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Are wild flowers and insect honeydews potential food resources for adults of the olive moth, *Prays oleae*?

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Abstract The use of non-crop resources by natural enemies and their potentialities to enhance their effectiveness as pest control agents is increasing as a method for conservation biological control. Nevertheless, the effect of consumption of non-crop resources by pests has been generally overlooked being this knowledge crucial to favor natural enemies but not pests. In the present work, insect honeydews and flowers suitability as food resources for the olive tree key-pest *Prays oleae* were analyzed under laboratory conditions. The selected honeydews were excreted by *Saissetia oleae* and *Euphyllura olivina*, two olive pests, and the selected plants were seven abundant species in the olive grove agroecosystem that bloom simultaneously with the flight period of the anthophagous generation of *P. oleae*. In this work, some of these resources were identified as potential food sources for *P. oleae*. Despite the general findings, which indicate that honeydews have less nutritional value for insects than nectar, *P. oleae* reached the best survival and reproduction performance with the insects' honeydews. Several of the tested flowers were identified as potential food resources for *P. oleae*, being *Malva sylvestris* the one that originated the best performance. Moreover, our results suggest that *P. oleae* females are synovigenic and emerge with nutritional reserves for reproduction. We highly recommend accomplishing further research before establishing these resources in biological control methods in order to confirm their effect on pests in fields.

Keywords Insect feeding · Non-crop resources · Praydidae · *Saissetia oleae* · *Euphyllura olivina* · Survival analysis · Reproduction

Key message

- Pests may feed on non-crop resources (pollen, nectar or honeydews) in some development phases; however, in the case of the olive moth, this knowledge has been overlooked.
- This is the first time that honeydews and flowers from the olive grove agroecosystem are identified as potential food sources for olive moth adults.
- These results constitute an important contribution to understand the nutritional needs of olive moth adults and will help approach more efficiently the conservation biological control of this pest.

Introduction

Habitat management is a method of conservation biological control that consists of improving pest control through conserving or modifying the environment to enhance survival, reproduction, and behavior of natural enemies

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(Landis et al. 2000). During some phases of their development, they need non-crop resources, such as pollen, nectar, insect honeydews, shelter or alternative preys and hosts (Jervis et al. 1993; Wäckers 2005). Pests feeding causes crop damages/economic losses, and in some phases of their life cycle, pests can use the same non-crop resources consumed by natural enemies (Kevan and Baker 1983; Baggen et al. 1999; Wäckers et al. 2007). Non-crop resources are sometimes enhanced to improve pest control, but the knowledge about the effect of those resources on pests is crucial before increasing their presence in the field in order to hamper pests performance (Baggen and Gurr 1998; Lavandero et al. 2006; Winkler et al. 2009a, b). Many studies analyzed the effect of different food resources (pollen, nectar, insect honeydews, and sugar solutions) on different natural enemies and on pests survival, reproduction, efficiency, or attractiveness (Jervis et al. 1993; Baggen and Gurr 1998; Géneau et al. 2012; Aguilar-Fenollosa and Jacas 2013; Balzan and Wäckers 2013; Beltrà et al. 2013; Gonzalez et al. 2015; Saeed et al. 2015). However, knowledge about the use of non-crop resources by most of the adult pests is still insufficient and as far as we know it has never been studied for the olive moth, *Prays oleae* (Bernard) (Lepidopteta: Praydidae).

The olive moth diet and development during its larval stage are well known. This is a monophagous herbivorous that feeds on the olive tree. It has three generations per year: i) the phylophagous generation that feeds on leaves and develops during autumn and winter; ii) the anthophagous generation that feeds on flowers and develops during the olive tree blooming; and iii) the carpophagous generation that feeds on fruits and develops during summer. Adult feeding habits are poorly known and they might be a determining factor for the survival and reproduction of the olive moth. Such information is crucial and needs to be investigated. Most adults of Lepidoptera order feed on floral nectar although they may also feed on a variety of other liquids such as honeydews (Kevan and Baker 1983; Jervis et al. 2005; Krenn 2010), with implications on conservation biological control, with risks or benefits of using these non-crop resources for Lepidoptera pests control (Lee and Heimpel 2005; Mevi-Schütz and Erhardt 2005; Begum et al. 2006; Lavandero et al. 2006 Winkler et al. 2009b; Balzan and Wäckers 2013). One hypothesis, which needs to be investigated, is that *P. oleae* feed on pollen and nectar provided by non-crop natural vegetation flowers or on insect honeydews from olive groves and surrounding areas.

Moreover, many studies about pests and natural enemies feeding on non-crop vegetation use a similar set of plants (Araj and Wratten 2015) and these plants are chosen due to their proved positive effect on many natural enemies and sometimes on biological control. For example, *Lobularia maritima* (L.) Desv., *Fagopyrum esculentum* M. or *Phacelia tanacetifolia* Benth were frequently studied (Lee et al. 2004; Lavandero et al. 2006; Balzan and Wäckers 2013; Araj and Wratten 2015). However, these plants are not always native and the potential for biological control of many other species in different agroecosystems are unknown. Some authors have already pointed out the importance of using native plants (Jervis et al. 1993; Fiedler and Landis 2007; Araj and Wratten 2015) that can be better adapted to the local environmental conditions, their use may reduce the risk of non-native plants invasion, and the economic inputs for farmers. Pollen and nectar provided by these plants might be used as food resources by the olive moth. Additionally, the olive moth might consume honeydews produced by two secondary hemipteran pests which feed on the olive tree, the black scale, *Saissetia oleae* (Olivier) and the olive psyllid, *Euphyllura olivina* (Costa) and both co-occur with the anthophagous generation of the olive moth. *E. olivina* larvae and adults perforate tender tissues of the olive tree and suck the sap of buds (Tzanakakis 2003). *E. olivina* overwinters as an adult, and oviposition starts in the beginning of spring (coincident with the development of new shoots) and can have various generations per year (Tzanakakis 2003 and references therein).

Here, we studied natural vegetation and honeydews produced by the black scale, *S. oleae*, and the olive psyllid, *E. olivina*, as potential food resources for adults of *P. oleae* in laboratory assays. The objectives were to investigate the effect of these non-crop resources, occurring in olive groves during the anthophagous generation of olive moth, on the survival and reproduction of the adults of this Lepidoptera pest. Implications of adult feeding on *P. oleae* biology and on biological control conservation are discussed.

Materials and methods

Experimental design

Non-crop resources in olive agroecosystems from the northeast of Portugal, Mirandela region, were used to determine their potentiality as food resources for *P. oleae* adults. The food resources selected were *S. oleae* and *E. olivina* honeydews and flowers of the following local plants: *Anthemis arvensis* L., *Andryala integrifolia* L. and *Crepis capillaris* (L.) Wallr. (Asteraceae), *Conium maculatum* L. (Apiaceae), *Jasione montana* L. (Campanulaceae), *Malva sylvestris* L. (Malvaceae) and *Trifolium repens* L. (Fabaceae). These plant species bloom during spring and are abundant during the anthophagous generation of the olive moth. The flowers were collected in the campus of the Polytechnic Institute of Bragança, northeast of Portugal. Their stems were submerged in water in 15-mL plastic jars and closed with parafilm. Honeydews were collected overnight by placing a Parafilm© strip under infested leaves of olive trees grown in climatic chambers in the laboratory. Given the complex life cycle of the olive moth, there is no rearing methodology of this pest in laboratorial conditions. Therefore, larvae of the anthophagous generation of the olive moth were collected in 15 orchards from the region and in each orchard, 20 larvae were randomly collected in 10 olive trees, in order to avoid clustering in conditions in which the larvae were grown and ensure randomization in the treatment assignment. In laboratory, larvae were transferred into tubes and placed in climatic chambers at 21 °C (±2 °C) and a 16:8 h L:D (light:dark) photoperiod until adults emergence. Newly emerged couples were transferred into 220-mL cages. Between 28 and 30 replicates per treatment (22 in *C. capillaris* treatment) was assembled. All cages were provided with water. Each treatment replicate was provided with flowers of one of the plant species or with honeydews of one of the insects. Approximately, 5 cm^2 of flower surface were used by treatment, which correspond to approximately 4 or 5 inflorescences of *A. arvensis*, *A. integrifolia*, *C. capillaris*, *J. montana*, *M. sylvestris*, and *T. repens* and two of *C. maculatum* (which presents bigger inflorescences), and a Parafilm© strip of approximately 5 cm^2 with honeydew was provided. Foods were replaced three times a week, accordingly to the flowers durability. A negative control (water) and a positive control [water-honey solution 10 % (m/v)] were assembled in jars of 15 mL, with a strip of filter paper as dispenser and closed with Parafilm©. Daily mortality and oviposition were recorded. Eggs laid in the cages were counted and marked with a dot to avoid over-counting and eggs laid in the jars were counted and removed.

Data analysis

Survival

Survival curves for each treatment were drawn using the Cox estimates of the survival function. Individuals that escaped during the experiment were right censored. Death hazard differences between treatments were checked separately by sexes using Cox's proportional hazard regression model (Cox PHM) through likelihood ratio test and using *coxph* function of the "*survival*" package (Therneau 2014) in R (R Core Team 2014). Efron's partial likelihood was used to estimate the parameters of the Cox PHM. The proportional hazard assumption of the Cox regression was confirmed testing the no correlation between the Schoenfeld's residuals and the survival time using the *cox.zph* function of the same package. Differences between death hazards among sexes for each diet treatment were analyzed following the same procedure performing one different analysis for each diet treatment.

Reproduction

Firstly, the following parameters were calculated: i) the number of fertile females (percentage of females that laid eggs per treatment in relation to the total number of females); ii) mean pre-oviposition period by fertile couple [±Standard Error (SE)]; iii) the mean oviposition period by fertile couple (±SE); iv) the mean lifetime fecundity by fertile couple (±SE); v) the total lifetime fecundity per treatment (the sum of all eggs laid by the females within each treatment).

Generalized Linear Mixed Models (GLMM) were used to analyze the influence of treatments on *P. oleae* pre-oviposition and oviposition periods with treatment as fixed factor and fertile female as random effect. The negative binomial distribution was used for the response variable to account with the over-dispersion. The Log-link was used between the expected value of the response variable and the systematic part of the model. The *glmmadmb* function from the "*glmmADMB*" package was used (Skaug et al. 2015). Overall differences were checked using Wald Chi square test with the *Anova* function from the *car* package.

Generalized Estimated Equations were used to estimate the autocorrelation between observations ($\alpha = 0.536$) and to account with the repeated sampling in the same subjects using the *geeglm* function with "AR1" correlation structure from the "*geepack*" package (Højsgaard et al. 2006). Then, a GLMM was used to fit the fecundity by treatment with treatment as fixed factor and fertile females as random effect and the function *corAR1* from the "*nlme*" package (Pinheiro et al. 2014) was used to impose the correlation previously calculated. Then, the same procedure used in the previous point was followed.

Following Balzan and Wäckers (2013), a series of generalized linear models (GLM) (with Poisson distribution, or negative binomial distribution to account with overdispersion when needed) were developed to fit the total lifetime fecundity as a function of female longevity for each treatment. The same procedure was followed to analyze the oviposition period as a function of female longevity for each treatment. One outlier was eliminated in the case of *T. repens* treatment.

Results

Longevity

Death hazard ratio by diet treatment

The Cox's proportional hazard regression model showed that female and male death hazard were significantly different among diet treatments (females: likelihood ratio = 259.3, df = 10, $p < 0.001$; males: likelihood ratio = 258.1, df = 10, $p < 0.001$). Death hazard for females fed on *A. arvensis*, *A. integrifolia*, *C. capilllaris* and *J. montana* did not differ significantly from the water treatment (negative control). *M. sylvestris*, *C. maculatum*, *T. repens* flowers and *E. olivina* honeydews showed significantly lower death hazards than the water treatment but higher than *S. oleae* and honey treatments (positive control) (Fig. 1a). Death hazard for males fed on *C. capillaris*, *A. integrifolia* and *T. repens* did not differ significantly from the water treatment but was significantly lower than those treatments with *A. arvensis* and *J. montana* and significantly higher than treatments with *C. maculatum*, *M. sylvestris* and *E. olivina* honeydew. Male death hazard with *S. oleae* honeydew did not differ significantly from the honey treatment and both showed a significant lower death hazard than the rest of the treatments (Fig. 1b).

Death hazard ratio among sexes within treatments

The Cox's proportional hazard regression models did not find significant differences among males and females for the death hazards on water, *C. maculatum*, *T. repens*, *E. olivina* and *S. oleae* honeydew (hazard ratio > 0.883; df = 1; $p > 0.09$ in all cases). On the other treatments, death hazard was higher for males than for females (Hazard ratio > 1.703; df = 1, $p < 0.05$ in all cases).

Reproduction

Daily oviposition (number of eggs) by fertile females through the experiment is shown in the Appendix (Fig. A1 in Supplementary material). The percentage of fertile females varied between 21 and 95 % among treatments and the mean of eggs laid by females varied between 34.7 (±8.5) and 230.5 (±21.8). The pre-oviposition period varied between 2.1 (±0.5) days with honey, and 8.8 (±1.6) days with *C. maculatum*. The longest oviposition period was accomplished with honey, with 21.3 (±2.1) days followed by *S. oleae*, with 20.09 (±2.54) days and the lowest with *A. arvensis,* with 2.4 (±0.4) days. *S. oleae* honeydew and honey led to the highest mean number of eggs per fertile female and to the highest total eggs laid per treatment (Table 1).

Pre-oviposition period, oviposition period, and lifetime fecundity

GLMM outputs fitted for pre-oviposition and oviposition periods and for the lifetime fecundity of *P. oleae* fertile females are shown in the Appendix (Table A1 in Supplementary material). These three variables were significantly affected by the food source (pre-oviposition period: $\chi^2 = 37.7$, df = 10, p value < 0.001; oviposition period: $\chi^2 = 10$, df = 195.7, p value < 0.001; lifetime fecundity: $\chi^2 = 89.9$, df = 10, p value < 0.001). *C. maculatum* was the only treatment that caused a significant increase of the

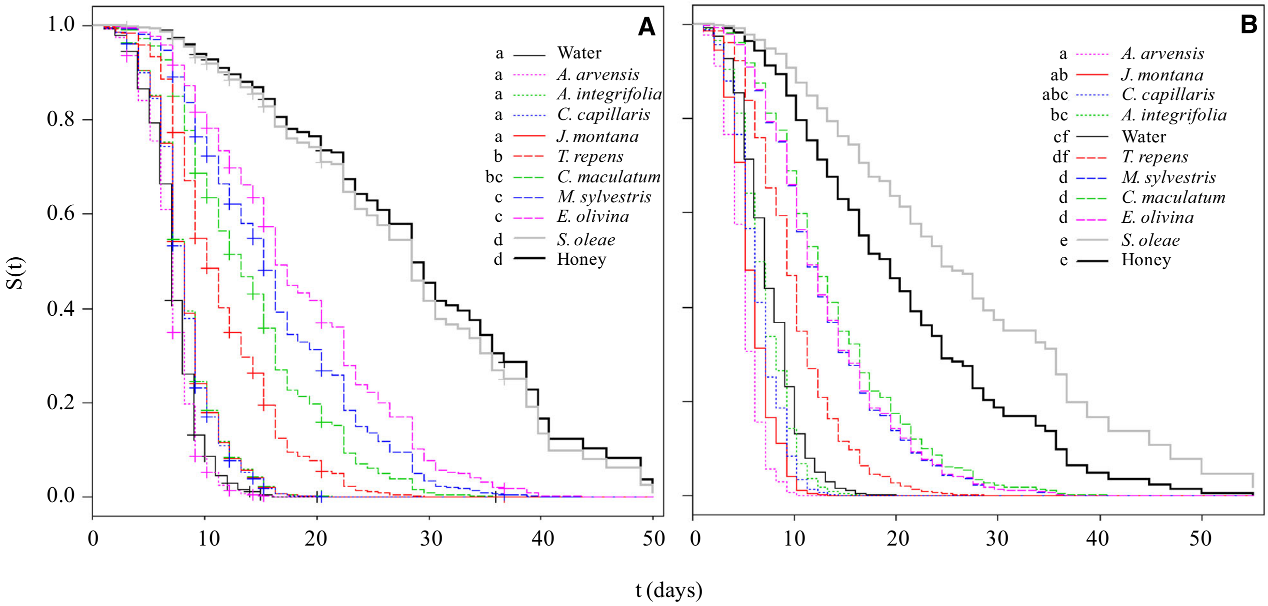

Fig. 1 Cox estimates of the survival function, S(t), for females (**a**) and males (**b**). *Different letters* on the legend indicate significant differences in death hazard among treatments (significance level < 0.05). *Crosses* indicate censored data

Table 1 Reproduction parameters of *Prays oleae* reared on different food sources

Treatments	% Fertile females[a]	Pre-oviposition period (±SE) (days)	Oviposition period (±SE) (days)	Mean eggs/fertile couple (±SE)	Lifetime fecundity
Water	90.00 (27/30)	3.04 (±0.30) a	3.74 (±0.32) a	56.89 (±7.78)	1536 a
A. arvensis	66.67 (20/30)	3.15 (±0.51) ab	2.40 (±0.37) a	34.70 (±8.48)	694 a
A. integrifolia	80.00 (24/30)	3.71 (±0.62) ab	3.37 (±0.42) ab	41.71 (±7.04)	1001 a
C. capillaris	95.45 (21/22)	4.09 (±0.59) ab	5.33 (±0.56) ab	56.81 (±12.48)	1193 a
C. maculatum	21.43 (6/28)	8.67 (±1.55) b	6.83 (±1.06) ab	83.00 (±28.40)	498 a
J. montana	60.00 (18/30)	2.33 (±0.37) ab	3.06 (±0.52) ab	37.78 (±11.23)	680 a
M. sylvestris	58.62 (17/29)	5.06 (±1.04) ab	5.53 (±1.19) ab	70.06 (±15.50)	1191 ab
T. repens	41.38 (12/29)	5.67 (±1.04) ab	6.33 (±1.64) ab	53.67 (±11.43)	644 a
E. olivina	56.67 (17/30)	5.94 (±1.20) ab	7.18 (±1.37) b	80.88 (±19.00)	1375 ab
S. oleae	70.00 (21/30)	5.81 (±1.53) ab	20.09 (±2.45) c	230.57 (±21.78)	4842 c
Honey	93.33 (28/30)	2.01 (±0.51) ab	21.29 (±2.08) c	195.79 (±29.76)	5482 bc

Different letters indicate significant differences ($p < 0.05$) between treatments after pairwise comparison

[a] The number of fertile females is bar left-sided within brackets and the total number of females is right-sided

pre-oviposition period when compared with water that instead did not significantly differ from the other treatments. Oviposition period on *S. oleae* honeydew and honey treatments was significantly higher than with the other treatments. The oviposition period was significantly higher on *E. olivina* honeydew than on water and *A. arvensis* treatments but did not significantly differ from the other treatments. Fecundity on *S. oleae* honeydew and honey treatments was significantly higher than on all the other treatments (Table 1).

Oviposition period and lifetime fecundity as a function of longevity

GLMs showed that the oviposition period was significantly prolonged with the longevity in females fed on *M. sylvestris*, *E. olivina*, *S. oleae* and honey (Fig. 2, Table 2). The lifetime fecundity significantly decreased with the longevity on the *C. maculatum* treatment (Fig. 3; Table 2).

Discussion

Insect feeding is determined by several aspects as availability, appearance or detectability, accessibility, and nutritional suitability of foods (Wäckers 2005). In the present work, the tested food resources are available during the flight period of the anthophagous generation of the olive moth. The selected plants bloom during the middle/end of spring and usually occur within and/or around olive groves. During this period, both *S. oleae* and *E. olivina* produce high amount of honeydew, the former because is in its latest stages of development (Pereira 2004) and the latter because is mainly in the juvenile stages.

Most of the food sources tested resulted suboptimal. This fact is not surprising as many adult insects use more than one food source to fulfill their dietary needs. However, honeydew from *S. oleae* was as good as honey solution (positive control) for *P. oleae*. *E. olivina* showed also good results. The fact that *S. oleae* honeydew alone (also *E. olivina* in some degree) were enough to maximize *P. oleae* potential survival and reproduction points at the importance of controlling this scale and psyllid insects when in co-occurrence with *P. oleae*.

Nectar concentration, viscosity, composition and amount, the floral architecture and the insect mouthpart structure affect the rate of energy obtained by butterflies (May 1985; Krenn 2010; Winkler et al. 2009a). Many Lepidoptera species can present difficulties to feed on crystalline or more viscous sugary liquids (May 1985; Winkler et al. 2009a). In our work, viscosity could be a reason for the differences found among treatments. Particularly, the lower viscosity of *S. oleae* honeydew than the *E. olivina* one could explain a better *P. oleae* survival and reproduction with the former. The open corolla of *M. sylvestris* and *C. maculatum* flowers facilitate nectar consumption by insects. *T. repens* produces high quality nectar and is highly attractive to pollinators (Jackobsen and Kristjiansson 1994), however Fabaceae flower architecture may not allow *P. oleae* to properly reach the nectaries.

Honeydew differs from nectar because it contains oligosaccharides synthesized by the insects from the dietary sugars (Wäckers 2000, 2001; Pacini and Nicolson 2007). Generally, nectar has been described to be a better food resource for insects than honeydew (Lee et al. 2004; Wäckers et al. 2008; Vollhardt et al. 2010). Nevertheless, in some cases no differences were found in longevity among insects fed on honeydews and insects fed on sucrose

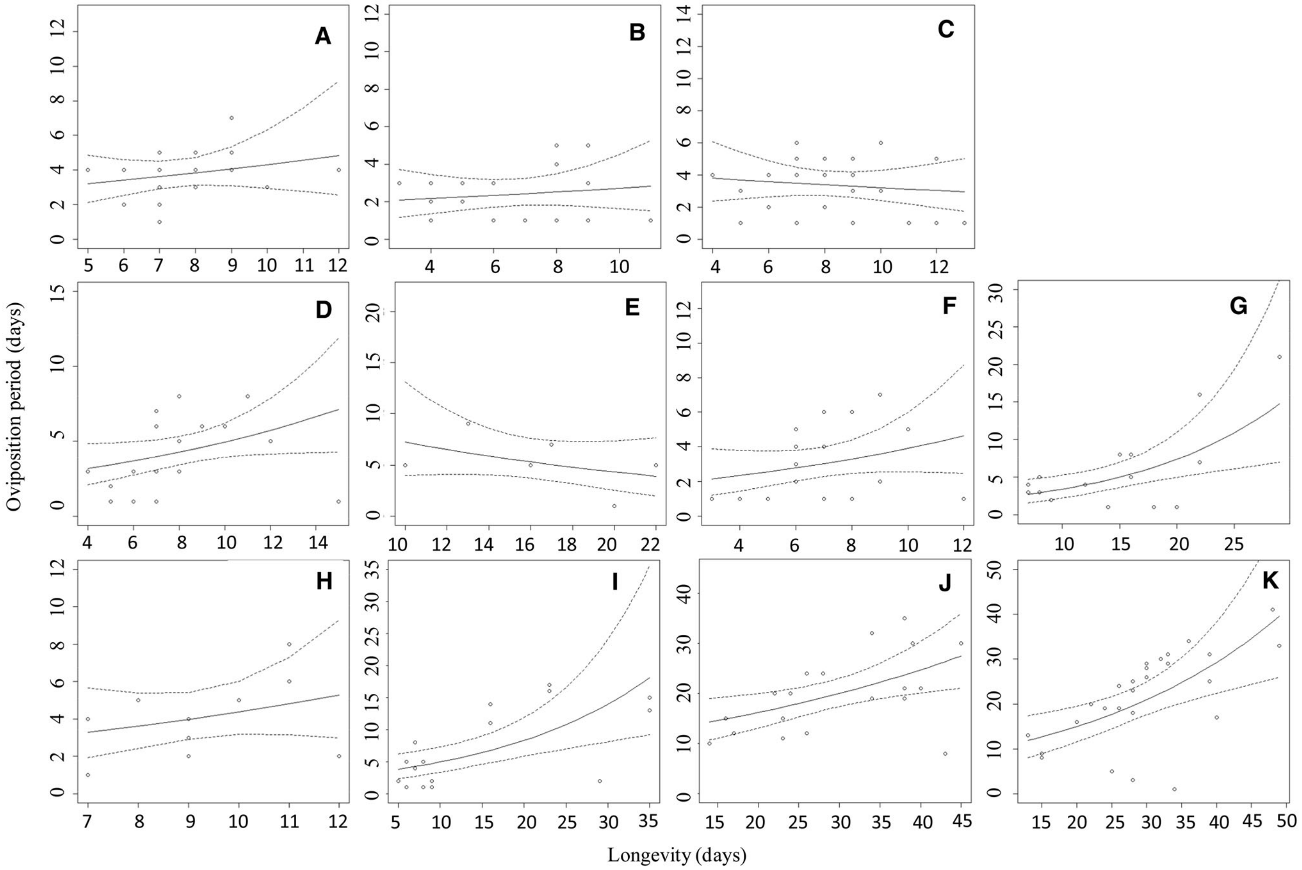

Fig. 2 GLMs plots for oviposition period variation as a function of longevity in each treatment. **a** Water; **b** *A. arvensis*; **c** *A. integrifolia*; **d** *C. capillaris*; **e** *C. maculatum*; **f** *J. montana*; **g** *M. sylvestris*; **h** *T. repens*; **i** *E. olivina*; **j** *S. oleae*; **k** Honey

and honey solution (Wäckers et al. 2008) and in others cases honeydew seemed to provide higher nutritional level (Lee et al. 2006). Additionally, honeydews from different species caused different increase in longevity (Wäckers et al. 2008). The sugar composition of hemipteran honeydew depends on both the insect and the plant species (Hendrix et al. 1992). The honeydew composition from *S. oleae* growing on *Citrus sinensis* L. contained fructose, sucrose and glucose, but no other carbohydrates (Byrne et al. 2003). Wang et al. (2011) found a positive effect of a single meal of *S. oleae* honeydew on the longevities of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) and the parasitoids *Psyttalia humilis* (Silvestri) (Hymenoptera: Braconidae) and *Scutellista caerulea* (Fonsc.) (Hymenoptera: Pteromalidae). Furthermore, the longevity was not different when fed on black scale honeydew than when fed on clover honey. The predator *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae) also feed on *S. oleae* honeydew during its adult phase (Sheldon and MacLeod 1971). To our knowledge, no studies have been performed to analyze the effect of *E. olivina* honeydew on insects. In this work, honeydews were generally better food resources for *P. oleae* than flowers. *S. oleae* honeydew was the best food resource for the olive moth, improving male and female survival, the oviposition period and the daily fecundity with respect to the other treatments and being the only treatment that was not different from the positive control. Moths fed on *E. olivina* honeydew presented also high values in these parameters, being better than the flowers in most cases. Accordingly to Wäckers (2001), evolution would favor sugars that reduce suitability of honeydews when natural enemies of the insect producing honeydew vary in their responses to different honeydew sugars. In olive groves, the populations of *S. oleae* and *E. olivina* probably are not affected by the consumption of their honeydews by *P. oleae*. Moreover, the olive tree canopy is a habitat shared by *P. oleae* adults and larvae, *S. oleae* and *E. olivina*. This may increase the profitability and consumption of honeydews by saving energy spent in foraging other resources.

Bogg (1997) indicated four lepidopteran categories according to the importance of the adult diet quality to the proportion of mature eggs at adult emergence. Adults from the A category do not feed, emerge with the eggs already

Table 2 GLMs outputs for estimated regression parameters and standard errors of oviposition period variation and lifetime fecundity as a function of longevity in each treatment

	Fixed effect	Oviposition period				Lifetime fecundity			
		Estimate	SE	*z*-value	*p*-value	Estimate	SE	*z*-value	*p*-value
Water	Intercept	0.88	0.54	1.64	0.10	4.04	0.90	4.47	<0.001
	Longevity	0.06	0.07	0.84	0.40	−0.0001	0.12	−0.001	0.99
A. arvensis	Intercept	0.62	0.47	1.31	0.19	3.56	0.68	5.21	<0.001
	Longevity	0.04	0.07	0.58	0.56	−0.003	0.10	−0.03	0.98
A. integrifolia	Intercept	1.45	0.42	3.47	<0.001	4.58	0.72	6.35	<0.001
	Longevity	−0.03	0.05	−0.57	0.57	−0.10	0.08	−1.24	0.21
C. capillaris	Intercept	0.87	0.34	2.52	0.01	2.63	0.80	3.27	<0.001
	Longevity	0.07	0.04	1.95	0.05	0.16	0.09	1.77	0.076
C. maculatum	Intercept	2.49	0.70	3.55	<0.001	7.94	0.90	8.82	<0.001
	Longevity	−0.05	0.04	−1.17	0.24	−0.24	0.05	−4.36	<0.001
J. montana	Intercept	0.52	0.46	1.12	0.26	4.84	0.92	5.25	<0.001
	Longevity	0.08	0.06	1.40	0.16	−0.18	0.13	−1.39	0.16
M. sylvestris	Intercept	0.46	0.43	1.06	0.29	3.70	0.72	5.17	<0.001
	Longevity	0.08	0.02	3.04	<0.001	0.04	0.05	0.78	0.43
T. repens	Intercept	0.53	0.89	0.60	0.55	3.88	1.50	2.59	0.01
	Longevity	0.09	0.09	1.01	0.31	0.02	0.16	0.13	0.90
E. olivina	Intercept	1.08	0.30	3.56	<0.001	4.44	0.44	10.11	<0.001
	Longevity	0.05	0.02	3.32	<0.001	−0.003	0.02	−0.13	0.89
S. oleae	Intercept	2.36	0.25	9.60	<0.001	5.15	0.37	13.97	<0.001
	Longevity	0.02	0.01	2.72	<0.001	0.01	0.01	0.81	0.42
Honey	Intercept	2.04	0.31	6.52	<0.001	4.79	0.69	6.96	<0.001
	Longevity	0.03	0.01	3.33	<0.001	0.02	0.02	0.73	0.46

In the models fitted for oviposition period Poisson distribution was used for water, *A. arvensis*, *A. integrifolia*, *C. capillaris*, *C. maculatum*, *J. montana* and *T. repens* treatments, and negative binomial distribution for *M. sylvestris*, *E. olivina*, *S. oleae* and honey treatments. In the models fitted for lifetime fecundity negative binomial distribution was used for all the treatments

mature and have shorter lifespans. The adult nutrition importance increases progressively in the other categories. Adults in the C and D emerge without mature eggs and feed on nectar (C category) or nectar and pollen (D category). The fecundity keeps constant for longer times. Jervis et al. (2001) assigned the A category to pro-ovigeny, B to weak synovigeny and C and D to synovigeny. For example, Berndt and Wratten (2005) analyzed the relation between lifetime fecundity and longevity of *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) with several food resources and found that the lifetime fecundity increase was due to the positive effect of the food resource in longevity rather than a direct increase in fecundity. This suggested that *D. tasmanica* is at least partially pro-ovigenic. In the present study, the lifetime fecundity increase was never related to the increase in longevity (Fig. 3; Table 2) and the moths did not lay eggs just after emergence. This suggests that *P. oleae* females may be synovigenic, emerging with no mature eggs. In future research, this should be verified by dissecting recently emerged adult moths to search for mature eggs. According to Boggs (1997), synovigenic Lepidoptera would feed on nectar, and in the case of the olive moth, likely in insect honeydews as well.

In this study, females fed on water (negative control) laid eggs suggesting that they already emerge with nutritional reserves. This would allow them to mature a minimum of eggs without feeding. Moreover, some of the treatments with better survival performances (*C. maculatum*, *T. repens*, *E. olivina*, *S. oleae*) did not cause differences in the survival among *P. oleae* sexes but in general the treatments that did not significantly increase the survival compared to water treatment (*A. integrifolia*, *A. arvensis*, *C. capillares*, *J. montana*) caused a higher death hazard for males. This means that, in general, treatments with poorer nutritional value, affect more negatively males than females, suggesting a better nutritional status of females after emergence. This effect would be diluted after males feeding. Exceptions were *M. sylvestris* treatment and honey, where males also showed a higher death hazard. The nutritional reserves of newly emerged females likely proceed from larval nutrition (Boggs 1997).

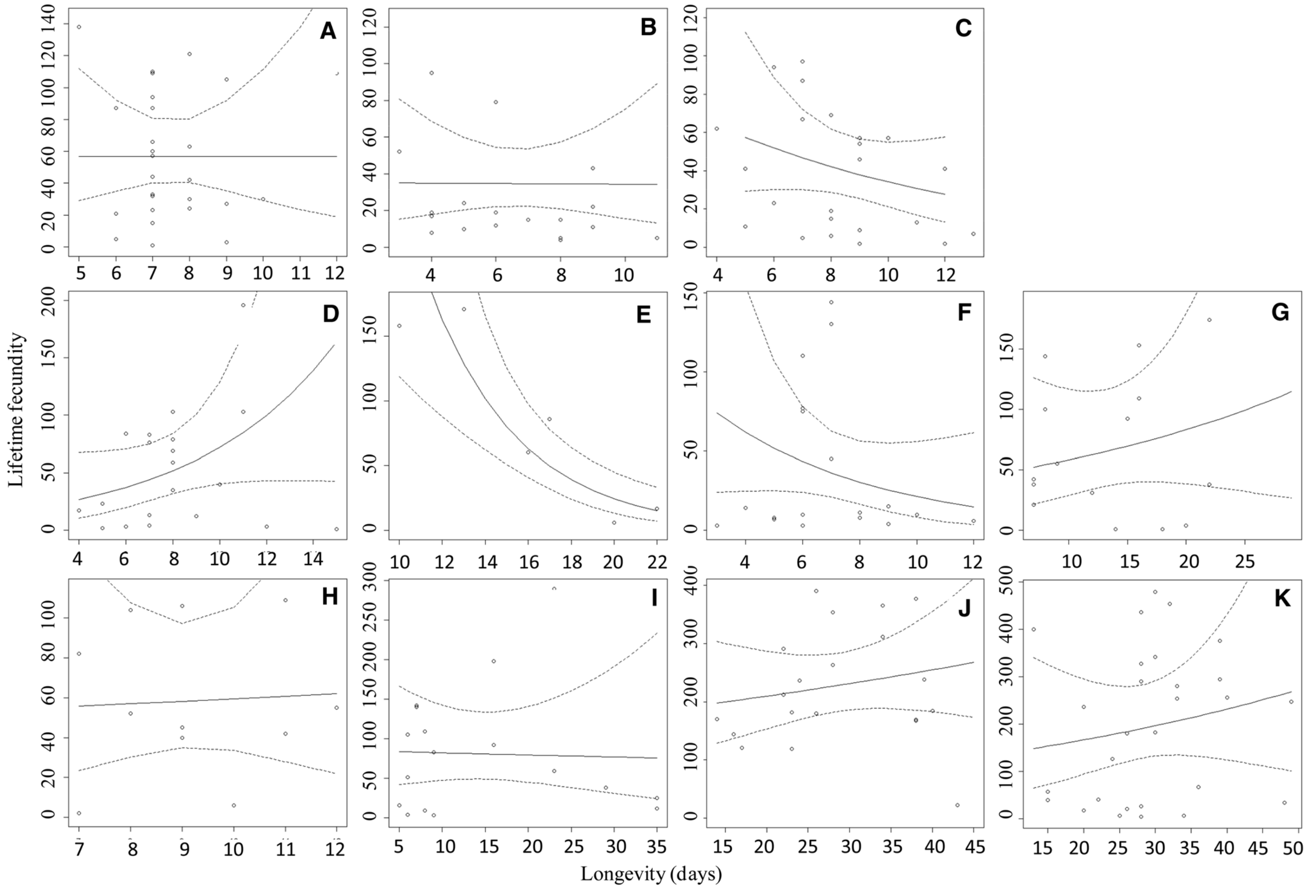

Fig. 3 GLMs plots for lifetime fecundity variation as a function of longevity in each treatment. **a** Water; **b** *A. arvensis*; **c** *A. integrifolia*; **d** *C. capillaris*; **e** *C. maculatum*; **f** *J. montana*; **g** *M. sylvestris*; **h** *T. repens*; **i** *E. olivina*; **j** *S. oleae*; **k** Honey

The egg production with *C. maculatum* was less constant and presented the lowest percentage of fertile couples, being that only six females laid eggs. In this case, the lifetime fecundity even decreased with longevity and it was the only treatment that originated a longer oviposition period than the water treatment. *C. maculatum* is one the most poisonous plants for many organisms due to the alkaloids production (Vetter 2004). Lepidopterans did not pollinate plants containing alkaloids (Kevan and Baker 1983), and in our work *C. maculatum* seemed to prolong *P. oleae* survival but caused some disruption on reproduction. However, when collecting the plant for the assays, we observed many potential natural enemies, as parasitoids or ladybirds apparently feeding on *C. maculatum* as well as lacewings eggs. This makes it a potential candidate for deeper studies.

Generally, the oviposition period increased with longevity in the treatments that caused longer longevities (honey solution, *S. oleae* and *E. olivina* honeydews and *M. sylvestris*), that can be translated to longer *P. oleae* oviposition periods with higher nutritional reserves.

This study was focused in potential food resources for adults of the anthophagous generation of the olive moth, however the adults feeding of phyllophagous and carpophagous generations have never been investigated. Further studies should address this topic.

Once insects may respond differently to food resources in laboratory and in field, laboratory experiments should be complemented with field assays. Lee et al. (2004) found nectar of *F. esculentum* to be a better food resource than honeydew of *Aphis glycines* Matsumura (Homoptera: Aphididae) for *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae) in laboratory experiments. The same group (Lee et al. 2006) found honeydew feeding to provide higher nutrient levels in field experiments. Also laboratory studies establishing nectar exploitation under controlled conditions did not elevate sugar contents of the *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and its parasitoid *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae) but in both insects their average overall sugar content increased in flowering margins (Winkler et al. 2009a, b). In our case: i) *P. oleae* may not fly frequently from the tree canopy to the ground cover, given that, *S. oleae* and *E. olivina* honeydews seem to be good quality foods for *P. oleae* and are already in that habitat; ii) the food resources that, when studied individually, did not have effect in laboratory, when complemented with other resources occurring in the field, could improve *P. oleae* performance. In caged experiments, insects could be deprived of some essential nutrients and mask the real effect of the tested food resources; iii) Intra and inter-specific competition and other trophic relationships are not considered in laboratory experiments. For example, the presence of ants foraging on *S. oleae* honeydew can influence the abundance of some *S. oleae* parasitoids (Barzman and Daane 2001) and could also influence *S. oleae* honeydew feeding by *P. oleae*; iv) in caged experiments, the flight energy spent in searching oviposition and foraging sites are not considered (May 1985; Winkler et al. 2006); v) in this study excised flowers were presented to the moths. Excised and intact flowers generally did not affect the parasitoid *Aphidius ervi* Hal. (Hymenptera: Braconidae) longevity, and excised flowers present some advantages in laboratory experiment related to space, manipulation and number of replicates issues. However, the effect of the flower presentation depends on the insect species and the studied variable. Physiological condition changes with subsequent nectar flow rates, concentration or composition changes could occur (Wade and Wratten 2007).

In conclusion, we found some potential natural foods for *P. oleae* in olive groves from the northeast of Portugal. In general, hemipteran honeydews were better food resources than flowers, pointing at the importance of controlling these insects when co-occurring with the olive moth. Particularly important was *S. oleae* honeydew once it originated as good performance as the positive control. Among the flowers, *M. sylvestris* caused the best survival and reproduction parameters. *C. maculatum* increased the longevity but disrupted some reproduction parameters. This species should be deeper investigated in a conservation biological control perspective, since, in the field, it seems to be highly attractive to natural enemies (unpublished observation). At the light of these results, we suggest that *P. oleae* females are synovigenic, emerging with no mature eggs and with reserves for reproduction. Finally, with high nutritional foods, *P. oleae* increased its survival, fecundity and oviposition period. We highly recommend further researches before maintaining, enhancing or introducing these resources in order to confirm their effects on *P. oleae* in the field.

Author contributions

MV, AM, AB, and JAP conceived and designed the research, MV and RM conducted the experiments, MV analyzed the data, MV and JAP wrote the manuscript. All authors read, revised and approved the manuscript.

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