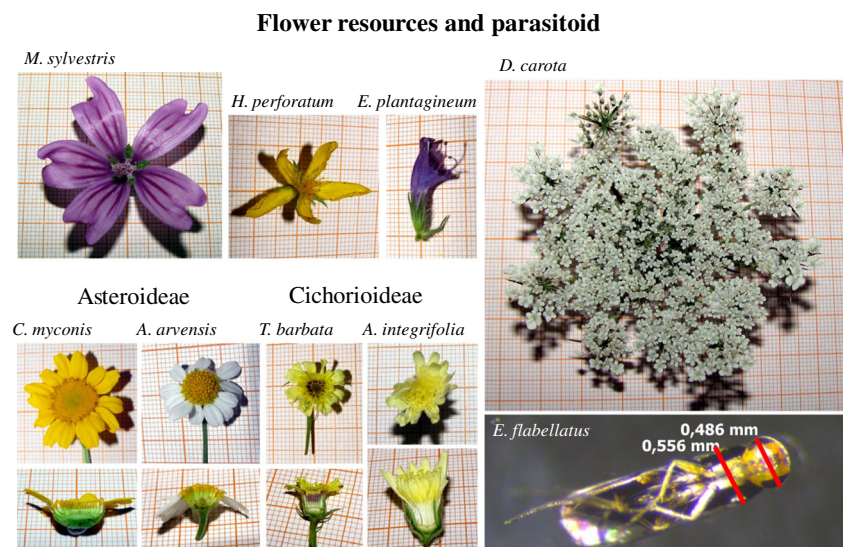
The relationship between floral architecture and the maximum width of *E. flabellatus* determines the accessibility to resources, in particular to nectar. The flower resources and the parasitoid used in this study are shown in Fig. 4.

The maximum width of *E. flabellatus* head and thorax was  $0.496 \pm 0.013$  mm and  $0.519 \pm 0.014$  mm, respectively. These results are similar to those found by Nave et al. (2016) (head =  $0.42 \pm 0.01$  mm; thorax =  $0.53 \pm 0.02$  mm). The floral



**Fig. 3** Lifespan (mean + SE) (days) for females of *Elasmus flabellatus* fed on different flower and insect honeydew food sources

**Fig. 4** Inflorescences of the plant species used in the experiments and *Elasmus flabellatus* females showing head and thorax widths. Sections of Asteroideae and Cichorioideae (Asteraceae) are shown



architecture can explain higher survivorships given by plants with open corollas and exposed nectaries, such as *M. sylvestris* and *D. carota*, as well as easily exploited resources, such as honeydews and honey, or the Cichorioideae *T. barbata* and *A. integrifolia* (Table 1). According to van Rijn and Wäckers (2016), the accessibility to nectar from Cichorioideae is likely limited by the close packing of the florets. The highest survival of *E. flabellatus* on *T. barbata* in relation to *A. integrifolia* could also be related to its shorter corolla.

The death hazard for females fed on *A. arvensis* ( $3.83 \pm 0.19$  days), *C. myconis* ( $3.06 \pm 0.28$  days), *E. plantagineum* ( $3.33 \pm 0.35$  days), *H. perforatum* ( $3.67 \pm 0.31$ ), and only water ( $3.94 \pm 0.22$  days) did not show significant differences among them ( $p > 0.05$ ). These plant treatments were gathered in a Cox PHM in order to obtain a unique survival curve (this procedure was adopted to facilitate the reading of the graphic once these curves were overlapped) (Fig. 2). In our work, survivorship given by the Asteroideae *A. arvensis* and *C. myconis* did not differ from water since the nectaries of these plants can be inaccessible for *E. flabellatus* because they are usually set at the base of the style in the disc floret (Sulborska and Weryszko-Chimielewska 2007) and the

size of the parasitoid did not allow to reach them. The no effects on lifespan for *E. plantagineum* could be due to the long tube depth compared to the size of the parasitoid (Table 1, Fig. 4). In the case of *H. perforatum*, although it has a single and open flower, the no effect probably is due to the absence of nectaries (Stevens 2007).

In this study, dissections showed that none of the analyzed females consumed pollen and neither contained eggs. This suggests that *E. flabellatus* is synovigenic and requires additional food to produce eggs. The species *Elasmus schmitti* Ruschka showed to be provigenic (Gumovsky et al. 2007) and *Elasmus nudus* (Nees) was referred as synovigenic (Mehrnejad 2012). None of them performed host feeding. Studies of host feeding on *P. oleae* is currently not possible due to its complicated life cycle (three generations a year, each one feeding on different tissues of the olive tree), making it difficult to rear this moth and its parasitoids under laboratory conditions. However, Redolfi and Campos (2010) reared *Elasmus steffani* Viggiani, a *P. oleae* parasitoid, on the alternative host *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) and observed host feeding. They found significant longer longevity for *E. steffani* females when fed on different

**Table 1** Inflorescence type and mean  $\pm$  SE (mm) of inflorescence measurements of plant species tested in the experiment with *Elasmus flabellatus*

Species	Type	Inflorescence diameter	Corolla diameter	Corolla length	Tube diameter	Tube width
<i>M. sylvestris</i>	Single and open	—	$23.55 \pm 0.86$	—	—	—
<i>H. perforatum</i>	Single and open	—	$21.40 \pm 0.61$	—	—	—
<i>E. plantagineum</i>	Single tubular	—	—	—	$13.45 \pm 0.48$	$1.77 \pm 0.06$
<i>D. carota</i>	Compound umbel	$44.85 \pm 2.27$	$3.45 \pm 0.25$	—	—	—
<i>C. myconis</i>	Disc capitulum	$15.80 \pm 0.83$	—	—	$2.92 \pm 0.08$	$0.38 \pm 0.01$
<i>A. arvensis</i>	Disc capitulum	$15.20 \pm 0.61$	—	—	$2.20 \pm 0.04$	$0.46 \pm 0.01$
<i>T. barbata</i>	Ligulate capitulum	$15.30 \pm 0.69$	—	$5.45 \pm 0.12$	—	—
<i>A. integrifolia</i>	Ligulate capitulum	$13.50 \pm 0.47$	—	$7.74 \pm 0.29$	—	—

food items in a descending order: honey solution > honey solution plus host larvae > without food. Similarly, for *Tricogramma turkestanica* Meyer (Hymenoptera: Trichogrammatidae), host feeding decreased its longevity, and this was attributed to the allocation of carbohydrates to egg production at the expenses of somatic maintenance (Ferracini et al. 2006). This could explain the lowest longevity of *E. steffani* with honey solution plus host larvae in Redolfi and Campos (2010) and provide an insight for *E. flabellatus* biology: probably *E. flabellatus* did not produce eggs because it needs to feed on the host, but this idea needs further research.

The selection of the most suitable food resources should be considered before implementing conservation biological control strategies. On the one hand, flower attractiveness and the accessibility of floral resources can influence *E. flabellatus* population dynamics in the field. Visual and olfactory attractiveness can determine the rate of visits to a given plant species or honeydew (Wäckers 2005 and reference therein), and these factors should be studied in future works. On the other hand, these food sources and floral cues may also benefit co-inhabiting pests. Lavandero et al. (2006) found that some flowering plants enhanced the fitness of both the parasitoid and the herbivore. Balzan and Wäckers (2013) found that flowers differently influenced both pest and parasitoid lifetables. In our case, the studied food resources could also influence *P. oleae* population in the olive grove. Special attention should be given to *M. sylvestris* because its flowering peak coincides with the flight period of both *E. flabellatus* and *P. oleae* (Villa, personal observation). In addition, some attention should be given to *D. carota*; although its flowering peak is slightly later, it starts to bloom during the flight period of anthophagous generation of *P. oleae* (Villa, personal observation). Also, honeydews secreted by *S. oleae* and *E. olivina* occur simultaneously with *P. oleae* (Villa, personal observation). *M. sylvestris* and both honeydews improved survival and reproduction of *P. oleae* in laboratory experiments (Villa et al. 2016a). These food items should be tested in the field to assess the benefits for the parasitoid and for the pest. In the case of the hemipterans, it is also needed to evaluate the yield loss associated to these insects: although they usually are not responsible for substantial damages, they feed on the crop and constitute a risk. Despite these threats, this is the first time that potential food resources for *E. flabellatus* females were identified. In sustainable pest control, this novel knowledge can be an important starting point that will allow selecting food resources to be improved and investigated in the field.

## 4 Conclusion

In summary, in this study, several potential natural foods for *E. flabellatus* were identified in the olive grove

agroecosystem. The best survival was accomplished with *S. oleae* and *E. olivina* honeydews, followed by *M. sylvestris*, *D. carota*, *T. barbata*, and *A. integrifolia* flowers. Thus, these food resources could be considered for conservation biological control. Our results also suggest that *E. flabellatus* is synovigenic and requires additional food resources for egg production. However, future investigation should be focused on studying the effect of these food resources on *E. flabellatus* and pest dynamics in the field, giving special attention to the relationships established between the secondary pests *S. oleae* and *E. olivina* and other elements of the agroecosystem, since they may result in a positive or negative effect in the final crop yield.

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