# Evaluation of native plant flower characteristics for conservation biological control of *Prays oleae*

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# Abstract

Several studies have shown that manipulating flowering weeds within an agroecosystem can have an important role in pest control by natural enemies, by providing them nectar and pollen, which are significant sources of nutrition for adults. The aim of this study was to assess if the olive moth, Prays oleae (Bernard, 1788) (Lepidoptera: Praydidae), and five of its main natural enemies, the parasitoid species Chelonus elaeaphilus Silvestri (Hymenoptera: Braconidae), Apanteles xanthostigma (Haliday) (Hymenoptera: Braconidae), Ageniaspis fuscicollis (Dalman) (Hymenoptera: Encyrtidae) and Elasmus flabellatus (Fonscolombe) (Hymenoptera: Eulophidae), as well as the predator Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae), can theoretically access the nectar from 21 flowering weeds that naturally occur in olive groves. Thus, the architecture of the flowers as well as the mouthpart structure and/or the head and thorax width of the pest and its enemies were analyzed. The results suggested that all beneficial insects were able to reach nectar of the plant species from Apiaceae family, i.e. Conopodium majus (Gouan) Loret, Daucus carota L. and Foeniculum vulgare Mill., as well as Asparagus acutifolius L., Echium plantagineum L., Capsella bursa-pastoris (L.) Medik., Raphanus raphanistrum L., Lonicera hispanica Boiss. et Reut., Silene gallica L., Spergula arvensis L., Hypericum perforatum L., Calamintha baetica Boiss. et Reut, Malva neglecta Wallr. and Linaria saxatilis (L.) Chaz. P. oleae was not able to access nectar from five plant species, namely: Andryala integrifolia L., Chondrilla juncea L., Dittrichia viscosa (L.) Greuter, Sonchus asper (L.) Hill and Lavandula stoechas L.

**Keywords:** habitat management, pest control, floral architecture, natural enemies, insect's morphology

(Accepted 4 December 2015; First published online 19 January 2016)

# Introduction

The olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae), is one of the most destructive pests of the

\*Author for correspondence Tel: 00351 259 350 475 Fax: 00351 259 350 629 E-mail: ana.nave@hotmail.com Mediterranean olive groves. This insect develops three generations per year that damage different components of the olive tree (Alvarado *et al.*, 2008). The leaf or phyllophagous generation develops to adulthood from September to March, consuming the inner side of the leaves. The flower or anthophagous generation, present from March to June, feeds on the flower bud devouring anthers, stigma and ovaries. The fruit or carpophagous generation occurs from June to September, when the larvae penetrate the developing fruit



and feed on the gelatinous tissue of the developing stone causing its premature fall (Bento *et al.*, 2007; Gonçalves *et al.*, 2007).

Although olive moth populations can be controlled by a rich complex of antagonists, it sometimes assumes a key pest status. It is recognized that this situation is largely due to these antagonists not being sufficiently effective due to, a spatial or temporal lack of food sources (such as pollen and nectar) (Jervis *et al.*, 1992).

The use of plants to provide nectar and pollen resources for natural enemies through habitat management is an increasing focus of conservation biological control. Some authors argue that this can be expedited by sowing flowering plants in the crop or in its vicinity (Maingay *et al.*, 1991; Lövei *et al.*, 1993). Moreover, studies also suggest that, when properly selected, autochthonous plants can be as valuable as exotic species to manage the habitat, with the advantage of not only being locally adapted, but also due to the fact that their use can also contribute to increasing the natural biodiversity and multifunctionality (Nentwig, 1998; Nicholls *et al.*, 2000; Fiedler & Landis, 2007*a*, *b*) with a consequent local economic return (Bertacchini, 2009).

Many adult parasitoids and predators benefit from shelter and food through combinations of flowering plants (Root, 1973; Hickman & Wratten, 1996; Harmon *et al.*, 2000; Landis *et al.*, 2000). Floral resources help the fitness of a wide range of agriculturally beneficial invertebrates, including lacewings (Robinson *et al.*, 2008), parasitoids (Irvin *et al.*, 2007), coccinellids (Bertolaccini *et al.*, 2008), spiders (Taylor & Bradley, 2009) and hoverflies (Van Rijn *et al.*, 2006; Pinheiro *et al.*, 2013*a*, *b*).

The selection of the most appropriate plant species for habitat management may be influenced by multiple factors, such as: nectar availability (Idris & Grafius, 1995) and accessibility (Wäckers *et al.*, 1996), duration of flowering (Lövei *et al.*, 1993) and time of flowering (Wratten & Van Emden, 1995). Negative aspects, such as the potential for pest populations also benefiting, resulting in an increased crop damage, must also be considered (Gurr *et al.*, 1998).

An important aspect of nectar exploitation by insects is the morphological fit between their mouthparts and the floral architecture (Jervis, 1998). Based on the structure of the flowers and the mouthparts of the pest and its natural enemies, nectar accessibility can be theoretically studied (Wäckers *et al.*, 2005; Vattala *et al.*, 2006). Natural enemies with short mouthparts may be unable to reach flowers' nectar when there are deep corollas, and therefore benefit from flowers that have open, shallow corollas (Wratten *et al.*, 2003).

Accessibility is important to ensure not only that the natural enemies can access floral resources, but also ensures that energy is not wasted through futile foraging (Winkler *et al.*, 2009). Hence, assuming that the system is resourcelimited, ensuring that the plants are correctly distributed spatially and temporarily is important to guarantee the optimal provision of the floral resources for the fulfillment of pest control goals (Tompkins, 2010).

Clearly, the flowering period needs to coincide with the presence of the natural enemy in the system (Landis *et al.*, 2000). Notwithstanding this, there is also the need to consider when the natural enemy is most resource limited, as it is then that supplementation of its diet would allow for its continued presence in the system (Jonsson *et al.*, 2009). Based on this without a thorough understanding of an agricultural system's invertebrate complex (and the various abiotic factors interacting) the effectiveness of conservation biological control manipulations will be unpredictable.

Identifying floral resources native to each country, which can also enhance natural enemy fitness, would add their value within agricultural landscapes and provide more Ecological Services 'for the price of one' (Tompkins, 2010). These services include for example, crop pollination and pest control, which help to maintain agricultural productivity and reduce the need for pesticide inputs (Isaacs *et al.*, 2009)

The present work was developed to identify a set of weed species from olive groves, that may enhance the impact on both the olive moth pest, and its natural enemies such as the hymenopteran parasitoids species *Ageniaspis fuscicollis* (Dalman), *Apanteles xanthostigma* (Haliday), *Chelonus elaeaphilus* Silvestri and *Elasmus flabellatus* (Fonscolombe) and the chrysopid predator species *Chrysoperla carnea* (Stephens), according to the guidelines of Böller *et al.* (2004).

These natural enemies were selected because they are known to be important in the control of the olive moth, either due to the frequency at which they occur in the Mediterranean region (Torres, 2007), namely in the interior Centre and North East regions of Portugal (Bento *et al.*, 1998; Soares *et al.*, 2005).

In the Mediterranean region, A. fuscicollis is the most important parasitoid of P. oleae showing parasitism rates that can sometimes exceed 80% (Torres, 2007). A. xanthostigma is a parasitoid whose biological cycle is synchronized with that of the phytophagous stage of *P. oleae* (Campos & Ramos, 1981) and leads to parasitism of up to 30% of the population (Tominic, 1969 cit. by Stavraki, 1984). C. elaeaphilus is a common parasitoid of P. oleae, in Portugal in regions such as Trás-os-Montes and Beira Interior (Bento et al., 1998; Soares et al., 2005), where it can parasitize up to 79% of the carpophagous generation population of the pest (Bento et al., 2004). E. flabellatus can parasitize up to 11% of the P. oleae phyllophagous generation (Campos & Ramos, 1981; Bento, 1999). C. carnea was the most common chrysopid found in Portuguese olive groves (Bento, 1999), and the larvae are the major oophagous predators of the olive moth accounting for over 90% of egg predation in some regions and years (Ramos & Ramos, 1990).

#### Material and methods

## Insects

*P. oleae* and parasitoids were obtained in olive groves from Beira Interior and Trás-os-Montes regions (respectively, Centre and North Eastern of Portugal) between March 2009 and September 2012. Samples of leaves and flowers with larvae (3rd, 4th and 5th instars) and pupae of the *P. oleae* were collected, in March, during the overwintering (phyllophagous) and the first (anthophagous) generations in June. In September, insects of the carpophagous generation were obtained from recently fallen fruit.

In the laboratory, mined leaves, flowers and fruit with larvae and pupae of *P. oleae*, were maintained in a climate controlled chamber at  $25 \pm 2^{\circ}$ C,  $60 \pm 5^{\circ}$  relative humidity (RH), photoperiod of 16:8 (L:D) h and daily observed until emergence of either *P. oleae* or parasitoids adults, identified and separated by species and gender.

*C. carnea* was obtained from the Koppert Biological Systems (The Netherland) and reared in UV-sterilized frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) purchased from Biotop (Livron-sur-Drôme, France), in a climate-controlled chamber under the same conditions as those describe above for *P. oleae*.

#### Plant species

Twenty-one plant species were chosen (table 1) to be characterized and evaluated in terms of their interests for habitat management, from floristic inventories carried out in olive groves in the Beira Interior region (Nave *et al.*, 2009), based on a group of criteria listed in the bibliography namely: (a) flowering phenology (Rebek *et al.*, 2005; Winkler, 2005), (b) potential role in supporting natural enemies (Bugg *et al.*, 1989; Maingay *et al.*, 1991; Lavandero *et al.*, 2005; Ambrosino *et al.*, 2006) and (c) multifunctionality (Rogers & Potter, 2004; Shrewsbury *et al.*, 2004; Fiedler *et al.*, 2008).

Twelve families were represented, in the plants considered in most cases with one or two species except for Apiaceae and Asteraceae family, which had three and four species, respectively, given their importance in feeding natural enemies.

Associating the characteristics of flowering (duration, period of the year) with the success of parasitoids, it was our decision to select species with different flowering periods.

Stipulating three flowering periods, from February to April, from May to July and from August to November, there were seven species (33% of total) in the 1st of these periods, 19 species (86% of total) in the 2nd period, and 17 species (81% of total) in the 3rd period. Further six of the seven flowering plants from the first period were also found to be present in the other two periods; these were: *C. bursa-pastoris, Linaria saxatilis, Raphanus raphanistrum, Silene gallica, Spergula arvensis* and *Trifolium repens*. There were also species with shorter flowering periods concentrated in the warmer months of the year, including *Foeniculum vulgare, Hypericum perforatum* and *Lavandula stoechas*.

## Measurements of insects

Male and female insect morphology was measured, under a stereoscopic microscope with an ocular micrometer using the computer software Digital Imaging Solutions (Olympus Soft Imaging Solutions GmbH, Olympus Europa).

To measure *P. oleae* proboscis length (fig. 1a) (N = 66, 33 per sex) the proboscis of individuals was unrolled with a needle and measured from the tip to the front of the compound eye. For *C. carnea* (fig. 1b) and parasitoids (fig 1c–f) that do not have elongated mouthparts, the head width or the thorax width are the limiting factor in exploiting nectar from deep, narrow flower corollas. Thus, the predator and parasitoid head and thorax ( $N \ge 12$ ) were measured between the extreme lateral margins of, respectively, the eyes and thorax.

## Flower morphology

At least, twelve samples of flowering plants were measured for each plant species. Corolla width and corolla depth of the flowers were measured. The latter was taken from the most proximal point of the corolla to the observed location of the nectar at the corolla base. Corolla width was measured at the width of the narrowest part of the corolla tube (fig. 2) (Baggen *et al.*, 1999; Winkler *et al.*, 2003; Krenn *et al.*, 2005; Vattala *et al.*, 2006).

## Data analysis

Statistical analyses were performed with IBM-SPSS statistics, version 20.0.0 (SPSS Inc., IBM Company, 2010). All data were checked for normality and homogeneity of variance with the Kolmogorov–Smirnov Test and the Levene's Test, respectively. Male and female measurements were compared, using a Student's *t* test. All statistical tests were performed at 5% significance level. Data are presented as mean values  $\pm$  standard error (SE).

## Results

#### Measurements of insects

In all the species studied, the measurements show that the thorax width is larger than the head width and, in both cases, they are higher in females (table 2).

The head width varied between  $0.34 \pm 0.01$  mm in *A. fuscicollis* and  $1.21 \pm 0.01$  mm in *C. carnea* and thorax width varied between  $0.48 \pm 0.01$  mm in *A. fuscicollis* and  $1.59 \pm 0.08$  mm in *C. carnea*.

#### Measurements of flowers

Of the 21 plants species studied, eight had flat corollas (no depth), 12 had deep corollas, and one was intermediate, with its nectar being partially hidden in small, cub-shaped flowers (table 3).

In those species with exposed floral nectaries, the smallest corolla opening was found in *Capsella bursa-pastoris* ( $2.08 \pm 12.11 \text{ mm}$ ) and the largest in *Malva neglecta* ( $19.46 \pm 0.65 \text{ mm}$ ).

The corolla depth was between  $2.31 \pm 0.09$  mm in *T. repens* and  $34.09 \pm 0.82$  mm in *Lonicera hispanica*, while the corolla width was between  $0.42 \pm 0.03$  mm in *Sonchus asper* and  $5.79 \pm 0.29$  mm in *Echium plantagineum*.

*R. raphanistrum* is the only example where the nectar is partly protected in small cub-shaped flowers (corolla depth:  $7.55 \pm 0.37$  mm and corolla width:  $3.07 \pm 0.13$  mm).

#### Theoretical nectar accessibility

The combinations of the floral measurements with the insect mouthpart structure and the thorax width of the insect species, allowed conclusions to be drawn on the theoretical nectar accessibility amongst the species considered (table 4).

Of the selected plants, eight were found to have exposed nectaries, which allow their nectar to be easily accessed by all the studied insects. These comprised three Apiaceae (*Conopodium majus, Daucus carota, F. vulgare*), one species of Asparagaceae (*Asparagus acutifolius*), one species of Brassicaceae (*C. bursapastoris*), one species of Caryophyllaceae (*S. arvensis*), one species of Hypericaceae (*H. perforatum*) and one species of Malvaceae (*M. neglecta*).

For *P. oleae* its relatively short proboscis  $(1.38 \pm 0.02 \text{ mm})$ , prevented access to all of the protected and partly protected nectaries. Thus theoretically, *P. oleae* would not be able to access nectar from 13 out of 21 plant species considered, i.e. *Anarrhinum belli-difolium, Andryala integrifolia, Calamintha baetica, Chondrilla juncea, Dittrichia viscosa, E. plantagineum, L. hispanica, L. saxatilis, L. stoechas, R. raphanistrum, S. gallica, S. asper and T. repens. However, the relatively narrow width of the thorax would probably allow it to access the nectar of <i>A. bellidifolium, C. baetica, E. plantagineum, L. saxatilis, L. hispanica, R. raphanistrum, S. gallica, R. raphanistrum, S. gallica, and T. repens.* 

Data from either the head or the thorax width of *C. elaeaphilus* and *A. xanthostigma* show that both species can theoretically

Plant species	Common name	Family	Criteria to selec- tion <sup>1</sup>		a ec-	Flowering period (Castroviejo, 1997)									
			1	2	3	February	March	April	May	June	July	August	September	October	Novembe
A. bellidifolium (L.) Willd.	Daisy leaved toadflax	Scrophulariaceae	_	_	x					x	x				
A. integrifolia L.	Common andryala	Asteraceae	x	-	x					x	x	x	x		
A. acutifolius L.	Wild asparagus	Asparagaceae	x	-	x							x	x		
Calamintha baetica Boiss. et Reut	Calamint	Lamiaceae	x	-	x				x	x	x	x	x	x	
C. bursa-pastoris (L.) Medik.	Shepherd's-purse	Brassicaceae	x	-	x			x	x	x	x	x	x	x	
C. juncea L.	Rush skeletonweed	Asteraceae	-	x	x				x	x	x	x	x	x	
C. majus (Gouan) Loret	Pignut	Apiaceae	x	x	x			x	x	x					
D. carota L.	Wild carrot	Apiaceae	-	x	x				x	x	x	x	x		
D. viscosa (L.) Greuter	False yellowhead	Asteraceae	x	x	-					x	x	x	x		
E. plantagineum L.	Paterson's curse	Boraginaceae	x	-	x				x	x	x	x	x		
F. vulgare Mill.	Wild fennel	Apiaceae	x	x	x						x	x	x		
H. perforatum L.	St. John's wort	Hypericaceae	x	x	x					x	x	x			
L. stoechas L.	Spanish lavender	Lamiaceae	x	-	x				x	x					
L. saxatilis (L.) Chaz.	Dalmatian toadflax	Scrophulariaceae	x	_	x		x	x	x	x	x	x	x		
L. hispanica Boiss. et Reut.	Common honeysuckle	Caprifoliaceae	_	_	x				x	x	x				
M. neglecta Wallr.	Common mallow	Malvaceae	x	x	x				x	x	x	x	x		
R. raphanistrum L.	Cadlock	Brassicaceae	x	_	x		x	x	x	x	x	x	x	x	x
S. gallica L.	Catchfly	Caryophyllaceae	x	_	_			x	x	x	x	x	x		
S. asper (L.) Hill	Sow thistle	Asteraceae	x	x	_				x	x	x	x	x	x	
S. arvensis L.	Corn spurry	Caryophyllaceae	x	_	_	x	x	x	x	x	x	х	x	x	x
T. repens L.	White clover	Fabaceae	x	-	-			x	x	x	x	x	x	x	x

Table 1. Plant species selected in this study, corresponding criteria and flowering period.

<sup>1</sup>(1) Flowering phenology, (2) Increasing the role natural enemies, (3) Multifunctional role.



P. oleae proboscis length



E. flabellatus head width



C. carnea head width



A. xanthostigma head width



C. elaeaphilus head width



A. fuscicollis head and thorax width

Fig. 1. Measurements of P. oleae and its studied natural enemies morphological parameters.



Fig. 2. Measurements of the flower morphology, shown for the case of *R. raphanistrum*.

access the nectar of all the selected plants, with the exception of three Asteraceae (*A. integrifolia, C. juncea* and *S. asper*).

*A. fuscicollis*, due to its small head width, is also theoretically capable to access the nectaries of all plant species studied, but the access to *S. asper* and *C. juncea* would be prevented by the *A. fuscicollis* thorax width.

Regarding *E. flabellatus*, either the head or the thorax width would prevent it access to *S. asper*, while its thorax width would prevent it from accessing to *A. integrifolia* and *C. juncea*.

The *C. carnea* head width would prevent access to the nectar of four Asteraceae, *A. integrifolia*, *C. juncea*, *D. viscosa* and *S. asper*, while the thorax width prevents its access to three other plant species, i.e. *A. bellidifolium*, *L. stoechas* and *T. repens*.

#### Discussion

The suppression of weeds associated with olive groves, practiced in many regions, negatively affects the performance of pest enemies, and consequently their biological control potential. Hence regional research efforts are needed to screen plants for their potential to conserve beneficial arthropods.

The use of native plant species ensures local adaptation, reduces the risk of one plant becoming particularly weedy or invasive, and increases the potential success of conservation efforts (Alpert *et al.*, 2000; Isaacs *et al.*, 2009). Bugg & Waddington (1994) and (Boller, 2001) have observed that the natural ground cover used as ecological infrastructure can have several positive aspects in agroecosystems.

This study, focusing on the theoretical accessibility to *P. oleae* and six of its most important natural enemies (the parasitoids *C. elaeaphilus, A. xanthostigma, A. fuscicollis, E. flabellatus* and the predator *C. carnea*), of nectar in 21 native plants found in the olive grove ecosystem, allowed to include these plants into four groups.

One group consisted of 14 plant species whose nectar is theoretically accessible to all the studied natural enemies as well as to *P. oleae*. These are, *A. acutifolius, C. bursa-pastoris, C. majus, D. carota, F. vulgare, H. perforatum, M. neglecta* and *S. arvensis,* that have an open corolla, as well as *E. plantagineum, R. raphanistrum, L. hispanica, S. gallica, C. baetica* and *L. saxatilis.* 

A second group consists of three plant species (*D. viscosa*, *L. stoechas* and *A. integrifolia*), which are importance because their nectar is accessible to the parasitoids studied but not to *P. oleae*. However they have a disadvantage in that *C. carnea* cannot also access them. This situation has been reported by Van Rijn (2012) for the Asteraceae, who found that the mouthparts of *Chrysoperla* lacewings (that do not show any adaptation towards feeding on floral nectar) may prohibit entry into the narrow tubular florets of these plants, which are also partly blocked by the anthers.

Insect species	Massuramont (M)	Malo	Fomalo	Specie	Statistical analysis		
	Weasurement (IV)	Mean $\pm$ SE (mm)	remate	Specie	T values	P values	
P. oleae	Proboscis length (33)	$1.37 \pm 0.03$	$1.40 \pm 0.02$	$1.38 \pm 0.02$	T(64) = -0.606	0.547*	
	Thorax width (14)	$1.39 \pm 0.02$	$1.42 \pm 0.02$	$1.40 \pm 0.02$	T(26) = -0.907	0.373*	
A. fuscicollis	Head width (16)	$0.34 \pm 0.01^{1}$					
	Thorax width (24)	$0.48 \pm 0.01^{1}$					
A. xanthostigma	Head width (12)	$0.62 \pm 0.01$	$0.66 \pm 0.01$	$0.64 \pm 0.01$	T(22) = -2.784	0.011	
0	Thorax width (12)	$0.88 \pm 0.02$	$0.90 \pm 0.02$	$0.89 \pm 0.01$	T(22) = -0.675	0.506*	
C. elaeaphilus	Head width (34)	$0.89 \pm 0.01$	$0.96 \pm 0.01$	$0.93 \pm 0.01$	T(66) = -5.249	0.000	
1	Thorax width (10)	$0.99 \pm 0.02$	$1.03 \pm 0.03$	$1.01 \pm 0.02$	T(18) = -1.049	0.308*	
E. flabellatus	Head width (20)	$0.42 \pm 0.01$	$0.46 \pm 0.01$	$0.44 \pm 0.01$	T(38) = -3.717	0.001	
	Thorax width (20)	$0.53 \pm 0.02$	$0.62 \pm 0.02$	$0.57 \pm 0.01$	T(38) = -4.076	0.000	
C. carnea	Head width (30)	$1.20 \pm 0.01$	$1.22 \pm 0.01$	$1.21 \pm 0.01$	T(58) = -1.435	0.157*	
	Thorax width (10)	$1.52 \pm 0.10$	$1.65\pm0.12$	$1.59\pm0.08$	T(18) = -0.778	0.447*	

Table 2. Measurements of insect morphology of the insect species studied.

*N*, number of insets measured.

\*Not significantly different (P > 0.05).

<sup>1</sup>Insects not separated by sex, due to their small size.

Table	<ol><li>Flower</li></ol>	species	used	and	floral	architecture
measur	rements.					

Plant species (N)	Nectaries	Floral architecture (mean ± SE (mm))			
	i tecuites	Corolla depth	Corolla opening		
A. bellidifolium (20)	Protected	$2.88\pm0.05$	$1.45\pm0.08$		
A. integrifolia (16)	Protected	$5.80 \pm 0.23$	$0.49 \pm 0.04$		
A. acutifolius (16)	Exposed	$0.00 \pm 0.00$	$5.87 \pm 0.12$		
C. baetica (20)	Protected	$8.84 \pm 0.30$	$2.47 \pm 0.16$		
C. bursa-pastoris (20)	Exposed	$0.00 \pm 0.00$	$2.08\pm0.11$		
C. juncea (20)	Protected	$5.09 \pm 0.14$	$0.45 \pm 0.03$		
C. majus (20)	Exposed	$0.00 \pm 0.00$	$2.68 \pm 0.08$		
D. carota (23)	Exposed	$0.00 \pm 0.00$	$2.24 \pm 0.12$		
D. viscosa (20)	Protected	$5.91 \pm 0.09$	$1.19 \pm 0.03$		
E. plantagineum (20)	Protected	$12.36\pm0.49$	$5.79 \pm 0.29$		
F. vulgare (20)	Exposed	$0.00 \pm 0.00$	$2.12 \pm 0.09$		
H. perforatum (18)	Exposed	$0.00 \pm 0.00$	$17.22 \pm 1.00$		
L. stoechas (18)	Protected	$4.53 \pm 0.17$	$1.25 \pm 0.09$		
L. saxatilis (12)	Protected	$2.71 \pm 0.13$	$2.44 \pm 0.27$		
L. hispanica (20)	Protected	$34.09 \pm 0.82$	$5.19 \pm 0.17$		
M. neglecta (19)	Exposed	$0.00 \pm 0.00$	$19.46 \pm 0.65$		
R. raphanistrum	Partly	$7.55\pm0.37$	$3.07 \pm 0.13$		
(20)	protected				
S. gallica (17)	Protected	$7.20 \pm 0.21$	$4.53 \pm 0.28$		
S. asper (20)	Protected	$8.05 \pm 0.33$	$0.42 \pm 0.03$		
S. arvensis (20)	Exposed	$0.00 \pm 0.00$	$5.92 \pm 0.44$		
T. repens (17)	Protected	$2.31 \pm 0.09$	$1.47 \pm 0.08$		

*N*, number of plants measured.

A third group comprises *A. bellidifolium* and *T. repens.* These species while they could well improve the populations of parasitoids they may also benefit *P. oleae.* Also their nectaries are not accessible to *C. carnea.* This finding is consistent with the finding of Van Rijn (2012), who stated that this insect cannot apparently use Fabaceae flowers. Further, species of Fabaceae family, such as *T. repens*, can theoretically provide accessible nectar but only in case where the parasitoids are able to push parts of the corolla (Winkler, 2005). The fourth group comprises two species, *C. juncea* and *S. asper*, whose nectar is not accessible to any of the insects studied, so they offer little potential in terms of increasing populations.

Other studies involving optimizing the value of hoverflies and lacewings, have shown useful to dedicate a substantial part of the field margin vegetation to flowers with well exposed nectaries (depth <1 mm) (Van Rijn, 2012). In this study, it was been shown that the same approach could be taken with eight of the species studied, namely A. acutifolius, C. majus, C. bursa-pastoris, D. carota, F. vulgare, H. perforatum, M. neglecta and S. arvensis. However, this group could probably be extended further. Some of the potentially valuable plant species identified here have also been reported in the literature as sources of nectar, pollen, honeydew and even as an alternative food sources for beneficial insects (Heil, 2015). These are for example, D. carota and F. vulgare (Bugg & Waddington, 1994; Bugg et al., 1998; Domínguez Gento et al., 2002; Fiedler et al., 2008; Sivinski, 2014). In which concern C. majus, C. bursa-pastoris, R. raphanistrum, S. arvensis and L. saxatilis, because they bloom early, they can play an important role in the early establishment of natural enemy populations (Nentwig, 1998). Also Böller et al. (2004) reported that some of these species can be used in mixtures of seeds for ground cover, namely D. carota, F. vulgare, H. perforatum and M. neglecta. As stated by Van Rijn (2012), since flowers with easily accessible nectar are much less common than those with accessible pollen, raising them in or around agricultural fields will likely relieve an important limitation to natural pest control.

It should be noted that, in addition to the accessibility, the concentration of nectar can also prevent the insect to feed it, since due to proboscis morphology, Lepidoptera often have difficulties in imbibing high viscosity nectars (Daniel *et al.*, 1989).

Results from this study provide information on the suitability of native olive grove flowering species that can probably enhance conservation biological control of *P. oleae*. Nineteen of the 21 weed species are potentially able to enhance conservation biological against *P. oleae*, namely: *C. majus*, *D. carota*, *F. vulgare*, *A. acutifolius*, *A. integrifolia*, *D. viscosa*, *E. plantagineum*, *C. bursa-pastoris*, *R. raphanistrum*, *L. hispanica*, *S. gallica*, *S. arvensis*, *T. repens*, *H. perforatum*, *C. baetica*,

Plant species	Theoretical nectar accessibility – mouthpart structure <sup>1</sup> /thorax width								
	P. oleae	A. fuscicollis	A. xanthostigma	C. elaeaphilus	E. flabellatus	C. carnea			
A. bellidifolium	N/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/N			
A. integrifolia	N/N	Y/Y	N/N	N/N	Y/N	N/N			
A. acutifolius	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
C. baetica	N/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
C. bursa-pastoris	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
C. juncea	N/N	Y/N	N/N	N/N	$Y^2/N$	N/N			
C. majus	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
D. carota	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
D. viscosa	N/N	Y/Y	Y/Y	Y/Y	Y/Y	N/N			
E. plantagineum	N/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
F. vulgare	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
H. perforatum	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
L. stoechas	N/N	Y/Y	Y/Y	Y/Y	Y/Y	Y/N			
L. saxatilis	N/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
L. hispanica	N/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
M. neglecta	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
R. raphanistrum	N/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
S. gallica	N/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
S. asper	N/N	Y/N	N/N	N/N	N/N	N/N			
S. arvensis	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y			
T. repens	N/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/N			

Table 4. Theoretical accessibility to floral nectar of the plant species studied by the insect species.

Theoretical access to floral nectar: Y, possible. N, not possible.

<sup>1</sup>Proboscis length in the case of *P. oleae*, head width for the others insects.

<sup>2</sup>Male.

*L. stoechas, M. neglecta, A. bellidifolium* and *L. saxatilis*. However, further research is required to verify that these plant species do indeed increase the effectiveness of the studied natural enemies. Conversely for plant species whose nectar was found to be accessed by *P. oleae*, the risk of benefiting the pest should also be carefully evaluated.

## Acknowledgements

This study was supported by FEDER Funds throughout Programa Operacional Factores de Competitividade – COMPETE and National Funds throughout FCT – Fundação para a Ciência e Tecnologia, within project PTDC/AGR-AAM/100979/2008 –'Increasing functional biodiversity in olive groves to enhance conservation biological control of insect pests', as well as PhD grant, SFRH/BD/34394/2008 granted to the first author. This manuscript is part of A. Nave's Ph.D. dissertation. The authors are grateful to the olive grove owners who permitted access to their groves for this study, with support to Associação de Agricultores para Produção Integrada de Frutos de Montanha. Thanks are due to Köppert Biological Systems that kindly provided *Chrysoperla carnea* used in this study.

# References

- Alpert, P., Bone, E. & Holzapfel, C. (2000) Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in Plant Ecology, Evolution and Systematics* 3, 52–66.
- Alvarado, M., Civantos, M. & Durán, J.M. (2008) Plagas in. pp. 511–593 in Barranco, D., Fernández-Escobar, R. & Rallo, L. (*Eds*) *El cultivo del olivo*. 6th edn. Madrid, Ediciones Mundi-Prensa.

- Ambrosino, M., Luna, J., Jepson, P. & Wratten, S. (2006) Relative frequencies of visits to selected insectary plants by predatory hoverflies (Diptera, Syrphidae), other beneficial insects, and herbivores. *Environmental Entomology* **35**, 394–400.
- Baggen, L.R., Gurr, G.M. & Meats, A. (1999) Flowers in tri-trophic systems, mechanisms allowing selective exploitation by insect natural enemies for conservation biological control. *Entomologia Experimentalis et Applicata* 91, 155–161.
- Bento, A. (1999) Contribuição para o estabelecimento de um programa de protecção integrada contra a traça-da-oliveira, *Prays oleae* (Bern.) em Trás-os-Montes. Tese de Doutoramento em Engenharia Agrícola, UTAD, Vila Real, 277 p.
- Bento, A., Lopes, J., Campos, M. & Torres, L. (1998) Parasitismo associado à traça da oliveira *Prays oleae* Bern. em Trás-os-Montes (Nordeste de Portugal). *Boletín Sanidad Vegetal Plagas* 24, 949–954.
- Bento, A.A., Cabanas, J.E., Pereira, J.A., Torres, L., Herz, A. & Hassan, S.A. (2004) Effects of different attractive sources on the abundance of olive predatory arthropods and possible enhancement of their activity as predators on eggs of *Prays oleae* Bern. 5th International Symposium on Olive Growing. Izmir, 73 pp.
- Bento, A., Torres, L., Cabanas, J.E. & Pereira, J.A. (2007) A traça-da-oliveira, *Prays oleae* (Bernard) in Torres, L. (Coord.). pp. 202–229 in Azevedo, J. (Ed.) Manual de protecção integrada do olival. Mirandela, João Azevedo.
- Bertacchini, E. (2009) Regional legislation in Italy for the protection of local varieties. *Journal of Agriculture and Environment* for International Development 103(1/2), 51–63.
- Bertolaccini, I., Nunez-Perez, E. & Tizado, E.J. (2008) Effect of wild flowers on oviposition of *Hippodamia variegata* (Coleoptera, Coccinellidae) in the laboratory. *Journal of Economic Entomology* **101**(6), 1792–1797.

- Boller, E.F. (2001) Functional biodiversity and agro-ecosystems management: identified information gaps. *Integrated Fruit Protection. IOBC/WPRS Bulletin* 24(5), 1–4.
- Böller, E., Häni, F. & Poehling, H. (2004) Ecological infrastructures, ideabook on functional biodiversity at the farm level – temperate zones of Europe. IOBCwprs Comm. Integr. Prod. Guid. Endors, Lindau, Switzerland, LBL. 220 p.
- Bugg, R.L. & Waddington, C. (1994) Using cover crops to manage arthropod pests of orchards: a review. Agriculture, Ecosystems & Environment 50, 11–28.
- Bugg, R., Ellis, R. & Carlson, R. (1989) Ichneumonidae (Hymenoptera) using extrafloral nectar of faba bean (*Vicia faba* L., Fabaceae) in Massachusetts. *Biological Agriculture and Horticulture* 6, 107–114.
- Bugg, R.L., Anderson, J.H.Thomsen, C.D. & Chandler, J. (1998) Farmscaping in California: managing hedgerows, roadside and wetland plantings, and wild plants for biointensive pest management. pp. 337–374 in Picket, C.H. & Bugg, R.L. (Eds) Enhancing Biological Control. Berkeley, CA, University of California Press.
- Campos, M. & Ramos, P. (1981) Contribución al estudio de la entomocenosis de *Prays oleae* Bern. (Lep. Hyponomeutidae) en Granada (España). *Acta Oecologica, Oecologia Applicata* 2(1), 27–35.
- **Castroviejo, S.** (1997) Flora iberica, plantas vasculares de la Península Ibérica e Islas Baleares Santiago Castroviejo, Real Jardín Botánico, Madrid, CSIC.
- Daniel, T.L., Kingsolver, J.G. & Meyhofer, E. (1989) Mechanical determinants of nectar-feeding energetics in butterflies, Muscle mechanics, feeding geometry, and functional equivalence. *Oecologia* 79, 66–75.
- Domínguez Gento, A., Roselló Oltra, J. & Aguado Sáez, J. (2002) Cubiertas vegetales: hierbas adventicias y abonos verdes. pp. 47–77 in Sociedad Española de Agricultura Ecológica (Eds), Diseño y manejo de la diversidad vegetal en agricultura ecológica. Valencia, Phytoma-España.
- Fiedler, A. & Landis, D. (2007a) Attractiveness of Michigan native plants to Arthropod natural enemies and herbivores. *Entomological Society of America* 36(4), 751–765.
- Fiedler, A. & Landis, D. (2007b) Plant characteristics associated with natural enemy attractiveness to Michigan native plants. *Environmental Entomology* 36, 878–886.
- Fiedler, A., Landis, D. & Wratten, S. (2008) Maximizing ecosystem services from conservation biological control, the role of habitat management. *Biological Control* 45, 254–271.
- Gonçalves, F., Oliveira, A. & Torres, L. (2007) Contribuição para o conhecimento do ciclo biológico da traça-da-oliveira, *Prays oleae* (Bernard), na Terra Quente Transmontana II. Colóquio Nacional de Horticultura Biológica, Lisboa, 19 e 20 de Abril, pp. 283–290.
- Gurr, G.M., Van Emden, H.F. & Wratten, S.D. (1998) Habitat manipulation and natural enemy efficiency, implications for the control of pests in. pp. 155–183 in Barbosa, P. (Ed.) *Conservation Biological Control*. San Diego, Academic Press.
- Harmon, J.P., Ives, A.R., Losey, J.E., Olson, A.C. & Rauwald, K.S. (2000) *Coleomegilla maculata* (Coleoptera, Coccinellidae) predation on pea aphids promoted by proximity to dandelions. *Oecologia* **125**, 543–548.
- Heil, M. (2015) Extrafloral nectar at the plant-insect interface: a spotlight on chemical ecology, phenotypic plasticity, and food webs. *Annual Review of Entomology* **60**, 213–232.
- Hickman, J.M. & Wratten, S.D. (1996) Use of *Phacelia tanacetifolia* strips to enhance biological control of aphids by hoverfly larvae in cereal fields. *Journal Economic Entomology* 89, 835–840.

- Idris, A.B. & Grafius, E. (1995) Wildflowers as nectar sources for Diadegma insulare (Hymenoptera, Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera, Yponomeutidae). Environmental Entomology 24, 1726–1735.
- Irvin, N.A., Hoddle, M.S. & Castle, S.J. (2007) The effect of resource provisioning and sugar composition of foods on longevity of three *Gonatocerus* spp., egg parasitoids of *Homalodisca vitripennis*. *Biological Control* **40**, 69–79.
- Isaacs, R., Tuell, J., Fiedler, A., Gardiner, M. & Landis, D. (2009) Maximizing arthropod-mediated ecosystem services in agricultural landscapes: the role of native plants. *Frontiers in Ecology and the Environment* 7, 196–203.
- Jervis, M. (1998) Functional and evolutionary aspects of mouthpart structure in parasitoid wasps. *Biological Journal of the Linnean Society* 63, 461–493.
- Jervis, M., Kidd, N., McEwen, P., Campos, M. & Lozano, C. (1992) Biological control strategies in olive pest management. *Research Collaboration in European IPM Systems* 52, 31–39.
- Jonsson, M., Wratten, S.D., Robinson, K.A. & Sam, S.A. (2009) The impact of floral resources and omnivory on a four trophic level food web. *Bulletin of Entomological Research* 99, 275–285.
- Krenn, H., Plant, J. & Szucsich, N. (2005) Mouthparts of flowervisiting insects. Arthropod Structure and Development 34, 1–40.
- Landis, D., Wratten, S. & Gurr, G. (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45, 175–201.
- Lavandero, B., Wratten, S., Shishehbor, P. & Worner, S. (2005) Enhancing the effectiveness of the parasitoid *Diadegma semiclausum* (Helen), movement after use of nectar in the field. *Biological Control* 34, 152–158.
- Lövei, G., Hodgson, D., Macleod, A. & Wratten, S. (1993) Attractiveness of some novel crops for flower-visiting hoverflies (Diptera, Syrphidae), comparison from two continents in S.D. pp. 368–370 in Corey, S. & Milne, W. (Eds) Pest Control and Sustainable Agriculture. Canberra, Australia, CSIRO.
- Maingay, H., Bugg, R., Carlson, R. & Davidson, N. (1991) Predatory and parasitic wasps (Hymenoptera) feeding at flowers of sweet fennel (*Foeniculum vulgare* Miller Var Dulce Battandier and Trabut, Apiaceae) and spearmint (*Menthas picata* L, Lamiaceae) in Massachusetts. *Biological Agriculture* and Horticulture 7, 363–383.
- Nave, A., Crespí, A., Campos, M. & Torres, L.M. 2009. Olive grove weeds with potential interest in the natural control of the olive moth, *Prays oleae*. XII Congresso da Sociedad Española de Malherbologia (SEMh)/XIX Congresso da Asociacion Latinoamericana de Malezas (ALAM)/II Congresso Iberico de Ciencias de las Malezas (IBMC), Lisboa – 11, 12 e 13 de Novembro 2009, pp. 39–42.
- Nentwig, W. (1998) Weedy plant species and their beneficial arthropods, potential for manipulation in field crops. pp. 49–71 *in* Pickett, C.H. & Bugg, R.L. (*Eds*) *Enhancing Biological Control*. Berkeley, CA, University of California Press.
- Nicholls, C., Parrella, M. & Altieri, M. (2000) Reducing the abundance of leafhoppers and thrips in a northern California organic vineyard through maintenance of full season floral diversity with summer cover crops. *Agricultural and Forest Entomology* 2, 107–113.
- Pinheiro, L., Torres, L.M., Raimundo, J. & Santos, S.A.P. (2013a) Effect of seven species of the family Asteraceae on longevity and nutrient levels of *Episyrphus balteatus*. *BioControl* 58, 797–806.
- Pinheiro, L.A., Torres, L., Raimundo, J. & Santos, S.A.P. (2013b) Effect of floral resources on longevity and nutrient levels of *Episyrphus balteatus* (Diptera, Syrphidae). *Biological Control* 67, 178–185.

- Ramos, P. & Ramos, J.M. (1990) Veinte años de observaciones sobre la depredación oófaga en *Prays oleae* Bern. Granada (España), 1970–1989. *Boletín Sanidad Vegetal Plagas* 16, 119–127.
- Rebek, E., Sadof, C. & Hanks, L. (2005) Manipulating the abundance of natural enemies in ornamental landscapes with floral resource plants. *Biological Control* 33, 203–216.
- Robinson, K.A., Jonsson, M., Wratten, S.D., Wade, M.R. & Buckley, H. (2008) Implications of floral resources for predation by an omnivorous lacewing. *Basic and Applied Ecology* 9, 172–181.
- Rogers, M. & Potter, D. (2004) Potential for sugar sprays and flowering plants to increase parasitism of white grubs (Coleoptera, Scarabaeidae) by Tiphiid wasps (Hymenoptera, Tiphiidae). *Environmental Entomology* 33, 619–626.
- Root, R.B. (1973) Organization of a plant-arthropod association in simple and diverse habitats, the fauna of collards (*Brassica* oleracea). Ecology Monographs 43, 95–124.
- Shrewsbury, P., Lashomb, J., Hamilton, G., Zhang, J., Patt, J. & Casagrande, R. (2004) The influence of flowering plants on herbivore and natural enemy abundance in ornamental landscapes. *International Journal of Ecology and Environmental Sciences* 30, 23–33.
- Sivinski, J. (2014) The attraction of lepidoptera to flowering plants also attractive to parasitoids (Diptera, Hymenoptera). *Florida Entomologist* 97(4), 1317–1327.
- Soares, M.F., Gomes, P., Simão, P., Veiga, C., Bento, A. & Torres, L. (2005) Parasitismo associado à traça-da-oliveira, Prays oleae Bernard, na Beira Interior Norte. Actas do VII Encontro Nacional de Protecção Integrada, Instituto Politécnico de Coimbra, Coimbra, 6 e 7 de Dezembro de 2005, pp. 371–378.
- SPSS Inc., IBM Company (2010) IBM, SPSS Statistic for Windows, version 19.0.0. 492, New York.
- Stavraki, H.G. (1984) Use of *Trichogramma* spp. against the carpophagous generation of *Prays oleae* (Bern.) in Greece. In Integrated Pest Control in Olive-Grove. Proceedings of the CEC/FAO/IOBC International Joint Meeting, Pisa, pp. 242–246.
- Taylor, R.M. & Bradley, R.A. (2009) Plant nectar increases survival, molting, and foraging in a wandering spider. *Journal of Arachnology* 37, 232–237.
- Tompkins, J-M. (2010) Ecosystem services provided by native New Zealand plants in vineyards. PhD Thesis. Universidade de Lincoln. 282 p.

- Torres, L. (2007) A protecção integrada do olival no contexto da produção integrada. pp. 31–125 in Torres, L. & Azevedo, J. (coord) (*Eds*) Manual de protecção integrada do olival. Portugal, Mirandela.
- Van Rijn, P.C.J. (2012) The suitability of field margin flowers as food source for *Chrysoperla* lacewings. *IOBC/WPRS Bulletin* 75, 213–216.
- Van Rijn, P.C.J., Kooijman, J. & Wäckers, F.L. (2006) The impact of floral resources on syrphid performance and cabbage aphid biological control. *IOBC/WPRS Bulletin* 29(6), 149–152.
- Vattala, H.D., Wratten, S.D., Phillips, C.B. & Wackers, F.L. (2006) The influence of flower morphology and nectar quality on the longevity of a parasitoid biological control agent. *Biological Control* 39(2), 179–185.
- Wäckers, F.L., Björnsen, A. & Dorn, S. (1996) A comparison of flowering herbs with respect to their nectar accessibility for the parasitoid *Pimpla turionellae*. *Proceedings of the Section Experimental and Applied Entomology of The Netherlands Entomological Society* 7, 177–182.
- Wäckers, F.L., Van Rijn, P.C.J. & Bruin, J. (2005) Plant-provided Food for Carnivorous Insects: A Protective Mutualism and its Applications. UK, Cambridge University Press, 356 p.
- Winkler, K. (2005) Assessing the risks and benefits of flowering field edges, Strategic use of nectar sources to boost biological control. PhD Thesis. Laboratory of Entomology. Wageningen University, Wageningen. 118 p.
- Winkler, K., Wäckers, F., Valdivia, L., Larraz, V. & Van Lenteren, J. (2003) Strategic use of nectar sources to boost biological control. Landscape Management for Functional Biodiversity. *IOBC/WPRS Bulletin* 26, 209–214.
- Winkler, K., Wäckers, F.L., Kaufman, L.V. & Van Lenteren, J.C. (2009) Nectar exploitation by herbivores and their parasitoids is a function of flower species and relative humidity. *Biological Control* 50, 299–306.
- Wratten, S.D. & Van Emden, H.F. (1995) Habitat management for enhanced activity of natural enemies of insect pests. pp. 117– 145 in Glen, D.M., Geaves, M.P. & Anderson, H.M. (Eds) Ecology and Integrated Farming Systems. Chichester, John Wiley.
- Wratten, S.D., Lavandero, B.I., Tylianakis, J., Vattala, D., Cilgi, T. & Sedcole, R. (2003) Effects of flowers on parasitoid longevity and fecundity. *New Zealand Plant Protection* 56, 233–238.