INSTITUTO SUPERIOR DE AGRONOMIA
Universidade de Lisboa

Ecological infrastructures in sustainable olive growing: studies about *Prays oleae* (Bernard) and its natural enemies

ANA MARÍA VILLA SERRANO

ORIENTADOR: Prof. Doutor José Alberto Cardoso Pereira
COORIENTADORES: Prof. Doutor Albino António Bento
Prof. Doutor António Maria Marques Mexia

TESE ELABORADA PARA OBTENÇÃO DO GRAU DE DOUTOR EM ENGENHARIA AGRONÓMICA

2016

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y no pudiera ser yo, sino tu música
en los mismos instantes que dura una verdad;
una verdad que pasa por un cuerpo
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Resumo

A oliveira (*Olea europaea* L.) é um elemento caraterístico da paisagem e da cultura da bacia Mediterrânica. Na atualidade, esta espécie é cultivada em todas as regiões com condições climáticas que permitem o seu estabelecimento e onde, na generalidade das situações, constitui uma importante fonte de rendimento económico. Esta planta serve de alimento a uma ampla e diversa entomofauna fitófaga, contudo, na generalidade das situações, apenas um reduzido número de espécies pode causar estragos com importância económica. Nesta tese, o estudo foi centrado num dos principais fitófagos da oliveira, a traça-da-oliveira *Prays oleae* (Bernard), praga que provoca grandes prejuízos em muitas regiões olivícolas, entre as quais Trás-os-Montes.

No olival, associadas a *P. oleae*, existe um considerável número de espécies de parasitoides e predadores que se alimentam desta praga. Vários destes inimigos naturais necessitam de recursos que não são proporcionados pela cultura (hospedeiro), como sejam fontes alimentares ricas em açúcares e aminoácidos, e/ou áreas de refúgio. Para satisfazer estas necessidades, os inimigos naturais recorrem a infraestruturas ecológicas existentes no próprio olival ou em áreas circundantes. Estas infraestruturas podem ser utilizadas, através de estratégias de proteção biológica de conservação (PBC), com o objetivo de conservar e melhorar o hábitat dos inimigos naturais e potenciar o seu estabelecimento, sobrevivência e reprodução com benefícios para a proteção da cultura contra as pragas. Contudo, as mesmas infraestruturas ecológicas podem exercer também um papel benéfico sobre a praga-alvo, neste caso a traça da oliveira, cuja fase adulta terá à sua disposição os mesmos recursos para se alimentar.

No presente trabalho, foram estudadas as infraestruturas ecológicas associadas ao olival com o objetivo de contribuir para o estabelecimento de estratégias de PBC contra a traça-da-oliveira. O trabalho foi realizado seguindo duas abordagens: na primeira, estudou-se a influência das infraestruturas ecológicas sobre a traça-da-oliveira e seus inimigos naturais, nomeadamente parasitoides e predadores. Nesta abordagem os objetivos específicos foram: (i) descrever a curva de voo da traça-da-oliveira e a sua capacidade de dispersão para as parcelas adjacentes ao olival compostas por vegetação herbácea e arbustiva; (ii) analisar os potenciais efeitos da complexidade da paisagem sobre *P. oleae* e sobre os crisopídeos que ocorrem no olival; (iii) avaliar o efeito da gestão da cobertura vegetal em *P. oleae* e nos seus parasitoides; (iv) determinar a abundância e diversidade de sirfídeos que ocorrem no olival, e suas infraestruturas ecológicas, durante períodos de escassez de alimento. Numa segunda abordagem foi analisada a potencialidade de alguns recursos, que fazem parte das infraestruturas ecológicas do olival, como alimento para adultos de *P. oleae* e dos seus inimigos naturais. Esta segunda parte teve por objetivos específicos estudar, a nível laboratorial, os efeito de diferentes recursos alimentares na sobrevivência e/ou reprodução dos (i) adultos da geração antófaga de *P. oleae*, (ii) do seu parasitoide *Elasmus flabellatus* (Fonscolombe) e (iii) do seu predador *Chrysoperla carnea s. l.* (Stephens). Os recursos selecionados para os estudos foram as meladas excretadas por pragas secundárias da oliveira, a cochonilha-negra *Saissetia oleae* (Olivier) e o algodão-da-oliveira *Euphyllura olivina* (Costa), e

plantas cuja floração é coincidente com a fase adulta dos insectos estudados, e (iv) no caso dos sirfídeos, foi analisado o consumo de recursos polínicos durante períodos de carência de recursos alimentares em estudos de campo.

A curva de voo de *P. oleae* e a diversidade e abundância dos inimigos naturais estudados (parasitoides, crisopídeos e sirfídeos) foi muito influenciada pelas condições climáticas do ano de 2012, quando ocorreu um período muito longo de seca extrema, baixas temperaturas no inverno e altas temperaturas na primavera e no verão. A geração carpófaga no ano 2012 e a filófaga no ano 2013 foram praticamente inexistentes e a diversidade de crisopídeos e parasitoides foi inferior ao que seria espectável.

Em relação à traça-da-oliveira, pela primeira vez foram estudados alguns aspetos referentes ao efeito que diferentes manchas paisagísticas podem ter na sua dispersão sendo que as parcelas de vegetação arbustiva e, em menor grau, as parcelas de vegetação herbácea, não actuaram como barreiras ao movimento da praga. Foi registada a existência de uma sincronia entre as diferentes gerações de *P. oleae* e *C. carnea*, no entanto, foi observado um pico de *C. carnea* na ausência de geração carpofaga da traça, o que sugere que devido aos seus hábitos alimentares *C. carnea* consome outras presas.

O parasitoide mais abundante, *Ageniaspis fuscicollis* (Dalman), foi positivamente afetado pela presença de cobertura vegetal no olival e negativamente pela aplicação de herbicida. No entanto, a mobilização dos olivais não afectou esta espécie, o que poderá estar relacionado com a heterogeneidade da paisagem que caracteriza a região, com a vegetação presente nas bordaduras ou com aquela que pode permanecer no olival depois da mobilização. O segundo parasitoide mais abundante, *E. flabellatus*, não foi afetado pelos diferentes tipos de gestão da cobertura vegetal.

Relativamente à biodiversidade de sirfídeos, foi capturado um maior número de sirfídeos em áreas abertas (parcelas de vegetação herbácea) do que em áreas arbóreas ou arbustivas (parcelas de olival e mato), sendo que poucos indivíduos foram capturados nos olivais. Contudo, durante a primavera os sirfídeos encontram-se em grande abundância no olival. Este facto, em conjunto com os resultados do consumo polínico, sugere que os sirfídeos voam entre diferentes tipos de parcelas para se alimentar. As espécies mais abundantes alimentaram-se de vegetação herbácea e arbustiva mostrando selecção por várias plantas e procurando alimento nas proximidades do olival.

No que respeita aos estudos sobre recursos alimentares, pela primeira vez foram identificados diferentes recursos presentes de forma natural no olival e disponíveis para todos os organismos estudados. Dos alimentos testados, a melada de *S. oleae* seguida da melada de *E. olivina* foram as fontes alimentares que proporcionaram melhor sobrevivência e reprodução de *P. oleae.* Os resultados mais favoráveis quando utilizadas as meladas de *S. oleae* em relação às meladas de *E. olivina* poderão estar relacionados com a menor viscosidade das primeiras, o que pode explicar os resultados obtidos. Entre as flores, o melhor desempenho foi obtido com *Malva sylvestris* L.. Por outro lado, *Conium maculatum* L. incrementou a longevidade mas prejudicou alguns parâmetros relacionados com a reprodução. Os resultados obtidos sugerem que *P. oleae* é uma espécie sinovigénica, emergindo sem ovos maduros e com reservas para a reprodução.

Quer a melada de *S. oleae* quer a de *E. olivina* aumentaram a sobrevivência de *E. flabellatus*, não se encontrando diferenças significativas entre os dois recursos. Entre as flores, a que resultou numa maior sobrevivência foi *M. sylvestris* seguida de *Daucus carota* L. Relativamente a *Andryala integrifolia* L., *Jasione montana* L. e*Tolpis barbata* (L.), não houve diferenças significativas entre estes tratamentos e o controlo negativo, mas também não foram observadas diferenças com *D. carota*.

Ambas as meladas de *S. oleae* e de *E. olivina* e as flores de três plantas (*Veronica persica* Poir, *M. sylvestris* e *Lamium purpureum* L.) incrementaram a sobrevivência de *C. carnea*. As flores de *Ranunculus ollissiponensis* Pers., *Lonicera etrusca* Santi, *Foeniculum vulgare* L. e *D. carota* também resultaram num relativo incremento da sobrevivência. Os resultados sugerem que os valores baixos obtidos para as variáveis reprodutivas estiveram relacionados com uma dieta pobre em proteínas.

Os sirfídeos mais abundantes, *Eupeodes corollae* (Fabricius) e *Episyrphus balteatus* (De Geer) consumiram e seleccionaram tipos polínicos de plantas herbáceas (Asteraceae, Ranunculaceae, tipo *Corrigiola telephiifolia* e Apiaceae, e Caryophyllaceae no caso de *E. corollae*) e de plantas arbustivas (tipo *Daphne gnidium*, tipo *Cytisus*/*Ulex*, *Arbutus unedo* e *Salix*) durante o outono, um período de escassez de recursos.

Em conclusão, no que respeita ao efeito das infraestruturas ecológicas, conclui-se que (i) *P. oleae* é capaz de se dispersar através de manchas de vegetação diferentes do olival, mas as implicações na PBC ainda necessitam de ser melhor investigadas, (ii) paisagens heterogéneas compostas por vegetação herbácea e arbustiva, circundantes ao olival, poderão favorecer os sirfídeos e (iii) a presença de cobertos vegetais no olival poderá beneficiar a taxa de parasitismo de *P. oleae*, enquanto a aplicação de herbicida exercerá um efeito oposto. Em relação à potencialidade dos recursos alimentares oferecidos pelas infraestruturas ecológicas associadas ao olival, as meladas de *S. oleae* e *E. olivina* e as flores de *M. sylvestris* serão potenciais recursos quer para os inimigos naturais, quer para *P. oleae.* Sendo assim, estes recursos alimentares deverão ser geridos no olival mediante alguma precaução. *V. persica* e *L. purpureum* são potenciais fontes alimentares para *C. carnea* no final do inverno e início da primavera. A floração destas plantas é coincidente com a geração filófaga de *P. oleae* pelo que o potencial efeito sobre esta deverá ser estudado. *D. carota* melhorou ligeiramente o desempenho de vários inimigos naturais estudados. *C. maculatum* poderá ser uma espécie candidata para aumentar a PBC. Por último, uma vez que *P. oleae* pareceu ser afectada positivamente por soluções açucaradas, tais como as meladas de insectos e o néctar de flores, é aconselhável dar atenção no uso deste tipo de recursos em estratégias de PBC.

A informação obtida permitirá o desenvolvimento de estratégias mais focalizadas em futuros trabalhos no âmbito da PBC contra a traça-da-oliveira através do estabelecimento de infraestruturas ecológicas que potenciem os inimigos naturais sem aumentar a praga. No entanto, e com o objectivo de aplicar este novo conhecimento no campo e possibilitar a realização de uma eficiente PBC, as várias questões que surgem a partir deste trabalho deverão ser investigadas de forma mais pormenorizada.

Palavras chave: *Prays oleae* (Bernard), predadores, parasitoides, recursos alimentícios, proteção biológica de conservação

Abstract

The olive tree (*Olea europaea* L.) has been shaping and characterizing landscape and culture in the Mediterranean basin. This tree is attacked by pest that can cause significant losses. This thesis was focused on the conservation biological control of one of its most damaging pests: the olive moth, *Prays oleae* (Bernard). Naturally, a large number of parasitoid and predator species are associated to this pest, and in order to satisfy their needs they make use of ecological infrastructures occurring in the olive grove agroecosystem. However, how these ecological infrastructures can be used to enhance the olive moth conservation biological control needs to be disentangled. For that, two approaches were followed. First, the influence of ecological infrastructures on *P. oleae* and its natural enemies was analyzed through field surveys. Then, different food resources were evaluated as potential foods for *P. oleae* and its natural enemies, i.e., the parasitoid *Elasmus flabellatus* (Fonscolombe) and the predator *Chrysoperla carnea s. l.* (Stephens). In the first approach, *P. oleae* was found to disperse throughout non-crop patches, although the implications for the pest population are unknown. The heterogeneous landscapes composed by herbaceous and woody vegetation around the olive groves were exploited by syrphids as food sources. Finally, ground covers within the olive groves favored the parasitoid *Ageniaspis fuscicollis* (Dalman) while the herbicide application diminished it. In the second approach, insects' honeydews [*Saissetia oleae* (Olivier) and *Euphyllura olivina* (Costa)] and the flowers of *Malva sylvestris* L. favored the survival and/ or reproduction of *P. oleae*, *C. carnea* and *E. flabellatus*. Several plant species such as *Daucus carota* L., *Veronica persica* Poir, and *Lamium purpureum* L. showed to be potential food resources for the studied natural enemies. From the results obtained in this research various applications for managing the olive grove agroecosystem are proposed.

Key words: *Prays oleae* (Bernard), predators, parasitoids, food resources, conservation biological control

Index

Agradecimentos 7

Resumo..15

Abstract...19

1. Introduction 3

1.1. Olive crop 3

1.2. Olive pests 4

1.3. Natural enemies of olive pests... 6

1.4. Conservation biological control 7

1.5. Conservation biological control in the olive agroecosystem 8

1.6. References... 10

2. Objectives and thesis structure 17

2.1. Objectives... 17

2.2. Thesis structure... 18

Dynamic of *Prays oleae* (Bernard) and chrysopids in olive groves surrounded by a heterogeneous landscape..21

Abstract.. 21

3.1. Introduction 23

3.2. Material and Methods 24

3.2.1. Study areas.. 24

3.2.2. *Prays oleae* flight activity 25

3.2.3. Sampling of chrysopids 25

3.2.4. Landscape metrics.. 25

3.3. Results.. 26

3.3.1. *Prays oleae* flight period 26

3.3.2. Chrysopids... 27

3.3.3. Landscape metrics.. 30

3.4. Discussion... 30

3.5. References... 32

Are wild flowers and insects honeydews potential food resources for adults of olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae)? A conservation biological control approach 37

Abstract 37

4.2. Material and Methods 40

4.2.1. Experimental design 40

4.2.2. Data Analysis 41

4.2.2.1. Survival 41

4.2.2.2. Reproduction 41

4.3. Results 42

4.3.1. Longevity 42

4.3.1.1. Death hazard ratio by diet treatment 42

4.3.1.2. Death hazard ratio among sexes within treatments 44

4.3.2. Reproduction 44

4.3.2.2. Pre-ovipostion period, oviposition period and lifetime fecundity 46

4.3.2.3. Oviposition period and lifetime fecundity as a function of longevity 47

4.4. Discussion 50

4.5. References 54

Effect of ground cover management on *Prays oleae* (Bernard) and its parasitoids 61

Abstract 61

5.1. Introduction 63

5.2. Material and methods 64

5.2.1. Study sites and sampling design 64

5.2.2. Data Analysis 66

5.3. Results 68

5.4. Discussion 71

5.5. References 73

Are wild flowers and insect honeydews potential food resources for *Elasmus flabellatus*, a parasitoid of the olive moth? A conservation biological control approach 79

Abstract 79
6.1. Introduction 81
6.2. Material and methods 82
    6.2.1. Parasitoids 82
    6.2.2. Selected foods 82
    6.2.3. Experimental design 82
    6.2.4. Data analysis 83
6.3. Results 84
    6.3.1. Lifespan 84
    6.3.2. Death hazard ratio by diet treatment 84
    6.3.3. Death hazard ratio among sexes within treatments 85
6.4. Discussion 88
6.5. References 90

Life-history parameters of *Chrysoperla carnea s. l.* fed on spontaneous plant species and insect honeydews: importance for conservation biological control 95

Abstract 95
7.1. Introduction 97
7.2. Material and Methods 98
    7.2.1. Insects 98
    7.2.2. Non-prey foods 98
    7.2.3. Experimental design 99
    7.2.4. Data analyses 99
        7.2.4.1. Survival 99
        7.2.4.1. Reproduction 99
        7.2.4.2. Development time 100
7.3. Results 100
    7.3.1. Survival 100
    7.3.2. Reproduction 102
    7.3.3. Development time 103
7.4. Discussion 105
7.5. References 107

Pollen feeding habits of syrphids in heterogeneous agricultural landscapes during autumn season, a period of food scarcity 113

Abstract 113

8.1. Introduction 115

8.2. Material and methods 116

8.2.1. Study areas 116

8.2.2. Syrphid sampling and identification 116

8.2.3. Pollen analyses 116

8.2.4. Flowering plant inventories 117

8.2.5. Data analyses 117

8.3. Results 117

8.3.1. Syrphids diversity 117

8.3.2. Plant diversity and ground cover 118

8.3.4. Pollen types 119

8.3.5. Pollen selection 123

8.4. Discussion 123

8.5. References 127

9. General discussion, application and future perspectives 137

9.1. General discussion 137

9.2. Applications 140

9.3. Future perspectives 141

Accepted Papers 143

CHAPTER 1

Introduction

1. Introduction

1.1. Olive crop

The olive tree (*Olea europaea* L.) is among the oldest and most widespread crops. It has been at the forefront of mankind becoming an integral part of traditions, cultures and myths. The history of the olive tree cultivation and olive oil have merged with the history of the great Mediterranean civilizations which did consider this tree as a symbol of human continuity, hegemony, braveness, wisdom, peace or divinity. It is believed that it was firstly cultivated in Near East about 6 millenniums ago and then spread through the Mediterranean basin, shaping and determining the Mediterranean landscapes (Bartolini and Petruccelli 2002) and characterizing economically, socially and culturally the population of the Mediterranean basin (Loumou and Giourga 2003). Nowadays, it is cultivated in all regions with climatic conditions that allow its establishment (Bartolini and Petruccelli 2002).

Portugal is one important olive producer country occupying the eighth position after Spain, Italy, Greece, Turkey, Tunisia, Syrian Arab Republic and Egypt with 309 706.04 tones of production per year (FAOSTAT 2015). Trás-os-Montes region, in the northeastern Portugal, in 2014 was responsible for the production of 58 946 t of olives in 80 159 ha of groves (INE 2015). The particular landscape configuration of Trás-os-Montes, with a considerable abrupt topography, together with the rural abandonment and the rural population aging of the last decades, are factors that determined the small size of the agricultural properties, being that the mean surface per agriculture property in the north of Portugal was 5.8 ha in 2009 and in Mirandela 6.6 ha (INE 2015). As a consequence, the olive production in this region did not experience the typical intensification from other producing regions, and traditional methods with low impact practices remain as the most common management. In general, olive groves are non-irrigated, the planting pattern range from 7x 7 to 10 x 10 meters approximately. Usually, they are pruned every two or three years. Soil usually is tilled or herbicide is applied, but in the last years many farmers started to keep ground covers until spring, when they are cut to reduce the competition for water and the fire risk. Additionally, Cobrançosa, Madural and Verdeal Transmontana are the most relevant cultivars produced in the region. All these features determine that the olive tree products from this region possess particular attributes that make them economically valuable in the market. There are various examples of success in this topic in the region, for example, the extra virgin olive oil “Casa de Santo Amaro” was awarded with the first prize in the light green extra virgin olive oil (*Verde ligeiro*) category by the International Oil Council (IOC) and the extra virgin olive oil of “Cooperativa dos Olivicultores de Valpaços, C.R.L.”, was a finalist for the category intense green extra virgin olive oil (*Verde intenso*) in the international competition for extra virgin olive oils “Mario Solinas” Quality Award (IOC 2015).

1.2. Olive pests

The olive tree has a large and diverse phytophagous entomofauna associated. At least 116 species of insects and 30 of mites are known to infest it (Tzanakakis 2003). In Trás-os-Montes region, several of these arthropods are responsible for large amounts of olive production losses, achieving the status of pests, being the most harmful pests:

- The olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae)

Prays oleae belongs to Yponomeutoidea superfamily, and in the last few years this superfamily has been subjected to several modifications (Lewis and Sohn 2015). Some previous subfamilies of Yponomeutidae were separated in independent families following results from molecular studies (Mutanen et al. 2010; Nieukerken et al. 2011) and Praydidae is now considered a family, including 51 species, where *P. oleae* was included. In Trás-os-Montes region, this moth is the most important olive tree pests, causing large production losses (Bento et al. 2001). The insect has three generations a year and their larval stages attacks different organs of the olive tree. Eggs of the anthophagous generation are laid on flower buds and after hatching, larvae feed on flowers. Its adult flight period occurs at the end of spring, laying the eggs of the carpophagous generation on the olive calyx. The carpophagous generation larvae bore into the olive stone and feed on the seed. At the end of summer and begging of autumn, adults emerge and lay the phyllophagous generation eggs on the olive leaves. The phyllophagous larvae dig galleries and fed on the leaves, where it overwinters until the following spring (Fig. 1.1) (Arambourg and Pravalorio 1986).

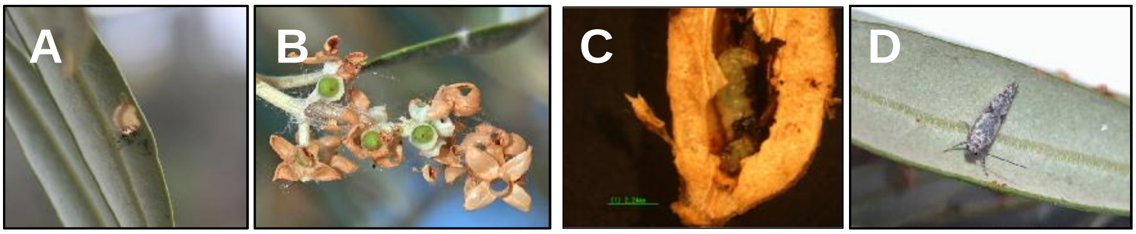

Fig. 1.1. Phyllophagous generation (A), anthophagous generation (B), carpophagous generation (D) and adult of the olive moth.

- The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae)

Bactrocera oleae is a very serious threat for olive trees in most of the olive producers regions (Danne et al. 2010). In general, *B. oleae* overwinters as pupae buried in the soil (Neuenschwander et al. 1986). Adults emerge during spring and oviposit in the olives when the fruits are suitable for oviposition, at the beginning of summer. Larvae feed on the olive mesocarp and its development is highly dependent on the temperature. In Trás-os-Montes region a peak of the flight period usually occurs from the middle of September till harvesting in November (Fig. 1.2) (Bento et al. 1999; Malheiro et al. 2015).

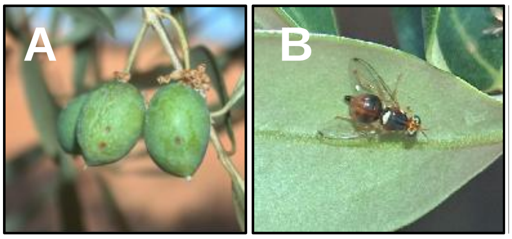

Fig. 1.2. Olives attacked by the olive fly (A), adult female of olive fly (B).

- The black scale, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae)

Saissetia oleae (Fig 1.3A) is a polyphagous insect living on over 150 species of plants (Tzanakakis 2003). In olive trees, it attacks the branches and leaves, sucking the olive tree sap. In Trás-os-Montes one generation, and sometimes the beginning of a second generation, have been identified. However, usually the damage is not considered important. The most relevant problem caused by *S. oleae* is derived from the fungi colonization of its honeydew that can cause difficulties to photosynthesis (Pereira 2004).

- The olive psyllid, *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae)

Euphyllura olivina (Fig 1.3B) larvae and adults perforate tender tissues of the olive tree and suck the sap of buds, tender shoots and floral axes as well as the fluid contents of inflorescences and young fruits (Tzanakakis 2003). It overwinters as an adult, oviposition start at the beginning of the spring (coincident with the development of new shoots) and can have various generations per year (Tzanakakis 2003 and references therein), although in Trás-os-Montes, commonly only two generation are observed during spring and begging of the summer (Pereira et al. 2001). The juveniles produce abundant honeydew droplets and a white waxy secretion (Tzanakakis 2003).

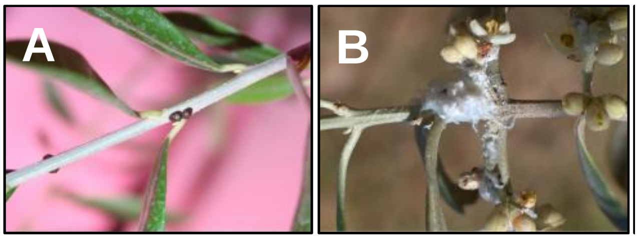

Fig. 1.3. Black scale (A) and Olive psyllid (B).

1.3. Natural enemies of olive pests

A large number of predators, parasitoids and entomopathogens, natural enemies of olive tree phytophagous, are associated to the olive groves.

Many important parasitoids naturally parasitizing *P. oleae*, *B. oleae* and *S. oleae* have been described. Among the *P. oleae* parasitoids some generalist and specific parasitoid, such as *Ageniaspis fuscicollis* (Dalman) (Hymenoptera: Encyrtidae), *Chelonus elaeaphilus* Silvestri (Hymenoptera: Braconidae) and *Elasmus flabellatus* (Fonscolombe) (Hymenoptera: Eulophidae), have been frequently observed (Bento et al. 1998; Herz et al. 2005). Several species of *Psyttalia* (Hymenoptera: Braconidae), *Utetes africanus* (Szepligeti) (Hymenoptera: Braconidae) and *Bracon celer* Szepligeti (Hymenoptera: Braconidae) are relevant parasitoids for *B. oleae* (Danne et al. 2010). Some *Coccophagus* (Hymenoptera: Aphelinidae), *Metaphycus* (Hymenoptera: Encyrtidae) and *Scutellista* (Hymenoptera: Pteromelidae) species are important parasitoids of *S. oleae* (Pereira 2004).

Among predators, the relevance of chrysopid larvae as predators of *P. oleae* is well documented, being particularly important *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) due to its abundance (Corrales and Campos 2004; Arambourg 1984; Ramos et al. 1987; Bento 1999; Porcel et al. 2013; Paredes et al. 2015). Other works pointed out the potential predation on immature stages of *S. oleae* (Arambourg 1984) and *E. olivina* (Pantaleoni et al. 2001; Gharbi et al. 2012). Larvae of syrphids have been found also to feed on olive pests, such as *P. oleae* (Sacchetti 1990; Silvestri 1908), *E. olivina* (Ksantini 2003), *Palpita vitrealis* (Rossi) (Lepidoptera: Crambidae) or *Euphyllura straminea* Loginova (Hemiptera: Psyllidae) (Rojo et al. 2003). Heteroptera (Paredes et al. 2015; Morris et al. 1999b), ants, and Coleoptera were found to prey on *P. oleae* (Morris et al. 1999b). Coccinelids are predators of *S. oleae* (Santos et al. 2009). Spiders are euryphagous predators feeding on many invertebrates, among them, pest. They are described as an abundant and diverse group in olive groves (Morris et al. 1999a; Cardenas et al. 2015). *B. oleae* is potentially predate by soil arthropods when is buried as a pupa in the soil (Danne et al. 2010). Dinis (2014) showed that some species of Carabidae from the olive grove prey on *B. oleae* in laboratory conditions being potential predators in the field.

Some entomopathogenic fungi have been also described as potential agents to control olive pests, such as *Beauveria bassiana* (Bals.) Vuill., *B. brongniartii* (Sacc.) Petch, *Metarhizium anisopliae* (Metschn.) Sorokin for *B. oleae* (Mahmoud 2009) or *B. bassiana*, *Cladosporium cladosporioides* and *Cladosporium oxysporum* (Oliveira 2013).

Some interactions among natural enemies and pests are showed in the figure 1.4.

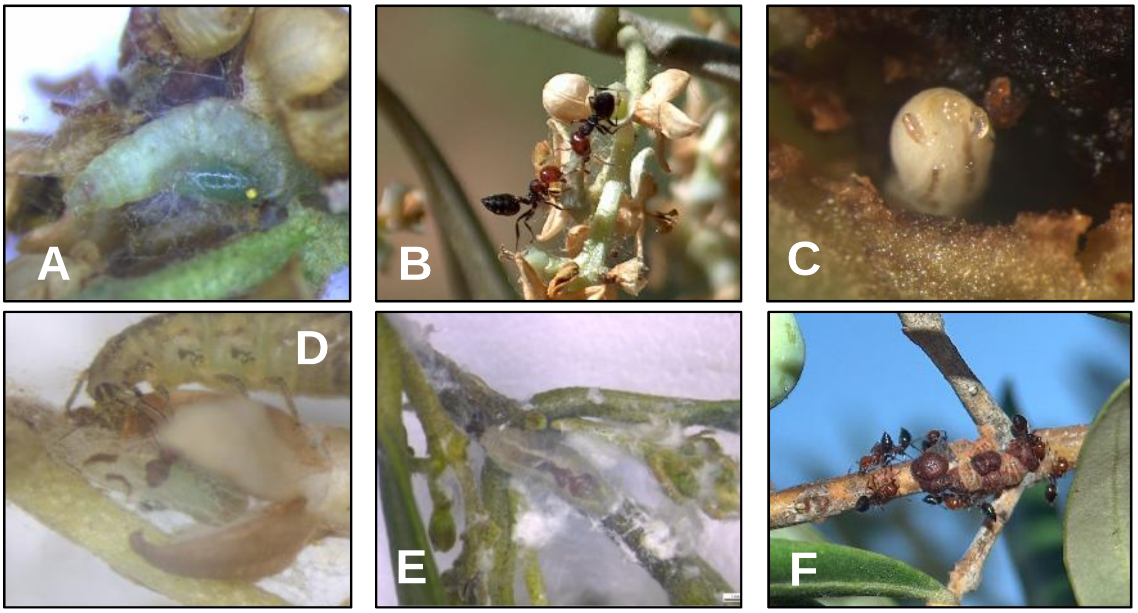

Fig.1.4. Parasitized olive moth larva (A), ants feeding on the olive moth larva (B), parasitized olive fly larva (C), chrysopid feeding on the olive moth larva (D), Syrphid feeding on the olive psylla (E), ants feeding on black scale honeydew (F).

1.4. Conservation biological control

Organic production has increased from 11 million hectares in the year 1999 to 43.1 million hectares in the year 2013, and the organic market size from 15.1 billion euro in 1999 to 54 billion euro in 2013 (IFOAM, 2014). Therefore, clearly the organic products consumption, free of synthetic pesticides, is increasing. However, pest control in conventional agriculture is done by using pesticides, what makes alternative strategies to be required for a sustainable agriculture. The biological control is one of the alternatives and is applied through several approaches: (i) conservation biological control; (ii) classical biological control; (iii) inoculation biological control; and (iv) inundation biological control (Gurr et al. 2002). This work will be focused in conservation biological control.

Conservation biological control is defined as *the modification of the environment or existing practices to protect and enhance specific natural enemies of other organisms to reduce the effect of pest* (Eilenberg et al. 2001) and is accomplished by: (i) reducing the use of pesticides (Gurr et al. 2002); (ii) habitat manipulation to create ecological infrastructures that provide resources to natural enemies and enhance their performance and effectiveness (Gurr et al. 2002; Landis et al. 2000).

Ecological infrastructures have to be suitable for natural enemies but not for pests (Lavandero et al. 2006; Winkler et al. 2010), and have to be suitable with the crop management system.

Different studies have focused in different requisites for natural enemies and have tried to find ecological infrastructures that supply natural enemies with alternative foods or shelter (Landis et al. 2000). The ecological infrastructures can be located outside or inside the crops, can be constituted by hedgerows, wildflower strips, grassland, groundcovers or anything that provide natural enemies with their life requisites (Boller et al. 2004). In this context, landscape structure can have a determinant role in pest control once in many cases was observed that natural enemies population were higher and pest pressure lower in complex landscape that in simple landscapes (Bianchi et al. 2006) being that this effect can vary with the scale (Chaplin-Kramer et al. 2011).

Importantly, parasitoids, many predators (such as chrysopids and syrphids) and various pests feed on non-crop resources in some phases of their life cycles (Jervis et al. 1993; Wäckers 2005; Lavandero et al. 2006; Wäckers et al. 2007; Winkler et al. 2010). These foods can be pollen, nectar, insects' honeydews, alternative preys and hosts (Landis et al. 2000) and can be provided by the ecological infrastructures in the agroecosystem.

The goal is to efficiently apply conservation biological control strategies in the agroecosystem through maintaining, conserving or establishing ecological infrastructures in order to enhance the natural enemies without favoring pests. The knowledge about what natural potential foods are the most suitable for these natural enemies but at the same time do not favor pests is crucial, as well as the knowledge about the influence that the crop and landscape characteristics and management have on these organisms.

1.5. Conservation biological control in the olive agroecosystem

In the olive agroecosystem, several researches have revealed some cues for establishing efficient conservation biological control strategies being the most recent works pointed in Table 1.1. According to these studies, in general, it seems that more complex and biodiverse landscapes and less impact management practices improve the diversity and abundance of natural enemies and reduce the pests. However, the knowledge about ecological infrastructures as well as the landscape and crop management more appropriated for favoring biological control is still insufficient. For example, food resources of the adult olive pests that do not consume crop-sources, such as *P. oleae* or *B. oleae*, is practically unknown. *P. oleae,* as most of Lepidoptera species, may feed on floral nectar and a variety of other liquids such as honeydews (Kevan and Baker 1983; Jervis et al. 2005; Krenn 2010). But the food resources used by *P. oleae* adults in the olive agroecosystem are still unidentified. Likewise occurs with the adult feeding of *B. oleae*. They are known to feed frequently on insect honeydews, flower nectar and pollen and other plant products in order to survive and reproduce (Tzanakakis 2003 and references therein) but the specific resources used are unknown. Also the knowledge about the use of resources by natural enemies in the olive agroecosystem is insufficient to design biological control strategies.

Table 1.1. Studies about the effect of relevant aspects for conservation biological control on natural enemies or pests in olive agroecosystems.

Organism	Response variable	Analyzed Factor	Effect	Reference
Natural Enemies				
Spiders	Abundance and diversity on the canopy	Management system	Positive on abundance with organic system	Cárdenas et al. 2015
		Plowing	Negative on abundance and diversity	
		Hedge vegetation	Positive on abundance	
C. carnea	Survival and reproduction	Natural occurring sugars	Positive with some sugars	Gonzalez et al. 2015
Carabidae	Diversity and abundance	Different types of ground cover management	General positive effect of ground cover presence	Oliveira, 2013
Spiders, ants, predatory heteroptera, parasitoids	Abundance	Non-crop vegetation	Positive effect of ground cover on spiders, parasitoids, and one heteroptera	Paredes et al. 2013a
			Positive effect of herbaceous and large woody vegetation modulated by ground cover	Paredes et al. 2013a
E. balteatus	Longevity and nutrient status	Flower resources	Positive effect	Pinheiro et al. 2013
Chrysopids	Abundance and biodiversity	Management system	In general, negative effect of more intensive practices	Porcel et al. 2013
Natural enemies	Abundance	Cereal cover crop	Positive on canopy parasitoids, particularly *A. fuscicollis*	Rodriguez et al. 2012
S. carulea *P. humilis*	Foraging behavior Survival	*S. oleae* honeydew	Positive	Wang et al. 2011
Parasitoids of *B. oleae*	Parasitoid emergence	Landscape connectivity at a large scale	Positive	Boccacio and Petacchi 2009
C. carnea	Abundance	Management system	Positive effect with organic and integrated	Corrales and Campos 2004
C. carnea	Longevity and Reproduction	Management system	Positive with organic	Corrales and Campos 2004
Arthropods	Abundance	Management system	Positive on integrated management	Ruano et al. 2004
Predators of *P. oleae*		Phenology	Synchrony with *P. oleae* anthophagous oviposition	Morris et al. 1999b
Ants	Abundance and biodiversity	Management system	Positive	Redolfi et al. 1999
C. carnea	Searching behavior	*S. oleae* honeydew contact	Behavior altered	McEwen et al. 1994
Pests				
B. oleae	Oviposition	Olive fruit volatiles	Positive correlation in some olives varieties	Malheiro et al. 2015
E. olivina,	Biological control	Single predator species or assemblages?	Single best predator	Paredes et al. 2015
P. oleae	Biological control	Single predator species or assemblages?	The most effective assemblage	Paredes et al. 2015
E. olivina, *P. oleae*	Abundance	Non-crop vegetation	Negative effect of herbaceous vegetation and woody vegetation near to the crop, and small patches of woody vegetation within the crop	Paredes et al. 2013b
B. oleae	Foraging behavior Survival	*S. oleae* honeydew	Positive	Wang et al. 2011
Several olive pest	Adult populations and damages	Cereal cover crops	Different effects and in some cases in opposite direction	Rodriguez et al. 2009
P. oleae	Eggs laid on shoots with *E. olivina* and *S. oleae*	Management system	Positive with integrated and organic management	Corrales and Campos 2004

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CHAPTER 2

Objectives and thesis structure

2. Objectives and thesis structure

2.1. Objectives

The main objective of this thesis was to analyze the importance of different ecological infrastructures in the olive grove agroecosystem to *Prays oleae* (Bernard) and its natural enemies. Two different approaches were followed:

1) Studies about the ecological infrastructures influence on *P. oleae* and their natural enemies. In this approach the specific objectives were:

i) Determine the capability of *P. oleae* adults to disperse over non-crop patches surrounding the olive groves (chapter 3).

ii) Analyze potential effects of landscape complexity on *P. oleae* and chrysopids from the olive groves (chapter 3).

iii) Evaluate the effect of different ground cover management on *P. oleae* and its parasitoids (chapter 5).

iv) Determine the abundance and diversity of syrphids in olive groves and surrounding ecological infrastructures during food scarcity periods (chapter 8).

2) The potentiality of non-crop, non-host and non-prey foods present within ecological infrastructures from the olive agroecosystem in *P. oleae* and its natural enemies. In this approach the specific objectives were:

i) Analyze nutritional suitability of non-crop sources for adults of the anthophagous generation of the olive moth (chapter 4).

ii) Analyze nutritional suitability of non-host for adults of *E. flabellatus* during the olive moth anthophagous generation flight period (chapter 6).

iii) Analyze nutritional suitability of non-prey sources along the year for adults of *C. carnea s. l.* (chapter 7).

iv) Analyze the pollen sources used by syrphids during food scarcity periods (chapter 8).

2.2. Thesis structure

In order to achieve this goal the thesis was organized as follows:

Introduction and objectives

- In **chapter 1** an introduction to the conservation biological control in olive groves is presented.
- In the **chapter 2** the objectives and thesis structure are described.

Studies about the pest

- In the **chapter 3**, through a descriptive work, *P. oleae* flight period and abundance and diversity of chrysopids in a heterogeneous landscape are analyzed with the aim of present the problem. The influence of scrubland and herbaceous patches vegetation on the landscape connectivity for *P. oleae,* the influence of the weather conditions and potential relationships predator-prey are discussed.
- In the **chapter 4** the suitability of several non-crop foods, present during the anthophagous generation of the olive moth in ecological infrastructures within and around the olive grove are analyzed as potential natural food resources for *P. oleae*. Implications of adult feeding on *P. oleae* biology and on conservation biological control are discussed.

Studies about parasitoids

- In the **chapter 5** the effect of different ground cover management, namely, spontaneous ground cover conservation, tillage and herbicide application, on (i) the olive moth emergence rate (ii) the parasitoid community composition and (iii) the parasitism rate, are analyzed.
- In the **chapter 6** the suitability of several non-host foods occurring during the flight period of *E. flabellatus* and occurring in ecological infrastructures within and around the olive grove are analyzed as potential natural food resources for this olive moth parasitoid.

Studies about predators

- In the **chapter 7** the suitability of several non-prey foods occurring along the year in ecological infrastructures within and around the olive grove, namely several flowering plant species and insect honeydews were analyzed as potential natural food resources for *C. carnea s. l.*
- In the **chapter 8** the plant species exploited by syrphids as pollen sources in olive groves and surrounding landscape during food scarcity periods are analyzed.

General discussion, application and future perspectives

- In the **chapter 9** a general discussion, application and future perspective are presented.

CHAPTER 3

Dynamic of *Prays oleae* (Bernard) and chrysopids in olive groves surrounded by a heterogeneous landscape

Dynamic of *Prays oleae* (Bernard) and chrysopids in olive groves surrounded by a heterogeneous landscape

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Abstract

Complex landscapes have been suggested to be more resilient to adverse conditions, affecting both the pests and their natural enemies. Therefore, the knowledge about the influence of weather conditions and landscape characteristics on arthropods emerges as a valuable tool for establishing efficient pest control strategies. The objective of this work was to analyze the flight period of the olive moth, *Prays oleae* (Bernard), and of its Chrysopidae predators. The study was carried out during 2012 and 2013 in different patches: olive grove, scrubland and herbaceous vegetation. For the first time, aspects related to the landscape connectivity for *P. oleae* were highlighted, being that scrublands and, in less degree herbaceous patches, do not seem to constitute a barrier for the *P. oleae* dispersion. Nevertheless, more complex and heterogeneous landscape presented lower number of captures of *P. oleae*. On the other hand, differences obtained between years for *P. oleae* captures can be due to the extreme weather conditions registered in 2012, such as low precipitations, low winter temperatures and high temperatures in spring and summer that negatively affected the pest population. *Chrysoperla carnea s. l.* was the most abundant species of chrysopids and was apparently more related with the occurrence of prey than with the climatic or landscape characteristics. This study contributes to the knowledge about *P. oleae* and *C. carnea s. l.* dynamics under adverse weather conditions and heterogeneous landscapes, and discloses new queries about the *P. oleae* dispersion and movement between patches.

Key words: olive moth, predator, olive grove, connectivity, pest control, landscape complexity

Manunscript to be submitted

CHAPTER 3

3.1. Introduction

The olive tree (*Olea europaea* L.) is a widespread crop in Mediterranean areas, with important social-economic and landscape impact, but pests can cause significant losses and reduce profits of the growers (Arambourg 1986; Ramos et al. 1998). In Trás-os-Montes region (northeast of Portugal), the olive moth *Prays oleae* (Bernard), is one of the most important pests of the olive tree (Bento et al. 2001). It has three generations a year and their larval stages feed on different organs of the olive tree. Eggs of the anthophagous generation are laid on floral buds and, after hatching, larvae feed on the flowers. The flight period of adults occurs at the end of spring, laying the eggs on the olive calyx and larvae of the carpophagous generation, bore into the olive stone and feed on the seed. At the end of summer and beginning of autumn, adults emerge and lay the eggs of the phyllophagous generation on the olive leaves. Larvae of the phyllophagous generation dig galleries and feed on leaves, where they overwinter till the beginning of spring (Arambourg, 1986).

Prays oleae has several natural enemies in olive groves and chrysopids are amongst the most important (Ramos et al. 1978; Neuenschwander and Michelakis 1980; Bento 1999; Porcel 2012; Paredes et al. 2015). In Trás-os-Montes region, six chrysopid species were previously identified by Bento et al. (1999) being *Chrysoperla carnea s. l.* (Stephens) and *Pseudomallada* (=*Dichochrysa*) *flavifrons* (Brauer) the most abundant species.

In the field, the synchrony between the population of *P. oleae* and chrysopid species should be considered for implementing successful biological control strategies. Once the development of chrysopid larvae occurs simultaneously with the oviposition period of *P. oleae*, this can be seen as a good indication for pest control. The abundance of both pest and predators can be influenced by several factors, such as landscape structure and composition (Thies and Tscharntke, 1999; Koh and Holland, 2014). In particular, adult chrysopids and *P. oleae* may feed on non-crop resources that can be provided by the vegetation occurring in the agricultural area and their surroundings that, in addition, can be used as shelter. Several studies suggest that heterogeneous landscapes, such as those of Trás-os-Montes, possess more potential for maintaining and enhancing the biological control of pests (Bianchi et al. 2006; Rusch et al. 2013). Studies performed with other olive pest, the olive fruit fly *Bactrocera oleae* (Rossi), showed that the reduction of the abundance of the pest was correlated with landscape complexity (Ortega and Pascual 2014). On the other hand, Boccaccio and Petacchi (2009) showed that parasitoids of *B. oleae* were positively affected by landscape connectivity.

Despite the importance of *P. oleae* as olive pest, its interaction with the surrounding landscape along its flight period is poorly known. In this context, the objectives of this study were: (i) to determine the flight period of *P. oleae*; (ii) to analyze its capability to disperse throughout non-crop patches; (iii) to determine the abundance and diversity of chrysopids during the same period, and (iv) to describe the synchrony between the pest and predators populations. Additionally, some aspect about the potential effect of the landscape structure and weather conditions on *P. oleae* and chrysopids are discussed.

3.2. Material and Methods

3.2.1. Study areas

The study was conducted in Mirandela municipality (northeastern Portugal), during 2012 and 2013, in three olive groves (Cedães: 41°29’16" N, -7°07’34" W, Paradela: 41º32’8’’N, -7º07’29’’W, and Guribanes: 41°34’12" N, -7°09’59" W) and two surrounding field areas (a herbaceous vegetation patch and a scrubland) next to each olive grove (Fig. 3.1). During the experimental years, the olive groves were not tilled and were not sprayed with pesticides.

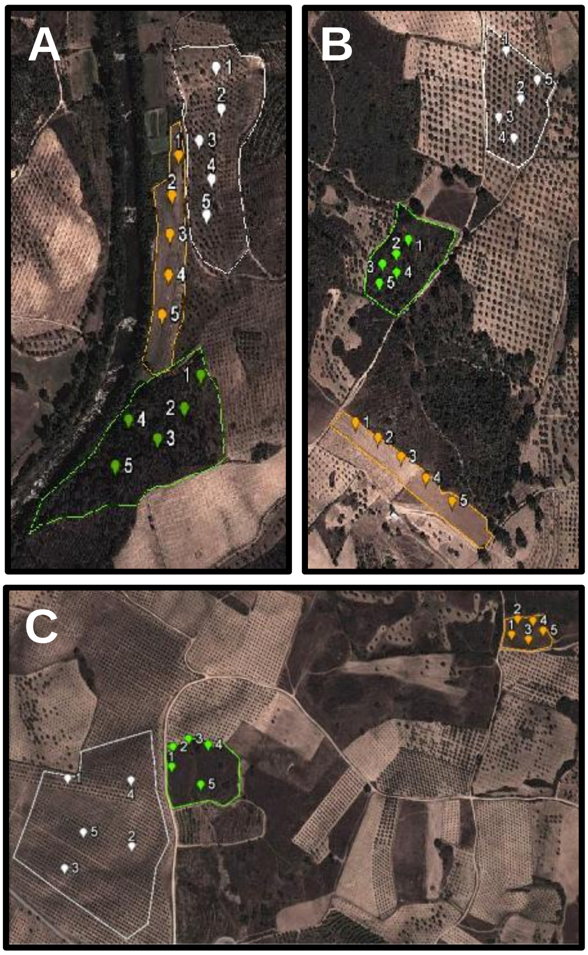

Fig. 3.1. Study sites (A: Guribanes: 41°34’12" N, 7°09’59" W; B: Paradela: 41º32’8’’N, 7º07’29’’W, and C: Cedães: 41°29’16" N, 7°07’34" W). Olive orchards are indicated in white, herbaceous vegetation patches in orange and scrubland patches in green. Numbers represent the Delta traps location. Images @ 2015 Google.

Scrubland patches were composed by three vegetation strata: herbaceous, shrub and tree strata derived from agriculture abandonment. Herbaceous vegetation patches were composed by cereal or grass mixture for livestock food. The areas of the three olive groves have about 2 ha and the surrounding patches 1 ha. The field selection was based on the most frequent field types occurring in the region.

3.2.2. *Prays oleae* flight activity

The flight activity of *P. oleae* was monitored from the end of March to December of 2012 and 2013. For that, five Delta traps, baited with *P. oleae* sex pheromone ((Z)-7-tetradecenal (Biosani, Palmela, Portugal), were installed in each olive grove, scrubland and herbaceous patch and separated about 50 m from each other (Fig. 3.1). In olive groves and scrublands the traps were hung on trees (at about 2 m height) and in the herbaceous vegetation patches were hung on a T-structure made of wood (at 70 cm height). Captures were recorded on a weekly basis.

3.2.3. Sampling of chrysopids

From the end of March to December of 2012 and 2013, chrysopids were captured on a weekly basis, with a sweep net in each olive grove by shaking olive tree branches during 30 minutes. Collected specimens were identified to species level and preserved in alcohol (70%).

3.2.4. Landscape metrics

A circular area with radius of 500 m was generated around each olive grove. Data from “Carta de Uso e Ocupação do Solo de Portugal Continental para 2007” (COS2007) were used to identify the land uses and proportions. The software Patch Analyst for ArcGIS, version 9.3.1 (ESRI, Redlands, California) was use to calculate the landscape indices.

The selection and description of landscape metrics were based on Ortega and Pascual (2014). The analyzed indexes were:

(i) Shannon landscape diversity index, sensitive to richness (number of patch types) and in less degree to the evenness (distribution of areas among different types) (McGarigal and Marks 1995);

(ii) Edge density which indicates the abundance of transition zones between different land uses, measure as meters of edge per hectare of sample area (Eiden et al*.* 2000);

(iii) Mean patch fractal dimension, a measure of the patch shape complexity, with values between 1 (shapes with very simple perimeters) and 2 (shapes with highly convoluted, plane filling perimeters) (McGarigal and Marks 1995);

(iv) Mean patch edge, which is measured as mean amount of edge per patch (m) (McGarigal and Marks 1995);

(v) Mean shape index, which is the average shape index of patches of the corresponding patch type. Shape index is minimum for circular patches and increases as patches become increasingly noncircular (McGarigal and Marks 1995);

(vi) Mean perimeter area ratio, measured as mean ratio of the patch perimeter (m) to area (m^2) (McGarigal and Marks 1995);

(vii) Mean patch size, as the sum of the areas (m^2) of all patches of the corresponding patch type, divided by the number of patches of the same type (McGarigal and Marks 1995);

(viii) Number of patches, as the number of patches per sample (McGarigal and Marks 1995);

(ix) Patch size standard deviation, a measure of absolute variation; it is a function of the mean patch size and the difference in size among patches (McGarigal and Marks 1995).

3.3. Results

3.3.1. *Prays oleae* flight period

Prays oleae captures in 2012 are shown in Figure 3.2A. The olive grove located in Cedães was the patch with the highest number of captures followed by the olive grove in Paradela. Guribanes olive grove and the herbaceous patches and scrublands in all locations presented low numbers of captures, barely exceeding 10 individuals per trap. The first adults of the phyllophagous generation were captured during April showing a peak in mid-May (olive groves means: Cedães = 165.8 and Paradela = 116). Captures of the individuals of the anthophagous generation increased during June with a peak at the end of that month (olive grove means: Cedães = 160; Paradela = 124.8). The number of individuals of the carpophagous generation was very low in every patch.

Prays oleae captures in 2013 are shown in Figure 3.3A. The number of individuals of the phyllophagous generation was extremely low in all patches. Individuals belonging to the anthophagous generation were captured in all the patches, being higher in olive groves, followed by scrublands and herbaceous vegetation patches. Additionally, the highest captures were registered in Cedães followed by Paradela and finally Guribanes. First individuals of the anthophagous generation were captured in the beginning of June and reached a peak at the beginning of July in all patches (olive grove means: Cedães = 210.60; Paradela = 156.40; Guribanes = 171.40 / scrubland: Cedães = 191.00; Paradela = 186.40; Guribanes = 146.00/ herbaceous patches: Cedães = 111.25; Paradela = 57.75; Guribanes = 116.00). The number of individuals of the carpophagous generation started to increase at the end of September and reached a peak at the beginning of October. Captures of this generation in Paradela and Guribanes were lower than in Cedães, being more noticeable in herbaceous and scrubland patches (olive grove means: Cedães = 291.00; Paradela = 194.20; Guribanes = 104.60/ scrubland: Cedães = 106.40; Paradela = 16.80; Guribanes = 13.00/ herbaceous patches: Cedães = 37.20; Paradela = 13.60; Guribanes = 0.80).

3.3.2. Chrysopids

In 2012, a total of 228 specimens of adult chrysopids were captured (Cedães = 141; Paradela = 22; Guribanes = 65) (Fig 3.2B, Fig 3.2C). Two taxa were identified, *C. carnea s. l.* and *Pseudomallada* sp. (Cedães: *C. carnea s. l.* = 110 and *Pseudomallada* sp. = 31; Paradela: *C. carnea s. l.* = 19 and *Pseudomallada* sp. = 3; Guribanes: *C. carnea s. l.* = 45 and *Pseudomallada* sp. = 20). In Cedães, the olive grove with the highest number of chrysopids, registered a first peak of *C. carnea s. l.* at the end of April, a second peak in mid-June and increased along the summer reaching a peak in the first week of October. Paradela and Guribanes olive groves presented a similar pattern but with much lower captures. *Pseudomallada* sp. showed a peak in May and on the first week of the autumn in Cedães and Guribanes while only three individuals were captured in Paradela.

In 2013, a total of 273 specimens of adult chrysopids were captured (Cedães = 118; Paradela = 110; Guribanes = 54) (Fig 3.3B, Fig 3.3C). Two taxa were identified, *C. carne*a and *Pseudomallada* sp. (Cedães: *C. carnea s. l.* = 99 and *Pseudomallada* sp. = 19; Paradela: *C. carnea s. l.* = 90 and *Pseudomallada* sp. = 11; Guribanes: *C. carnea s. l.* = 51 and *Pseudomallada* sp. = 3). *C. carnea s. l.* was relatively frequent during the spring in Cedães and Paradela olive groves, presenting a peak in June. During the summer, captures decreased and started to increase at the end of September with a peak in October. Captures in Guribanes grove showed a similar pattern but with lower captures. *Pseudomallada* sp. presented a similar pattern to that observed in 2012, but with general lower captures.

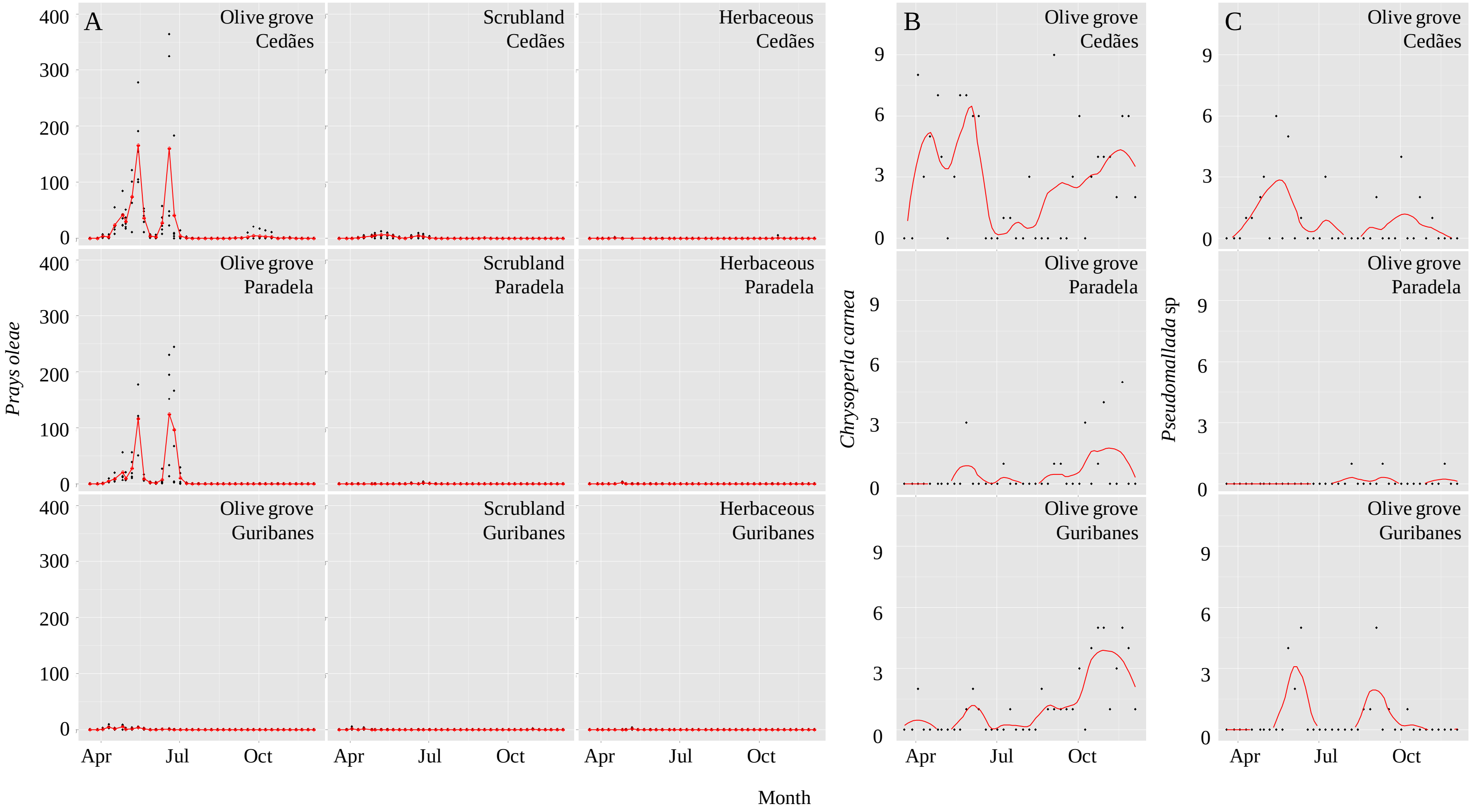

Fig. 3.2. Number of captures in each site (Cedães, Paradela and Guribanes) in 2012. (A) Number of *P. oleae* by patch (olive grove, scrubland and herbaceous vegetation). Black points indicate the number of *Prays oleae* captured in each delta trap along the experiment. Red points indicate the mean number of *P. oleae* males captured in each data. Red lines represent the flight period of *P. oleae* males. Number of *Chrysoperla carnea s. l.* (B) and *Pseudomallada* sp. (C) captured with the sweep net in olive along the experiment. Red lines represent smooth curves (local polynomial regression method – span = 0.3) of the number of captures along the year.

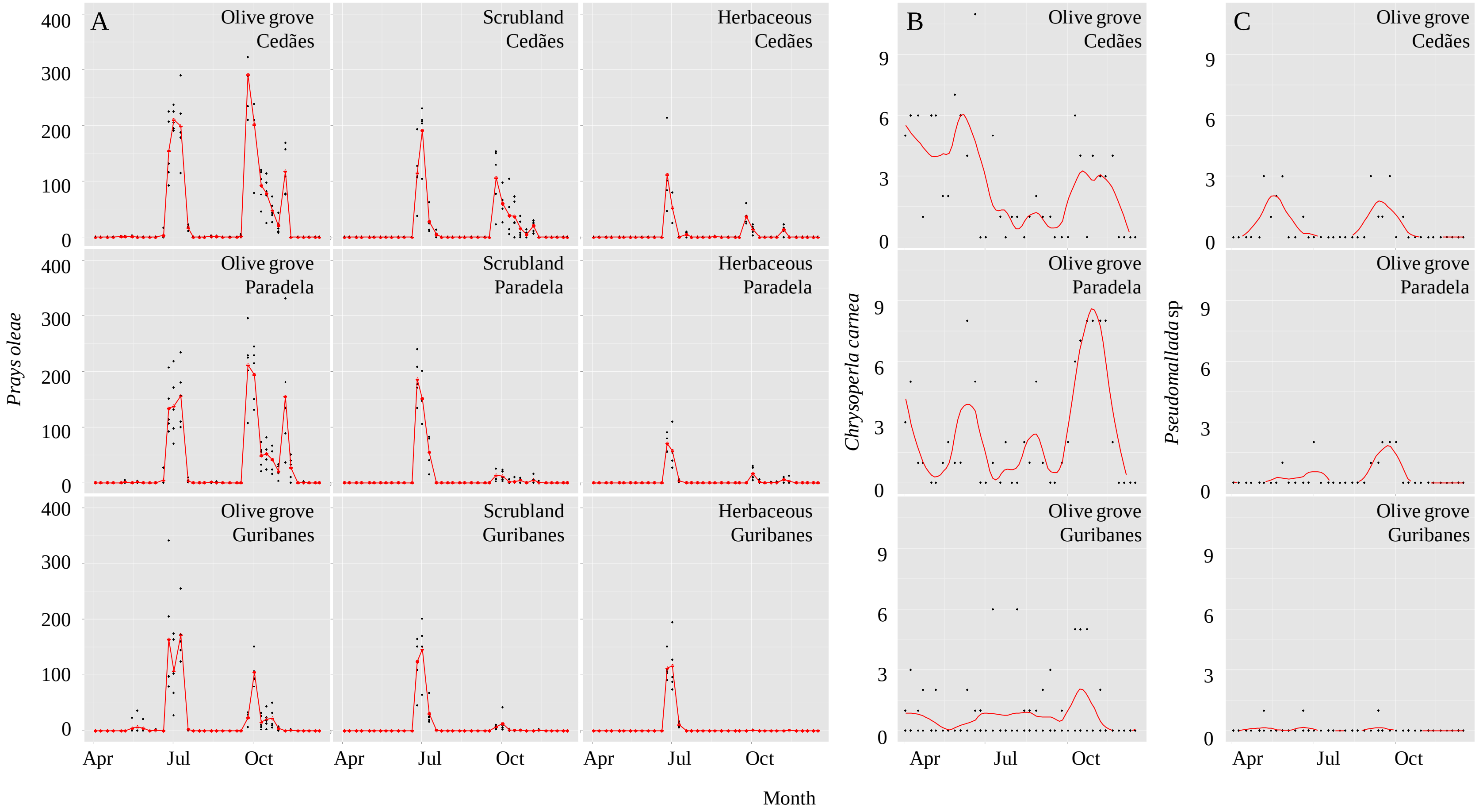

Fig. 3.3. Number of captures in each site (Cedães, Paradela and Guribanes) in 2013. (A) Number of *Prays oleae* by patch (olive grove, scrubland and herbaceous vegetation). Black points indicate the number of *P. oleae* captured in each delta trap along the experiment. Red points indicate the mean number of *P. oleae* captured in each data. Red line represent the flight period of *P. oleae* males. Number of *Chrysoperla carnea s. l.* (B) and *Pseudomallada* sp (C) captured with the sweep net in olive along the experiment. Red lines represent smooth curves (local polynomial regression method – span = 0.3) of the number of captures along the year.

3.3.3. Landscape metrics

The values of the landscape metrics are shown in the Table 3.1. Guribanes and Paradela location presented higher Shannon diversity index than Cedães. Guribanes presented the highest edge density, mean patch fractal dimension, mean shape index, mean perimeter area ratio and number of patches followed by Paradela and Cedães, except in the mean perimeter area ratio that was higher in Cedães than in Paradela. The highest mean patch edge, mean patch size and patch size standard deviation was showed by Cedães location followed by Paradela and Cedães.

Table 3.1. Landscape index values for the studied areas.

Name	Guribanes	Paradela	Cedães
Shannon diversity index	1.491	1.535	0.971
Edge density (m/ha)	576.229	218.786	138.969
Mean patch fractal dimension (m^2)	1.456	1.388	1.388
Mean patch edge (m)	530.666	859.327	1212.416
Mean shape index	2.118	1.562	1.500
Mean perimeter (m)-area (m^2) ratio	1682.051	1075.920	1275.644
Mean patch size (m^2)	0.921	3.928	8.724
Number of patches	85	20	9
Patch size standard deviation (m^2)	1.109	9.221	14.312

3.4. Discussion

Prays oleae captures observed in this study showed some differences in relation to other works (Ramos et al. 1989; Pereira et al. 2004), with a general low number of captures in both years and a nearly absence of the carpophagous generation in 2012 and phyllophagous in 2013. In northeastern Portugal other studies found medium values of more than 400 individuals in the phyllophagous generation and about 550 in the antophagous (Pereira et al. 2004).

Weather annual variations strongly affect *P. oleae* dynamics (Gonzales et al. 2015) and accordingly to the Portuguese Sea and Atmosphere Institute, I. P. (IPMA 2012), during the winter, the spring and the summer 2012, an extreme drought situation ravaged Portugal mainland. The winter was the driest since the first records in 1931, and in February, Mirandela registered 28 days with minimum temperatures equal or lower than 0. During the spring, the drought remained (softened by some precipitation in May) and temperatures in the spring and the summer were higher than the mean. At the end of the autumn the drought was finished in almost all the northern locals. A decrease of *P. oleae* larvae growth has been described under unfavorable weather conditions (Tzanakakis 2003 and references therein). Moreover, low temperatures in winter increased the mortality of *P. oleae* larvae (Ramos et al. 1978; Kumral 2005) and high temperature and low relative humidity during the anthophagous and carpophagous generation caused high mortality of eggs and larvae (Civantos 1998). Therefore, in this study the extreme weather conditions observed in 2012 could have lead to an increase of *P. oleae* mortality and/or the larvae dormancy, resulting in the low the number of anthophagous and phyllophagous captures and the nearly absence captures of carpophagous adults. The 2012/2013 winter was colder and drier than the mean (IPMA 2013), and this together with the extreme conditions of the previous year probably weakened *P. oleae*, leading to the almost absence of phyllophagous generation in 2013. The spring 2013 registered the highest precipitations in the last 50 years, probably causing the observed recovery of *P. oleae* populations. The weather conditions in the summer and autumn 2013 remained close to normal values (IPMA 2013). In agreement with our results, the high variability in the response of *P. oleae* to the surrounding vegetation was attributed to climatic variability between years (Paredes et al. 2013b).

Results obtained in 2013 indicated that scrubland and, in less degree, herbaceous patches did not act as barriers to the movement of *P. oleae*, especially during the anthophagous generation. The landscape connectivity is defined as “the degree to which the landscape facilitates or impedes movement among resource patches” (Tailor et al. 1993) and our results suggest that these patches, particularly scrublands, did not affect landscape connectivity for *P. oleae*. However, if the capability of *P. oleae* to penetrate non-olive grove patches is positive, negative or null for its dispersion toward other olive groves needs to be clarified. These type of patches, herbaceous and woody vegetation areas near and within olive groves, were found to decrease the abundance of *P. oleae* and *E. olivina* (Paredes et al. 2013b) and Paredes et al. (2013a) found that herbaceous and large woody vegetation adjacent to de crop influence the abundance of natural enemies, being this effect modulated by ground cover.

In this study, we captured more *P. oleae* individuals in Cedães, followed by Paradela and Guribanes. Furthermore, the landscape indexes indicated that Guribanes landscape was more complex presenting: (i) a higher Shannon diversity index than Cedães which indicates higher richness and evenness; (ii) the highest edge density which indicates higher abundance of transition zones between land uses; (iii) the highest mean patch fractal dimension which indicates a more complex patch shape, and; (iv) the highest mean shape index indicating less circular patches (see McGarigal and Marks, 1995). These values, in conjunction with the highest mean patch edge, mean patch size and patch size standard deviation in Cedães showed that apparently the most heterogeneous and complex landscape was represented by Guribanes, followed by Paradela and Cedães. This suggests that the lowest *P. oleae* captures obtained in Guribanes could be related with the higher landscape heterogeneity and complexity. These results are in agreement with Ortega and Pascual (2014) who found the edge density, the mean patch size and the patch size standard deviation related with *B. oleae* captures at short distances. Further research should be developed to elucidate the effect on landscape parameters on *P. oleae* at larger scales.

Regarding chrysopids, the diversity observed in this work was lower than the diversity found by other authors (Bento 1999; Porcel 2012). This could be related with the different methods of capture employed. *C. carnea s. l.* peaks were in general registered just before *P. oleae* peaks, suggesting that *C. carnea s. l.* may be synchronized with *P. oleae*, fact that has been already described in other works (Neuenschwander and Michelakis 1980; Bento 1999). Nevertheless, in 2012, an autumn peak was registered despite the absence of the carpophagous generation, therefore *C. carnea s. l.* possibly fed on other preys. In Cedães were registered not only the highest number of *P. oleae* captures but also the highest abundance of chrysopids. Nevertheless, in spite of the climatic conditions improvement in 2013, *C. carnea s. l.* captures did not increase. Apparently, occurrence and abundance of this species could be more related to the prey resources occurrence than to the weather conditions or landscape characteristics. Nevertheless, in this study all locations are characterized by heterogeneous and complex landscapes, and the differences among locations could be not sufficient to influence *C. carnea s. l*.

Pseudomallada sp. captures were in general very low. It presented two peaks, one in spring and one at the begging of the autumn. Other authors (Bento et al. 1999) observed that sometimes this taxon exceeded *C. carnea s. l.* In this study, *Pseudomallada* sp. was observed in the beginning of the autumn in Guribanes.

In conclusion, the variation between years of *P. oleae* captures were strongly related with the weather conditions, being negatively affected by low precipitations along the year, low winter temperatures and high temperatures in spring and summer. For the first time, landscape connectivity aspects were identified for *P. oleae*, being that was clearly able to disperse over a heterogeneous landscape composed by scrublands and herbaceous patches, fact particularly noticeable in scrubland patches. More complex and heterogeneous landscape presented less number of captures, being that many interactions among pests, natural enemies and landscape may be taking place simultaneously. *C. carnea s. l*. was apparently more related with the occurrence of prey than with the weather conditions or landscape characteristics. This study provides new data that contributes to the knowledge about *P. oleae* and *C. carnea s. l.* dynamics under adverse weather conditions and heterogeneous landscapes, and discloses new queries about the *P. oleae* dispersion and movement between patches.

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CHAPTER 4

Are wild flowers and insects honeydews potential food resources for adults of olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae)? A conservation biological control approach

Are wild flowers and insects honeydews potential food resources for adults of olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae)? A conservation biological control approach

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Abstract

The use of non-crop resources by natural enemies and their potentialities to enhance their effectiveness as pest controllers is an increasing strategy of conservation biological control. Nevertheless, the effect of non-crop resources consumption by pests has been generally overlooked being this knowledge crucial to implement strategies that 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 insects honeydews were from *Saissetia oleae* and *Euphyllura olivina*, two olive pests, and the selected plants were abundant species in the olive agroecosystem that bloom simultaneously with the existence anthophagous generation of *P. oleae* adults. Some of these resources were identified as potential foods for *P. oleae*. Despite the general findings indicating honeydews to 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 showed to be potential food resources for *P. oleae*, being *Malva sylvestris* the most efficient. Moreover, our results suggest that *P. oleae* females are synovigenic and emerge with nutritional reserves for reproduction. We highly recommend accomplishing further researches before establishing these resources in biological control strategies in order to confirm their effect on pests in fields.

Keywords: insect feeding, non-crop resources, *Saissetia oleae*, *Euphyllura olivina*, survival analysis, reproduction

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4.1. Introduction

Habitat management is a strategy of conservation biological control that consists in improving the pest control through conserving or modifying the environment to enhance the natural enemies' survival, reproduction and behavior (Landis et al. 2000). Many natural enemies, during some phases of their development, need non-crop resources that are provided by vegetation or insects, such as pollen, nectar, insects' 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 feed on 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 the pest control, but the knowledge about the effect of those resources on pests is crucial before increasing them in fields to avoid benefit pests (Baggen and Gurr 1998; Lavandero et al. 2006; Winkler et al. 2009a; 2009b). Many studies analyzed the effect of different food resources (flowers, 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; Lee et al. 2004; Berndt and Wratten 2005; Lee et al. 2006; Winkler et al. 2006; Pfiffner et al. 2009; Winkler et al. 2009a; Winkler et al. 2009b; Géneau et al. 2012; Aguillar-Fenollosa and Jacas 2013; Balzan and Wäckers 2013; Beltrà et al. 2013; Gonzalez et al. 2015; Saeed et al. 2015). However, the knowledge about the use of non-crop resources by most of the adult pests is still insufficient. This is the case of the olive moth, *Prays oleae* (Benard, 1788).

The olive moth diet and development during its larvae stage is well known. This is a monophagous herbivorous that feed on the olive tree. It has three generations a year: i) the phylophagous generation that feeds on leaves and develop during the autumn and winter; ii) the anthophagous generation that feeds on flowers and develop during the olive tree blooming; and iii) the carpophagous generation that feeds on the fruits and develop during the summer. Adult feeding is poorly known and it is probably a determining factor for the survival and reproduction of the olive moth. Most adult Lepidoptera 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 (Baggen and Gurr 1998; Baggen et al. 1999; Lee and Heimpel 2005; Mevi-Schütz and Erhardt 2005; Begum et al. 2006; Lavandero et al. 2006; Pfiffner et al. 2009; Winkler et al. 2009a; Winkler et al. 2009b; Géneau et al. 2012; Balzan and Wäckers 2013). *Prays oleae* could be feeding on non-crop natural vegetation flowers or on insect honeydews from surrounding and within the olive groves.

Moreover, many of the referenced studies about feeding of pest and natural enemies on non-crop vegetation use a similar set of plants and these plants are chosen due to their proved positive effect on many natural enemies and sometimes in biological control. For example, *Lobularia maritima* (L.) Desv., *Fagopyrum esculentum* M. or *Phacelia tanacetifolia* Benth were frequently studied. However, these plants are not always native and the potential in biological control of many other species in different agroecosystems are unknown. Some authors have already pointed out the importance of native plants (Jervis et al. 1993; Fiedler and Landis 2007; Araj and Wratten 2015) that can be better adapted to the local environment conditions, their use may reduce the risk of non-native plants invasion and the economic inputs for farmers.

Here, we studied natural vegetation and honeydews produced by the black scale, *Saissetia oleae* (Olivier 1791), and the olive psyllid, *Euphyllura olivina* (Costa 1839), secondary pests of the olive tree in the studied region, 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 the 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.

4.2. Material and Methods

4.2.1. Experimental design

Abundant 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., *Crepis capillaris* (L.) Wallr., *Conium maculatum* L., *Jasione montana* L., *Malva sylvestris* L. and *Trifolium repens* L. These plant species bloom during the spring and commonly are present in abundance during the anthophagous generation of the olive moth. The flowers were collected in the campus of Polytechnic Institute of Bragança, northeast of Portugal. Their stems were submerged under water in 15 mL plastic jars and closed with parafilm. Honeydews were collected from infested olive trees grown in climatic chambers in the laboratory. Larvae of the anthophagous generation of olive moth were collected in olive orchards from the same region. In laboratory, the larvae were transferred into tubes and located in climatic chambers at 21ºC (± 2 ºC) and 16:8 h L:D (light:dark) until adults emergence. Newly emerged couples were transferred into 220 mL cages. Between 28 and 30 replicates per treatment (22 in *C. capillaris* treatment) were 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 flowers surface were used by treatment and a homogeneous amount of honeydew was used. 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. Daily mortality and egg numbers were recorded.

4.2.2. Data Analysis

4.2.2.1. 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.

4.2.2.2. 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 treatment 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 overdispersion. 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. 2014). 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 female 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 the females longevity for each treatment. The same procedure was followed to analyze the oviposition period as a function of the females longevity for each treatment. One outlier was eliminated in the case of *T. repens* treatment.

4.3. Results

4.3.1. Longevity

4.3.1.1. Death hazard ratio by diet treatment

The Cox's PHM showed that females and males hazard of death were significantly different among diet treatments (Females: Likelihood ratio = 259.3, df = 10, $p < 0.001$; Males: Likelihood ratio = 258.1, df = 10, $p < 0.001$). Regarding to the females, the death hazard for females fed on *A. arvensis*, *A. integrifolia*, *C. capilllaris* and *J. montana* did not differed significantly from the water (negative control) treatment. The treatments with *M. sylvestris*, *C. maculatum*, *T. repens* flowers and with *E. olivina* honeydews showed significantly lower death hazard than the water treatment but higher than the *S. oleae* and the honey (positive control) ones (Fig. 4.1A, Table 4.1). With respect to the males, the death hazard under *C. capillaris*, *A. integrifolia* and *T. repens* diets did not differ significantly from the water treatment but was significantly lower than with *A. arvensis* and *J. montana* and significantly higher than 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. 4.1B, Table 4.1).

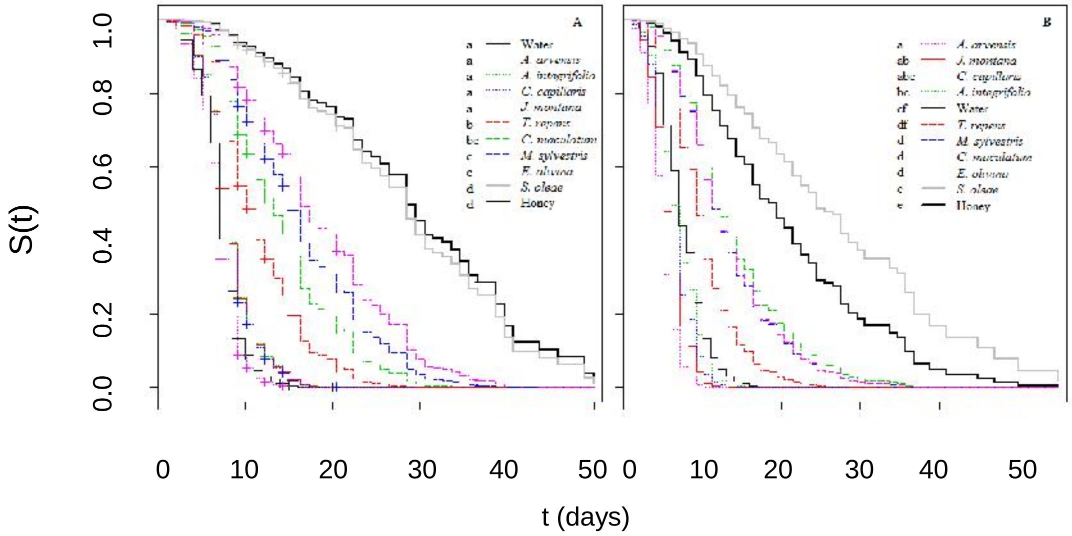

Fig. 4.1. Cox estimates of the survival function, S(t), for females (A) and males (B). Different letters on the legend indicate significant death hazard differences among treatments (significance level < 0.05). Crosses indicate censored data.

Table 4.1. Hazard ratio, exp (β_k), for comparison group relative to reference group, i.e. the comparison group has exp (β_k) times the death hazard than the reference group. The hazard ratio for groups k and j is exp (β_k-β_j). Bold letters indicate that the difference between two groups is significant. Bonferroni correction was applied (significance level < 0.05).

Comparison group		Reference group									
	Diet treatment	*A. arvensis*	*A. integrifolia*	*C. capillaris*	*C. maculatum*	*J. montana*	*M. sylvestris*	*T. repens*	*E. olivina*	*S. oleae*	Honey
Females	Water	0.827	1.443	1.384	**5.377**	1.425	**7.512**	**3.382**	**9.947**	**29.019**	**32.265**
	A. arvensis		1.745	1.673	**6.500**	1.723	**9.080**	**4.088**	**12.023**	**35.077**	**39.000**
	A. integrifolia			0.959	**3.726**	0.988	**5.205**	**2.343**	**6.892**	**20.107**	**22.356**
	C. capillaris				**3.885**	1.030	**5.427**	**2.443**	**7.187**	**20.966**	**23.311**
	C. maculatum					**0.265**	1.397	0.629	1.850	**5.397**	**6.000**
	J. montana						**5.270**	**2.373**	**6.979**	**20.360**	**22.637**
	M. sylvestris							**0.450**	1.324	**3.863**	**4.295**
	T. repens								**2.941**	**8.581**	**9.540**
	E. olivina									**2.917**	**3.244**
	S. oleae										1.112
Males	Water	**0.285**	0.757	0.595	**3.944**	**0.456**	**3.490**	1.930	**3.536**	**15.162**	**8.900**
	A. arvensis		**2.654**	2.086	**13.836**	1.599	**12.242**	**6.771**	**12.404**	**53.188**	**31.220**
	A. integrifolia			0.786	**5.212**	0.602	**4.612**	**2.551**	**4.673**	**20.038**	**11.762**
	C. capillaris				**6.632**	0.766	**5.868**	**3.245**	**5.946**	**25.494**	**14.964**
	C. maculatum					**0.116**	0.885	0.489	0.897	**3.844**	**2.257**
	J. montana						**7.656**	**4.235**	**7.758**	**33.265**	**19.526**
	M. sylvestris							0.553	1.013	**4.345**	**2.550**
	T. repens								1.832	**7.855**	**4.611**
	E. olivina									**4.288**	**2.517**
	S. oleae										0.587

4.3.1.2. 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 under water (Hazard ratio = 0.833; Likelihood ratio = 0.47, df = 1, p = 0.50), *C. maculatum* (Hazard ratio = 1.154; Likelihood ratio = 0.29, df = 1, p = 0.59), *T. repens* (Hazard ratio = 1.264; Likelihood ratio = 0.78, df = 1, p = 0.38), *E. olivina* honeydew (Hazard ratio = 1.580; Likelihood ratio = 2.95, df = 1, p = 0.09) and *S. oleae* honeydew (Hazard ratio = 1.084; Likelihood ratio = 0.09, df = 1, p = 0.76) diet treatments. The death hazard under *A. integrifolia* was higher for males than for females with a slight significance (Hazard ratio = 1.703; Likelihood ratio = 3.87, df = 1, p = 0.05). Males showed significant higher death hazard than females under *A. arvensis* (Hazard ratio = 2.458; Likelihood ratio = 9.5, df = 1, p = 0.002), *C. capillaris* (Hazard ratio = 2.112; Likelihood ratio test = 5.35, df = 1, p = 0.02), *J. montana* (Hazard ratio = 2.425; Likelihood ratio = 10.13, df = 1, p = 0.001), *M. sylvestris* (Hazard ratio = 1.909; Likelihood ratio test = 5.41, df = 1, p = 0.02) and honey (Hazard ratio = 2.880; Likelihood ratio = 13.46, df = 1, p < 0.001).

4.3.2. Reproduction

Daily oviposition (number of eggs) laid by fertile females through the experiment are showed in the Fig. 4.2.

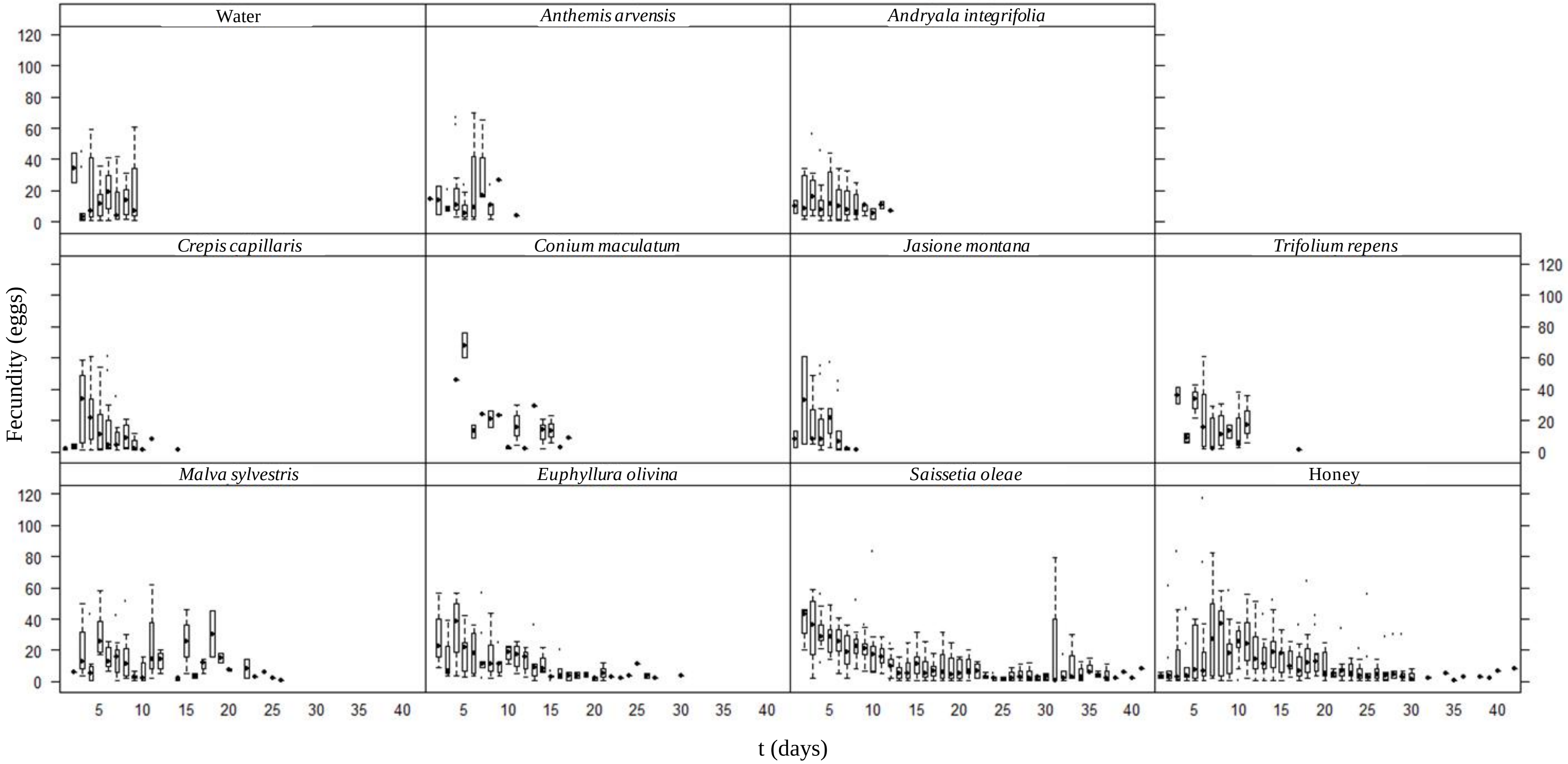

Fig. 4.2. Boxplot of daily oviposition (eggs number) of *P. oleae* females fed with each treatment. Points are the medians of laid eggs by day.

The percentage of fertile females varied between 21% and 95% among treatments and the mean eggs laid by females varied between 34.70 (± 8.48) and 230.57 (± 21.78). The pre-ovipostion period varied between 2.07 (± 0.51) days with honey, and 8.67 (± 1.55) days with *C. maculatum*. The longest oviposition period was accomplished with honey, with 21.29 (± 2.08) days followed by *S. oleae*, with 20.09 (± 2.45) days and the lowest with *A. arvensis,* with 2.4 (± 0.37) days. The *S. oleae* honeydew and honey treatments led to the highest mean eggs per fertile female and to the highest total laid eggs per treatment (Table 4.2).

Table 4.2. Reproduction parameters of *Prays oleae* reared with different food sources.

Treatments	% fertile females[1]	Pre-oviposition period (±SE) (days)	Oviposition period (±SE) (days)	Mean eggs/fertile couple (±SE)	Total eggs per treatment
Water	90.00 (27/30)	3.04 (±0.30)	3.74 (±0.32)	56.89 (±7.78)	1536
A. arvensis	66.67 (20/30)	3.15 (±0.51)	2.40 (±0.37)	34.70 (±8.48)	694
A. integrifolia	80.00 (24/30)	3.71 (±0.62)	3.37 (±0.42)	41.71 (±7.04)	1001
C. capillaris	95.45 (21/22)	4.09 (±0.59)	5.33 (±0.56)	56.81 (±12.48)	1193
C. maculatum	21.43 (6/28)	8.67 (±1.55)	6.83 (±1.06)	83.00 (±28.40)	498
J. montana	60.00 (18/30)	2.33 (±0.37)	3.06 (±0.52)	37.78 (±11.23)	680
M. sylvestris	58.62 (17/29)	5.06 (±1.04)	5.53 (±1.19)	70.06 (±15.50)	1191
T. repens	41.38 (12/29)	5.67 (±1.04)	6.33 (±1.64)	53.67 (±11.43)	644
E. olivina	56.67 (17/30)	5.94 (±1.20)	7.18 (±1.37)	80.88 (±19.00)	1375
S. oleae	70.00 (21/30)	5.81 (±1.53)	20.09 (±2.45)	230.57 (±21.78)	4842
Honey	93.33 (28/30)	2.01 (±0.51)	21.29 (±2.08)	195.79 (±29.76)	5482

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

4.3.2.2. Pre-ovipostion period, oviposition period and lifetime fecundity

The GLMM outputs fitted for pre-oviposition and oviposition periods and for lifetime fecundity of *P. oleae* fertile females are shown in the Table 4.3. These three variables were significantly affected by the food source (pre-oviposition period: χ^2 = 37.689, df = 10, p-value < 0.001; oviposition period: χ^2 = 10, df = 195.72, p-value < 0.001; lifetime fecundity: χ^2 = 89.9, df = 10, p-value < 0.001). *C. maculatum* was the only treatment that caused a significant increase of the pre-oviposition period with respect to the water treatment but this treatment was not significantly different from the other treatments. Oviposition period under *S. oleae* honeydew and honey treatments was significantly higher than with the rest of the treatments. With *E. olivina* honeydew the oviposition period was significantly higher than with water and *A. arvensis* treatments but did not significantly differ from the other treatments. Fecundity under *S. oleae* honeydew and honey treatment was significantly higher to all the other treatments.

Table 4.3. GLMM output for estimated regression parameters and standard errors with negative binomial distribution for pre-oviposition period, oviposition period and lifetime fecundity. Food resource is the fixed factor and the fertile female identity the random factor. Fecundity of *Prays oleae* in cages under water treatment is the baseline. Different letters indicate significant differences ($p < 0.05$) between treatments after pairwise comparison.

	Pre-oviposition period				Oviposition period				Lifetime fecundity			
Fixed effect	Estimate	SE	z-value	p-value	Estimate	SE	z-value	p-value	Estimate	SE	z-value	p-value
Intercept	1.11	0.15	7.28	<0.001a	1.32	0.13	10.23	<0.001 a	0.05	0.15	0.36	0.72a
A. arvensis	0.05	0.23	0.22	0.83ab	-0.41	0.28	-1.90	0.06 a	-0.53	0.23	-2.27	0.02a
A. integrifolia	0.22	0.21	1.05	0.29ab	-0.11	0.19	-0.57	0.57 ab	-0.32	0.22	-1.49	0.14a
C. capillaris	0.12	0.23	0.54	0.59ab	0.11	0.20	0.54	0.59 ab	-0.04	0.23	-0.19	0.85a
C. maculatum	1.01	0.30	3.40	0.001b	0.35	0.28	1.27	0.20 ab	0.36	0.35	1.04	0.30a
J. montana	-0.14	0.25	-0.56	0.57ab	-0.24	0.22	-1.09	0.27 ab	-0.42	0.24	-1.78	0.07a
M. sylvestris	0.59	0.23	2.59	0.01ab	0.43	0.20	2.12	0.03 ab	0.20	0.24	0.83	0.40ab
T. repens	0.56	0.25	2.26	0.02ab	0.02	0.23	0.11	0.92 ab	-0.09	0.27	-0.34	0.73a
E. olivina	0.65	0.22	2.96	0.003ab	0.65	0.19	3.46	0.001 b	0.33	0.24	1.36	0.17ab
S. oleae	0.60	0.21	2.86	0.004ab	1.68	0.17	10.11	<0.001 c	1.41	0.22	6.34	<0.001c
Honey	-0.12	0.23	-0.52	0.60ab	1.75	0.17	10.56	<0.001 c	1.20	0.21	5.78	<0.001bc

4.3.2.3. Oviposition period and lifetime fecundity as a function of longevity

The GLMs showed that the oviposition period was significantly prolonged with the longevity in females fed with *M. sylvestris*, *E. olivina*, *S. oleae* and honey (Fig. 4.3, Table 4.4). The lifetime fecundity significantly decreased with longevity with the *C. maculatum* treatment (Fig. 4.4, Table 4.4).

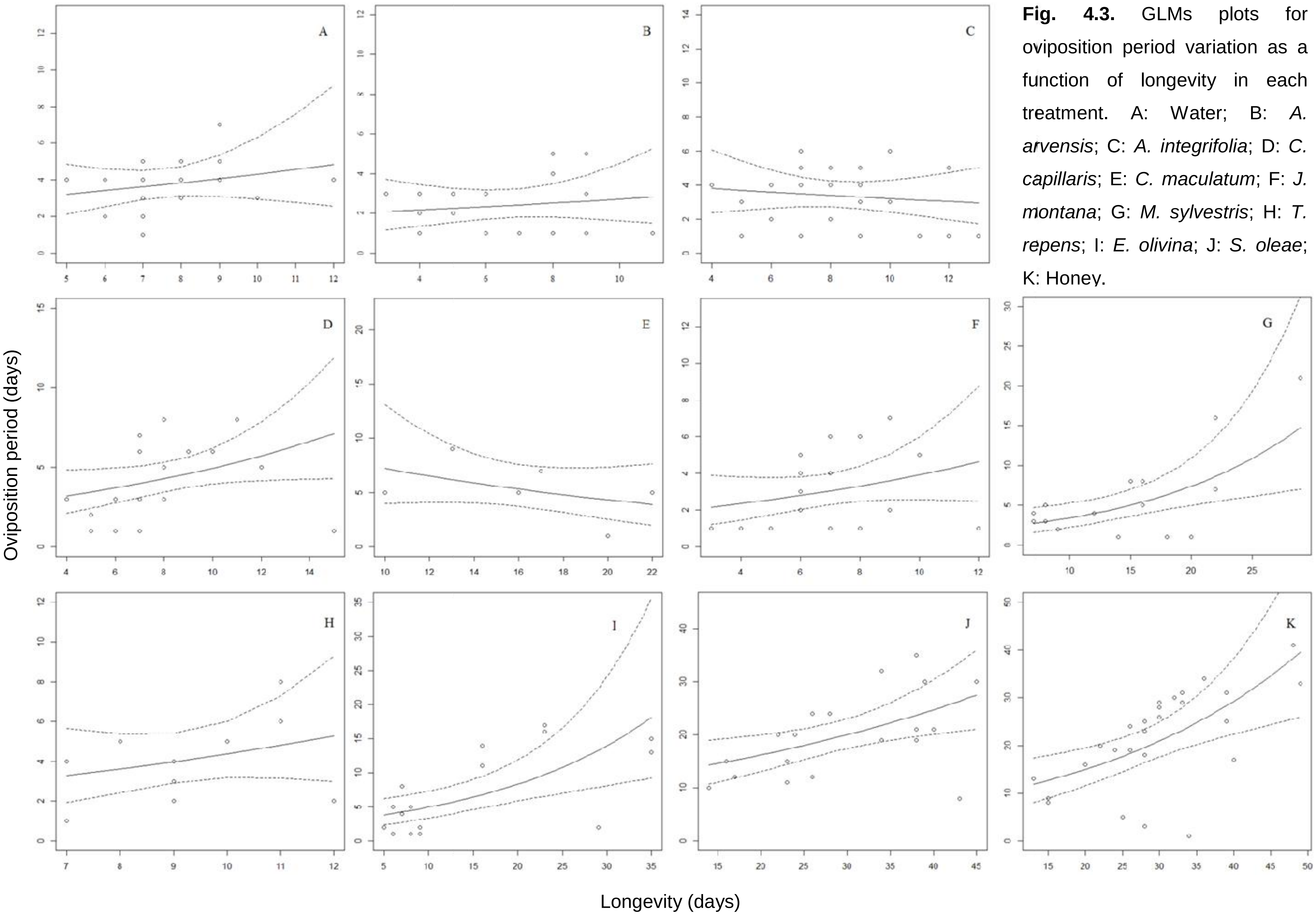

Fig. 4.3. GLMs plots for oviposition period variation as a function of longevity in each treatment. A: Water; B: *A. arvensis*; C: *A. integrifolia*; D: *C. capillaris*; E: *C. maculatum*; F: *J. montana*; G: *M. sylvestris*; H: *T. repens*; I: *E. olivina*; J: *S. oleae*; K: Honey.

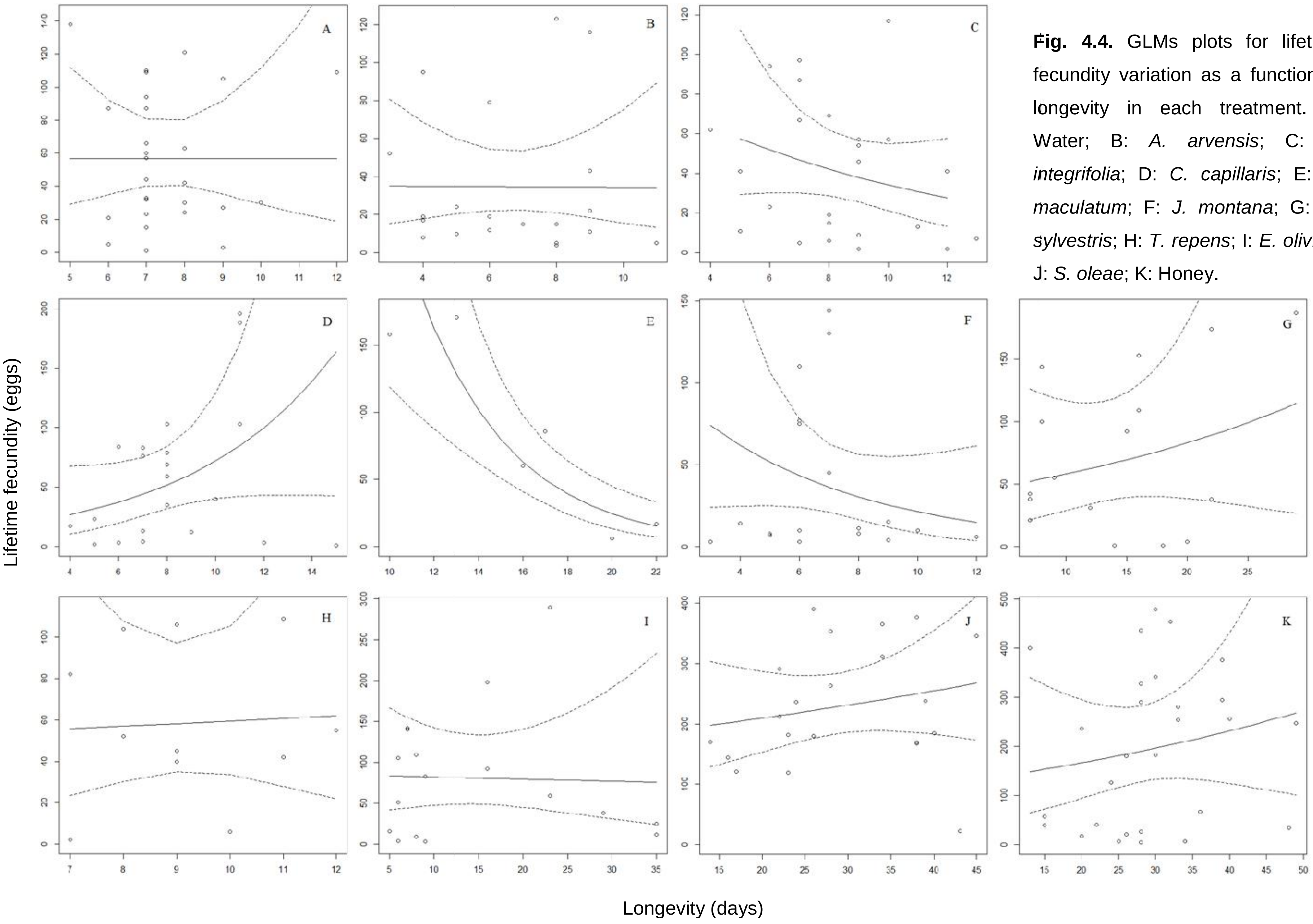

Fig. 4.4. GLMs plots for lifetime fecundity variation as a function of longevity in each treatment. A: Water; B: *A. arvensis*; C: *A. integrifolia*; D: *C. capillaris*; E: *C. maculatum*; F: *J. montana*; G: *M. sylvestris*; H: *T. repens*; I: *E. olivina*; J: *S. oleae*; K: Honey.

Table 4.4. GLMs outputs for estimated regression parameters and standard errors of oviposition period variation and lifetime fecundity as a function of longevity in each treatment. 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.

		Oviposition period				Lifetime fecundity			
	Fixed effect	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

4.4. 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 anthophagous generation of the olive moth. The selected plants bloom at the middle/end of spring and usually occur within and/or around olive orchards. In this period, *S. oleae* is also in advanced development stages that coincide with the most abundant honeydew production (Pereira 2004) and *E. olivina* is mainly in juveniles that produce abundant honeydew droplets.

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 sugar 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 the better *P. oleae* survival and reproduction with the former. The open corolla of *M. sylvestris* and *C. maculatum* flowers facilitate insects' nectar consumption. *T. repens* produces a high quality nectar and is highly attractive to pollinators (Jackobsen and Kristjansson 1994), however the Fabaceae flower architecture may not allow *P. oleae* properly reach the nectaries.

Nutritional suitability depends on the food resource composition and the insect capability to process these components (Wäckers 2005). The nectar main components are water and the sugars sucrose, glucose and fructose, although contain other minor components (monosaccharides, disaccharides, oligosaccharides, amino acids and proteins, ions, antioxidants, lipids, terpenoids, secondary compounds as toxic compounds and cytoplasmic remnants) (Kevan and Baker 1983; Pacini and Nicolson 2007). The honeydew produced by homopteran insects is the excretory product from the excess sugar and water in their diet (Pacini and Nicolson 2007). Honeydew differs from nectar given that contains oligosaccharides synthesized by the insects from the dietary sugars (Wäckers 2000; Wäckers 2001; Pacini and Nicolson 2007). Oligosaccharides in homopteran honeydews are thought to be involved in osmoregulation functions (Wäckers 2000; Byrne et al. 2003), but also in evasive strategies to avoid honeydew consumption from parasitoids and non-mutualism predators (Wäckers 2001). Wäckers (2001) found that some common oligosaccharides in honeydews but not in nectar reduced the lifespan of *Cotesia glomerata* (L.). In increasing order of lifespan reduction, these oligosaccharides were: erlose, melezitose, trehalose and raffinose. However, in Lepidoptera few studies have been accomplished to elucidate the role or individual sugars in their performance, but for example melezitose and melibiose had a negative effect in *Pieris brassicae* L. lifespan, while sucrose, fructose and glucose had a positive effect (Romeis and Wäckers 2002). 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 longevity differences were found among insects fed on honeydews and insects fed on sucrose 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 homopteran 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) and the parasitoids *Psyttalia humilis* (Silvestri) and *Scutelista caerulea* (Fonscolombe). Furthermore, the longevity was as long when fed with black scale honeydew as with clover honey. The predator *Chrysoperla carnea* (Steph.) also feed on *S. oleae* honeydew during its adult phase (Sheldon and McLeod 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 the males and females survival, the oviposition period and the daily fecundity with respect to the rest of the treatments. Moths fed with *E. olivina* honeydew presented also high values in these parameters, being better than the flowers in the most of the cases. *S. oleae* and *E. olivina* growing on olive trees probably do not produce honeydews with detrimental components for the olive moth. The survival and reproduction improvements of the olive moth with the honeydew in relation to the flowers could be due to a better concentration of fructose, sucrose and glucose or to the presence of other beneficial components. Accordingly to Wäckers (2001), evolution would favor sugars that reduce the nutritional and kairomonal suitability of honeydews when natural enemies of the insect producing honeydew vary in their responses to different honeydew sugars. In olive orchards, the *P. oleae* feeding on *S. oleae* or *E. olivina* honeydews probably does not affect positively or negatively to these homopterans populations. Thus, there is not an apparent reason for the evolution favors low honeydew nutritional value for *P. oleae*. Moreover, it could be possible that *P. oleae* would be naturally adapted to feed on these honeydews. The olive tree canopy is at the same time the place where *P. oleae* adults oviposite and larvae feed, and where *S. oleae* and the *E. olivina* develop. This may make feeding on the honeydews more profitable in terms of energy by saving foraging travel costs.

Bogg (1997) indicated four lepidopteran categories according to the importance of the adult diet quality to the proportion of eggs mature at adult emergence. Adults from the A category do not feed, emerge with the eggs already 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) 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. 4.4, Table 4.4) and the moths did not laid eggs just after emergence. This suggests that *P. oleae* females may be synovigenic, emerging with no mature eggs. According to Boggs (1997) they would feed on nectar, and in the case of the olive moth, likely in insects' honeydews as well.

In this study, even females supplied only with water laid eggs suggesting that females already emerge with nutritional reserves. This would allow them to mature a minimum of eggs without feeding. Moreover, some of the treatments with the better survival performance (*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 males were in general more prejudiced under the treatments with poorer nutritional value, suggesting a better nutritional status for 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).

The egg production with *C. maculatum* was less constant and presented the lowest percentage of fertile couples, being that only 6 females laid eggs. In this case, the lifetime fecundity even decreased with longevity and it was the only treatment that originated a longer ovipostition period than the water treatment. *C. maculatum* is one the most poisonous plants for many organisms due to the alkaloids production (Vetter 2004). Lepidoterans 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 signify longer *P. oleae* oviposition periods with higher nutritional value of foods.

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 to be a better food resource than honeydew in laboratory experiments, and 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 Lepidoptera *Plutella xylostella* (L.) and its parasitoid *Diadegma semiclausum* (Hellen) but both insects were shown to increase their average overall sugar content in flowering margins (Winkler et al. 2009a; 2009b). In our case: i) *P. oleae* may not travel frequently from the trees 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 the olive trees canopies; ii) food resources, that isolated, do not to have effect in laboratory, complemented with other resources present in the field, could improve the *P. oleae* performance. In caged experiments, insects could be deprived of some essential nutrients and mask the real effect of the tested food resource; iii) Intra and interspecific 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 the *P. oleae* approximation to *S. oleae* honeydews; iv) in caged experiments, the travel energy costs for 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 to the parasitoid *Aphidius ervi* Haliday longevity, and excised flowers present some advantages in laboratory experiment relate 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 orchards from the northeast of Portugal. In general, Homopteran honeydews were better food resources than flowers. *S. oleae* honeydew originated a better performance, probably due to its lower viscosity. Among the flowers, *M. sylvestris* caused the better 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 field seem to highly attract natural enemies (unpublished observation). In the light of this study results, we suggest that *P. oleae* females are synovigenic, emerging with no mature eggs and with reserves for reproduction. Finally, with high nutritional value foods *P. oleae* increased its survival, fecundity and oviposition period. We highly recommend accomplishing further researches before maintaining, enhancing or introducing these resources in biological control strategies in order to confirm their effect on *P. oleae* in fields.

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4.5. References

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CHAPTER 5

Effect of ground cover management on *Prays oleae* (Bernard) and its parasitoids

Effect of ground cover management on *Prays oleae* (Bernard) and its parasitoids

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Abstract

Spontaneous ground covers may be used as ecological infrastructures to provide food, alternative hosts and shelters for parasitoids in olive groves, contributing to the biological control of pests. This study investigated the effect of herbicide application, tilling, and conservation of spontaneous ground covers on the anthophagous generation of the olive moth, *Prays oleae* (Bernard), and its parasitism. The study was performed in 2011 and 2013 in 14 and 15 olive groves, respectively, with different management types. Generalized estimation equations (GEE) were used to analyze the olive moth emergence, the overall parasitism rate, the most abundant parasitoid species and the number of parasitoid specimens emerged by each olive moth larvae. *Ageniaspis fuscicollis* (Dalman) accounted for the majority of the parasitism, followed by *Elasmus flabellatus* (Fonscolombe). In both years, ground cover management type did not influence the emergence rate of *Prays oleae*. However, results were different for the overall parasitism rate, the emergence of *A. fuscicollis* and number of *A. fuscicollis* emerged by each olive moth larvae, in each study year. In 2011, those variables were significantly higher in groves with spontaneous ground covers than treated with herbicide, showing a negative effect of herbicides on parasitoids and in 2013, tilled groves obtained higher values for the above mentioned variables and this could be related with the landscape heterogeneity that characterizes the studied region.

Key words: Conservation biological control, olive grove, non-crop vegetation, *Ageniaspis fuscicollis*, *Elasmus flabellatus*

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CHAPTER 5

5.1. Introduction

Habitat management through the establishment and maintenance of ecological infrastructures, such as diversified ground covers, is a strategy of conservation biological control that aims to conserve or manipulate the environment in order to enhance the effectiveness of natural enemies (Landis et al. 2000; Boller et al. 2004). Regarding parasitoids, nectar and pollen from flowers are essential foods for many adults (Jervis et al. 1993, Vattala et al. 2006). Flowers can promote the abundance and longevity of parasitoids as well as the parasitism rate (Díaz et al. 2012) by providing them with food resources, alternative hosts for generalist parasitoids and shelter (Landis et al. 2000), but may also benefit pests (Baggen and Gurr 1998; Lavandero et al. 2006).

The olive grove has a relevant economic, social and landscape importance in the Mediterranean area where the olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae), is considered one of the most important olive pests. *P. oleae* develops three generations per year: the phyllophagous that feeds on the olive leaves from October to April, the anthophagous that feeds on floral buttons from April to June, and the carpophagous, that penetrates the fruit and feeds on the stone from June to October. The carpophagous generation causes the major damage to the crop (Bento et al. 2001). Several generalist and specific parasitoids wasps, such as *Ageniaspis fuscicollis* (Dalman) (Hymenoptera: Encyrtidae), *Chelonus elaeaphilus* Silvestri (Hymenoptera: Braconidae) and *Elasmus flabellatus* (Fonscolombe) (Hymenoptera: Eulophidae), attack the olive moth (Bento et al. 1998; Herz et al. 2005).

In perennial agroecosystems spontaneous vegetation can be removed through farming practices such as tillage or herbicide application. Soil erosion and pollution are two consequences of those practices that could also influence parasitoid communities (Vanwalleghem et al. 2011; Egan et al. 2014). Previous studies carried out in olive groves showed that spiders, parasitoids and the predatory Heteroptera species *Deraeocoris punctum* (Rambur) were positively influenced by ground covers when compared with tilled groves (Lousão et al. 2007; Herz et al. 2005; Cárdenas et al. 2012; Rodriguez et al. 2012; Paredes et al. 2013a). However, results obtained for olive pests were inconsistent. Paredes et al. (2013b) found that ground covers were associated with the reduction of abundance of two olive pests, *P. oleae* and *Euphyllura olivina* (Costa), but a long term analysis at a regional scale performed by Paredes et al. (2015) showed that ground covers did not influence the abundance of *Bactrocera oleae* (Rossi), *P. oleae*, *E. olivina* and *Saissetia oleae* (Olivier). Both local scale factors, such as the intensity of pesticide application or micro-climatic features, and landscape scale factors, such as the landscape diversity or the patch size can affect pests in olive groves (Rodriguez et al. 2009; Boccaccio and Petacchi 2009; Ortega and Pascual 2014).

In a sustainable agriculture perspective, studies are needed to establish the most appropriate management practices considering factors such as the biological control of pests. In this context, the objective of this work was to study the effect of different management practices (spontaneous ground cover conservation, tillage, and herbicide application) on parasitoids emerged from *P. oleae*. In particular, we hypothesized that farming practices influence: (i) the olive moth emergence rate (ii) the parasitoid community composition and (iii) the parasitism rate.

5.2. Material and methods

5.2.1. Study sites and sampling design

The studied groves are located around Mirandela municipality (northeast of Portugal) (Fig. 5.1), a broad and heterogeneous olive grove area. Fifteen groves with different ground cover management practices were selected in 2011 (six tilled olive groves, five groves with spontaneous ground cover and four olive groves with herbicide application) and 14 groves were selected in 2013 (five tilled groves, five groves with spontaneous ground cover and four groves with herbicide application) (Table 5.1). Groves were selected with a spatial random distribution according to the different management practices in order to minimize the spatial dependency. The mean area of these groves is about 2 ha, they are not irrigated and no pesticides were applied during the anthophagous generation of the olive moth. The herbicide used was glyphosate. In 2012, sampling was not possible due to the low population levels of olive moth, caused probably by the extreme drought and abnormally high temperatures that occurred during the anthophagous generation.

To ensure a heterogeneous distributed sampling within each grove, 10 olive trees were randomly selected and 20 larvae of the anthophagous generation of the olive moth were hand-collected in each tree at the end of May. A total of 200 larvae were collected in each grove. In the laboratory, larvae were transferred into tubes and placed in a climatic chamber (21ºC and 16:8 h L:D) until emergence. Adult olive moths and parasitoids emergence was recorded. Parasitoids were identified and sexed.

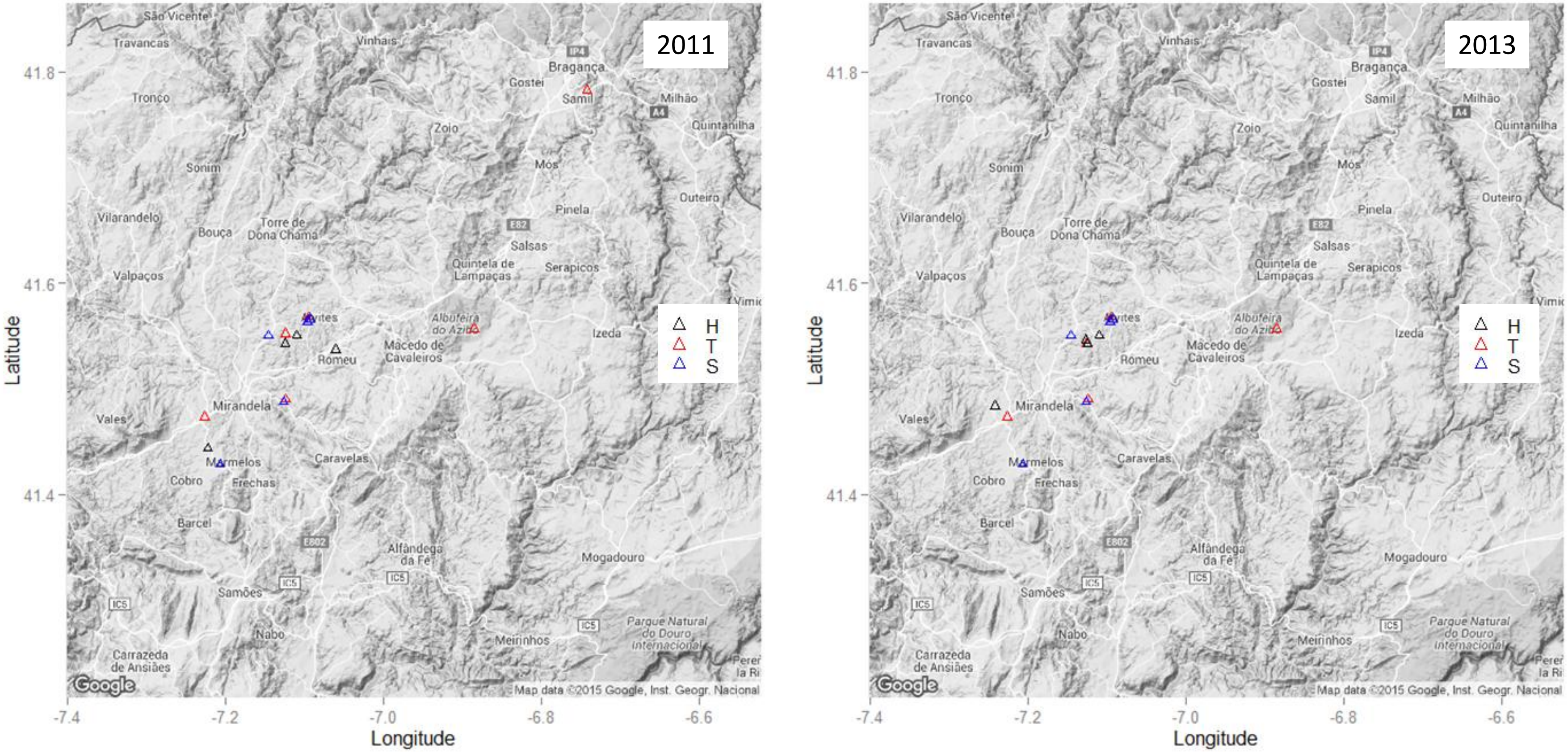

Fig. 5.1. Location of the sampled groves. A: 2011; B: 2013. H: Herbicide application; T: Tillage; S: Ground cover.

Table 5.1. Geographic coordinates, sampling year and management type associated with each olive grove.

Olive grove	Geographic coordinates	Sampling year	Spontaneous ground cover	Herbicide	Tillage
São Pedro	41°25'44.19"N; 7°12'22.70"W	2011/2013	yes		
Cedães 1	41°29'16.92"N; 7° 7'31.95"W		yes		
Valbom	41°33'2.19"N; 7°8'41.21"W		yes		
Alvites 1	41°33'55.21"N; 7°5'40.29"W		yes		
Alvites 2	41°33'57.46"N; 7°5'35.70"W		yes		
Aeródromo	41°28'24.70"N; 7°13'30.96"W				yes
Cedães 2	41°29'25.69"N; 7°7'24.35"W				yes
Paradela 1	41°32'35.56"N; 7°7'26.28"W			yes	
Paradela 2	41°33'2.42"N; 7°6'31.70"W			yes	
Alvites 3	41°34'4.13"N; 7°5'38.76"W				yes
Salselas	41°33'24.36"N; 6°53'6.07"W				yes
Samil	41°46'58.48"N; 6°44'29.55"W	2011			yes
São Pedro	41°26'37.63"N; 7°13'18.02"W			yes	
Romeu	41°32'14.19"N; 7°3'35.58"W			yes	
Paradela 3	41°33'7.80"N; 7°7'24.52"W				yes
Paradela 4	41°32'35.62"N; 7°7'30.87"W	2013			yes
Sucães	41°29'1.41"N; 7°14'28.62"W			yes	
Paradela 5	41°32'47.45"N; 7°7'32.65"W			yes	

5.2.2. Data Analysis

Generalized Estimation Equations (GEE) were used to fit the response variables. Generalized estimating equations (GEE) are a tool for the analysis of correlated non-normally distributed response variables. A major advantage of GEE is that they can cope with misspecifications of the entire distribution and only require the main structure. Correct inference about regression coefficients is possible even if variances and correlations are erroneously specified (Ziegler and Vens 2010). The explanatory variable, X_{is}, was ground cover management with three levels: tilled groves (T), groves with spontaneous ground cover (S) and groves treated with herbicide application (H).

The olive moth adult emergence, overall parasitoid emergence and the most abundant parasitoid species response variables are binary, with value 1 for success and 0 for failure. The variance structure is binomial type and the relationship between the conditional mean and the systematic component is logit link, therefore,

$$E\ (Y_{is}|\ X_{is}) = e^{\alpha+\beta 1 X_{is}} / \ 1+e^{\alpha+\beta 1 X_{is}}$$

or

$$E\ (Y_{is}|\ X_{is}) = \pi_{is} \text{ and } var\ (Y_{is}|\ X_{is}) = \pi_{is} \times (1 - \pi_{is}),$$

where Y_{is} the value of response variable where i=1,...,200 larvae and s the grove and π_{is} the probability of success of the response variable (Zuur et al. 2009).

Exchangeable correlation structure was used because correlation between two observations from the same grove is expectable. The scale parameter was fixed to 1 because binary data cannot be overdispersed.

The number of parasitoids emerged from the olive moth larvae (separately analyzed for the most abundant parasitoid species) are count data. The variance structure is Poisson type and the relationship between the conditional mean and the systematic component is log link, therefore,

$E\ (Y_{is} | X_{is}) = e^{\alpha+\beta 1 X_{is}}$

or

$E\ (Y_{is} | X_{is}) = \mu_{is}$ and $\mathrm{var}\ (Y_{is} | X_{is}) = \phi \times \nu(\mu_{is})$

where ν() is the variance function and ϕ the scale parameter. Also in this case exchangeable correlation structure was used. Some outliers were eliminated to minimize heterogeneity in the models residuals.

Data analyses were performed using R software (R Core Team 2014). *geeglm* function from geepack package (Højsgaard et al. 2006) was used. *anova* function from *stats* package was applied to assess significantly differences between management levels and followed by pairwise comparison with *lsmeans* function from lsmeans package (Lenth et al. 2015).

The model validation for the binary dependent variable models was performed using the heat map plot and heat map statistics using *heatmapFit* package (Esarey et al. 2014). In the heat map plot our model predicted probabilities are plotted versus an in-sample empirical frequencies (obtained from non parametric smoothing) and a heat map line is drawn. Then one-tailed p-value is obtained from comparing the original heat map line with its parametrically bootstrapped distribution (obtained by the simulation of 1000 draws of the response variable from the fitted model). If more than 20% of the p-values of observations on the heat map line are less than or equal to 0.1, the specification is rejected. Otherwise, it is accepted (Esarey and Pierce 2012; Esarey and Du 2014). In the present work the heat map statistics indicated that 0% of in-sample predictions have a bootstrapped $p < 0.1$ for all models, being therefore accepted.

Graphic model validation was performed to assess the models for the count dependent variables following Zuur et al. (2009). Residual were plotted against fitted values to identify violation of homogeneity. Residuals were plotted against the explanatory variable ground cover management. Histograms of the residuals were plotted to assess the normality of the residuals.

5.3. Results

The emergence of olive moth adults increased from 2011 to 2013 (Fig. 5.2A, 5.2B), although no differences were found among management practices in both years (2011: $\chi^2 = 5.04$, df = 2, $p = 0.08$; 2013: $\chi^2 = 4.44$, df = 2, $p = 0.11$). In 2011, the emergence of olive moth adults increased from tilled groves ($\pi_T = 0.156$) to groves with spontaneous ground covers ($\pi_s = 0.160$) and treated with herbicide ($\pi_H = 0.205$). In 2013 the emergence of olive moth adults were higher for groves with spontaneous ground covers ($\pi_s = 0.696$) followed by groves treated with herbicide ($\pi_H = 0.685$) and tilled ($\pi_T = 0.548$).

Regarding the overall parasitism rate, differences among managements were observed in both years (Fig. 5.2C, 5.2D). In 2011, the parasitism rate was higher in olive groves with spontaneous ground covers ($\pi_s = 0.500$) than in tilled groves ($\pi_T = 0.412$) or in groves treated with herbicide ($\pi_H = 0.362$); significant statistical differences were found between groves with spontaneous ground covers and those treated with herbicides ($\chi^2 = 8.91$, df = 2, $p = 0.012$), and in 2013 the highest value of parasitism was estimated for the tilled groves ($\pi_T = 0.129$), that did not significantly differ from groves treated with herbicide ($\pi_H = 0.050$) but differed from groves with spontaneous ground covers ($\pi_s = 0.045$) ($\chi^2 = 7.15$, df = 2, $p = 0.028$).

In both years, the most abundant parasitoid emerged from olive moth larvae was *A. fuscicollis* that followed the same pattern observed for the overall parasitism (Fig. 5.2E, 5.2F). In 2011, the estimated probability for this parasitoid was $\pi_s = 0.378$ in groves with spontaneous ground covers followed by tilled groves ($\pi_T = 0.252$) and groves treated with herbicide ($\pi_H = 0.194$) showing significant statistical differences between groves with spontaneous ground covers and groves treated with herbicide ($\chi^2 = 9.23$, df = 2, $p = 0.0099$). In 2013, the estimated probability in tilled groves ($\pi_T = 0.113$) did not significantly differ from groves treated with herbicide ($\pi_H = 0.040$) but differed from groves with spontaneous ground cover ($\pi_s = 0.032$) ($\chi^2 = 6.92$, df = 2, $p = 0.031$).

The second most abundant species of parasitoids emerging from the olive moth larvae was *E. flabellatus*. The estimated probabilities for this species decreased from 2011 to 2012 (Fig. 5.2G, 5.2H). In 2011, they were higher in olive groves treated with herbicide ($\pi_H = 0.152$) followed by tilled groves ($\pi_T = 0.142$) and groves with spontaneous ground covers ($\pi_s = 0.115$) and in 2013 they were higher in groves with spontaneous ground covers ($\pi_s = 0.014$) followed by tilled groves ($\pi_T = 0.012$) and groves treated with herbicide ($\pi_H = 0.01$) but these differences were not significant (2011: $\chi^2 = 0.922$, df = 2, $p = 0.63$; 2013: $\chi^2 = 0.466$, df = 2, $p = 0.79$).

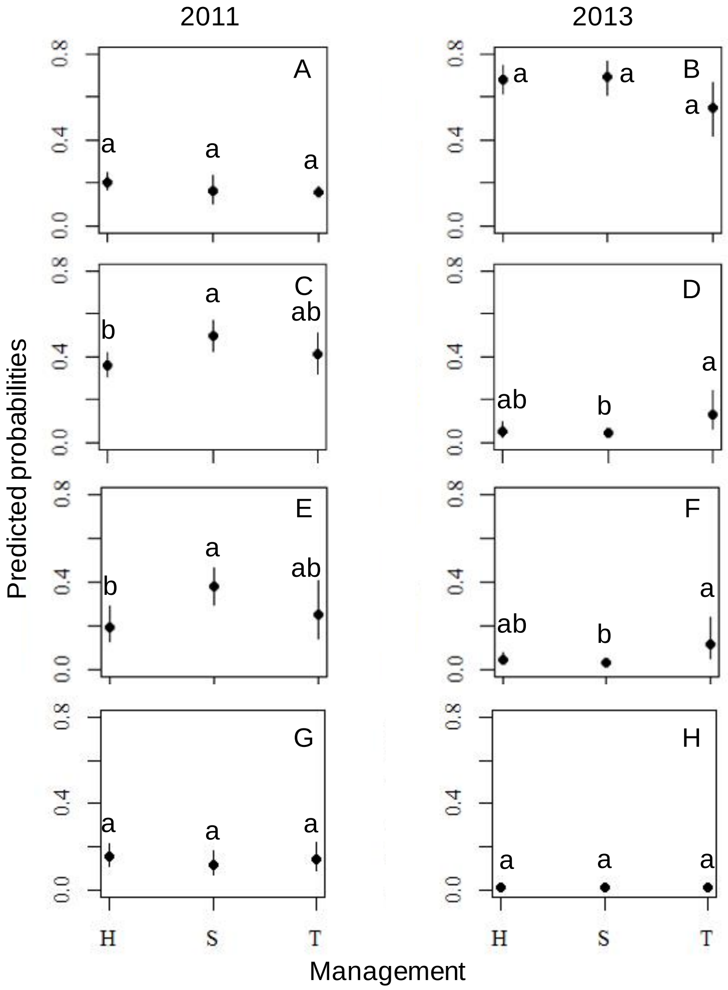

Fig. 5.2. Plotted Generalized Estimation Equations for the predicted probabilities by management type of: A and B: emergence of the olive moth; C and D: overall parasitism rate; E and F: *Ageniaspis fuscicollis* emerged; G and H: *Elasmus flabellatus* emerged. The dots are the fitted values and the bands the 95% confidence intervals. H: Herbicide application; T: Tillage; S: Ground cover.

The mean number of *A. fuscicollis* emerged from each *P. oleae* larvae in 2011 (Fig. 5.3A, 5.3B) was higher in groves with spontaneous ground covers ($\mu_s = 13.96$) followed by tilled groves ($\mu_T = 12.71$) and groves treated with herbicide ($\mu_H = 11.83$); significant differences were found between groves with spontaneous ground covers and groves treated with herbicide ($\chi^2 = 12$, df = 2, $p < 0.0025$). In 2013 the higher mean number of *A. fuscicollis* emerged from each *P. oleae* larvae was estimated for the groves treated with herbicide ($\mu_H = 12.38$) followed by the groves with spontaneous ground covers ($\mu_s = 11.94$) and tilled groves ($\mu_T = 11.56$) but no significant differences were found between management practices ($\chi^2 = 0.462$, df = 2, $p = 0.79$).

The mean number of *E. flabellatus* emerged from *P. oleae* larva in 2011 (Fig. 5.3C) was higher in olive groves treated with herbicide ($\mu_H = 2.30$) followed by tilled groves ($\mu_T = 2.12$) and groves with spontaneous vegetation ($\mu_S = 2.04$) but no significant statistical differences were found among managements ($\chi^2 = 2.36$, df = 2, $p = 0.31$). In 2013, due to the low parasitation by *E. flabellatus*, it was not possible to elaborate a model concerning the number of *E. flabellatus* emerged by parasitized moth.

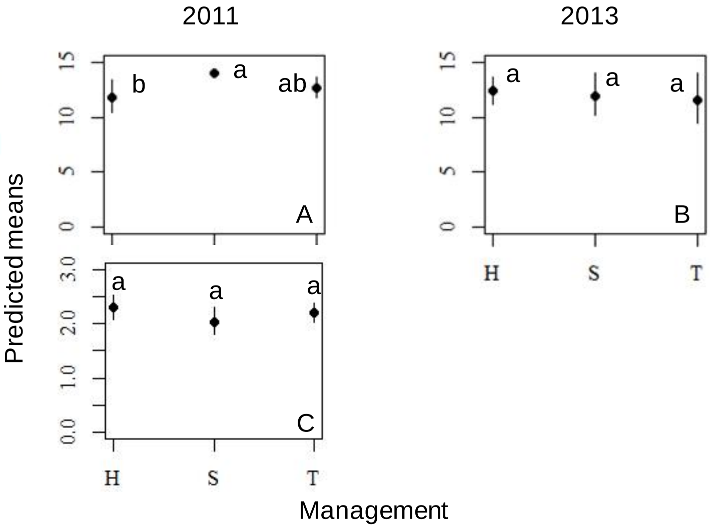

Fig. 5.3. Plotted Generalized Estimation Equations for the predicted means of parasitoids emerged per parasitized moth by management type: A and B: *Ageniaspis fuscicollis*; C: *Elasmus flabellatus*. The dots are the fitted values and the bands the 95% confidence intervals. H: Herbicide application; T: Tillage; S: Ground cover.

In 2011, the estimated probability of olive moth females (Fig. 5.4A) was higher in groves with spontaneous vegetation ($\pi_S = 0.559$) than in tilled groves ($\pi_T = 0.524$) and groves treated with herbicide ($\pi_H = 0.446$) and in 2013 (Fig. 5.4B) it was higher in groves treated with herbicide ($\pi_H = 0.490$) that in tilled groves ($\pi_T = 0.478$) and groves with spontaneous vegetation ($\pi_S = 0.465$) but these difference were not significant (2011: $\chi^2 = 5.29$, df = 2, $p = 0.071$; 2013: $\chi^2 = 0.513$, df = 2, $p = 0.77$).

The estimated probabilities for *E. flabellatus* females in 2011 (Fig. 5.4C) were higher in groves with spontaneous vegetation ($\pi_S = 0.764$) than in tilled groves($\pi_T = 0.732$) and in groves treated with herbicide ($\pi_H = 0.701$) but these difference were not significant ($\chi^2 = 2.5$, df = 2, $p = 0.29$). This model residuals are more negative than positive, indicating that it could be over predicting the proportion of females. But it not shows heterogeneity. In 2013, due to the low parasitation by *E. flabellatus* was not possible to elaborate a model concerning the proportion of *E. flabellatus* females emerged by parasitized moth.

Other parasitoids emerged in low numbers in both years. In 2011, these less common taxa were found mostly in tilled groves and seven taxa were identify: *Apanteles xanthostigma* (Haliday) (H: 2, S: 9, T: 11); Chalcididae (H: 11, T: 2), *Pnigalio* sp. (T: 1); *Angitia armillata* Grav.(T: 2), Ichneumonidae (T: 1); Pteromelidae (T: 2), *Chelonus elaeaphilus* Silvestri (T: 2, H: 1), and in tilled groves 2 parasitoids were not identified. In 2013 two taxa were identified: *A. xanthostigma* (T: 1, H: 1), *C. elaeaphilus* (T: 2, H: 1) and few specimens were not identified (H: 1, S: 1, T: 2).

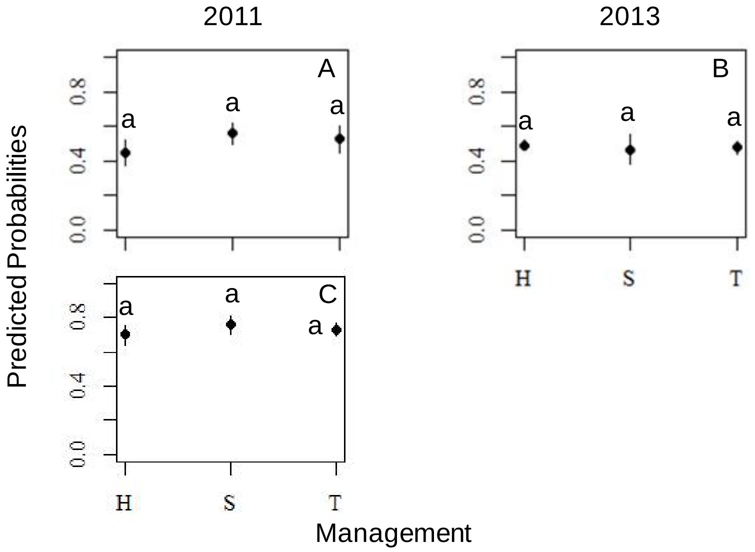

Fig. 5.4. Plotted Generalized Estimation Equations for the predicted probabilities of female proportion by management type of: A and B: *Prays oleae*; C: *Elasmus flabellatus*. The dots are the fitted values and the bands the 95% confidence intervals. H: Herbicide application; T: Tillage; S: Ground cover.

5.4. Discussion

In this study, *A. fuscicollis* was the most abundant parasitoid species emerged from *P. oleae* larvae of the anthophagous generation which is in agreement with the general pattern found in the Mediterranean region by Campos and Ramos (1982), Bento et al. (1998), Herz et al. (2005) and Rodriguez et al. (2012). Bento et al.(1998) and Herz et al. (2005) described *C. eleaphilus* as the second and sometimes the most abundant parasitoid species, but in this study only few individuals emerged. These authors also found a more diverse parasitoid complex and *E. flabellatus* as having less importance than in the present work.

Our results suggest that the effect of management practices on the overall parasitism rate, the parasitism rate by *A. fuscicollis* and the number of *A. fuscicollis* emerged from each parasitized olive moth larvae can change with the sampling year. Spontaneous ground covers favored those variables in 2011, but the pattern changed in 2013 and tilled groves showed higher values. From 2011 to 2013, the emergence rate of the olive moth increased while the parasitism rate decreased. Weather conditions, such as drought, can influence this variation between years affecting both the olive moth and, consequently, its parasitoids (Montiel Bueno 1981; Campos and Ramos 1982;

Paredes et al. 2013b). In 2011, the levels of parasitism rate were high and contributed to a lower level of emergence of the olive moth, likely decreasing its population in field. We only analyzed the effects on the anthophagous generation, but pest level reduction due to parasitism might be mainly noticeable in the carpophagous generation because *A. fuscicollis* has a great synchronism with the olive moth (Campos and Ramos 1982), and a high abundance of *A. fuscicollis* in groves could have contributed to reduce the carpophagous generation. Corroborating this idea is the fact that *A. fuscicollis* was found to be an important parasitoid of the carpophagous generation parasitizing until 55% of the larvae (Bento et al. 1998).

In 2012, the low levels of the olive moth observed might have affected the abundance of parasitoids in the agroecosystem and the pest increased its population in the following year. Also, low levels of the pest could have derived in an abrupt diminution of its parasitoids that will not be represented in the beginning of 2013 and the effect of ground cover could not be observed. Paredes et al. (2013a) observed a reduction of the olive moth population (although the reduction was slight when pest population was low) mainly attributed to an indirect effect on natural enemies.

Landscape heterogeneity can also influence the effect of management practices in olive groves. In our work, the study area is surrounded by different patches of herbaceous and woody plants that could favor the abundance of parasitoids in olive groves and reduce the potential effects of tillage or herbicide application. This effect was previously observed by Paredes et al. (2013a), Rodriguez et al. (2012) and Pak et al. (2015) who found that parasitoids responded to their local environment and to the landscape in which they are embedded and Paredes et al. (2015) suggested that ground cover is not effective in reducing pest abundance when considered as a single factor. Moreover, *P. oleae* and its parasitoids could respond differently to the plant composition occurring in an agroecosystem as a consequence of flower morphology that determines the accessibility to pollen and nectar by different insects.

In 2011, the overall parasitism rate, the parasitism rate by *A. fuscicollis* and the number of *A. fuscicollis* emerged from each olive moth was lower in olive groves treated with herbicide but no differences were found both for the parasitism rate by *E. flabellatus* and the number of *E. flabellatus* emerged from each olive moth in both years. *A. fuscicollis* is a specialist parasitoid of *Prays* spp. moths (Campos and Ramos, 1982; Mineo et al. 1975) and *E. flabellatus* behaves as hiperparasitoid and can be considered as an undesirable parasitoid (Bento et al. 1998), nevertheless, in 2011 in groves treated with herbicide, this species was responsible for almost half of the overall parasitism (Fig. 5.2G). Therefore, in some conditions, *E. flabellatus* could have more importance than usually considered. Negative impacts of herbicide on reproduction and survival of parasidoids have been already described by Menezes et al. (2012). In this case, potential toxicity of herbicide on *A. fuscicollis* can also be considered.

In sum, a positive effect of spontaneous ground covers on the *A. fuscicollis* parasitism rate of the olive moth and on the number of *A. fuscicollis* emerged from each olive moth larvae, and a negative effect of herbicide application on *A. fuscicollis* parasitism was observed. Surrounding vegetation could also influence parasitoids in olive groves and reduce differences between management types.

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CHAPTER 5

CHAPTER 6

Are wild flowers and insect honeydews potential food resources for *Elasmus flabellatus*, a parasitoid of the olive moth? A conservation biological control approach

Are wild flowers and insect honeydews potential food resources for *Elasmus flabellatus*, a parasitoid of the olive moth? A conservation biological control approach

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Abstract

Adult parasitoids need non-host foods, such as nectar or honeydews for survival and reproduction. In a conservative biological control strategy, the knowledge about non-host feeding of parasitoid species is a key factor of success to increase their action. *Elasmus flabellatus* (Fonscolombe) (Hymenoptera: Eulophidae) is a parasitoid of the olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae), and its nutritional behavior in olive agroecosystem is completely unknown. In this work, the suitability of two secondary pest honeydews and 13 flower species were analyzed as potential foods for *E. flabellatus* through survival analysis in laboratory assays. 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) and *Daucus carota* L. (Apiaceae). Implications on the conservation biological control of *P. oleae* and future research approaches are discussed.

Key words: Nutritional ecology, conservation biological control, survival analysis, *Elasmus flabellatus* (Fonscolombe) (Hymenoptera: Eulophidae)

Manuscript submitted

6.1. Introduction

Insect feeding is determined by several aspects such as availability, detectability, accessibility and nutritional suitability of foods (Wäckers 2005). Identifying the main food sources exploited by pests and natural enemies in agroecosystems constitute a crucial knowledge in order to establish efficient conservation biological control strategies. Therefore, the occurrence of suitable food in agroecosystems is considered an important factor for the efficiency of natural enemies as pest control agents (Landis et al. 2000; Bianchi et al. 2006).

Adult parasitoids need energy for maintenance, locomotion and reproduction that are provided by non-host foods (Jervis et al. 1993; Jervis et al. 2008). Several studies have been conducted to determine the influence of non-host feeding (such as flowers, insect honeydews and sugar solutions), on various parasitoid species. Different traits related with survival and reproduction (e.g. potential fecundity, realized fecundity, egg load) under laboratory and field conditions were evaluated (Jervis et al. 1993; Baggen and Gurr 1998; Lee et al. 2006; Berndt and Wratten 2005; Irvin et al. 2006; Lavandero et al. 2006; Winkler et al. 2006; Lee and Heimpel 2008; Wäckers 2008; Luo et al. 2010; Winkler et al. 2010; Geneau et al. 2012; Balzan and Wäckers 2013; Beltrà et al. 2013; Belz et al. 2013; Tena et al. 2013; Zhu et al. 2015).

In the olive grove agroecosystem, the olive moth *Prays oleae* (Bernard) (Lepidoptera: Praydidae) is one of the most important pests (Arambourg 1986). It has three generations a year, the phyllophagous (feeding on leaves), the anthophagous (feeding on flowers) and the carpophagous (feeding on fruits). This pest is parasitized by several hymenopteran species; however, the information about food resources of both the olive moth and their parasitoids is scarce.

Elasmus flabellatus (Fonscolombe) (Hymenoptera: Eulophidae) is an ectophagous and gregarious parasitoid attacking larvae and pupae of Lepidoptera order (Gelechiidae, Tortricidae, Noctuidae, Yponomeutidae, Heliozelidae, Plutellidae, Psychidae, and Pyralidae families), and larvae of Hymenoptera order (Cephidae, Bethylidae, Braconidae, and Ichneumonidae families) (Yefremova and Strakhova 2010 and references therein). This is an idiobiont parasitoid, i.e., females paralyze *P. oleae* last instar larva, lay their eggs, and develop outside it (Bento 2007). This parasitoid species act as a facultative hyperparasitoid of other *P. oleae* parasitoids and the levels of parasitism on this pest were found to vary between 10% for the phyllophagous generation (Bento et al. 1998) and 19% for the anthophagous generation (Villa, unpublished data). Therefore, this species could be considered a good candidate to control *P. oleae* in the field and additional studies are needed in order to elucidate which food resources could contribute for enhancing survival and reproduction of *E. flabellatus* in the olive grove. Among the spontaneous plants occurring in agroecosystems some unexploited species could have an important role in parasitoids feeding (Araj and Wratten, 2015). 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) could have a final positive effect by feeding parasitoids and improving their performance. In this context, this work aims at studying the effect of 13 spontaneous plant species and honeydews produced by *S. oleae* and *E. olivina* on the survival of *E. flabellatus*. Most of the tested plants are common species in several European agroecosystems, occurring during the spring and beginning of the summer. The selected species belong to Apiaceae family (*Conopodium majus* (Gouan) Loret. and *Daucus carota* L.), Asteraceae (*Anthemis arvensis* L., *Andryala integrifolia* L., *Crepis capillaris* (L.) Wallr., *Coleostephus myconis* (L.) Rchb.f.,*Chrysanthemum segetum* L. and *Tolpis barbata* (L.)), Boraginaceae (*Echium plantagineum* L.), Campanulaceae (*Jasione montana* L.), Caryophyllaceae (*Spergularia purpurea* (Pers.) G. Don and Gaertn.), Hypericaceae (*Hypericum perforatum* L.) and Malvaceae (*Malva sylvestris* L.). For the first time, several potential natural foods showed to enhance *E. flabellatus* survival.

6.2. Material and methods

6.2.1. 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). In the laboratory, they were transferred into tubes (1.7 cm diameter and 12 cm high) and placed in a climatic chamber at 21ºC (± 2 ºC) and 16:8 h L:D (light: dark) until the emergence of adults.

6.2.2. Selected foods

Selected plants were: *A. arvensis*, *A. integrifolia*, *C. capillaris*, *C. majus*, *C. myconis*, *C. segetum*, *D. carota*, *E. plantagineum*, *H. perforatum*, *J. montana, M. sylvestris*, *S. purpurea* and *T. barbata*. The plant selection was based in a previous plant inventory of the flora of the olive groves in the Trás-os-Montes region (northeastern Portugal). Flowers were collected in the campus of the Polytechnic Institute of Bragança, northeastern Portugal. Honeydews produced by *S. oleae* and *E. olivina* were collected from infested small olive trees grown in climatic chambers in the laboratory.

6.2.3. Experimental design

Newly *E. flabellatus* adults were transferred into cages (2.7 cm diameter and 12 cm high) and provided with water and one of the treatments. Between 18 and 34 replicates were assembled for females. Due to the low number of emerged males, only *A. integrifolia*, *J. montana*, *D. carota* and *S. oleae* honeydew were tested. Thus, between 12 and 32 replicates were assembled in the case of males. Approximately, 4 cm^2 of flowers surface were used by treatment as well as a homogeneous amount of honeydew. The cut stems of the flowers were submerged under 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 intact flowers (Wade and Wratten 2007). Honeydews were placed on Parafilm© strips. Foods were replaced three times a week, accordingly to the flowers durability. A negative control (water) for females and males and a positive control (water-honey solution 10% (w/v)) for females were assembled. Daily mortality was recorded.

6.2.4. Data analysis

Firstly lifespan means and standard errors for females and males were calculated and plotted. Death hazard differences between treatments were checked separately by sexes using Cox's proportional hazard regression model (Cox PHM) (Equation 1)

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

Equation 1

Where $h\ (t, x)$ is the hazard function, $h_0\,(t)$ is the baseline and $exp\ (\beta_i\, x_i)$ is the death hazard ratio for each treatment (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 them. A death hazard ratio of 1 indicates that no differences exist among two treatments. A death hazard ratio < 1 indicates that a treatment has lower death risk than another comparison treatment. A hazard ratio > 1 means a higher death risk for the comparison treatment

The Cox PHM was estimated 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 $exp(\beta i)$ of the Cox PHM. The proportional hazard assumption of the Cox PHM 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.

After obtaining the Cox PHM for males and females, the Cox estimates were used for drawn the survival curves. For that, the *survfit* function using the Cox models as parent data was used.

6.3. Results

6.3.1. Lifespan

Mean lifespans, standard errors and number of replicates for *E. flabellatus* females and males are showed in Fig. 6.1. The higher females lifespan was observed under honey solution (59.08 ± 4.63 days) followed by *E. olivina* (27.96 ± 3.46 days) and *S. oleae* honeydews (19.37 ± 2.03 days). Females lifespan with all the tested flowers was lower, presenting means no higher than 10 days. The best performance with flowers was achieved under the *M. sylvestris* treatment (9.78 ± 0.68 days) followed by *D. carota* (5.31 ± 0.32 days). *A. integrifolia*, *C. capillaris*, *J. montana* and *T. barbata* flowers resulted in mean lifespans for females between 4 and 5 days. With the other flowers the mean lifespan for females was lower than 4 days being the lowest lifespan achieved with *C. majus* (3.04 ± 0.18 days) (Fig. 6.1A). Due to the low number of males, only 5 treatments were analyzed. The best performance was achieved with *S. oleae* honeydew (9.81 ± 1.19 days). The mean lifespan for males with the flowers varied between 3 and 3.5 days (Fig 6.1B).

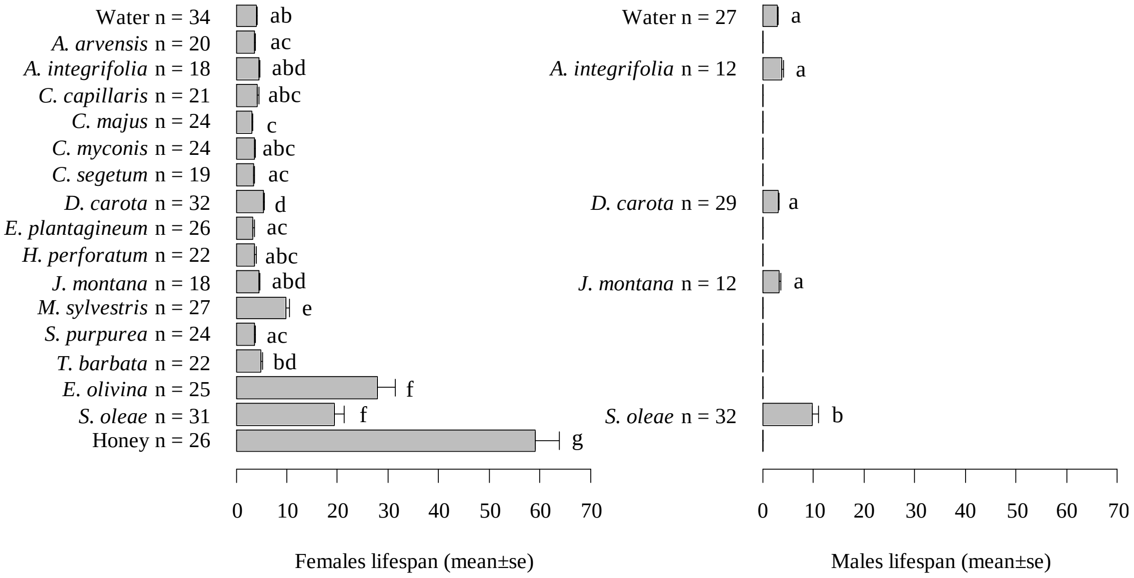

Fig. 6.1. Lifespan (mean ± se) (days) for females (A) and males (B) of *Elasmus flabellatus* under the different flowers and insect honeydews food sources. Different letters indicate significant death hazard differences among treatments (significance level < 0.05). n indicates the number of replicates per treatment.

6.3.2. Death hazard ratio by diet treatment

Cox estimates of the survival function for males and females are showed in Fig. 6.2. The Cox PHM showed that females and males hazard of death were significantly different among diet treatments (Females: χ^2 = 491, df = 16, p < 0.001; Males: χ^2 = 55.3, df = 16, p < 0.001).

Regarding to the females, the ones fed on honey solution, *E. olivina* and *S. oleae* honeydews and *M. sylvestris* and *D. carota* flowers showed a significantly lower death hazard than the ones under the water treatment. Females fed on honey solution showed the lowest death hazard, followed by the insect honeydews that did not differ among them. Following the honeydews, the lowest death hazard was caused by *M. sylvestris* and *D. carota*, being the latter significantly higher. The death hazard for females fed on *A. arvensis*, *C. segetum*, *E. plantagineum*, *S. purpurea*, *C. capillaris, C. myconis*, *H. perforatum*, *A. integrifolia*, *J. montana* and *T. barbata* did not significantly differ from the death hazard for females under the water treatment. Although hazard ratios with *A. integrifolia*, *J. montana*, *T. barbata* and did not differ significantly from water, they also did not differ from *D. carota*, being one of the flowers that caused longer lifespans. The death hazard for females fed on *C. majus* was significantly higher than the one for females under the water treatment (negative control) (Table 6.1, Fig. 6.2A).

The only treatment that caused a significantly lower death hazard for males than the negative control was *S. oleae* honeydew. The death hazard for males with the rest of the treatments (*A. integrifolia*, *D. carota* and *J. montana*) did not significantly differ from the one with water (Table 6.2, Fig. 6.2B).

6.3.3. Death hazard ratio among sexes within treatments

In general, the Cox PHM showed that males had significantly higher death hazard ratio than females (Water: Hazard ratio = 3.84, $\chi^2 = 18.4$, df = 1, $p < 0.001$; *D. carota*: Hazard ratio = 4.00, $\chi^2 = 22.2$, df = 1, $p < 0.001$; *J. montana*: Hazard ratio = 2.41, $\chi^2 = 4.76$, df = 1, $p = 0.03$; *S. oleae* honeydew*:* Hazard ratio = 3.2, $\chi^2 = 16.7$, df = 1, $p < 0.001$). *A. integrifolia* (Hazard ratio = 1.4; $\chi^2 = 0.79$, df = 1, $p = 0.37$) was the only treatment that did not show significant death hazards differences among males and females.

Table 6.1. Death hazard ratio for *Elasmus flabellatus* females, exp (β_k), for comparison group relative to reference group. The comparison group has exp (β_k) times the death hazard than the reference group: exp (β_k) = 1, means no differences among treatments; exp (β_k) < 1 means lower death risk for the comparison group; exp (β_k) > 1 means higher death risk for the comparison group. Bonferroni correction was applied (significance level < 0.05). p-values are indicated between brackets.

		Reference group															
	Diet treatment	*Anthemis arvensis*	*Andryala integrifolia*	*Crepis capillaris*	*Conopodium majus*	*Coleostephus myconis*	*Chrysanthemum segetum*	*Daucus carota*	*Echium plantagineum*	*Hypericum perforatum*	*Jasione montana*	*Malva sylvestris*	*Spergularia purpurea*	*Tolpis barbata*	*Euphyllura olivina*	*Saissetia oleae*	Honey
Comparation group	Water	0.784 (0.389)	1.619 (0.102)	1.473 (0.171)	0.440 (0.002)	0.982 (0.945)	0.701 (0.215)	2.972 (<0.001)	0.713 (0.194)	0.924 (0.772)	1.400 (0.251)	13.156 (<0.001)	0.793 (0.385)	2.251 (0.004)	144.37 (<0.001	66.396 (<0.001)	570.45 (<0.001)
	Anthemis arvensis		2.065 (0.047)	1.879 (0.047)	0.561 (0.576)	1.252 (0.461)	0.894 (0.727)	3.791 (<0.001)	0.909 (0.750)	1.178 (0.598)	1.785 (0.076)	16.78 (<0.001)	1.012 (0.970)	2.871 (<0.001)	184.14 (<0.001)	84.687 (<0.001)	727.6 (<0.001)
	Andryala integrifolia			0.910 (0.769)	0.272 (<0.001)	0.607 (<0.001)	0.433 (0.175)	1.836 (0.021)	0.44 (0.014)	0.571 (0.078)	0.865 (0.664)	8.128 (<0.001)	0.490 (0.024)	1.390 (0.302)	89.191 (<0.001)	41.02 (<0.001)	352.43 (<0.001)
	Crepis capillaris				0.299 (<0.001)	0.667 (0.175)	0.476 (0.021)	2.018 (0.014)	0.484 (0.015)	0.627 (0.128)	0.950 (0.875)	8.932 (<0.001)	0.539 (0.042)	1.528 (0.166)	98.022 (<0.001)	45.081 (<0.001)	387.32 (<0.001)
	Conopodium majus					2.231 (0.006)	1.593 (0.133)	6.753 (<0.001)	1.62 (0.09)	2.099 (0.013)	3.18 (0.001)	29.891 (<0.001)	1.802 (0.043)	5.113 (<0.001)	328.02 (<0.001)	150.86 (<0.001)	1296.1 (<0.001)
	Coleostephus myconis						0.714 (0.276)	3.028 (<0.001)	0.726 (0.261)	0.941 (0.836)	1.426 (0.256)	13.401 (<0.001)	0.808 (0.462)	2.292 (0.005)	147.06 (<0.001)	67.633 (<0.001)	581.08 (<0.001)
	Chrysanthemum segetum							4.239 (<0.001)	1.017 (0.957)	1.317 (0.381)	1.996 (0.036)	18.762 (<0.001)	1.131 (0.688)	3.210 (<0.001)	205.89 (<0.001)	94.691 (<0.001)	813.55 (<0.001)
	Daucus carota								0.240 (<0.001)	0.311 (<0.001)	0.471 (0.012)	4.426 (<0.001)	0.267 (<0.001)	0.757 (0.319)	48.572 (<0.001)	22.339 (<0.001)	191.93 (<0.001)
	Echium plantagineum									1.296 (0.373).	1.964 (0.028)	18.457 (<0.001)	1.113 (0.705)	3.157 (<0.001)	202.540 (<0.001)	93.150 (<0.001)	800.31 (<0.001)
	Hypericum perforatum										1.516 (0.192)	14.243 (<0.001)	0.859 (0.607)	2.437 (0.003)	156.300 (<0.001)	71.884 (<0.001)	617.61 (<0.001)
	Jasione montana											9.399 (<0.001)	0.567 (0.070)	1.608 (0.140)	103.140 (<0.001)	47.433 (<0.001)	407.53 (<0.001)
	Malva sylvestris												0.06 (<0.001)	0.171 (<0.001)	10.974 (<0.001)	5.047 (<0.001)	43.361 (<0.001)
	Spergularia purpurea													0.352 (<0.001)	0.005 (<0.001)	0.012 (<0.001)	0.001 (<0.001)
	Tolpis barbata														64.15 (<0.001)	29.503 (<0.001)	253.48 (<0.001)
	Euphyllura olivina															0.46 (0.007)	3.951 (<0.001)
	Saissetia oleae																8.592 (<0.001)

Table 6.2. Death hazard ratio for *Elasmus flabellatus* males, exp (β_k), for comparison group relative to reference group. The comparison group has exp (β_k) times the death hazard than the reference group: exp (β_k) = 1, means no differences among treatments; exp (β_k) < 1 means lower death risk for the comparison group; exp (β_k) > 1 means higher death risk for the comparison group. Bonferroni correction was applied (significance level < 0.05). p-values are indicated between brackets.

	Diet treatment	Reference group				
		Water	*A. integrifolia*	*D. carota*	*J. montana*	*S. oleae*
Comparation group	Water		1.640 (0.158)	1.080 (0.774)	1.262 (0.502)	9.044 (<0.001)
	A. integrifolia			0.659 (0.226)	0.770 (0.524)	55.144 (<0.001)
	D. carota				11.683 (0.651)	83.720 (0.158)
	J. montana					71.657 (0.158)

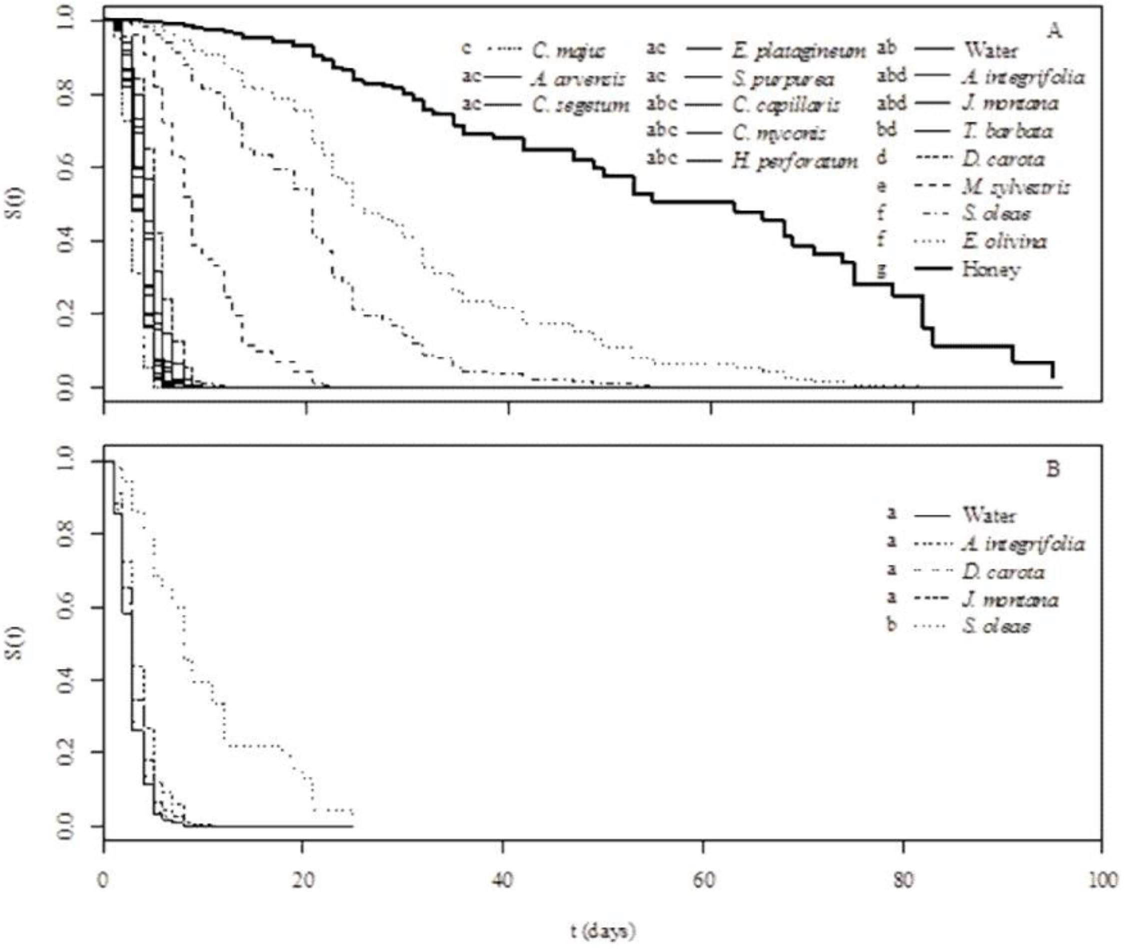

Fig. 6.2. Survival function, S(t), estimated using the Cox Proportional Hazard Models, for females (A) and males (B) of *Elasmus flabellatus*. Different letters indicate significant death hazard differences among treatments (significance level < 0.05).

6.4. Discussion

Food resources analyzed in this work are spatially and temporally coincident with *E. flabellatus* adults, which enables parasitoids feeding. Several of the analyzed food resources resulted in lower death hazards than the negative control (water). The death hazard for males was generally higher than for females, suggesting that, in general, females live longer. Redolfi and Campos (2010) also found significant longer females longevity for the *P. oleae* parasitoid, *Elasmus steffani* Viggiani when fed on honey solution or honey solution plus host larvae, but no differences were found without food.

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 (Wäckers 2000; Byrne et al. 2003), but also in evasive strategies to avoid honeydew consumption by parasitoids and non-mutualism predators (Wäckers 2001). Sugar composition of honeydews 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 *S. oleae* and *E. olivina* honeydews composition growing on olive trees. In this study, the natural foods that resulted in better reductions of *E. flabellatus* death hazard were *S. oleae* and *E. olivina* honeydews and, therefore, they seemed to be nutritionally suitable for *E. flabellatus*. Parasitoid feeding has been observed to be negatively influenced by the viscosity of sugary liquid foods (Wäckers 2000; Winkler et al. 2009; Williams III et al. 2015). In spite of the higher viscosity of *E. olivina* honeydew in comparison with *S. oleae,* females' hazard deaths were not significantly different when fed on both honeydews, suggesting that *E. flabellatus* is able to feed on viscous sugary liquids.

In a meta-analysis, Russel (2015) found that, in general, Brassicaceae and Apiaceae species tended to increase parasitoids longevity while some Asteraceae and Lamiaceae species enhanced the longevity and others did not. Plant families characterized by open flowers and free petals generally resulted in longer longevities than those with complex floral morphologies. Particularly, *D. carota* was reported to increase the longevity of several parasitoid species (Russel, 2015). Also in the field, the inflorescences of several Apiaceae, including *D. carota*, were found to be the most foraged by parasitoid species and Lamiaceae and Fabaceae (with more complex corolla) the least (Jervis et al. 1993). In our study, both *D. carota* and *M. sylvestris* flowers decreased the death hazard of *E. flabellatus* females, being that the longest lifespan observed between flowers was accomplished with *M. sylvestris*. However, *C. majus*, also belonging to Apiaceae, reached significantly shorter lifespan when compared with the negative control that could be probably related with the presence of toxic components, contrary to the general pattern found for this family. *A. integrifolia*, *J. montana* or *T. barbata* did not result in different hazard deaths from water. However, they also did not differ from *D. carota* and, when associated with other food resources (e.g. honeydew), they may result in longer lifespans. For the other tested plant species, no effects on lifespan were observed. This could be caused by: i) nutritional unsuitability, due to aspects as the lack of key nutrients or indigestible compounds; ii) inaccessibility of the food resources due to the corolla architecture; and/or iii) insufficient food production.

Accordingly to Jervis et al. (2008), only few parasitoids (Mutillidae, Scoliidae and some Bombyliidae) consume pollen. In this work, honey solution resulted in a longer lifespan than tested honeydews suggesting that honey contains components that benefit *E. flabellatus.* One hypothesis could be the presence of free aminoacids exuded from pollen into honey, a process that was observed in nectar (Erhardt and Baker 1990).

A query that arises from this research is whether the increases in longevity would trigger larger fecundities and better pest control. Generally, (i) increases in longevity improve the chances of insects to have enough time to lay all their available eggs (more likely in rich environment where the insect will not die due starvation or lack of hosts) and (ii) increases in fecundity reduces the chances of parasitoids becoming egg-limited (Jervis et al. 2008; Wade et al. 2008). For example, the increase in longevity of the parasitoid *Dolichogenidea tasmanica* (Cameron) was translated in the increase in fecundity in laboratory experiments (Berndt and Wratten 2005). Accordingly to Lane et al. (1999), the fecundity of parasitoids is positively correlated with their ability to suppress host populations in the host order Lepidoptera. Nevertheless, in field experiments this effect is not always clear and sometimes takes the opposite direction. For example, in the presence of flowers only males abundance of *D. tasmanica* increased but not females, and the relative parasitism rate of leafrollers was not significantly increased (Berndt et al. 2002). In some cases, the presence of flowering plants led to higher parasitism and less pest abundance (Irvin et al. 2006). But in others, longer longevities of females derived from sugar resources feeding did not result in higher parasitism rates (Lee and Heimpel 2008). *E. flabellatus* likely attacks larger host stages, as a general behavior quoted by Jervis et al. (2008) for idiobiont parasitoids. Accordingly to the balance mortality hypothesis, this would lead to lower mortality and would reduce the need to invest in higher fecundities early in life (Jervis et al. 2008). This type of parasitoids has longer lifespans, later reproduction and lower fecundities. They present lower ovigeny index, as a characteristic of synovigenic parasitoids, i.e. they emerge at least with some immature eggs and need to feed in order to mature the eggs (Jervis et al. 2008).

An important aspect to consider before implementing conservation biological control is that these food sources may also benefit coincident occurring pests. Lavandero et al. (2006) found that some flowering plants enhanced both the parasitoid and the herbivore fitness. Balzan and Wäckers (2013) found that flowers influenced differently both pest and parasitoid lifetables. In our case, the studied foods could also influence *P. oleae* population in the olive grove. Special attention should be given to *M. sylvestris* because its flowering peak is not only coincident with *E. flabellatus* but also with *P. oleae* flight period. Also some attention should be given to *D. carota*, although its flowering peak is slightly later (simultaneously with *E. flabellatus* flight period and during the beginning of the summer), starts to bloom during the flight period of anthophagous generation of *P. oleae*. Also, the potential benefits of honeydews as occurring simultaneously with *P. oleae*, should be evaluated with care.

Summarizing, in this study we identified several potential natural foods for *E. flabellatus* in the olive agroecosystem. The best performance was accomplished with *S. oleae* and *E. olivina* honeydews, followed by *M. sylvestris* and *D. carota* flowers. Thus, these food resources could be maintained in agroecosystems in order to establish conservation biological control strategies. However, future investigation should be focused on: i) identifying these foods nutrients and *E. flabellatus* metabolism involved to process them; ii) analyzing reproduction traits of *E. fablellatus*; iii) studying the effect of these foods on *E. flabellatus* and pest dynamics in 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; iv) identifying other potential foods (e.g. flowers or aphids honeydews) and the effect of mixture foods.

6.5. References

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CHAPTER 7

Life-history parameters of *Chrysoperla carnea s. l.* fed on spontaneous plant species and insect honeydews: importance for conservation biological control

Life-history parameters of *Chrysoperla carnea s. l.* fed on spontaneous plant species and insect honeydews: importance for conservation biological control

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Abstract

Chrysoperla carnea s. l. (Stephens) (Neuroptera: Chrysopidae) larvae are generalist predators feeding on many crop pests while adults feed on non prey-foods. The knowledge about the nutritional suitability of non-prey food for adults in agroecosystems is crucial to establish conservation biological control strategies and is poorly known in olive groves, where *C. carnea s. l.* larvae prey on different pests. In this study, the effect of honeydews secreted by two hemipteran olive tree secondary pests and 11 plant species on the life-history parameters (survival, reproduction and development time) of *C. carnea s. l*. were analyzed. For the first time insect pest honeydews from the olive agroecosystem and several plant species, blooming throughout the year, were found to improve *C. carnea s. l.* adults survival. Pollen consumption seems to be essential for reproduction. These observations constitute an important finding for implementing new conservation biological control approaches.

Key words: nectar, pollen, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae), *Chrysoperla carnea s. l.* (Neuroptera: Chrysopidae), survival analysis

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CHAPTER 7

7.1. Introduction

Conservation biological control aims to conserve and manage crop environment to enhance the effectiveness of natural enemies (Landis et al. 2000). Some natural enemies feed on non-prey foods (pollen, nectar or insect honeydews) in some stages of their development (Jervis and Heimpel 2005) and the lack of these resources in the agroecosystems may represent a limitation for their action in pest control (Gurr and Wratten 1999). Chrysopid family is a major group of natural enemies and, among them, *Chrysoperla carnea s. l.* (Stephens) is one of the most important species. The larval stage feed on many crop pests whereas adults are palino-glycophagous, consuming non-prey foods such as nectar and pollen, obtained from different plant species, or honeydews produced by hemiperan insects present in agroecosystems (Wäckers 2005; Petanidou et al. 2006). The nutritional level of available food resources might influence different life-history parameters (survival, reproduction or development time) of *C. carnea s. l.* and potentially, its effectiveness as a pest control agent. In previous field experiments the consumption of pollen from different flower species by *C. carnea s. l.* was observed by Villenave et al. (2005) and of honeydews by Sheldon and MacLeod (1971) and Hogervorst et al. (2007). However, to our knowledge, only Van Rijn (2012) compared the suitability of various plant species on longevity and fecundity of *C. carnea s. l*. concluding that flowers with well exposed nectaries enhanced survival. Recently, Gonzalez et al. (2015) analyzed the longevity and reproduction of this species fed on ten types of sugars and an artificial diet of honey and pollen, and obtained higher longevity but lower oviposition on fructose and higher fecundity in the artificial diet.

The olive grove is a widespread crop in Mediterranean areas with an important socio-economical impact. The relevance of chrysopid larvae as predators of *Prays oleae* (Bernard), one of the most important pests in this agroecosystem, is well documented (Arambourg 1984; Ramos et al. 1987; Bento 1999; Paredes et al., 2015). Also, a synchrony between *C. carnea s. l.* and *P. oleae* populations was found by Bento (1999). In addition, other works pointed out the potential predation on immature stages of two secondary pest, *Saissetia oleae* (Olivier) (Arambourg 1984) and *Euphyllura olivina* (Costa) (Pantaleoni et al. 2001; Gharbi et al. 2012).

Despite the importance of *C. carnea s. l.* in the olive agroecosystem, the role of non-prey foods occurring within and around olive groves on life-history parameters of *C. carnea s. l.* is relatively unknown. Porcel et al. (2013) obtained positive correlations between the abundance of *C. carnea s. l.* adults and the presence of weed covers, and McEwen and Ruiz (1994) found an association between non-crop vegetation and chrysopid eggs. On the other hand, Alrouechdi (1984) found that chrysopids laid eggs preferentially in areas with high densities of *S. oleae* honeydew, which can be attractive for *C. carnea s. l.* adults (McEwen et al. 1993).

In this context, the objective of this work was to study the effect of honeydews secreted by *S. oleae* and *E. olivina*, and 11 spontaneous plant species, occurring in Mediterranean areas, on life-history parameters (survival, reproduction and development time) of *C. carnea s. l.* in laboratory assays. For the first time, various natural non-prey foods in olive groves were identified as nutritionally suitable for *C. carnea* s. *l.* adults. This constitutes a new valuable knowledge that will enable to develop new approaches in conservation biological control strategies for olive pests.

7.2. Material and Methods

7.2.1. Insects

C. carnea s. l. was obtained from a colony maintained in a climatic chamber at 24ºC (±2ºC) and 16:8 h L: D. Initial specimens were purchased from Nutesca S.L. (Baeza, Spain). The colony was supplied with an artificial diet and water and larvae were gently transferred from the rearing culture using a brush, and placed individually in Petri dishes (5.5 cm in diameter x 1.8 cm height). Larvae were provided with *Ephestia kuehniella* Zeller eggs, purchased from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands), and with water in microcentrifuge tubes (0.5 mL), sealed with Parafilm© and a filter paper strip as a water dispenser, until adult emergence. Newly emerged couples were used in the experiments.

7.2.2. Non-prey foods

S. oleae and *E. olivina* honeydews were collected from small olive trees, infested with the pests, grown in a climatic chamber, 21ºC (±2ºC) and 16:8 h L: D, in the laboratory.

Eleven spontaneous plant species that bloom in different periods of the year in Mediterranean areas were selected: Asteraceae (*Calendula arvensis* L. and *Senecio vulgaris* L.), Apiaceae (*Daucus carota* L. and *Foeniculum vulgare* L.), Caprifoliaceae (*Lonicera etrusca* Santi), Lamiaceae (*Lamium purpureum* L. and *Rosmarinus officinalis* L.), Malvaceae (*Malva sylvestris* L.), Ranunculaceae (*Ranunculus ollissiponensis* Pers.), Caryophyllaceae (*Stellaria media* (L.) Vill.) and Plantaginaceae (*Veronica persica* Poir). Flowers were collected in the campus of the Polytechnic Institute of Bragança, northeastern Portugal. The blooming period of the selected plants in this area is: (i) *L. purpureum* and *V. persica*, blooming from January to June with a peak at the end of winter and early spring; (ii) *C. arvensis*, *R. officinalis*, *S. media* and *S. vulgaris*, blooming from November/December to June/July with a peak at the end of winter and early spring but extending longer than the previous plants; (iii) *R. ollissiponensis*, blooming from February to May with a peak in April; (iv) *L. etrusca*, blooming from March to July; (v) *D. carota* and *M. sylvestris*, blooming from April to September, with a peak at the end of the spring and during the summer but extending their flowering sometimes until November, in the case of *M. sylvestris*; (vi) *F. vulgare*, blooming from May to the begging of the autumn, with a peak during the summer.

7.2.3. Experimental design

Newly emerged couples were transferred into glass cages (1.5 L) closed with paper for ventilation. For each treatment, from 27 to 35 couples were assembled in each cage. Flowers were cut in field, transferred into small jars (2 x 4 cm), provided with water and sealed with Parafilm©. This procedure is considered adequate since previous studies did not find differences between observed lifespans with cut and intact flowers (Wade and Wratten 2007). Foods were replaced three times a week. Approximately, 8 or 9 cm^2 of flowers were used per treatment as well as a homogeneous amount of honeydew. A negative control (water) and two positive controls (water-honey solution 10% (w/v) and sucrose 1M) were assembled. Honey solution, sucrose solution and water were placed in small jars (2 x 4 cm), sealed with Parafilm© and with a filter paper strip as a water dispenser and were replace three times a week. Mortality and the number of eggs were recorded on a daily basis. Eggs were collected by cutting the pedicel with forceps, transferred into petri dishes (provided with water and food), and development was monitored.

7.2.4. Data analyses

7.2.4.1. Survival

Firstly, lifespan means and standard errors were calculated. Then, survival curves were drawn for each treatment with Kaplan-Meier estimates using the *surv* and *survfit* functions from the *survival* package (Therneau 2014) in R (R Core Team 2014). Statistical differences among curves were analyzed with the log-rank test using *survdiff* function from the same package and the same procedure was subsequently applied in order to perform a complete pairwise analysis comparing each pair of treatments. Due to the large number of multiple comparisons along the pairwise analysis the Bonferroni correction was applied (*p-value* < 0.05). The *survdiff* function was also applied to test if there were differences in survival between sexes within treatments.

7.2.4.1. Reproduction

Generalized linear models (GLM) for count data and negative binominal distribution to account with overdispersion (Zuur et al. 2009) were used to assess the effect of the different treatments for the pre-oviposition and oviposition period applying the *glm.nb* function from the *MASS* package (Venables and Ripley 2002). Then, a *post hoc* analysis was carried out in order to detect the differences between treatments using the *glht* function from the *multcomp* package (Hothorn et al. 2008). The Bonferroni correction was applied. Pre-oviposition and oviposition period were statistically analyzed only when more than three couples laid eggs.

The influence of each treatment on the egg number was estimated using the number of eggs laid by each female during the longest pre-oviposition and oviposition period altogether (37 days) as dependent variable (eggs/day) and the factor treatment as explanatory variable. Twelve females (maximum number of fertile females + 1) within each treatment were used as random factor. Since the eggs laid by each female were counted at regular intervals (days), firstly the auto-correlation within subject was estimated ($\alpha = 0.27$) applying generalized estimation equations using the *geeglm* function with the “AR1” correlation structure from the *geepack* package (Højsgaard et al. 2006). Then, considering the correlation value previously calculated and the large number of zeros in the dataset, a generalized linear mixed model was applied using the *glmmadmb* function from the *glmmADMB* package (Skaug et al. 2014). To assess the differences among treatment a *post hoc* analysis and the Bonferroni correction was applied.

7.2.4.2. Development time

The effect of the treatments on the development time was studied by analyzing: (i) the number of days spent in each stage (i.e. from the couple allocation in the cage to each egg laid, egg, L1, L2, L3 and pupa) and (ii) the proportion of individuals that reached alive each development stage in relation to the total analyzed eggs. Development parameters were statistically analyzed only when more than three couples laid eggs.

In the first case (i) a series of GLMs was applied using the *glm.nb* function followed by a *post hoc* analysis using the procedure exposed before. The Bonferroni correction was applied. Finally, a series of GLMs for proportional data with binomial distribution (Zuur et al. 2009) were developed using the proportion of individuals that reached alive each development stage as dependent variable. As above, a *post hoc* analysis and the Bonferroni correction was also applied. Eggs accidentally damaged during manipulation and missing larvae were not considered in these analyses.

7.3. Results

7.3.1. Survival

Lifespan of both males and females fed on water and *C. arvensis* was significantly lower when compared with the other food sources while honey, sucrose and honeydews secreted by both hemipteran species gave a significantly higher lifespan (Table 7.1). Survival functions were significantly different between treatments for both males (Fig. 7.1A) and females (Fig. 7.1B) after applying Bonferroni correction (significance level = 0.05/120) ($\chi^2 = 424$, df = 15, $P < 0.0004$ for males and $\chi^2 = 422$, df = 15, $P < 0.0004$ for females). Both sexes survival function showed the maximum performance under the positive controls, honey and sucrose treatments (high performance treatments). For males, survival functions on honeydews did not differ significantly from treatments with sucrose but showed a significant lower survival than with honey. For females fed on *S. oleae* and *E. olivina* honeydews (high/mid performance treatments), survival functions were not significantly different from positive controls. Nor females neither males survival functions fed on *M. sylvestris*, *V. persica* and *L. purpureum* flowers (mid performance treatments) significantly differed from those fed on *S. oleae* or *E. olivina* honeydews but under all the remaining treatments a significantly lower performance was showed. Nevertheless, both sexes survival functions fed on *R. ollissiponensis*, *L. etrusca*, and *F. vulgare* (low performance treatments) and females fed on *D. carota* (low performance treatments for females) showed significantly higher performance than the negative control. Males survival function fed on *D. carota* and both sexes survival function fed on *R. officinalis*, *S. media*, *S. vulgaris* and *C. arvensis* (no effect treatments) did not differ from the negative control.

Table 7.1. Lifespan (days) (mean ± se) for males and females of *Chrysoperla carnea s. l.* fed on different non-prey foods. Different letters in the column indicate significant differences in the Log-Rank test among treatments. Bonferroni correction was applied (*p-value* < 0.05). Between brackets is the number of individuals tested in each treatment (n).

Treatment	**Males**	**Females**
Honey	58.60 ± 4.61 (32) f	46.70 ± 3.56 (35) d
Sucrose	42.30 ± 3.47 (25) df	46.30 ± 4.24 (28) d
S. oleae honeydew	22.40 ± 3.96 (29) ad	30.10 ± 4.81 (29) ad
E. olivina honeydew	24.30 ± 4.16 (29) ad	29.00 ± 4.48 (29) ad
C. arvensis	2.28 ± 0.12 (29) c	2.21 ± 0.14 (29) e
D. carota	3.00 ± 0.27 (29) bc	3.93 ± 0.44 (30) bc
F. vulgare	4.79 ± 0.52 (28) be	4.82 ± 0.64 (28) bc
L. etrusca	3.89 ± 0.31 (28) be	4.00 ± 0.40 (29) bc
L. purpureum	11.90 ± 2.33 (28) ae	17.30 ± 2.75 (29) a
M. sylvestris	17.30 ± 2.25 (33) a	22.70 ± 3.02 (33) a
R. officinalis	2.81 ± 0.32 (27) bc	3.19 ± 0.40 (27) bce
R. ollissiponensis	4.54 ± 0.43 (28) be	4.86 ± 0.51 (28) c
S. media	2.83 ± 0.38 (30) bc	3.50 ± 0.73 (28) bce
S. vulgaris	3.07 ± 0.38 (29) bc	2.76 ± 0.26 (29) be
V. persica	15.70 ± 2.31 (29) a	23.70 ± 3.01 (30) a
Water	2.27 ± 0.12 (30)c	2.27 ± 0.12 (30) e

Differences between females and males survival functions were found with honey ($\chi^2 = 6.73$, df = 16, $P < 0.05$) and *V. persica* treatments ($\chi^2 = 3.97$, df = 1, $P < 0.05$), being better the females performance with *V. persica* and the males performance with honey. With *D. carota* the survival for females was higher than for males with a marginal significance ($\chi^2 = 3.84$, df = 1, $P = 0.05$). The other treatments did not cause significant differences between females and males survival functions (*C. arvensis*: $\chi^2 = 0.06$, df = 1, $P = 0.810$; *F. vulgare*: $\chi^2 = 0.010$, df = 1, $P = 0.979$; *L. etrusca*: $\chi^2 = 0.08$, df = 1, $P = 0.778$; *L. purpureum*: $\chi^2 = 2.47$, df = 1, $P = 0.116$; *M. sylvestris*: $\chi^2 = 2.63$, df = 1, $P = 0.105$; *R. officinalis*: $\chi^2 = 0.430$, df = 1, $P = 0.513$; *R. ollissiponensis*: $\chi^2 = 0.37$, df = 1, $P = 0.545$; *S. media*: $\chi^2 = 0.87$, df = 1, $P = 0.352$; *S. vulgaris*: $\chi^2 = 0.49$, df = 1, $P = 0.483$;

Sucrose: $\chi^2 = 1.68$, df = 1, P = 0.195; *E. olivina*: $\chi^2 = 1.02$, df = 1, P = 0.313; *S. oleae*: $\chi^2 = 1.28$, df = 1, P = 0.278).

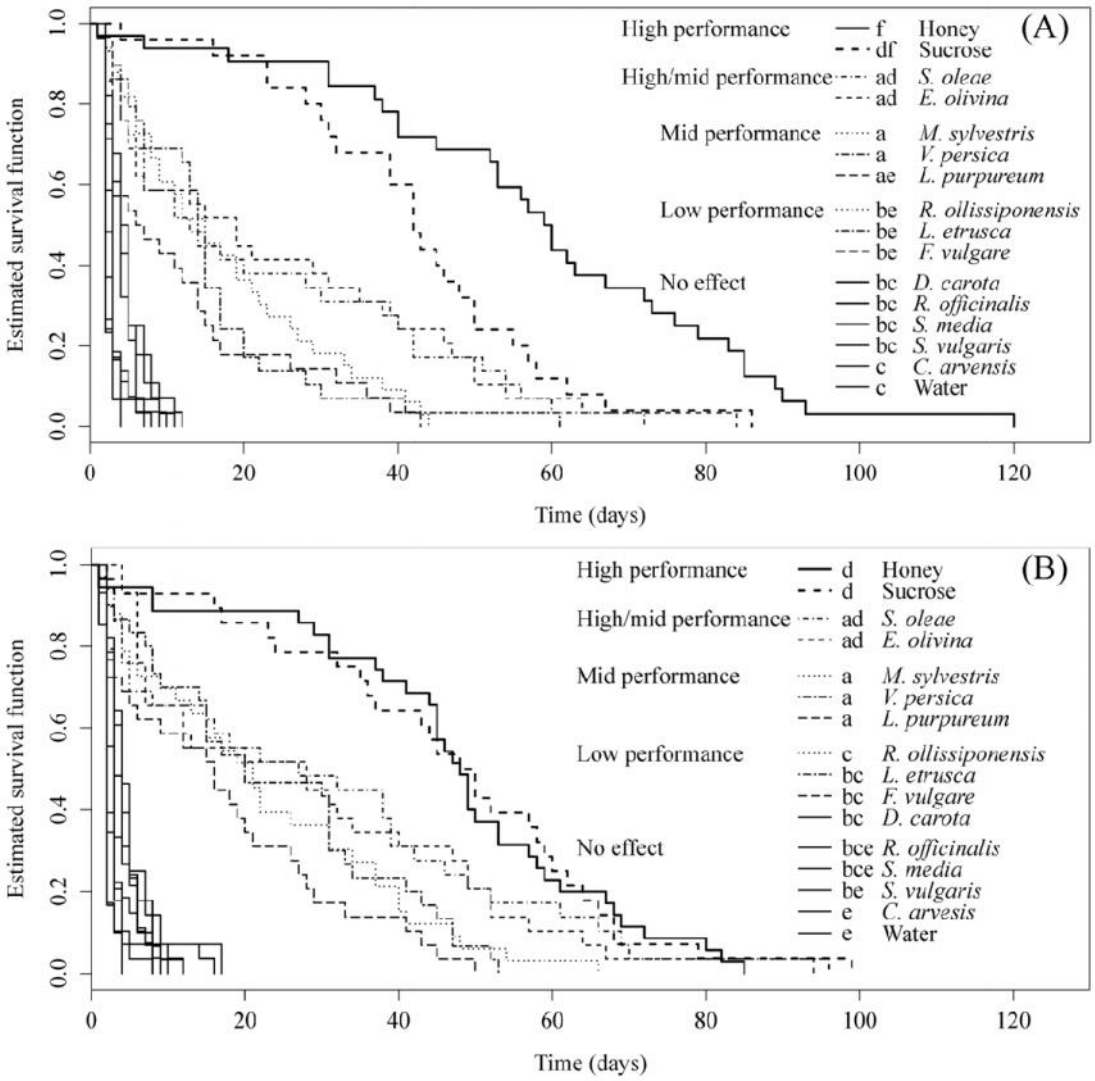

Fig. 7.1. Kaplan-Meier estimates of the survival functions for *Chrysoperla carnea s. l.* males (A) and females (B) reared with different non-prey foods. Different letters indicate significant differences in the Log-Rank test among treatments. Bonferroni correction was applied (*p-value* < 0.05).

7.3.2. Reproduction

Pre-oviposition and oviposition periods were statistically analyzed for honey, sucrose, *L. purpureum*, *M. sylvestris* and *V. persica*. Although *C. carnea s. l.* fed on *E. olivina* (2 fertile females) and *S. oleae* (1 fertile female) honeydews laid some eggs, they were not included in the statistical analysis due to the low number of individuals. GLM showed that pre-oviposition and oviposition periods were different between treatments after applying Bonferroni correction (significance level = 0.05/5) (pre-oviposition period: $\chi^2 = 25.43$, df = 4, P < 0.01; Oviposition period: $\chi^2 = 15.48$, df = 4, P

< 0.01). *M. sylvestris* and *V. persica* caused pre-oviposition periods significantly longer than honey and *L. purpureum* but did not significantly differ from sucrose (Fig. 7.2A). Oviposition period was significantly higher for *V. persica* than for *M. sylvestris*, but no significantly differences were found between the other treatments (Fig. 7.2B).

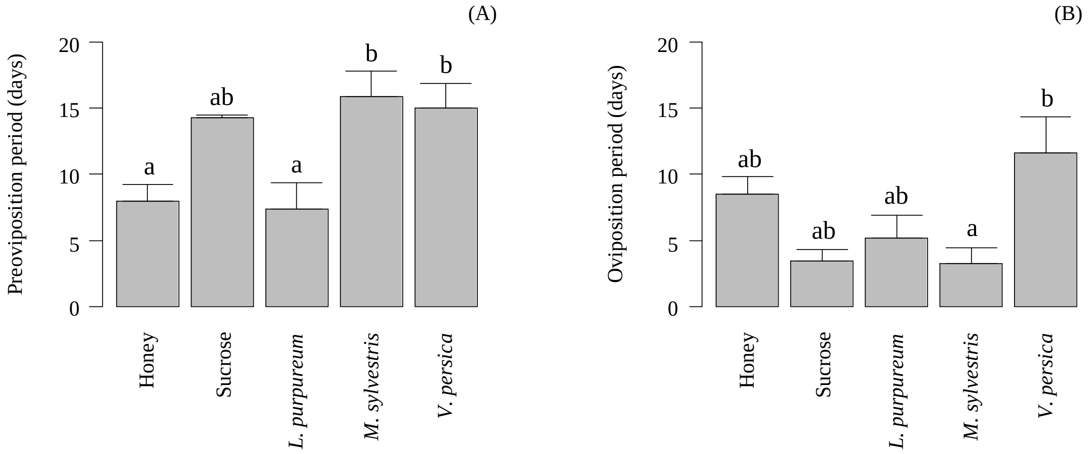

Fig. 7.2. Pre-oviposition (A) and oviposition (B) periods (days) of *Chrysoperla carnea s. l.* reared with different non-prey foods (mean ± se). Different letters indicate significantly differences among treatments.

GLMM indicated that the number of eggs laid per day by female were significantly different among treatments after applying Bonferroni correction (significance level = 0.05/5) (χ^2 = 27.80, df = 6, P< 0.01). The number of eggs laid per day was significantly higher for *V. persica* than for the insect honeydews and the other treatments did not significantly differ between them (Fig. 7.3).

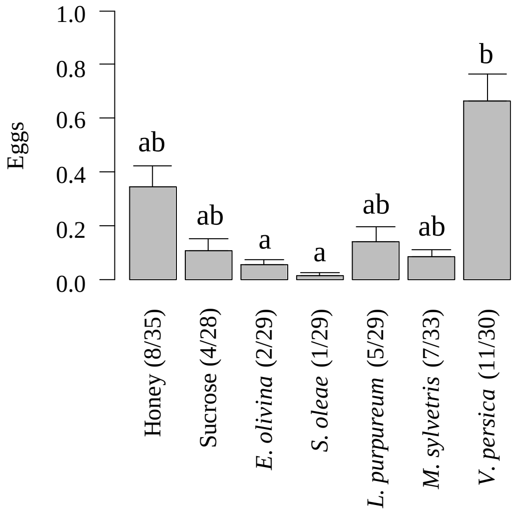

Fig. 7.3. Eggs/day laid by *Chrysoperla carnea s. l.* reared with different non-prey foods (mean ± se). Different letters indicate significantly differences among treatments. Between brackets are indicated the number of fertile females (on the left of the bar) and the total number of females (on the right of the bar).

7.3.3. Development time

The numbers of analyzed eggs for each treatment were: 48 for *L. purpureum*, 51 for *M. sylvestris*, 206 for *V. persica*, 142 for honey, and 37 for sucrose. The time (mean ± se) spent in each development stages is showed in Table 7.2. Bonferroni correction was applied (significance level = 0.05/6). GLM indicated that the time spent from the couple allocation in the cage to each egg lay varied between 10 to 24 days and was significantly different between treatments (χ^2 = 617.73, df = 4, P < 0.008), being significantly longer for *V. persica*, followed by *M. sylvestris* and sucrose and significantly shorter with honey. Eggs hatched in 4 or 5 days and this time was not significantly different among treatments (χ^2 = 5.40, df = 4, P = 0.249). Both L1 and L2 stages spent around 3 or 4 days for molting and treatments did not significantly differ (L1 for molting to L2: χ^2 = 0.40, df = 4, P = 0.982; L2 for molting to L3: χ^2 =.4.88, df = 4, P = 0.3). L3 spent between 8 and 16 days being significantly longer for *M. sylvestris* than for *V. persica* but no significant differences were found between the other treatments (χ^2 = 16.40, df = 4, P < 0.008). Adults spent around 11 and 12 days to emerge from the pupae and no differences were found among treatments (χ^2 = 2.80, df = 4, P = 0.591).

Table 7.2. Time spent (mean ± se) (days) at each development stage by *Chrysoperla carnea s. l.* offspring reared with different non-prey foods. L: time until egg laying; E: time spent as egg until hatching; L1: time spent as L1; L2: time spent as L2; L3: Time spent as L3; P: Time spent as pupa. Different letters indicate significant differences (*p-value* < 0.05). Bonferroni correction was applied.

Treatment	L	E	L1	L2	L3	P
Honey	9.77 ± 0.38 a	4.63 ± 0.10 a	3.59 ± 0.13 a	3.43 ± 0.12 a	9.34 ± 0.65 ab	11.69 ± 0.15 a
Sucrose	15.05 ± 0.73 b	3.52 ± 0.25 a	3.32 ± 0.21 a	3.29 ± 0.21 a	8.67 ± 1.09 ab	11.13 ± 0.26 a
M. sylvestris	15.24 ± 0.64 b	4.28 ± 0.32 a	3.40 ± 0.27 a	3.17 ± 0.17 a	16.00 ± 2.37 a	14.67 ± 3.28 a
L. purpureum	10.79 ± 0.63 a	4.31 ± 0.19 a	3.43 ± 0.20 a	2.67 ± 0.20 a	12.30 ± 1.27 ab	11.83 ± 0.39 a
V. persica	23.89 ± 0.54 c	4.23 ± 0.13 a	3.51 ± 0.12 a	2.83 ± 0.11 a	8.86 ± 0.53 b	12.07 ± 0.13 a

Table 7.3. Proportion of *Chrysoperla carnea s. l.* offspring (mean ± SE) reared with different non-prey foods reaching alive at each development stage in relation to the total analyzed eggs per treatment.

Treatment	L1	L2	L3	Pupa	Adults
Honey	68.47 ± 0.08 ab	44.05 ± 0.11 ab	40.87 ± 0.11 ab	34.88 ± 0.10 a	38.10 ± 0.09 a
Sucrose	60.07 ± 0.17 ab	54.34 ± 0.15 ab	50.72 ± 0.18 ab	47.50 ± 0.17 a	44.12 ± 0.17 a
M. sylvestris	35.59 ± 0.14 a	27.43 ± 0.14 a	22.25 ± 0.14 a	22.15 ± 0.14 a	17.58 ± 0.14 a
L. purpureum	83.45 ± 0.06 b	73.75 ± 0.90 b	69.71 ± 0.10 b	55.42 ± 0.17 a	41.25 ± 0.20 a
V. persica	52.38 ± 0.08 a	37.16 ± 0.70 a	35.05 ± 0.07 ab	25.28 ± 0.05 a	25.28 ± 0.05 a

The proportion (mean ± se) of individuals that reached each development stage in relation to the total analyzed eggs is shown in Table 7.3. Bonferroni correction was applied (significance level = 0.05/5). The L1 hatching proportion and the molting from L1 to L2 were significantly different among treatments being significantly higher for *L. purpureum* than for *M. sylvestris* and *V. persica* (hatching proportion: χ^2 = 18.23, df = 4, P < 0.01; L1 molting to L2: χ^2 = 17.43, df = 4, P < 0.01). The proportion of individuals that reached L3 was significantly higher for *L. purpureum* than *M. sylvestris* but was not significantly different from the other treatments (χ^2 = 18.67, df = 4, P < 0.01). Pupation proportion was not significantly different among treatments (pupation: χ^2 = 12.18, df = 4, P = 0.016). Finally, adult emergence was significantly lower with *M. sylvestris* than with honey (χ^2 = 16.06, df = 4, P < 0.01).

7.4. Discussion

In this study, several non-prey foods commonly found in olive groves were identified as potential resources for *C. carnea* adults. Honeydews of the secondary pests *S. oleae* and *E. olivina* and flowers of *V. persica*, *L. purpureum*, *M. sylvestris* showed to be nutritionally suitable for adults of *C. carnea s. l*. The three plant species bloom in southwestern Europe throughout the year: *V. persica* and *L. purpureum* bloom during winter and early spring and *M. sylvestris* from late spring to autumn. The occurrence of suitable winter flowering plants in agroecosystems is particularly relevant since they can enhance the nutritional status of *C. carnea s. l.*, which is essential for overwintering and reproduction at the end of winter (Sheldon 1975). Food of unsuitable quantity and quality maintain individuals in a quiescence state and retard the reproductive activity at the beginning of spring (Principi 1991).

These five non-prey foods improved *C. carnea s. l.* survival; however, reproduction parameters were lower when compared with other studies (Sundby 1967; Krishnamoorthy 1984; Venzon et al. 2006). Chrysopids were found to lay none or only few eggs when fed on low proteinaceous diets (Sheldon and MacLeod 1971; McEwen et al. 1994; Gibson and Hunter 2005; Venzon et al. 2006). Also, a certain amount of carbohydrates is needed for egg laying (Sheldon and MacLeod 1971; Venzon et al. 2006). Insect honeydews and flower nectar are carbohydrate sources (while poor in proteins) that most probably enhanced the lifespan of chrysopids This finding is particularly relevant once flowers with accessible nectaries are less frequent than those with accessible pollen (van Rijn 2012). Low protein contents in the diet could have resulted in the low number of eggs laid by *C. carnea s. l*. *V. persica* was the only plant species that improved reproduction likely due to pollen consumption. Also honey caused better reproduction fitness, probably because of the presence of pollen.

Both *M. sylvestris* and *L. purpureum* improved survival but not the reproduction which may suggest that *C. carnea s. l.* adults were not consuming enough amounts of pollen grains. In the case of *M. sylvestris*, reproduction did not improve in spite of its open corolla and the fact that it is an entomophilous plant (Comba et al. 1999). This plant has large and echinate pollen grains (measuring > 60 µm and sometimes > 100 µm in diameter) (Moore et al. 1991). *M. sylvestris* and cotton (Malvaceae: *Gossypium hirsutum* L.) have similar pollen shapes and sizes and, in a previous study Vaissière and Vinson (1994) found that bees, *Apis mellifera* L. (Hymenoptera: Apidae) did not consume cotton. They suggested that the size of the pollen spines of cotton probably impeded the bees, to groom and pack it. Also the big size of the cotton pollen grain may have influenced its consumption. In this work, *C. carnea s. l.* adults were observed to accumulate high quantities of pollen grains on the surface of their bodies (Villa, personal observation). These pollen agglomerations, together with the pollen spines and size, probably made difficult the pollen consumption, explaining the low reproduction performance.

L. purpureum is also an entomophilous plant and produces great amounts of pollen that aggregates in clumps and has quite long flowers (17.09 mm, SD ± 0.667) with petals that form a landing platform for insect visitors and a hooded part where anthers are located (Denisow and Bożek 2008). In our experiment, probably *L. purpureum* pollen was consumed in low quantities due to the difficult accessibility or the incapability to consume pollen from the clumps, originating low reproduction performance. Additionally, the long corolla, probably make difficult the consumption of nectar from the landing platform. However, in this study they were observed to consume nectar on the ovary or on the basis of the corolla once the flower was felt off (Villa, personal observation).

Plants of *D. carota*, *F. vulgare*, *R. ollissiponensis* and *L. etrusca* (low performance) slightly improved *C. carnea s. l.* survival but they did not generate egg production. These species, with the exception of *L. etrusca*, have well exposed nectaries. A lower nutritional quality or lower consumption of nectar could be responsible for *C. carnea s. l.* shorter lifespan. These results are in agreement with Gonzalez et al. (2015) that suggested that the reproductive success of *C. carnea s. l.* not only depends on foods rich in proteins but also on foods that provide energy to improve the lifespan and lifetime fecundity.

Females survival function was significantly higher with *V. persica* and honey, indicating that probably pollen consumption benefited more females than males. Accordingly, Villenave et al. (2005) found females to consume more pollen grains than males.

In relation to the development, differences in time and success were generally more noticeable in early stages. This could be related with nectar quality and quantity differences which could be translated in the eggs yolk quality for larvae. However, this effect was diluted in later stages.

In field, *C. carnea s. l.* was found to consume pollen from several plant species, (Villenave et al. 2005) and probably mixture of plants would result in better performance by supplying different types of nutrients. Additionally, under laboratory conditions, factors such as weather conditions or intra and interspecific resource competition are not taken into account. Therefore, mixture of plants and field experiments would be needed to complement this experience.

Finally, the effect of non-prey foods in crops not always leads to pest reductions. For example, Wyss et al. (1995) found a pest density reduction resulting from weed strips implantation in apple orchards that increased aphidophgous predators. However, Markó et al. (2013) found that the cover management affected *C. carnea s. l.* numbers but pests did not present different suppression. Importantly, the analyzed honeydews produced by two secondary pests of the olive tree could have a positive effect by feeding predators of olive pests. Therefore, the trophic relationships between non-prey foods, predators and olive pests need to be deeply investigated in the olive agroecosystem.

Summarizing, in these study we indentified for the first time several Mediterranean non-prey foods, that occur throughout the year, influencing *C. carnea s. l.* life-history parameters: two insect honeydews (*S. oleae* and *E. olivina*) and three plant species (*M. sylvestris*, *V. persica* and *L. purpureum*) that highly enhanced the survival of *C. carnea s. l.* and other four plants that also resulted in some survival improvement (*R. ollissiponensis*, *L. etrusca*, *F. vulgare* and *D. carota*). Additionally, foods rich in proteins together with foods rich in carbohydrates seem to be needed for reproduction. These findings will allow new approaches for conservation biological control strategies and management of olive agroecosystems.

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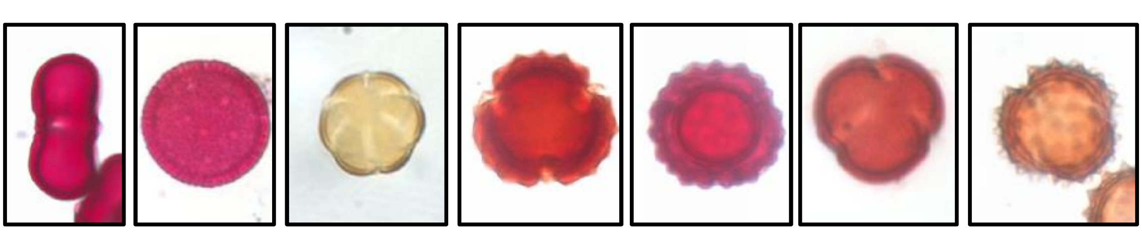
CHAPTER 7

CHAPTER 8

Pollen feeding habits of syrphids in heterogeneous agricultural landscapes during autumn season, a period of food scarcity



Pollen feeding habits of syrphids in heterogeneous agricultural landscapes during autumn season, a period of food scarcity

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Abstract

Many syrphid larvae are predators and have an important role as biological control agents of pests in agroecosystems. However, adults feed on non-prey resources such as pollen from flowers. Heterogeneous landscapes can provided syrphids with a larger biodiversity of plants and ensure the existence of food resources. This takes a relevant importance in food scarcity periods, like autumn, particularly for syrphid species that spend those periods as adults. Nevertheless, feeding habits of syrphid adults under adverse conditions is poorly known. In this study, the pollen consumption and selection by *Eupeodes corollae* (Fabricius) and *Episyrphus balteatus* (De Geer) were analyzed in olive groves and herbaceous and woody surrounding patches in 2012 and 2013 autumns. Guts were dissected and pollen types identified and compared with ground cover plants in the studied patches. Both species consumed and selected pollen types from herbaceous (such as Asteraceae, Ranunculaceae, *Corrigiola telephiifolia* type and Apiaceae and Caryophillaceae in the case of *E. corollae*) and woody vegetation (such as *Arbutus unedo*, *Cytisus/ Ulex* pollen type, *Daphne gnidium* type, and *Salix*) that occurred in different patches, indicating that they flew between patches. These results highlight the importance of conserving heterogeneous agricultural landscapes in order to guarantee the existence of food resources for syrphids in periods of scarcity.

Key words: *Eupeodes corollae*, *Episyrphus balteatus*, predator, non-crop vegetation, olive grove

Manuscript submitted

CHAPTER 8

8.1. Introduction

Adult syrphids (Diptera: Syrphidae) require nectar as source of carbohydrates for energy and pollen as source of amino acids, lipids, carbohydrates, vitamins and minerals for maturating their reproductive system (Haslett 1989a; Irvin et al. 1999; Lundgren 2009; Schneider 1948; Wratten et al. 1995) while many larvae act as natural control agents preying aphids, some moths and psyllids (van Veen 2010; Speight 2011). Pollen seems to be particularly important for females since they consume more pollen and less nectar than males (Haslett 1989a). Females require pollen to mature their eggs and for yolk deposition in the egg and males need nectar to provide energy for mate seeking, tissue maintenance and spermatogenesis (Haslett 1989a; Hickman et al. 1995; Irvin et al. 1999; Wratten et al. 1995).

Heterogeneous agricultural landscapes can positively influence syrphid abundance and diversity due to the occurrence of more diverse food items (flowering weeds, shrubs and trees) both in the crop field as well as in its surroundings, contrarily to more homogeneous agricultural landscapes or areas where vegetation has been removed by tillage or due to herbicide application (e.g. Cowgill et al. 1993; Haenke 2009; Lövei et al. 1993; Sajjad and Saeed 2010). In this context, the maintenance of heterogeneous areas is considered an essential issue for conservation biological control of pests by syrphids since, in some cases, a decrease in infestation levels was shown (e.g. Lövei et al. 1993).

In the Mediterranean region, spontaneous plants bloom mainly in spring and become less abundant from summer to winter seasons. Coinciding with plants blooming, syrphids are mainly active in spring remaining in diapause during the rest of the year, usually as larvae but sometimes as adults (Schneider 1948; Speight 2011). Probably, for this reason, syrphid diversity and feeding behavior was mainly studied during spring (e. g. Burgio et al. 2007; Hickman et al. 1995; Wratten et al. 1995). Few studies were carried out during seasons of flower scarcity, such as autumn, whereby pollen feeding habits is poorly known. During diapause periods, if they feed, the energy obtained is used for catabolism and storage of material in the fat body (Schneider 1948). Therefore, syrphid feeding behavior during low activity periods might be relevant for their development and maintenance and its knowledge could bring new insights about habitat management in order to enhance syrphid performance.

One of the techniques used for studying plant feeding behavior is to analyze pollen grains contained in guts. Syrphid species mostly digest pollen grains through enzymes in the midgut, afterward pollen exine remains visible (Gilbert 1981; Haslett 1983) which makes possible to identify pollen types consumed by syrphids through gut dissection. This technique has been followed by different authors as a mean of studying seasonal and sex feeding patterns (Hickman et al. 1995; Irvin et al. 1999; Wratten et al. 1995).

The olive tree is one of the most important crops in the Mediterranean region, with a widespread distribution and a high social-economic impact. In this crop, larvae of syrphids were found to feed on olive pests, such as *Prays oleae* (Bern.) (Lepidoptera: Praydidae) (Sacchetti 1990; Silvestri 1908), *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae) (Ksantini 2003), *Palpita vitrealis* (Rossi) (Lepidoptera:Crambidae) or *Euphyllura straminea* Loginova (Hemiptera: Psyllidae) (Rojo et al. 2003). The effect of adjacent vegetation (herbaceous and woody) to the olive grove on important natural enemies (e.g. spiders, ants, predatory heteropterans and hymenopteran parasitoids) was studied by Paredes et al. (2013), however, foraging habits of adult syrphid are poorly known and, as far as we know, any study was done in landscapes where the olive tree is dominant. Thus, the objective of this work was to identify the plant species exploited by syrphids as pollen sources in olive groves and surrounding landscape during periods of flowers scarcity. We tested whether syrphids selected herbaceous or woody plants and if they exploited several patches surrounding agricultural areas to determine the importance of heterogeneous landscapes in supplying food resources for syrphids.

8.2. Material and methods

8.2.1. Study areas

Field studies were conducted in the northeastern Portugal, Mirandela municipality, during 2012 and 2013, in three olive groves (Cedães: 41°29’16" N, -7°07’34" W, Paradela: 41º32’8’’N, -7º07’29’’W, and Guribanes: 41°34’12" N, -7°09’59" W) and two surrounding field areas (a herbaceous vegetation patch and a scrubland) next to each olive grove. During the experimental years the olive groves were not tilled and were not sprayed with pesticides.

Scrubland patches were composed by three vegetation strata: herbaceous, shrub and tree strata derived from agriculture abandonment. Herbaceous vegetation patches were composed by cereal or grass mixture for livestock food. The areas of the three olive groves have about 2 ha and the surrounding patches 1 ha. The field selection was based on the most frequent field types occurring in the region.

8.2.2. Syrphid sampling and identification

Five delta traps were installed in each patch aiming to monitor the flight cycle of the olive moth. Syrphid adults were collected from each delta trap in December 2012 and 2013, corresponding to the sampling period comprised between September and December. Syrphids species were identified according to Van Veen (2010) and kept in alcohol 96% until further analysis.

8.2.3. Pollen analyses

Each syrphid was washed in alcohol 96% to eliminate the external pollen. The abdomen was removed from the body and opened with the help of a scalpel, needles and fine forceps. The gut was released onto a glass slide, two drops of glycerin jelly:water (1:1) were added and a coverslip (22 x 22 mm) was applied. To prevent pollen contamination between samples scalpel, needles and forceps were washed after each dissection. Pollen grains were counted and identified to pollen type using an optical microscope. Identification was based on Valdés et al. (1987) and Moore et al. (1991) and supported by a reference pollen collection hosted at the School of Agriculture, Polytechnic Institute of Bragança. When pollen grains were more than 5000, half of the slide was counted and more than 15000, a quarter of the slide was counted and the total number of grains was estimated thereafter.

8.2.4. Flowering plant inventories

Five flowering plant inventories (25 m^2) were carried out in the olive groves and in the herbaceous patches and three (100 m^2) were carried out in scrubland patches. This makes a total of 39 plant inventories for characterizing the plant community of the olive grove agroecosystem. Inventories were accomplished every other week, from September to December, in each patch. Percentage ground cover for each flowering plant species was registered following the Daubenmire Cover Scale modified by Bailey (Mueller-Dombois and Ellenberg 1974).

8.2.5. Data analyses

The association between syrphid species and the three types of patches was analyzed by a contingency table. To analyze if the pollen types found in the gut of syrphids were consumed at random or were selected, a specific Z test was applied following Villenave et al. (2006). It was calculated $Z = (\bar{Y} - 1)/ \alpha$, where $\bar{Y}$ = average of Yi for a pollen type and α = standard error. Yi = percentage of the *i* pollen type consumed by syrphids / percentage of ground cover by the *i* pollen type flowers. Consumption is considered at random when $-1.96 < Z < 1.96$. This test is only significant if the number of observation is at least 15. Differences between total pollen grains consumed by females and males were analyzed through Mann–Whitney *U* test. Statistical analyses were performed with IBM-SPSS statistics, version 19.0.0 (SPSS Inc. IBM Company 2010).

8.3. Results

8.3.1. Syrphids diversity

Eupeodes corollae (Fabricius) and *Episyrphus balteatus* (De Geer) were the most abundant species collected in delta traps in both years. Other specimens belonging to seven species (*Eupeodes luniger* (Meigen), *Eupeodes nielseni* (Dusek and Laska), *Sphaerophoria scripta* (Linnaeus), *Melanostoma mellinum* (Linnaeus), *Melanostoma scalare* (Fabricius), *Eristalis similis*

(Fallen) and *Paragus* sp.) were also captured. Since few specimens of these species were collected, pollen feeding habits could only be analyzed for *E. corollae* and *E. balteatus*. Abundance and distribution throughout the patches and years for the least collected species are provided as supplemental material – Table A.1.

During the autumn 2012, *E. corollae* was the most abundant species representing 64.1% of the total specimens (herbaceous vegetation: 53 females and 59 males; scrubland patches: 14 females and 22 males; olive groves: 7 females and 4 males), followed by *E. balteatus* with 23.4% of the total specimens (herbaceous vegetation: 21 females and 29 males; scrubland: 8 males).

During the autumn 2013, *E. balteatus* was the most abundant species representing 71% of the total specimens (herbaceous vegetation: 20 females and 29 males; scrubland: 2 males) followed by *E. corollae,* representing 9% of the total specimens (herbaceous vegetation: 3 females and 3 males; olive orchard: 1 female).

In both years, the abundance of syrphids was higher in herbaceous patches, followed by scrublands and olive groves. The contingency table showed a statistical significant association between the patch type and both *E. corollae* and *E. balteatus* in 2012 and between the patch type and *E. balteatus* in 2013 ($\chi^2 = 19.66$, *p*-value < 0.05, df = 4). In particular, *E. balteatus* was positively associated with herbaceous patches since it was more abundant there than expected.

8.3.2. Plant diversity and ground cover

Plant inventories carried out in each patch resulted in the identification of 52 flowering plant species belonging to 21 families and they were grouped in 27 pollen types (detailed information is given as supplemental material – Table A.2). In the scrublands, five species were identified and grouped in five pollen types in both autumn seasons. The most abundant was *Arbutus unedo* L. (*Arbutus unedo* pollen), followed by *Daphne gnidium* L. (*Daphne gnidium* type) and *Foeniculum vulgare* L. (Apiaceae pollen). In herbaceous patches, during 2012, 28 plant species were identified and grouped in 17 pollen types. Species belonging to Cichorioideae subfamily dominated, namely *Leontodon taraxacoides* (Vill.) Mérat subsp. *longirostris* Finch and P.D. Sell, *Hypochaeris radicata* L. and *Chondrilla juncea* L. In 2013, 32 species were identified and grouped in 15 pollen types. The most abundant species was *Conyza sumatrensis* (Retz.) E.Walker (Cardueae pollen) followed by *Hypochaeris glabra* L. (Cichorioideae pollen) and *Brassica barrelieri* (L.) Janka (Brassicaceae pollen). In olive groves, in 2012, 14 species were identified and grouped in eight pollen types. The most abundant species was *C. juncea* followed by *C. sumatrensis* and *F. vulgare*. In 2013, 11 species were identified and grouped in seven pollen types. The most abundant species was *C. juncea* followed by *Capsella bursa-pastoris* (L.) Medik.(Brassicaceae pollen) and *F. vulgare*.

In 2012, herbaceous patches presented the highest percentage of ground cover (1.4%), followed by olive groves (0.5%) and scrublands (0.4%). In 2013, herbaceous patches presented the highest percentage (7.05%) followed by scrublands (3.6%). The lowest percentage was presented by olive groves (0.9%), being the total ground cover of flowering plants higher than in 2012. In herbaceous patches, species belonging to Brassicaceae, Fabaceae and Amaranthaceae accounted for 48% of the increment in the total ground cover. The differences found in scrubland patches were principally due to the higher ground cover of *A. unedo.*

8.3.4. Pollen types

A total of 40 pollen types were found in the gut of syrphids. The number of pollen grains varied between around 10 to several thousands.

Considering *E. corollae*, in 2012, the diversity of pollen types found in each specimen varied from one to five (Fig. 8.1) and 24 pollen types were identified. Pollen types belonging to Asteraceae were the most represented, followed by Fabaceae, *Corrigiola telephiifolia* type, *D. gnidium* type and Ranunculaceae (Fig. 8.2). 65.3% of the 72 females and 67.1% of the 85 males contained pollen grains in their guts but no differences were found (Z = -0.325, *p*-value = 0.745) between total pollen grains or pollen types consumed by females and males. In 2013, once seven individuals were captured, only a descriptive analysis is presented. The diversity of pollen types varied from one to seven and three out of four females and the three males analyzed had pollen in their guts. Fourteen pollen types were found being the most represented belonged to Asteraceae, Ranunculaceae and *Salix* type.

For *E. balteatus*, in 2012, the diversity of pollen types found in the gut of the specimens varied between one and seven (Fig. 8.1). Twenty pollen types were identified being Asteraceae the most represented, followed by *D. gnidium* type, Fabaceae, *Corrigiola telephiifolia* type, Ranunculaceae and *A. unedo* (Fig. 8.3). 68.4% of the 19 females and 63.9 % of the 36 males contained pollen in their guts and no differences were found (Z = -0.027; *p*-value = 0.978) between total pollen grains consumed by females and males. In 2013, the diversity of pollen types found per specimen varied between one and 11. Twenty seven pollen types were identified in the gut of the analyzed *E. balteatus*, being Asteraceae pollen types the most represented followed by *Salix* type, Ranunculaceae pollen, *Cytisus/ Ulex* type, *A. unedo* and *D. gnidium* type. 90% of the 20 females and 93% of 31 males contained pollen grains in their guts and no differences (Z = -1.187; *p*-value = 0.235) were found between total pollen grains consumed by females and males.

Several specimens contained pollen types consumed in a different patch from that where they were captured. Thus, in 2012, 60 out of 112 *E. corollae* specimens collected in herbaceous patches consumed pollen types not represented in these patches as well as 20 out of 36 specimens collected in scrubland patches and 5 out 11 specimens collected in olive groves; for *E. balteatus*, 24 out of 50 specimens collected in herbaceous patches consumed pollen in other patches and 2 out of 8 specimens collected in scrubland. In 2013, 5 out of 6 *E. corollae* specimens collected in herbaceous obtained pollen in other patches and 1 out 1 specimen collected in olive groves, while 42 out 49 *E. baletatus* specimens collected in herbaceous patches visited other areas as well as 1 out 2 specimens collected in scrublands.

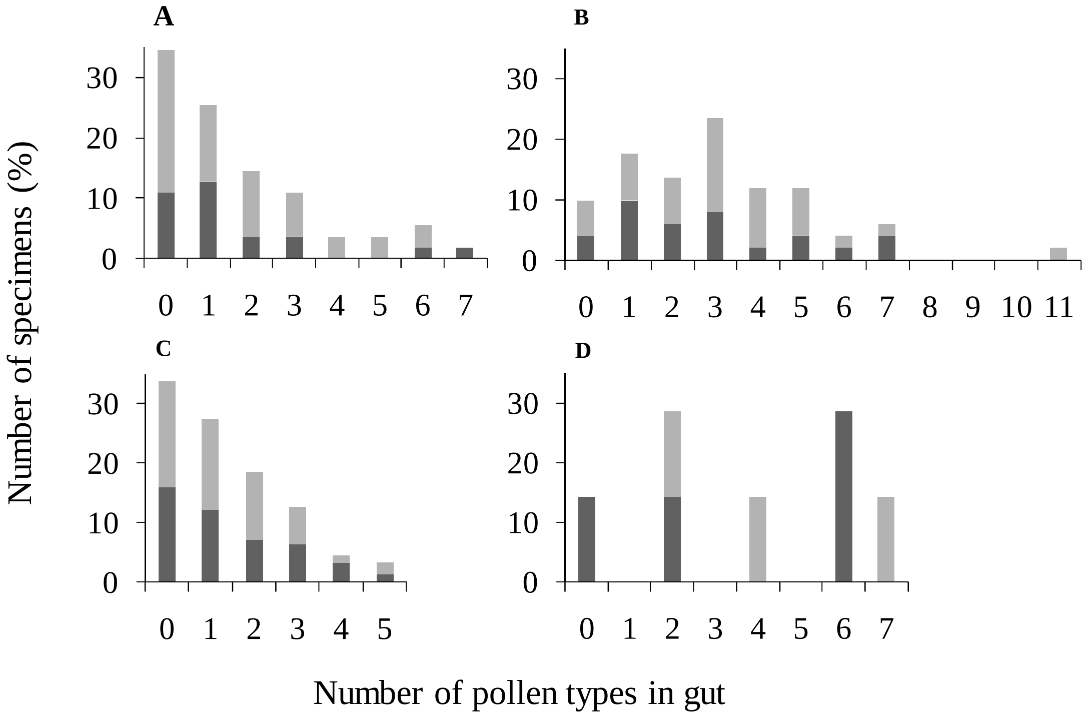

Fig. 8.1. Distribution of the number of different pollen types found in the gut of *Episyrphus balteatus* (A: 19 females and 36 males in 2012; B: 20 females and 31 males in 2013), *Eupeodes corollae* (C: 72 females and 85 males in 2012; D: 4 females and 3 males in 2013). Females ■; Males ▒.

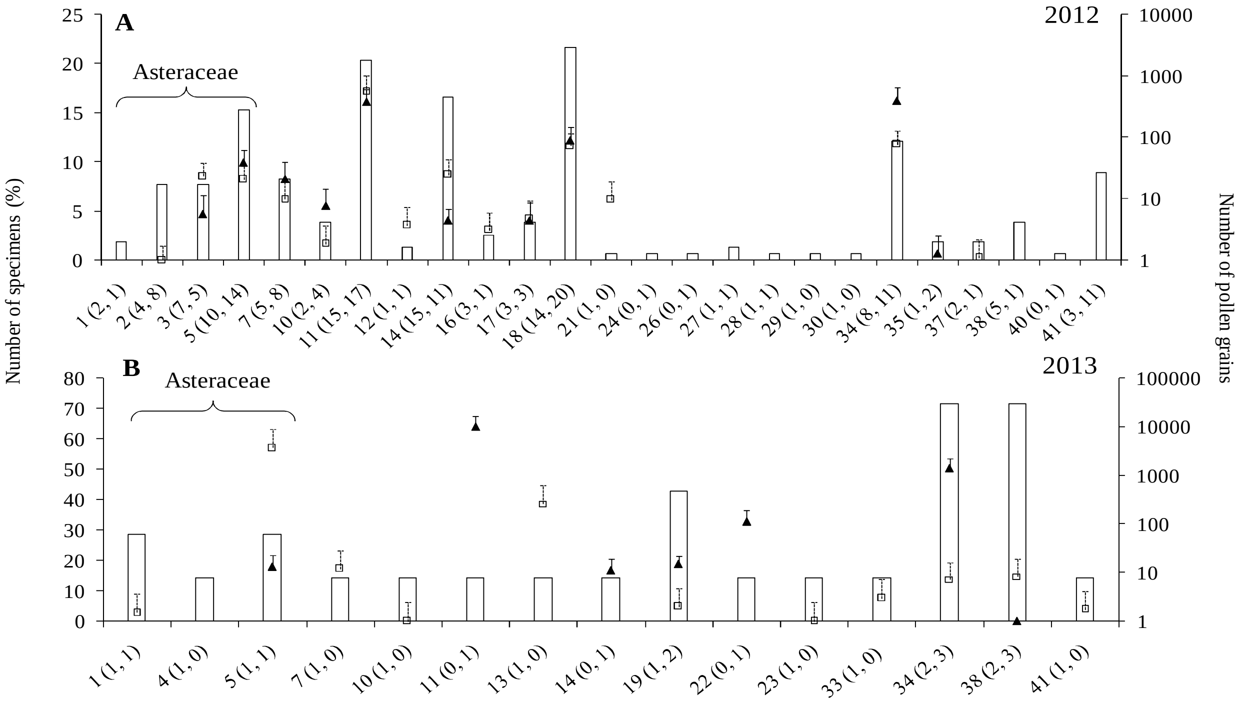

Fig. 8.2. Number of specimens (%) containing different pollen types (bars), number of pollen grains (mean + se) for females (☐) and males (▲), in autumn 2012 and 2013. *Eupeodes corollae*: A (72 females and 85 males), B (4 females and 3 males). In the xx –axis, total number of females and males with each pollen type are indicated between brackets after pollen type name, as follows (total number of females, total number of males). Pollen types - 1: *Anthemis* type; 2: Asteraceae (Other); 3: *Aster* type; 4: Cardueae; 5: Cichoriodeae; 7: Apiaceae; 10: Caryophyllaceae; 11: *Corrigiola telephiifolia* type; 12: Chenopodiaceae; 13: *Convolvulus arvensis* type; 14: *Daphne gnidium* type; 16: *Arbutus unedo*; 17: *Erica* type; 18: Fabaceae; 19: *Cytisus/Ulex* type; 21: *Hypericum*; 22: *Jasione* type; 23: Lamiaceae; 24: *Lonicera*; 26: *Mentha* type; 27: *Muscari comosum* type; 28: *Myrtus* type; 29: *Olea*; 30: *Pinus pinaster*; 33: Poaceae; 34: Ranunculaceae; 35: *Rhamnus* type; 37: *Rumex* type; 38: *Salix*; 40: *Viburnum* type; 41: Others.

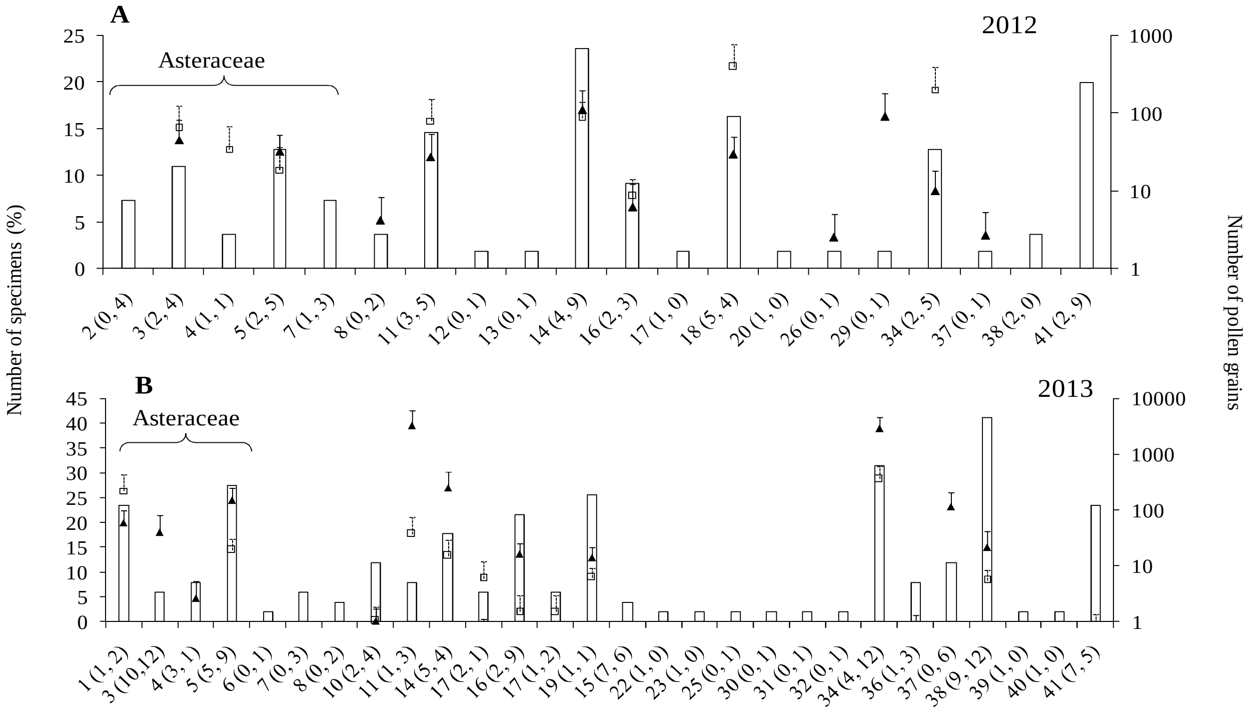

Fig. 8.3. Number of specimens (%) containing different pollen types (bars), number of pollen grains (mean + se) for females (☐) and males (▲), in autumn 2012 and 2013. *Episyrphus balteatus*: A (19 females and 36 males), B (20 females and 31 males). In the xx – axis, total number of females and males with each pollen type are indicated between brackets after pollen type name, as follows (total number females, total number males). Pollen types 1: *Anthemis* type; 2: Asteraceae (Other); 3: *Aster* type; 4: Cardueae; 5: Cichoriodeae; 6: *Alnus*; 7: Apiaceae; 8: *Betula*; 10: Caryophyllaceae; 11: *Corrigiola telephiifolia* type; 12: Chenopodiaceae; 13: *Convolvulus arvensis* type; 14: *Daphne gnidium* type; 16: *Arbutus unedo*; 17: *Erica* type; 18: Fabaceae; 19: *Cytisus/Ulex* type; 20: *Hippuris*; 22: *Jasione* type; 23: Lamiaceae; 25: *Malva sylvestris* type; 26: *Mentha* type; 29: *Olea*; 30: *Pinus pinaster*; 31: *Pinus pinea/halepensis* type; 32: *Pinus sylvestris* type; 34: Ranunculaceae; 36: Rosaceae; 37: *Rumex* type; 38: *Salix*; 39: Scrophulariaceae; 40: *Viburnum* type; 41: Others.

8.3.5. Pollen selection

Z-test was applied when more than 15 specimens were captured, i.e., *E. balteatus* in 2012 and 2013 and *E. corollae* in 2012. Z-test values indicated that some pollen types were consumed at random (-1.96 < Z < 1.96) and some were selected. *E. balteatus* selected *Aster* type, *C. telephiifolia* type, *D. gnidium* type, Fabaceae pollen and Ranunculaceae pollen in 2012 and *A. unedo*, Cichorioideae pollen, *Cytisus/Ulex* type, Ranunculaceae pollen and *Salix* pollen in 2013. Although the Z-test indicated that *Aster* type, *D. gnidium* type and Cichorioideae pollen were not selected in the both years their Z-test values were close to the selection value and a considerable number of specimens contained them in their guts (Table 1).

Regarding to *E. corollae* in 2012, Z-test showed selection for Apiaceae pollen, *Aster* type, not identified Astereaceae, Caryophyllaceae pollen, Cichorioideae pollen, *C. telephiifolia* type, *D. gnidium* type and Fabaceae pollen (Table 1).

8.4. Discussion

The most abundant species collected in this study, *E. balteatus* and *E. corollae*, are widely distributed in Europe (Van Veen 2010; Speigh 2011). Both are commonly related to open habitats (Branquart and Hemptinne 2000; Rojo et al. 2003; Speight 2011) and their flight period occurs from the beginning of spring until the end of autumn, being that in some regions they can overwinter as adults (Speight 2011). In our work, also a higher number of syrphids was collected in open (herbaceous) than in woody patches (olive groves and scrublands). Moreover, *E. balteatus* was equally abundant in both years while *E. corollae* was mainly found in 2012. This may indicate that the populations of *E. balteatus* could be more stable than *E. corollae*. Additionally, few specimens were captured in olive groves in this season. Nevertheless, during spring syrphids are easily observed hovering over the flowers within the olive groves. That could indicate the use of different types of patches seasonally by syrphids, being that herbaceous and woody vegetation patches around the groves would be selected during autumn.

Table 8.1. Ground cover (Cov %) for the pollen types consumed by *E. balteatus* and *E corollae* in autumn 2012 and 2013. Number of *E. balteatus* (N *E.b.*) and *E. corollae* (N *E.c.*) specimens containing the different pollen types in their guts in the studied periods, and Z test results for *E. balteatus* (Z *E.b.*) and *E. corollae* (Z *E.c.*) in the different periods of study (Z). Consumption is considered at random when -1.96 < Z < 1.96. Bold numbers indicate pollen type selection.

	2012					2013		
Pollen type	Cov (%)	N *E.b.*	Z *E.b.*	N *E.c.*	Z *E.c.*	Cov (%)	N *E.b.*	Z *E.b.*
Alnus							1	1.000
Anthemis type	0.009	4	1.410	3	1.220	0.036	3	1.060
Apiaceae	0.069	4	1.500	13	**2.764**	0.098	3	0.380
Arbutus unedo	0.105	5	1.180	4	1.511	1.000	11	**2.060**
Aster type	0.013	6	**2.070**	12	**2.959**	0.007	12	1.850
Asteraceae (other)		4	1.550	12	**2.507**			
Betula		2	1.000					
Brassicaceae						0.559	2	28.920
Cardueae	0.085	2	0.420			0.006	4	0.290
Caryophyllaceae	0.002			6	**2.061**	0.013	6	1.430
Chenopodiaceae		1	1.000	2	1.000			
Cichoriodeae	0.364	7	1.810	24	**3.563**	0.621	14	**2.780**
Convolvulus arvensis type		1	1.000					
Corrigiola telephiifolia type		8	**2.650**	32	**5.290**	0.002	4	1.790
Cytisus/Ulex type							13	**2.790**
Daphne gnidium type	0.025	13	**2.820**	26	**3.574**	0.156	9	1.760
Echium type						0.007	3	1.430
Erica type		1	1.000	6	1.756		3	1.160
Fabaceae	0.013	9	**2.850**	34	**5.453**	0.416	2	0.810
Hypericum	0.005			1	1.000			
Hippuris		1	1.000					
Jasione type							1	1.000
Lamiaceae							1	1.000
Lonicera				1	0.999			
Malva sylvestris type							1	1.000
Myrtus type				1	1.000			
Mentha type	0.057	1	0.960	1	21.239			
Muscari comosum type				2	1.016			
Olea		1	1.000	1	1.000			
Pinus pinaster				1	1.000		1	1.000
Pinus pinea/halepensis type							1	1.000
Pinus sylvestris type							1	1.000
Ranunculaceae		7	**2.020**	19	**3.880**		16	**3.980**
Rhamnus type				3	1.404			
Rosaceae							4	1.440
Rumex type		1	1.000	3	1.445		6	1.640
Salix		2	1.000	6	**2.279**		21	**3.160**
Scrophulariaceae							1	1.000
Viburnum type				1	1.000		1	1.000
N *E. balteatus* analyzed			58				51	
N *E. balteatus* with pollen			36				46	
N *E. corollae* analyzed			157					
N *E. corollae* with pollen			104					

Regarding to the pollen feeding habits in autumn, the amount of pollen consumed by females did not differ from males, contrarily to what was observed in spring by Haslett (1989a), probably because energy is spent for tissue maintenance purposes and not for reproduction. Both *E. balteatus* and *E. corollae* fed on different plant species revealing a certain degree of selectivity for herbaceous vegetation, such as Asteraceae, Ranunculaceae, *C. telephiifolia* type, and woody vegetation, such as *A. unedo*, *Cytisus/ Ulex* pollen type, *D. gnidium* type, and *Salix* and, in the specific case of *E. corollae*, also Apiaceae and Caryophillaceae. Consumption of pollen is the result between the compatibility of the floral morphology with the insect head and the structure of the mouthparts (Jervis and Heimpel 2005) and these traits can influence plant selection done by syrphids. According to Branquart and Hemptinne (2000), adults of the syrphinae subfamily did not show strong flower preferences but exploited pollen and nectar produced by native plants with large inflorescences and flat corollas, e.g. Apiaceae, Asteraceae, Ranunculaceae and Rosaceae. Among other species, they found *E. balteatus* and *E. corollae* to be highly polyphagous, which is in agreement with our study; nonetheless, we observed that they did not use all flowers available, selecting some pollen types in detriment of others. As referred by Jervis and Kidd (1996), generalist flower-visitors can visit some flower types more frequently than would be expected on the basis of their relative abundance and preferences can alter with different nutritional and environmental factors.

In this study, Asteraceae plants were commonly found in herbaceous and olive grove patches, while in the scrublands, they were less common. In *E. balteatus* and *E. corollae* guts, *Aster* pollen type and Cichorioideae pollen were the most abundant identified pollens (Fig. 8.2; Fig. 8.3) and in most of the cases they were selected (Table 8.1) showing to be important food resources for these syrphid species. On the contrary, *Anthemis* pollen type and Cardueae pollen were less abundant in the guts (Fig. 8.2; Fig. 8.3) and were not selected (Table 8.1). Plant species with these pollen types have been already referred in the literature to be consumed by *E. balteatus* and *E. corollae* (Lundgren, 2009 and references therein; Speight, 2011; Van Veen, 2010). Moreover, the Asteraceae species, *Chamaemelum nobile* (L.) All. (*Anthemis* pollen type) and *Crepis vesicaria* L. (*Cichorioideae* pollen type) showed a positive effect in *E. balteatus* longevity in laboratory studies (Pinheiro et al. 2013).

Considering Ranunculaceae and *C. telephiifolia* (in 2012), they were consumed and selected by syrphids although they were not inventoried in any of the studied patches. Thus, those specimens certainly visited other areas. *E. corollae* and *E. balteatus* were also referred to feed on species with Ranunculaceae pollen by Cowgill et al. (1993) and Speight (2011).

In this study, Apiaceae and Caryophyllaceae pollen were selected by *E. corollae* but not by *E. balteatus*. Some Apiaceae and Caryophyllaceae species are referred as being attractive to syrphids (Bugg et al. 2008; Speight 2011; van Veen 2010). Although Apiaceae were not selected by *E. balteatus* in this study, Laubertie et al. (2012), in a laboratory experiment, showed that species such as *Coriandrum sativum* L. can enhance *E. balteatus* reproduction.

In relation to the woody species, although *E. balteatus* and *E. corollae* were captured in low numbers in the scrubland patches, they consumed and selected bushes pollen (*D. gnidium* type, *Cytisus*/ *Ulex* type, *A. unedo* and *Salix*) independently on the patch where they were captured. In 2013, we found *E. balteatus* consuming and selecting *Cytisus*/ *Ulex* pollen type and some *E. corollae* consuming it. Our results are in agreement with Herrera (1988), who found both species visiting *D. gnidium* and *E. corollae* visiting *Ulex minor* Roth. (*Cytisus*/ *Ulex* type) and with Speight (2011), who referred *E. balteatus* to feed on *A. unedo* flowers. *Salix* is cited as being important in the early spring and attractive for the first emerging syrphids (van Veen 2010) and *E. corollae* is referred to feed on it (Speight 2011). In the current work, syrphids consumed and selected *Salix* but it was not present in the inventoried patches, showing that those specimens visited non-sampled areas.

Plant species belonging to Fabaceae (e.g. *Trifolium repens* L.), Lamiaceae (e.g. *Mentha suaveolens* Ehrh.) and Brassicaceae (e.g. *Raphanus raphanistrum* L. and *Brassica barrelieri* (L.) Janka) were identified in all the patches but were barely selected by *E. balteatus* and *E. corollae*. However, these families have been described to be attractive to syrphids (Bugg et al. 2008; Haslett 1989b; Van Veen 2010; Speight 2011) and, in some cases, to have a positive effect on *E. balteatus* longevity (Pinheiro et al. 2013).

These results suggest that syrphids flew among patches to forage, indicating that adult syrphid feeding may be affected by landscape and is in agreement with Ouin et al. (2006) who showed that greater patch areas, connectivity, and habitat heterogeneity had positive effects on syrphid richness. Moreover, Sarthou et al. (2005) found that landscape structure, length of forest edges and probably the presence of shrubs, influenced the abundance of *E. balteatus*. Ricarte et al. (2011) also highlighted the need to focus on the conservation of woodland remnants of grassland-dominated landscape and scrubland-dominated landscape in order to preserve a large proportion of the biodiversity of syrphids in their studied area, as well as on the maintenance of the mosaic landscape. Additionally, landscape heterogeneity could favor other biocontrol agents, as shown by Koh and Holland (2015) for Anthocoridae, Nabidae and Coccinellidae predatory families or by Lefebvre et al. (2016) for the spider species *Cheiracanthium mildei* C. L. Koch, resulting in complementary action against pests.

In the late summer, fewer plants are flowering and the number of active syrphids decreases (van Veen 2010). However, in this study, during the autumn, a considerable amount of syrphids was collected in white delta traps baited with *P. oleae* pheromone, although in the first instance the goal was not the syrphid capture. Several reasons to explain these captures may be: (1) the low abundance of flowers in association with the white color of the trap could have been a lure to syrphids, resulting in an abnormal number of specimens captured. This hypothesis is in agreement with both Schneider (1969) who suggested that the attractiveness of traps to insects increase when the availability of surrounding flowers decreases and Hickman et al. (2001) who found hungry syrphids flying around yellow water traps. Additionally, Wratten et al. (1995) already found white traps to be attractive for some syrphid species; (2) autumn weather (cold, wind and rain) may stimulate shelter search, being the shape of delta traps an appropriate refuge against adverse weather conditions; and finally, (3) syrphids may be attracted by *P. oleae* pheromone; however, this is the least plausible explanation once during spring and summer periods, syrphids were abundant in the studied area, and delta traps, that were already installed in the field, captured low numbers of syrphids (Villa, Personal observation). Delta traps are not a usual method to capture syrphids, nevertheless in this study they captured a high abundance of specimens.

Summarizing, in this work syrphids fed on both herbaceous and woody vegetation, showing selection for several plants and foraging in patches in the vicinities of the crop. Moreover, in seasons characterized by adverse weather conditions, these areas could act as overwintering sites. These results highlight the importance of conserving heterogeneous agricultural landscapes in order to ensure the existence of food resources and shelter for syrphids. Such observations could be a valuable asset, since syrphids act as biological control agents in several agroecosystems. Therefore, these studies are of major importance to determine what resources could contribute to improve and enhance natural enemies in the agricultural landscape.

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Appendix A

Table A.1. Total abundance of syrphid species collected in three olive groves and two surrounding fields types next to each olive grove in autumn 2012 and 2013.

	Syrphid Species	Olive grove		Herbaceous vegetation		Scrubland		**Total**
		Female	Male	Female	Male	Female	Male	
Autumn 2012	*Eupeodes corollae* (Fabricius, 1974)	7	4	53	59	14	22	**159**
	Eupeodes luniger (Meigen, 1822)	0	0	2	0	1	0	**3**
	Eupeodes nielseni (Dusek and Laska, 1976)	0	0	0	0	1	0	**1**
	Episyrphus balteatus (De Geer, 1776)	0	0	21	29	0	8	**58**
	Sphaerophoria scripta (Linnaeus, 1758)	0	0	6	0	2	1	**9**
	Melanostoma mellinum (Linnaeus, 1758)	0	0	1	1	0	1	**3**
	Melanostoma scalare (Fabricius, 1794)	0	0	2	0	0	0	**2**
	Not identified	0		12		1		**13**
	Total	**7**	**4**	**85**	**89**	**16**	**32**	**248**
Autumn 2013	*Eupeodes corollae* (Fabricius, 1794)	1	0	3	3	0	0	**7**
	Episyrphus balteatus (De Geer, 1776)	0	0	20	29	0	2	**51**
	Melanostoma mellinum (Linnaeus, 1758)	0	0	2	1	0	0	**3**
	Eristalis similis (Fallen, 1817)	0	0	2	0	1	0	**3**
	Paragus sp	0	0	0	1	0	0	**1**
	Not identified	0		4		0		**4**
	Total	**1**	**0**	**27**	**34**	**1**	**2**	**71**

Table A.2. Percentage of ground cover (mean) by flowering plants (family, pollen types and plant species) in scrubland, herbaceous and olive grove from September to December, 2012 and 2013.

Family	Pollen type	Species	Autumn 2012			Autumn 2013		
			Scrubland	Herbaceous	Olive grove	Scrubland	Herbaceous	Olive grove
Amaranthaceae								
	Chenopodiaceae							
		Amaranthus albus L.					0.067	
		Chenopodium album L.					0.020	0.115
Apiaceae								
	Apiaceae							
		Daucus carota L.		0.044	0.006		0.007	
		Eryngium campestre L.						
		Foeniculum vulgare L.	0.0556		0.100	0.100	0.007	0.180
Asparagaceae								
	Ruscus type							
		Ruscus aculeatus L.				0.013		
Asteraceae								
	Anthemis type							
		Chamaemelum mixtum (L.) All.		0.017			0.013	
		Chrysanthemum segetum L.		0.006			0.007	
		Coleostephus myconis (L.) Rchb.f.		0.006	0.0004		0.083	
	Aster type							
		Pulicaria paludosa Link		0.033	0.0004			
		Senecio jacobaea L.		0.007	0.000074			
		Senecio vulgaris L.						0.020
	Cardueae							
		Calendula arvensis L.						0.040
		Carlina hispanica Lam.	0.028					
		Conyza sumatrensis (Retz.) E.Walker		0.033	0.194		1.767	0.020
		Xanthium spinosum L.					0.007	
	Cichorioideae							
		Andryala integrifolia L.		0.011	0.006		0.033	0.007
		Chondrilla juncea L.		0.172	0.217		0.020	0.200
		Crepis capillaris (L.) Wallr.		0.089	0.001		0.027	
		Hypochaeris glabra L.		0.017	0.006	0.011	1.507	
		Hypochaeris radicata L.		0.272				

		Leontodon taraxacoides (Vill.) Mérat subsp. *longirostris* Finch&P.D.Sell		0.300	0.001		0.053	
		Sonchus asper (L.) Hill subsp. *glaucescens* (Jord.) Ball					0.007	
Boraginaceae								
	Echium type							
		Echium plantagineum L.					0.021	
	Heliotropium type							
		Heliotropium europaeum L.		0.017				
Brassicaceae								
	Brassicaceae							
		Brassica barrelieri (L.) Janka					0.815	
		Capsella bursa-pastoris (L.) Medik.					0.115	0.188
		Diplotaxis catholica (L.) DC.					0.233	
		Raphanus raphanistrum L.		0.022			0.327	
Caryophyllaceae								
	Corrigiolatelephiifolia type							
		Corrigiola telephiifolia Pourr.					0.007	
	Caryophyllaceae							
		Spergula arvensis L.						0.040
		Petrorhagia nanteuilii (Burnat) P.W. Bal & Heywood			0.006			
Ericaceae								
	Arbutus unedo							
		Arbutus unedo L.	0.31			3.000		
Euphorbiaceae								
	Euphorbiaceae							
		Chamaesyce sp		0.011				
		Euphorbia segetalis L.		0.017				
Fabaceae								
	Fabaceae indif							
		Trifolium pratense L.					0.015	
		Trifolium repens L.		0.039			1.233	
Geraniaceae								
	Erodium							
		Erodium cicutarium (L.) L'Hér.						0.028

Hypericaceae								
	Hypericum							
		Hypericum perforatum L.	0.009	0.006				
Lamiaceae								
	Mentha type							
		Mentha pulegium L.		0.006				
		Mentha suaveolens Ehrh.		0.167			0.080	
Phytolaccaceae								
	Phytolacca							
		Phytolacca americana L.			0.017			0.100
Plantaginaceae								
	Plantagocoronopus type							
		Plantago coronopus L.		0.039			0.013	
	Plantagolanceolata type							
		Plantago lanceolata L.		0.011			0.147	
Poaceae								
	Poaceae							
		Agrostis castellana Boiss. &Reut.		0.006			0.013	
		*Festuca arundinacea*Schreb.		0.006			0.027	
		Not identified					0.007	
		Lolium rigidum Gaudin					0.320	
Polygonaceae								
	Polygonum aviculare type							
		Polygonum aviculare L.					0.013	
Solanaceae								
	Solanaceae							
		Datura stramonium L.		0.006			0.047	
Thymelaeaceae								
	Daphne gnidium type							
		Daphne gnidium L.	0.074			0.467		
Verbenaceae								
	Verbena type							
		Verbena officinalis L.		0.011	0.006			
Zygophyllaceae								
	Tribulus type							
		Tribulus terrestris L.		0.017				

CHAPTER 9

General discussion, application and future perspectives

9. General discussion, application and future perspectives

The choice of the best management and type of ecological infrastructures depends on factors such as the synchrony with the natural enemies' life cycle and the suitability with the crop management practices. They also must not include resources that favor pests. In this thesis several ecological infrastructures from the olive agroecosystem have been identified as potential reservoirs of key requisites (foods and/or shelter) for natural enemies of *P. oleae*. Some elements of these ecological infrastructures, namely non-prey and non-host resources simultaneously occurring with the natural enemies, have been found to be potential natural foods for *P. oleae* natural enemies. The potential effect of these ecological infrastructures and some of the analyzed elements on *P. oleae* have been determined.

9.1. General discussion

Influence of ecological infrastructures on P. oleae *and its natural enemies*

The study of the potential use or influence of the ecological infrastructures on *P. oleae* and its natural enemies was addressed in field experiments in chapters 3 (*P. oleae* and chrysopids), chapter 5 (parasitoids) and 8 (syrphids). Also the effect of the ground cover management (herbicide, tilling or natural ground cover maintenance) on parasitoids and *P. oleae* was studied (chapter 5). In these experiences, the weather conditions of 2012 (extreme drought situation, low temperatures in winter and high temperatures in summer) determined the results. All the studied insects (*P. oleae*, chrysopids, syrphids and parasitoids) were affected by the weather conditions, leading to negligible values of the carpophagous generation of the olive moth in 2012 and the phyllophagous in 2013 (chapter 3), lower chrysopid (chapter 3) and parasitoid (chapter 5) diversity than expected. In the chapter 5 the high increase of emerged adults of the anthophagous generation of *P. oleae* and the strong diminution of parasitoids observed in 2013 could be related to the 2012 drought. The drought could have given origin to a pest-parasitoid disequilibrium and lead to a strong diminution of parasitoids in 2012 which in the following year would cause a high increase of the pest numbers. Also captures of syrphids (chapter 8) varied from 2012 to 2013, and this could have some relation with the weather conditions.

For the first time some aspects related with landscape connectivity for *P. oleae* were addressed (chapter 3) being that scrublands and in less degree herbaceous vegetation patches did not act as barriers to the movement of *P. oleae*. It remains to disentangle the direction of the effect of this capability on the dispersion of the pest to other olive groves. Apparently *P. oleae* captures were strongly influenced by the weather conditions but the most abundant chrysopid, *C. carnea s. l.*, seem to be more affected by the lack of prey. Some synchrony between *C. carnea s. l.* and *P. oleae* was observed, however in 2012 an autumn peak of *C. carnea s. l.* was registered despite the absence of *P. oleae* carpophagous generation, suggesting that *C. carnea* *s. l.* fed on other preys. Once *C. carnea s. l.* was the most abundant chrysopid, it was selected for analyzing non-prey foods for adults in the chapter 7.

Spontaneous ground covers positively affected the parasitism accomplished by the most abundant parasitoid, *A. fuscicollis*, as well as the number of individuals emerged per olive moth, while herbicide application negatively affected this parasioid (chapter 5). Potential toxicity of herbicide on *A. fuscicollis* should be analyzed. However the tillage did not affect these variables. These could be related with the landscape heterogeneity that characterized the region and/or to the edge vegetation that usually remains in tilled olive groves. *E. flabellatus* was not affected by the ground cover management.

In laboratory we have observed that the ability of *A. fuscicollis* to fly is reduced compared to *E. flabellatus*, which leads to hypothesize that *E. flabellatus* could take more advantage from the plant species present in the ecological infrastructures within the olive crop and around them. Additionally we have observed that *A. fuscicollis* is highly sensitive to temperature variations and has shorter longevities (unpublished data). Even thought the number of individuals emerged from a larva of *P. oleae* is higher with *A. fuscicollis* than with *E. flabellatus* (chapter 5), this apparent weakness of *A. fuscicollis* (more sensitive to temperature variations and to herbicide application) lead to think that its mortality could be elevated under adverse conditionin field, and in those situations *E. flabellatus* could gain certain relevance as *P. oleae* control agent. Under this hypothesis, in the chapter 6, *E. flabellatus* was selected to analyze the suitability of non-host foods for adults, although future research should focus on *A. fuscicollis* as well.

During autumn, a flower scarcity period in the Mediterranean areas, a higher number of syrphids was collected in open (herbaceous) than in woody patches (olive groves and scrublands) being that few specimens were captured in olive groves (chapter 8). In contrast, during spring syrphids are easily observed hovering over the flowers within the olive groves. Additionally the pollen consumption results suggest that syrphids flew among patches to forage. The most abundant syrphids during the autumn fed on both herbaceous and woody vegetation foraging in patches in the vicinities of the crop.These results could indicate the use of different types of patches seasonally by syrphids, being that herbaceous and woody vegetation patches around the groves would be selected during autumn.

In sum, the heterogeneous landscape composed by herbaceous and woody vegetation around the olive groves could favor syrphids. *P. oleae* is able to disperse over non-crop patches, although the implications for the pest populations are unknown. Additionally, the ground covers within the olive groves can favor parasitism on *P. oleae* while the herbicide application can diminish it. The weather conditions strongly affect the studied insects.

Food resources suitability for P. oleae *and its natural enemies*

The potentiality of non-crop, non-host and non-prey foods present within the studied infrastructures (olive grove ground covers, surrounding scrubland and herbaceous vegetation patches) to benefit *P. oleae* and some of their natural enemies was studied in the chapter 4 (*P. oleae*), in the chapter 6 (parasitoids), chapter 7 (chrysopids) and chapter 8 (syrphids).

Honeydews secreted by *S. oleae* followed by *E. olivina* honeydews were the foods that resulted in the better survival and reproduction performance of adults of the anthophagous generation of *P. oleae* (chapter 4). The lower viscosity of *S. oleae* than *E. olivina* could explain the better performance obtained with the former. Among the flowers, the better performance was accomplished with *M. sylvestris. C. maculatum* increased the longevity but disrupted some reproduction parameters. Once this species seem to be highly attractive for natural enemies, it should be deeper investigated. *T. repens* only improved the survival of females. The other plants (*A. arvensis*, *A. integrifolia*, *C. capillaris* and *J. montana*) did not affect *P. oleae* biological parameters. The results obtained suggest that *P. oleae* is sinovigenic, emerging without mature eggs and with reserves for reproduction.

Several non-host foods present in the olive agroecosystem infrastructures were identified as nutritionally suitable for *E. flabellatus* (chapter 6). The honeydews secreted by *S. oleae* and *E. olivina* showed to be the most suitable foods tested for *E. flabellatus*. No differences in survival were found with both insects honeydews, suggesting that *E. flabellatus* is able to feed on viscous sugary liquids. Among the flowers the best performance was observed with *M. sylvestris* and was followed by *D. carota*. *A. integrifolia*, *J. montana* and *T. barbata* did not show difference with negative control but also did not show differences with *D. carota*, therefore when associated with other food resources they may result in longer lifespan. For *A. arvensis*, *C. segetum, E. plantagineum*, *S. purpurea*, *C. capillaris*, *C. myconis* and *H. perforatum* no differences with the negative control were found. *C. majus* resulted in the shorter lifespan than the negative control.

Both *S. oleae* and *E. olivina* honeydews and flowers of the three plants that sequentially bloom during the year, *V. persica*, *M. sylvestris*, and *L. purpureum* highly enhance *C. carnea* survival (chapter 7). Four flowers (*R. olissiponensis*, *L. etrusca*, *F. vulgare*, and *D. carota*) also resulted in some improvement. The results obtained suggest that the low reproduction performance could be related with a poor diet in proteins, being that the food that originated better reproduction fitness were *V. persica* and honey solution. *R. officinalis*, *S. media*, *S. vulgaris* and *C. arvensis* did not show differences with the negative control.

E. balteatus and *E. corollae* consumed and selected both herbaceous (Asteraceae, Ranunculaceae, *C. telephiifolia* type and Apiaceae and Caryophyllaceae in the case of *E. corollae*) and woody vegetation (*D. gnidium* type, *Cytisus*/ *Ulex* type, *A. unedo* and *Salix*) showing selection for several plants during a food scarcity period (chapter 8).

In conclusion, *S. oleae* and *E. olivina* honeydews were the foods that resulted in better performance for *P. oleae*, *E. flabellatus* and *C. carnea s. l*. *P. oleae* seemed to be affected by the viscosity of sugary liquids, while its natural enemies did not. Flowers of *M. sylvestris* enhanced the performance of *P. oleae*, *E. flabellatus* and *C. carnea s. l. V. persica* and *L. purpureum* can be interesting plants for *C. carnea s. l.* in the beginning of the season. Regarding Apiaceae plants, *D. carota* improved the survival of *E. flabellatus* and slightly improved the survival of *C. carnea s. l.*, and Apicaece pollen type was selected by *E. corollae*. Syrphids seem to select Ranunculaceae, and *R. olissiponensis* belonging to this family also slightly improved *C. carnea s. l.* Also Asteraceae and bushes species were important for syrphids. Therefore, the maintenance of these resources in the agroecosystem could benefit more than one type of natural enemy, although some care should be taken particularly with the insect honeydews and *M. sylvestris* once also benefited *P. oleae*.

9.2. Applications

- Complex and heterogeneous landscapes (with herbaceous and woody areas vegetation around the olive groves) as well as low impact management practices (spontaneous ground cover and no application of herbicides) seem to favor some natural enemies of *P. oleae*. However more research is needed to understand the effect on the final pest control effect.
- Several foods that improved the performance of natural enemies (such as insect honeydews or *M. sylvestris*), also improved *P. oleae* in laboratory assays, therefore these foods should be used with care.
- Several foods that improved the performance of natural enemies (*V. persica* and *L. purpureum*) are not coincident with the anthophaous generation of the olive moth, however they are coincident with the phyllophagous generation and they could influence *P. oleae* populations.
- *D. carota* slightly improved some of the natural enemies. Its blooming period is coincident with the end of the anthophagous generation flight period of *P. oleae*. Therefore, although this plant can present some potentiality for conservation biological control strategies, some research is needed to analyze its effect on the pest.
- *C. maculatum* is a potential candidate for enhance biological control, but deeper investigation is needed to confirm it.
- Since *P. oleae* seem to be more positively affected for sugary liquids as insect honeydews and nectar of flowers special attention should be given to these types of foods in conservation biological control strategies.

9.3. Future perspectives

Several queries, which should be tackled in further researches, raised from the results obtained in this thesis:

Queries about P. oleae

- Once different landscape patches do not act as barriers for the *P. oleae* movement, it should be investigated how this could affect to the *P. oleae* capability to disperse to other olive groves and the final goal of pest control.
- A better understanding about the landscape structure on *P. oleae* is still needed.
- The adult feeding of the phyllophagous and carpophagous generations of the olive moth. Particularly the effect of *V. persica* and *L. purpureum* on the phyllophaous generation should be studied.
- The effect of non-crop feeding of *P. oleae* in the field studies should complement the laboratory experiment presented in this thesis.

Queries about the parasitoids

- The potential toxicity of herbicides and pesticides on *A. fuscicollis* should be investigated.
- Studies about reproduction traits of *E. flabellatus* should complement the results presented in this thesis.
- Suitability of natural foods for *A. fuscicollis* should be determined.
- The response of *E. flabellatus* and *A. fuscicollis* biological parameters to different scenarios of stress conditions should be investigated.
- The non-host feeding of *E. flabellatus* in field studies should complement the laboratory experiment presented in this thesis.

Queries about chrysopids

- A better understanding about the landscape structure on chrysopids is needed.
- The effect of non-prey feeding of *C. carnea s. l.* in field studies should complement the laboratory experiment presented in this thesis.

Queries about syrphids

- Syrphids feeding during spring and summer remains to be evaluated.
- The specific role of *E. balteatus* and *E. corollae* in the *P. oleae* control.

Queries about the food resources

- The nutrients of the suitable foods identified in this thesis should be analyzed.
- The metabolism involved to process the suitable foods in *P. oleae* and their natural enemies remains to be investigated.

- Mixture of foods should be tested in laboratory experiments in order to avoid masking the real effect of the tested food resources by the deprivation of essential nutrients.

Other queries

- Evaluate the positive or negative effect of *E. olivina* and *S. oleae* on the final crop yield
- Other important pests, such as *B. oleae*, should be tested to determine the effect of the ecological infrastructures and food resources studied in this thesis on their performance.
- Test the final effect on pest control of the identified ecological infrastructures and food resources.

The knowledge obtained as a result of the investigations accomplished in this thesis will allow to elaborate new and more efficient approaches to the research about conservation biological control of the olive moth through the study of ecological infrastructures designed to enhance the natural enemies but not the pest. However, and with the goal of applying this knowledge in the field and make possible the use of efficient conservation biological control strategies, the queries that arise from this work have to be studied in detail.

APPENDIX

Accepted Papers

Villa et al., 2016. Are wild flowers and insect honeydews potential food resources for adults of the olive moth, *Prays oleae*?, Journal of Pest Science, In Press doi: 10.1007/s10340-016-0745-8

Villa et al., 2016. Ground cover management affects parasitism of *Prays oleae* (Bernard), Biological Control, 96:72-77, doi:10.1016/j.biocontrol.2016.01.012

Villa et al., 2016. Life-history parameters of *Chrysoperla carnea* s.l. fed on spontaneous plant species and insect honeydews: importance for conservation biological control In Press, BioControl, doi: 10.1007/s10526-016-9735-2

J Pest Sci
DOI 10.1007/s10340-016-0745-8

ORIGINAL PAPER

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

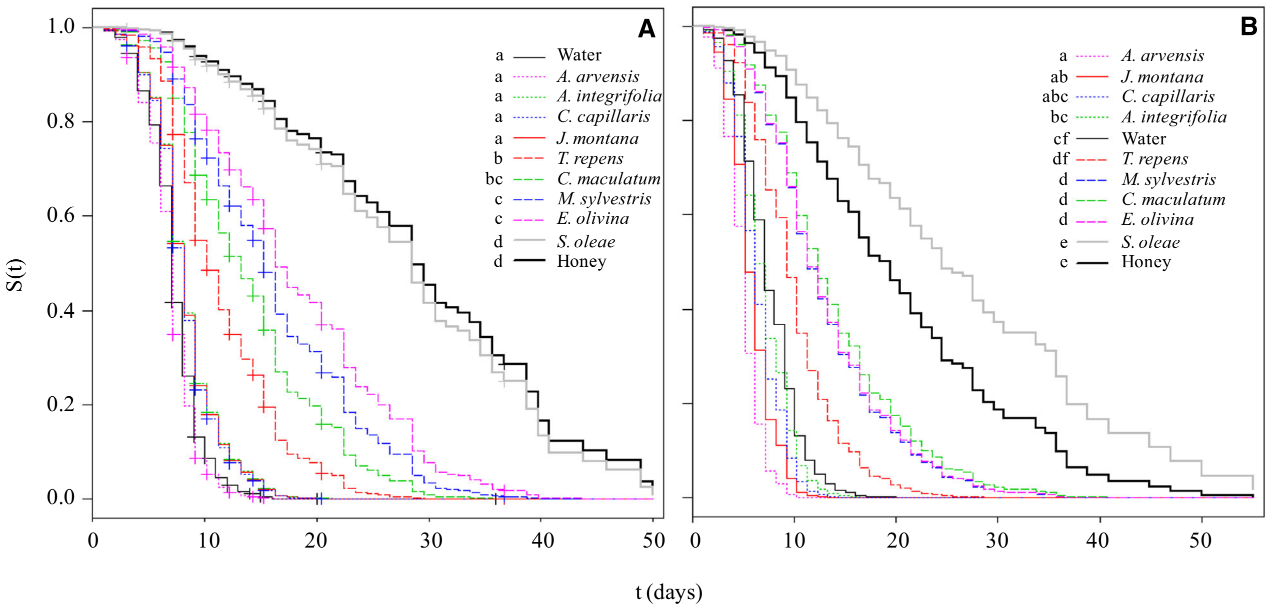

Fig. 1 Cox estimates of the survival function, S(t), for females (**a**) and males (**b**). *Different letters* on the legend indicate significant differences in death hazard among treatments (significance level < 0.05). *Crosses* indicate censored data

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

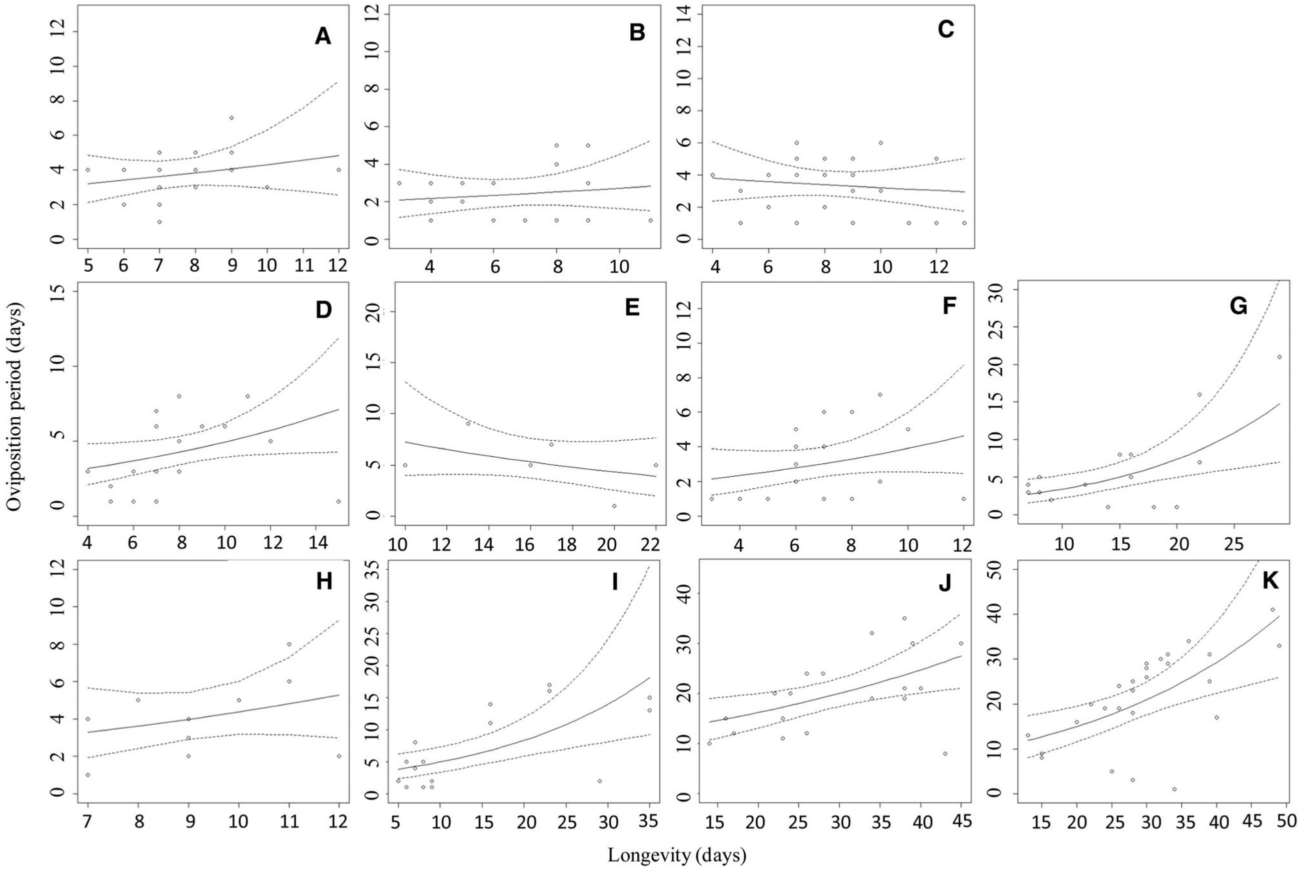

Fig. 2 GLMs plots for oviposition period variation as a function of longevity in each treatment. **a** Water; **b** *A. arvensis*; **c** *A. integrifolia*; **d** *C. capillaris*; **e** *C. maculatum*; **f** *J. montana*; **g** *M. sylvestris*; **h** *T. repens*; **i** *E. olivina*; **j** *S. oleae*;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

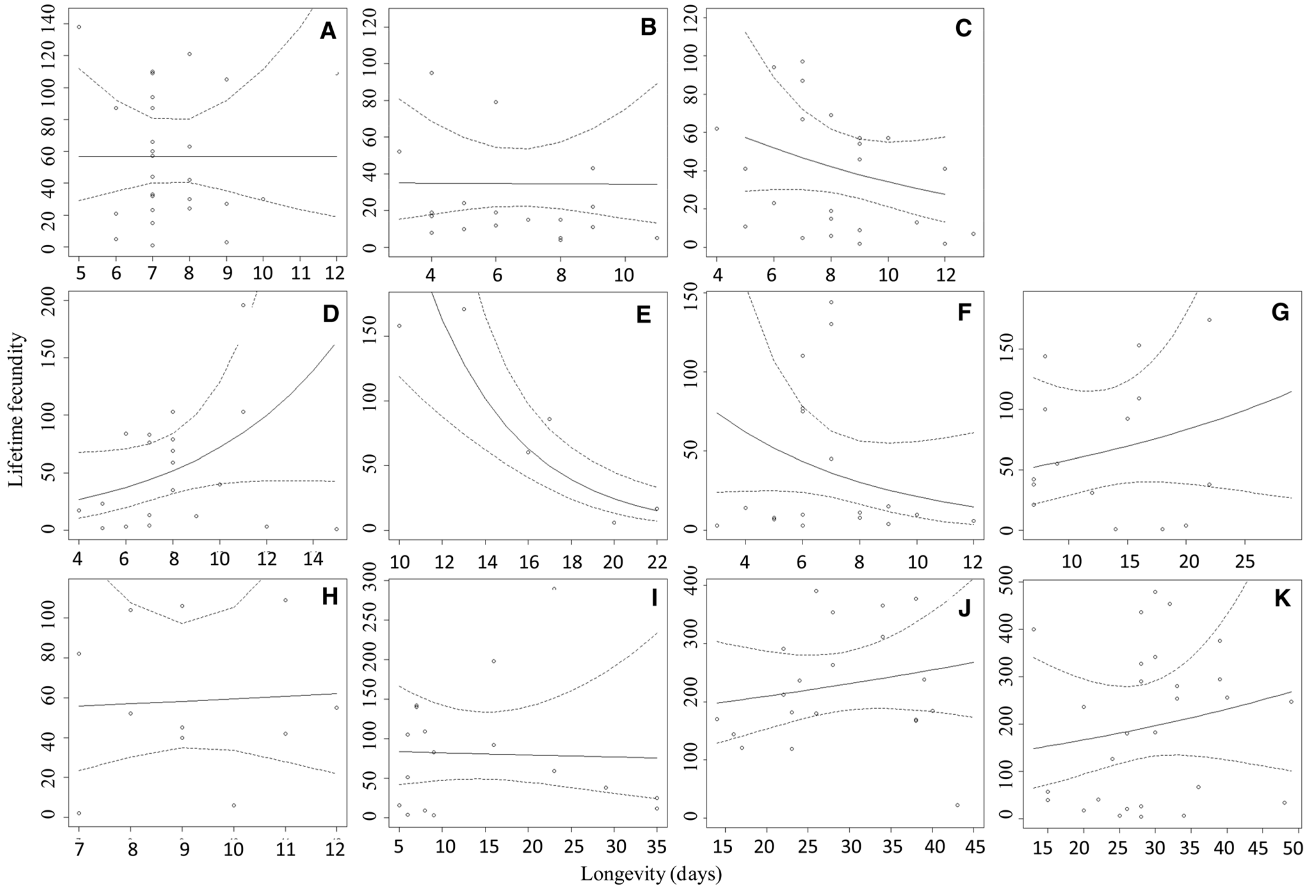

Fig. 3 GLMs plots for lifetime fecundity variation as a function of longevity in each treatment. **a** Water; **b** *A. arvensis*; **c** *A. integrifolia*; **d** *C. capillaris*; **e** *C. maculatum*; **f** *J. montana*; **g** *M. sylvestris*; **h** *T. repens*; **i** *E. olivina*; **j** *S. oleae*;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

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HIGHLIGHTS

- Ground cover management did not influence the emergence rate of *Prays oleae*.
- Spontaneous ground covers favored the overall parasitism and *Ageniaspis fuscicollis*.
- Herbicide application negatively affected the overall parasitism and *A. fuscicollis*.
- *Elasmus flabellatus* was not affected by the ground cover management.
- Surrounding vegetation areas may be important for maintaining parasitoids in the olive grove.

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ABSTRACT

Spontaneous ground covers comprise ecological infrastructures that may provide food, alternative hosts and shelter for parasitoids in olive groves, thus contributing to biological control of pests. This study investigated the effects of herbicide application, tillage, and conservation of spontaneous ground covers on parasitism of the anthophagous generation of the olive moth, *Prays oleae* (Bernard). The study was performed in northeast Portugal in 2011 and 2013 in 14 and 15 olive groves, respectively, with different management types. Generalized Estimating Equations (GEE) were used to analyze olive moth emergence, overall parasitism rate, relative abundance of parasitoid species, and total parasitism of olive moth larvae. *Ageniaspis fuscicollis* (Dalman) accounted for the majority of the parasitism, followed by *Elasmus flabellatus* (Fonscolombe). In both years, ground cover management type did not influence the emergence rate of *P. oleae*. However, overall parasitism rate, emergence of *A. fuscicollis*, and the number of *A. fuscicollis* emerging per olive moth larvae varied among years. In 2011, the latter response variables were significantly higher in groves with spontaneous ground cover than in those treated with herbicide, indicating a negative effect of herbicides on parasitoids. Although tilled groves obtained higher values for these variables in 2013, parasitism rates were generally very low. In sum, the management of ground covers seemed to influence the overall rate of *P. oleae* parasitism in some years, but longer-term experiments are needed to clarify this trend.

1. Introduction

Habitat management through the establishment and maintenance of ecological infrastructure, such as diversified ground cover, is a strategy of conservation biological control that aims to conserve or manipulate the environment in order to enhance the effectiveness of natural enemies (Landis et al., 2000; Boller et al., 2004). Because nectar and pollen are essential food for many adult parasitoids (Jervis et al., 1993; Vattala et al., 2006), flowers can promote the abundance and longevity of parasitoids as well as increase parasitism rates (Díaz et al., 2012). However, apart from providing shelter and alternative hosts for generalist parasitoids (Landis et al., 2000), flowers may also benefit pests (Baggen and Gurr, 1998; Lavandero et al., 2006).

Olive groves have relevant economic, social and landscape importance in the Mediterranean area and the olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae), is one of the most important olive pests. *P. oleae* has three generations per year: the phyllophagous generation feeds on olive leaves from October to April, the anthophagous generation feeds on floral buttons from April to June, and the carpophagous generation penetrates the fruit and feeds on the stone from June to October. The carpophagous generation causes the most damage to the crop (Bento et al., 2001). Several generalist and specialist parasitoid wasps, such as *Ageniaspis fuscicollis* (Dalman) (Hymenoptera: Encyrtidae),

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Chelonus elaeaphilus Silvestri (Hymenoptera: Braconidae) and *Elasmus flabellatus* (Fonscolombe) (Hymenoptera: Eulophidae), attack the olive moth (Bento et al., 1998; Herz et al., 2005).

In perennial agroecosystems, spontaneous vegetation can be removed through either tillage or herbicide application. Soil erosion and pollution are two consequences of these practices that could influence parasitoid communities (Vanwalleghem et al., 2011; Egan et al., 2014). Previous studies in olive groves showed that spiders, parasitoids and the predatory heteropteran *Deraeocoris punctum* (Rambur) were positively influenced by ground covers when compared with tilled groves (Lousão et al., 2007; Herz et al., 2005; Cárdenas et al., 2012; Rodríguez et al., 2012; Paredes et al., 2013a). However, results obtained for olive pests were inconsistent. Paredes et al. (2013b) found that areas of herbaceous and woody vegetation near olive crops, and smaller patches of woody vegetation within olive groves, were associated with reduced abundance of two olive pests, *P. oleae* and *Euphyllura olivina* (Costa), but inter-row ground covers had no effect on these pests. A long term analysis at a regional scale performed by Paredes et al. (2015) showed that ground covers did not influence the abundance of *Bactrocera oleae* (Rossi), *P. oleae*, *E. olivina* and *Saissetia oleae* (Olivier). Both local factors, such as the intensity of pesticide application or micro-climatic features, and larger-scale factors, such as landscape diversity or patch size, can affect pest abundance in olive groves (Rodríguez et al., 2009; Boccaccio and Petacchi, 2009; Ortega and Pascual, 2014).

From a sustainability perspective, studies are needed to establish the management practices that most favor the biological control of pests. The objective of the present study was to determine the effect of different management practices (conservation of spontaneous ground cover, tillage, or herbicide application) on the parasitoid species emerging from *P. oleae*. In particular, we hypothesized that farming practices would influence: (i) olive moth emergence rate (ii) parasitoid community composition and (iii) the overall rate of parasitism.

2. Material and methods

2.1. Study sites and sampling design

The studied groves were located in Bragança District in north-eastern Portugal (Fig. 1). Fifteen groves with different ground cover management practices were selected in 2011 (six tilled olive groves, five with spontaneous ground cover, and four with herbicide application) and 14 were selected in 2013 (five tilled groves, five with spontaneous ground cover, and four with herbicide application). A heterogeneous distribution of the plots according to the different management practices was used as criteria when choosing the groves to avoid spatial clustering of management types and thus results that might be more related to grove proximity than management practices. The minimum distance among plots was 300 m (from the center of the grove) and the maximum was 65 km. The mean area of these groves was about 2 ha; none were irrigated and no insecticides were applied during the anthophagous generation of the olive moth. According to farmers' information, 2 l/ha of the herbicide glyphosate (Roundup Ultra®, Bayer, aqueous solution with 360 g/l of glyphosate) was sprayed in the plantation row, in herbicide treatment groves, at the end of April. The distance between trees varied from seven to nine meters and the age of trees varied from 18 to 80 years. In 2012, sampling was not possible due to low population levels of olive moth region-wide, probably caused by extreme drought and abnormally high temperatures during the anthophagous generation.

To ensure a heterogeneous distribution of samples within each grove, 10 olive trees were randomly selected at the end of May and 20 olive moth larvae were hand-collected from each tree at a height of 1.5–1.7 m by walking around the tree canopy, for a total of 200 larvae from each grove. In the laboratory, larvae were isolated in plastic tubes (6.0 cm height × 1.0 cm in diameter) and held in a climatic-controlled chamber set to 21 °C and a 16:8 (L:D) day length until emergence. Adult olive moth and parasitoid emergence in each tube was recorded, as well as dead/non-emerged larvae. Parasitoids were identified to species and sexed.

2.2. Data analyses

Since the larvae within each grove probably experienced similar conditions, the values obtained with groves are not assumed to be independent, i.e., spatial autocorrelation exists between these samples (see Zuur et al., 2009). One method available for dealing with such interdependency among samples is the Generalized Estimating Equation (GEE). An advantage of GEEs is that they can cope with misspecifications of the entire distribution and require only the main structure. Thus, correct inferences about regression coefficients are possible even if variances and correlations are erroneously specified (Ziegler and Vens, 2010). In the present study, GEEs were used to analyze the data after model validation. The explanatory variable, X_{is}, was ground cover management with three levels: tillage (T), groves with spontaneous ground cover (S) and groves treated with herbicides (H). Binary response variables were adult moth emergence, overall parasitoid emergence, and most abundant parasitoid species, with values of 1 for success and 0 for failure. The variance structure was of binomial type and the relationship between the conditional mean and the systematic component was logit link, therefore,

$$E(Y_{is}|X_{is}) = e^{\alpha+\beta 1 X_{is}}/1 + e^{\alpha+\beta 1 X_{is}}$$

or

$$E(Y_{is}|X_{is}) = \pi_{is} \text{ and } \operatorname{var}(Y_{is}|X_{is}) = \pi_{is} \times (1 - \pi_{is}),$$

where Y_{is} the value of response variable where i = 1,...,200 larvae and s the grove and π_{is} the probability of success of the response variable (Zuur et al., 2009). Exchangeable correlation structure was used because correlation between two observations from the same grove is expected. The scale parameter was fixed to 1 because binary data cannot be overdispersed.

Because the numbers of parasitoids emerging from moth larvae (separately analyzed for the most abundant parasitoid species) are count data, the variance structure was Poisson type and the relationship between the conditional mean and the systematic component was log link, therefore,

$$E(Y_{is}|X_{is}) = e^{\alpha+\beta 1 X_{is}}$$

or

$$E(Y_{is}|X_{is}) = \mu_{is} \text{ and } \operatorname{var}(Y_{is}|X_{is}) = \phi \times v(\mu_{is})$$

where v() is the variance function and ϕ the scale parameter. Exchangeable correlation structure also was used in this case.

Data analyses were performed using the *geeglm* function from "*geepack*" package (Højsgaard et al., 2006) in R software (R Core Team, 2014) and the *anova* function from "*stats*" package was applied to test for differences between management treatments, followed by pairwise comparison with the *lsmeans* function from "*lsmeans*" package (Lenth and Hervé, 2015).

Model validation for binary dependent variables was performed using the heat map plot and heat map statistics in the "*heatmapFit*" package (Esarey et al., 2014). In the heat map plot, predicted probabilities are plotted versus within-sample empirical frequencies (obtained by nonparametric smoothing) and a heat map line is drawn. Then one-tailed p-value is obtained by comparing the original heat map line with its parametrically bootstrapped

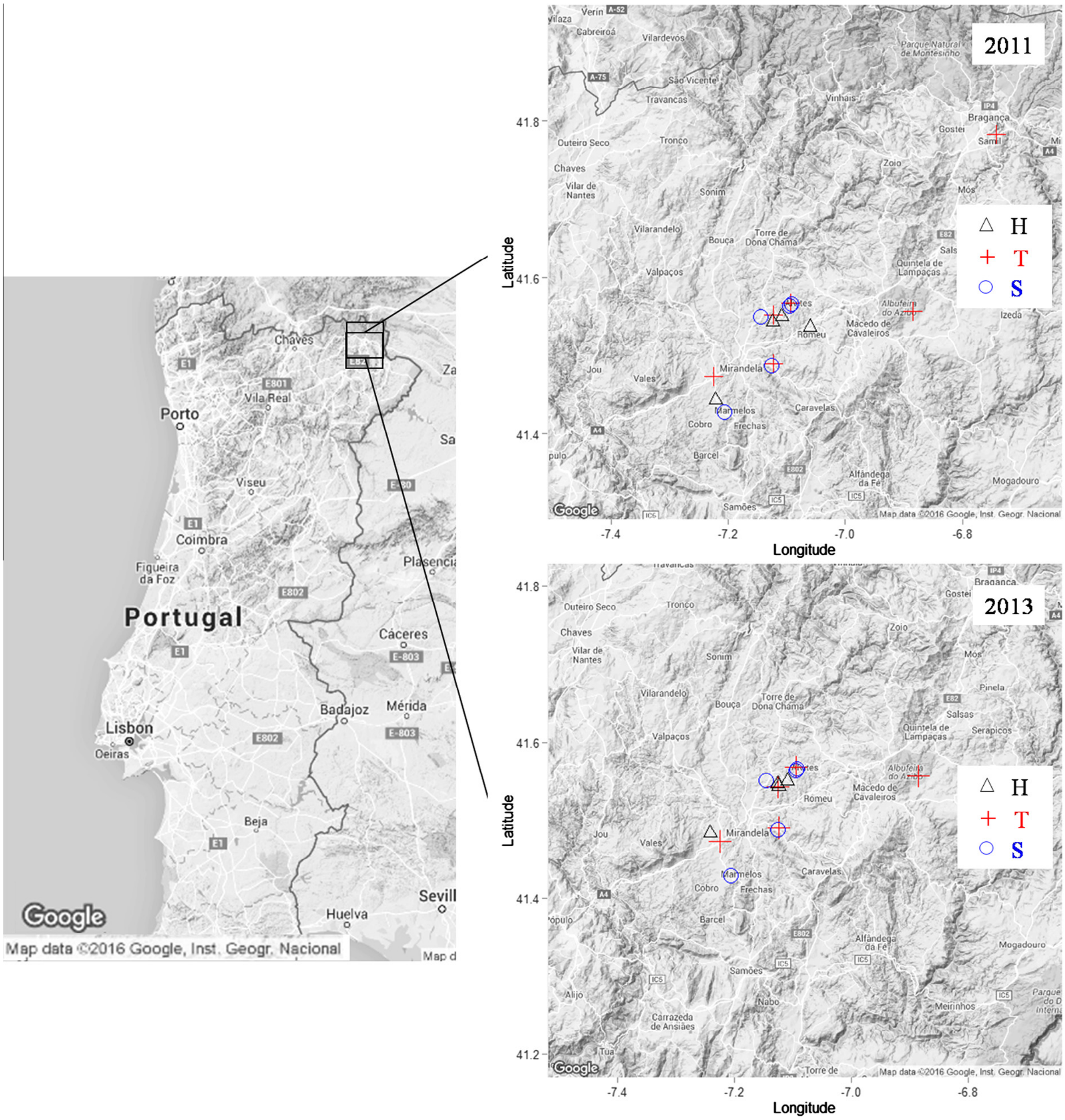

Fig. 1. Maps showing locations of the sampled groves. A: 2011; B: 2013. H: herbicide application; T: tillage; S: ground cover.

distribution (obtained by the simulation of 1000 draws of the response variable from the fitted model). If more than 20% of observations on the heat map line are $P \leqslant 0.1$, then the specification is rejected; otherwise, it is accepted (Esarey and Pierce, 2012; Esarey and Du, 2014). In the present work, heat map statistics indicated that 0% of within-sample predictions had a bootstrapped $P < 0.1$ for all models, thus being accepted.

Graphic model validation was performed for the count dependent variables following Zuur et al. (2009). Residuals were plotted against fitted values to identify any violations of homogeneity. Residuals were plotted against the explanatory variable 'treatment' (ground cover management). Histograms of residuals were plotted to assess their normality.

3. Results

Moth emergence in 2011 varied from 15.6% in tilled groves to 20.5% in groves treated with herbicide and in 2013 varied from 54.8% in tilled groves to 69.6% in groves with spontaneous ground cover. No differences were found among management practices in either year for moth emergence and for the proportion of emerged moth females (Table 1).

Differences in overall parasitism among management treatments were observed in both years. In 2011, parasitism rate was significantly higher in olive groves with spontaneous ground cover than in groves treated with herbicide. In 2013, parasitism was significantly higher in tilled groves than in groves with spontaneous ground cover, although not compared to groves treated with herbicide (Table 1).

In both years, *A. fuscicollis* was the most abundant parasitoid emerging from olive moth and the statistical differences were the same as those obtained for the overall parasitism (Table 1). The second most abundant parasitoid emerging from olive moth larvae was *E. flabellatus*. The emergence of this species varied from 11.5% in groves with spontaneous ground cover to 15.2% in groves treated with herbicide in 2011 and from 1.0% in groves treated

Table 1
Predicted probabilities (π) for response variables obtained with Generalized Estimating Equations by management type (H: herbicide application; T: tillage; S: ground cover). Predicted probabilities bearing different letters were significantly different among management types within rows (LS means, $\alpha = 0.05$; d.f. = 2).

Response variables	π_H (C.I. 95%)	π_S (C.I. 95%)	π_T (C.I. 95%)	χ^2	p
2011					
Prays oleae emergence	0.205 (0.168–0.248) a	0.160 (0.105–0.237) a	0.156 (0.135–0.180) a	5.04	0.08
Overall parasitism	0.362 (0.308–0.421) b	0.500 (0.428–0.573) a	0.412 (0.321–0.509) ab	8.91	0.012
Ageniaspis fuscicollis emergence	0.194 (0.126–0.286) b	0.378 (0.297–0.467) a	0.252 (0.142–0.409) ab	9.23	0.001
Elasmus flabellatus emergence	0.152 (0.106–0.215) a	0.115 (0.070–0.183) a	0.142 (0.089–0.221) a	0.99	0.63
Proportion of females – *Prays oleae*	0.446 (0.372–0.522) a	0.559 (0.495–0.620) a	0.524 (0.444–0.603) a	5.29	0.07
Proportion of females – *Elasmus flabellatus*	0.701 (0.640–0.756) a	0.764 (0.706–0.813) a	0.732 (0.693–0.768) a	2.50	0.29
2013					
Prays oleae emergence	0.685 (0.614–0.747) a	0.696 (0.611–0.770) a	0.548 (0.418–0.672) a	4.44	0.11
Overall parasitism	0.050 (0.025–0.100) ab	0.045 (0.032–0.064) b	0.129 (0.064–0.244) a	7.15	0.028
Ageniaspis fuscicollis emergence	0.040 (0.021–0.074) ab	0.032 (0.020–0.052) b	0.113 (0.049–0.239) a	6.92	0.031
Elasmus flabellatus emergence	0.010 (0.004–0.024) a	0.014 (0.008–0.023) a	0.012 (0.004–0.029) a	0.47	0.79
Proportion of females – *Prays oleae*	0.490 (0.464–0.517) a	0.465 (0.380–0.553) a	0.478 (0.440–0.515) a	5.13	0.77

C.I.: 95% confidence interval.

with herbicide to 1.4% in groves with spontaneous ground cover in 2013. There were no significant differences among management treatments in the numbers of *E. flabellatus* emerging in either year (Table 1). Females of *E. flabellatus* in 2011 had equal probability of emerging in all groves regardless of management treatment (Table 1), although model residuals were more negative than positive, indicating that it could be over-predicting the proportion female. In 2013, it was not possible to model the proportion of *E. flabellatus* that were female due to low parasitation by this species.

In 2011, significantly more *A. fuscicollis* emerged from each *P. oleae* larvae in groves with spontaneous ground cover than in groves treated with herbicide, with tilled groves not significantly different from either (Table 2). In 2013, there were no significant differences among management practices in the number of *A. fuscicollis* emerging (Table 2). In 2011, there were no significant differences in numbers of *E. flabellatus* emerging from *P. oleae* larvae among management treatments (Table 2), and in 2013, it was not possible to elaborate a model for *E. flabellatus* due to low levels of parasitism.

Other parasitoid species emerged in low numbers in both years. In 2011, these less common taxa were found mostly in tilled groves and seven taxa were identified: *Apanteles xanthostigma* (Haliday) (H: 2, S: 9, T: 11); Chalcididae (H: 11, T: 2), *Pnigalio* sp. (T: 1); *Angitia armillata* Grav. (T: 2), Ichneumonidae (T: 1); Pteromelidae (T: 2), *Chelonus elaeaphilus* Silvestri (T: 2, H: 1). Two parasitoid species recovered from tilled groves were not identified. In 2013, two taxa were recollected: *A. xanthostigma* (in T: 1 and H: 1), *C. elaeaphilus* (in T: 2 and H: 1) and several specimens remained unidentified (in H: 1, S: 1, and T: 2).

4. Discussion

The primary parasitoid *A. fuscicollis* was the most abundant species emerging from *P. oleae* larvae of the anthophagous generation in this study, in agreement with the general pattern described in the Mediterranean region by Campos and Ramos (1982), Bento et al. (1998), Herz et al. (2005) and Rodríguez et al. (2012). Bento et al. (1998) and Herz et al. (2005) described *C. eleaphilus* as the second most abundant parasitoid, but only a few individuals emerged in the present study. These authors also reported a more diverse parasitoid complex with *E. flabellatus* less prominent than in the present work.

Our results suggest that the effect of management practices on parasitism by *A. fuscicollis*, and the numbers emerging per parasitized moth larva vary with year. Spontaneous ground covers favored both these response variables in 2011, but the pattern changed in 2013 and tilled groves yielded higher values for parasitism by *A. fuscicollis*, but the ground cover management did not influence the number of emerged *A. fuscicollis*. The emergence rate of the olive moth varied from 15.6–20.5% in 2011 to 54.8–69.6% in 2013 while the parasitism rate varied from 36.2–50.0% in 2011 to 4.5–12.9% in 2013. Weather conditions, such as drought, probably influence this variation between years. According to The Portuguese Sea and Atmosphere Institute (www.impa.pt) the accumulated precipitation and minimum temperature in the region of Bragança during 2011 and 2013 presented normal values, while the winter of 2011/2012 presented particularly lower than normal values (i.e., ca. 50 mm and −4 °C, compared to ca. 220 mm and 1 °C). Similarly, Paredes et al. (2013b) inferred that the annual variability in abundance of two pest species (*E. olivina* and *P. oleae*) was suggestive of an important role of climatic conditions in modulating pest abundance. Montiel Bueno (1981) pointed to weather conditions and pest density (which influences parasitism rate) as factors that can influence olive moth mortality. Thus, the change in abundance of *P. oleae* in 2013, which was probably triggered by weather conditions in 2012, may have also affected parasitoid abundance and mitigated the effect of other factors, such as ground cover. In 2011, levels of parasitism were high and contributed to lower moth emergence, likely decreasing the field population. We analyzed only treatment effects on the anthophagous genera-

Table 2
Predicted means (μ) for response variables obtained with Generalized Estimating Equations by management type (H: herbicide application; T: tillage; S: ground cover). Predicted means bearing different letters were significantly different among management types within rows (LS means, $\alpha = 0.05$; d.f. = 2).

Response variables	μ_H (C.I. 95%)	μ_S (C.I. 95%)	μ_T (C.I. 95%)	χ^2	p
2011					
No. *Ageniaspis fuscicollis*	11.83 (10.46–13.37) b	13.96 (13.59–14.34) a	12.71 (11.83–13.67) ab	12	0.0025
No. *Elasmus flabellatus*	2.31 (2.11–2.54) a	2.29 (1.93–2.72) a	2.18 (1.99–2.38) a	0.899	0.64
2013					
No. *Ageniaspis fuscicollis*	12.42 (11.27–13.68) a	11.93 (10.15–14.02) a	12.18 (10.24–14.49) a	0.193	0.91

C.I.: 95% confidence interval.

tion, but pest reduction due to parasitism might have been more pronounced in the carpophagous generation because *A. fuscicollis* is well synchronized phenologically with the olive moth (Campos and Ramos, 1982). For example, *A. fuscicollis* was found to parasitize up to 55% of larvae in the carpophagous generation (Bento et al., 1998).

Landscape heterogeneity can also interact with the effects of management practices in olive groves. In our work, the study area was surrounded by different patches of herbaceous and woody plants that could have favored or disfavored the abundance of parasitoids in olive groves, thus clouding the potential effects of tillage or herbicide application. Similarly, Paredes et al. (2013a), Rodríguez et al. (2012) and Pak et al. (2015) who found that parasitoids responded to local environments and to the landscape within which that environment is embedded. Paredes et al. (2015) suggested that ground cover is not effective in reducing pest abundance when considered as a single factor. Moreover, *P. oleae* and its parasitoids could respond differentially to plant composition with an agroecosystem as a consequence of flower morphology that in turn determines the accessibility of pollen and nectar to different insects (Jervis et al., 1993; Wäckers, 2005).

In 2011, overall parasitism, parasitism by *A. fuscicollis*, and the number of *A. fuscicollis* emerging per olive moth were lower in olive groves treated with herbicide, but no differences were found for either parasitism by *E. flabellatus*, or the number of *E. flabellatus* emerging per olive moth, in either year. *E. flabellatus* behaves as a facultative hyperparasitoid, of other *P. oleae* parasitoids and, generally, is considered undesirable (Bento et al., 1998). Nevertheless, this species was responsible for almost half the overall parasitism in groves treated with herbicide in 2011. Therefore, in some conditions, *E. flabellatus* could have more importance than usually considered. Moderately negative impacts of glyphosate on the reproduction and survival of *Palmistichus elaeisis* (Delvare & LaSalle) (Hymenoptera: Eulophidae), an endoparasitoid of Lepidoptera and Coleoptera pupae, were described by Menezes et al. (2012) in a study conducted under controlled conditions. In our assay, the potential direct toxicity of herbicide to *A. fuscicollis* might also be considered. However, the absence of flowers, and therefore floral resources for *A. fuscicollis*, could also have been an important factor influencing the abundance of this parasitoid.

In summary, a generally positive effect of spontaneous ground cover on parasitism of the olive moth by *A. fuscicollis* was observed, and a generally negative correlation of herbicide use with levels of *A. fuscicollis* parasitism. Weather and surrounding vegetation could also influence parasitoids in olive groves and may have reduced differences between management types in the present study. Thus, longer-term experiments are needed to determine how ground cover management and other environmental conditions influence parasitim levels, given different results were obtained in different years.

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Life-history parameters of *Chrysoperla carnea s.l.* fed on spontaneous plant species and insect honeydews: importance for conservation biological control

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Abstract *Chrysoperla carnea s.l.* (Stephens) (Neuroptera: Chrysopidae) larvae are generalist predators feeding on many crop pests while adults feed on non-prey food. The knowledge about the nutritional suitability of non-prey food for adults in agroecosystems is crucial to establish conservation biological control strategies and is poorly known in olive groves, where *C. carnea s.l.* larvae prey on different pests. In this study, the effect of honeydew secreted by two hemipteran olive tree secondary pests and 11 plant species on the life-history parameters (survival, reproduction and development time) of *C. carnea s.l.* were analyzed. Insect pest honeydew from the olive agroecosystem and several plant species, blooming throughout the year, were found to improve *C. carnea s.l.* adult survival. Pollen consumption seems to be essential for reproduction. These findings are important for designing and implementing new conservation biological control approaches.

Keywords Nectar · Pollen · *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) · *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae) · Survival analysis

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Introduction

Conservation biological control aims to conserve and manage the crop environment to enhance the effectiveness of natural enemies (Landis et al. 2000). Some natural enemies feed on non-prey food (pollen, nectar or insect honeydew) in some stages of their development (Jervis and Heimpel 2005) and the lack of these resources in the agroecosystems may represent a limitation for their action in pest control (Gurr and Wratten 1999). The Chrysopidae family is a major group of natural enemies and, among them, *Chrysoperla carnea s.l.* (Stephens) is one of the most important species. The larval stage feeds on many crop pests whereas adults are palino-glycophagous, consuming non-prey food such as nectar and pollen, obtained from different plant species, or honeydew produced by hemipteran insects present in agroecosystems (Wäckers 2005; Petanidou et al. 2006). The nutritional level of available food resources for adults might influence different life-history parameters (survival, reproduction or offspring development time) of *C. carnea s.l.* and potentially its effectiveness as a pest control agent. In cabbage crops, Villenave et al. (2005) observed the occurrence of pollen from different plant families (e.g. Brassicaceae, Chenopodiaceae, Gramineae and Rosaceae) in *C. carnea* diverticula and Sheldon and MacLeod (1971) and Hogervorst et al. (2007) verified the consumption of honeydew. However, to our knowledge, only van Rijn (2012) compared the suitability of various plant species on longevity and reproduction of *C. carnea s.l.* concluding that flowers with accessible nectar (e.g. three Apiaceae, one Caryophyllaceae, one Polygonaceae and two Asteraceae species) benefited both parameters. Recently, Gonzalez et al. (2016) analyzed the longevity and reproduction of this species fed on ten types of sugars or a diet composed of honey and pollen (1:1) and obtained higher longevity, but low oviposition, on fructose and higher fecundity on the artificial diet.

The olive grove is a widespread crop in Mediterranean areas with an important socio-economical impact. The relevance of chrysopid larvae as predators of *Prays oleae* (Bernard), one of the most important pests in this agroecosystem, is well documented (Arambourg 1984; Ramos et al. 1987; Bento 1999; Paredes et al. 2015). Also, a synchrony between *C. carnea s.l.* and *P. oleae* populations was found by Bento (1999). In addition, other works pointed out the potential predation on immature stages of two secondary pests, *Saissetia oleae* (Olivier) (Arambourg 1984) and *Euphyllura olivina* (Costa) (Pantaleoni et al. 2001; Gharbi et al. 2012).

Despite the importance of *C. carnea s.l.* in the olive agroecosystem, the role of non-prey food occurring within and around olive groves on life-history parameters of *C. carnea s.l.* is relatively unknown. Porcel et al. (2013) obtained positive correlations between the abundance of *C. carnea s.l.* adults and the presence of weed covers, and McEwen and Ruiz (1994) found an association between non-crop vegetation and chrysopid eggs. On the other hand, Alrouechdi (1984) found that chrysopids laid eggs preferentially in areas with high densities of *S. oleae* honeydew, which can be attractive for *C. carnea s.l.* adults (McEwen et al. 1993).

In this context, the objective of this work was to study the effect of different food sources on life-history parameters related with survival, and reproduction of *C. carnea* adults and consequences on offspring development time and survival in laboratory assays. Selected food sources were honeydews secreted by *S. oleae* and *E. olivina*, and floral resources provided by 11 spontaneous plant species commonly found in Mediterranean areas and distributed in many other regions of the world. Various natural non-prey food occurring in olive groves were identified as nutritionally suitable for *C. carnea* s.*l.* adults. This constitutes novel valuable knowledge that will enable to develop new approaches in conservation biological control strategies for olive pests.

Materials and methods

C. carnea s.l. rearing

C. carnea s.l. adults used in the experiments were obtained from a stock colony established in a climatic chamber at 24 °C (±2 °C) and 16:8 h L:D. *C. carnea s.l.* larvae were initially purchased from Nutesca S.L. (Baeza, Spain) and, in the laboratory, they were isolated (to avoid cannibalism) in plastic Petri dishes (5.5 cm in diameter × 1.8 cm height) and fed ad libitum with *Ephestia kuehniella* Zeller eggs, purchased from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands), and with water in 0.5 ml microcentrifuge tubes sealed with Parafilm© and provided with a filter paper strip as water dispenser. Every day, newly emerged adults were transferred to a methacrylate cage (40 × 30 × 30 cm). The stock colony was supplied with at least 15 aliquots of 0.5 ml of an artificial diet for adults (supplied in lids that were removed from 1.5 ml microcentrifuge tubes), *E. kuehniella* eggs for larvae and water in 0.5 ml microcentrifuge tubes. The artificial diet was prepared with 15 ml of commercial condensed milk, one chicken egg, one chicken egg yolk, 30 g of honey, 20 g of D-(–)-fructose 99 %, 30 g of wheat germ and 45 ml of distilled water (Vogt et al. 1998). The stock mixture was divided into aliquots of 15 ml that were frozen and used when needed. Larvae of the first and subsequent generations were gently removed from the stock colony using a brush, placed individually in plastic Petri dishes (5.5 cm in diameter × 1.8 cm height) and reared as described before. Newly emerged individuals were paired and used in the experiments. Every four months, new individuals were purchased and introduced in the stock colony.

Non-prey food

S. oleae and *E. olivina* honeydews were collected overnight by placing Parafilm© strips (4 cm^2) under infested leaves of small olive trees (cv. "Cobrançosa"), grown in a climatic chamber at 21 °C (±2 °C) and 16:8 h L:D. Eleven spontaneous plant species that bloom in different periods of the year in Mediterranean areas were selected: Asteraceae (*Calendula arvensis* L. and *Senecio vulgaris* L.), Apiaceae (*Daucus carota* L. and *Foeniculum vulgare* L.), Caprifoliaceae (*Lonicera etrusca* Santi), Lamiaceae (*Lamium purpureum* L. and *Rosmarinus officinalis* L.), Malvaceae (*Malva sylvestris* L.), Ranunculaceae (*Ranunculus ollissiponensis* Pers.) Caryophyllaceae (*Stellaria media* (L.) Vill.) and Plantaginaceae (*Veronica persica* Poir). Flowers were collected in the campus of the Polytechnic Institute of Bragança, northeastern Portugal. The blooming period of the selected plants in this area is: (1) *L. purpureum* and *V. persica*, blooming from January to June with a peak at the end of winter and early spring; (2) *C. arvensis*, *R. officinalis*, *S. media* and *S. vulgaris*, blooming from November/December to June/July with a peak at the end of winter and early spring but extending longer than the previous plants; (3) *R. ollissiponensis*, blooming from February to May with a peak in April; (4) *L. etrusca*, blooming from March to July; (5) *D. carota* and *M. sylvestris*, blooming from April to September, with a peak at the end of spring and during summer but extending their flowering sometimes until November, in the case of *M. sylvestris*; (6) *F. vulgare*, blooming from May to early autumn, with a peak during summer. The blooming period of these plant species coincides with the occurrence of *C. carnea s.l.* in olive groves, since adults can be found throughout the year, increasing in March and reaching maximum abundances in July/August and October. The lacewings overwinter as adults (Bento 1999; Campos and Ramos 1983).

Experimental design

Couples of newly emerged individuals were transferred into glass cages (1.5 l) that were closed with paper for ventilation. For each treatment, 27–35 couples were tested with one of the following treatments: (1) water only (negative control), (2) water plus honey solution 10 % (w/v) (positive control), (3) water plus sucrose 1 M (positive control) and (4) water plus one of the plant species. Water, honey and sucrose solutions were provided by filling a glass vial (2 cm in diameter × 4 cm height), sealed with Parafilm© and a strip of filter paper, fitted through a hole, serving as dispenser. Commercial multifloral organic honey was chosen as mimic for nectar, i.e., a mixture of fructose, glucose, sucrose and other minor components such as amino acids, enzymes, minerals, phenolic acids and polyphenols (Bogdanov et al. 2004). Sucrose was chosen because it is one of the most abundant sugars in nectar (Pacini and Nicolson 2007). Flowers were cut in the field, and approximately 9 cm^2 of floral surface of each plant species was placed in a glass vial (2 cm in diameter × 4 cm height) filled with water and sealed with Parafilm©. All foods were replaced three times a week and cages were kept in a climatic chamber at 24 °C (±2 °C) and 16:8 h L:D. Every day, adult survival and the number of eggs laid were checked. Dead individuals and all deposited eggs were removed from cages. The egg pedicel was gently cut with forceps and isolated into plastic Petri dishes (5.5 cm in diameter × 1.8 cm height) and *E. kuehniella* eggs and water was also added in order to provide food for the larvae (as described before). Every day, each Petri dish was checked and survival and the development stage of the individual (egg, L1, L2 and L3 larvae, pupa and adult) were determined.

Data analysis

Survival

Survival curves were drawn for each treatment with Kaplan–Meier estimates using the surv and survfit functions from the "survival" package (Therneau 2014) in R (R Core Team 2014). Mean survival time and SE were extracted from the Kaplan–Meier curves using the print.survfit function from the same package. Statistical differences among curves were analyzed with the log-rank test using survdiff function from the same package and the same procedure was subsequently applied in order to perform a complete pairwise analysis comparing each pair of treatments. Due to the large number of multiple comparisons along the pairwise analysis (120) the Hochberg correction was applied to control the false discovery rate ($\alpha = 0.05$) (Benjamini and Hochberg 1995). The survdiff function was also applied to test if there were differences in survival between sexes within treatments. Individuals that escaped during the experiment were excluded from the analysis.

Reproduction

Generalized linear models (GLMs) for count data with negative binominal distribution to account for overdispersion and a log link between the predictor function and the mean of the distribution (Zuur et al. 2009) were used to assess the effect of the different treatments on pre-oviposition (as the number of days spent from female emergence to the first egg laid) and oviposition (as the number of days spent from the first to the last egg laid) periods and lifetime fecundity (as the total number of eggs laid per female during its lifetime) applying the glm.nb function from the "*MASS*" package (Venables and Ripley 2002). Then, a Tukey test for post-hoc analysis was carried out in order to detect the differences between treatments using the glht function from the "multcomp" package (Hothorn et al. 2008). The Bonferroni correction was applied. These variables were statistically analyzed only when more than three females laid eggs per treatment. Following Balzan and Wäckers (2013), a series of GLMs with Poisson distribution (for non-overdispersed data) or negative binomial distribution (for overdispersed data) and a log link were used to fit the total lifetime fecundity as a function of female lifespan for each treatment. The same procedure was followed to analyze the total lifetime fecundity as a function of the oviposition period for each treatment.

Offspring development time and survival

A series of GLMs for count data and negative binominal distribution to account for overdispersion and a log link was used to test the effect of the different treatments on the time (number of days) spent in each development stage (i.e., time spent from female emergence to the oviposition of each egg, time spent as egg, L1, L2 and L3 larvae and pupae) using the glm.nb function followed by a Tukey test for post-hoc analysis using the procedure mentioned before. The Bonferroni correction was applied. Finally, a series of GLMs for proportional data with binomial distribution and a logit link (Zuur et al. 2009) was used to test the effect of the different treatments on the percentage of offspring that reached each development stage in relation to the number of eggs laid per female. A Tukey test for post-hoc analysis and the Bonferroni correction were applied as mentioned before. Eggs accidentally damaged during manipulation and missing larvae were dropped from these analyses that were only performed when more than three females per treatment laid eggs.

Results

Survival

Survival curves were significantly different among treatments for both males (Fig. 1a) and females (Fig. 1b) ($\chi^2 = 424$, $df = 15$, $P < 0.05$ for males and $\chi^2 = 422$, $df = 15$, $P < 0.05$ for females). In both sexes, survival curves showed better results for the positive controls (honey and sucrose treatments). For males, survival curves on *S. oleae* and *E. olivina* honeydews did not differ significantly from treatments with one of the positive controls (sucrose) but showed a significantly lower survival than with the other positive control (honey). For females fed on honeydew, survival curves were not significantly different from positive controls. Survival curves of females fed on *M. sylvestris*, *V. persica* and *L. purpureum* significantly differed from those fed on *S. oleae* or *E. olivina* honeydews but survival curves of females fed on these three floral resources and of males fed on *M. sylvestris* and *V. persica* were significantly higher than on all the remaining flowers and the negative control. The survival curve of females fed on *L. purpureum* did not differ from those fed on *V. persica*, *M. sylvestris*, *F. vulgare* and *R. ollissiponensis*. Survival curves of both sexes fed on *R. ollissiponensis*, *L. etrusca*, and *F. vulgare* and of females fed on *D. carota* were significantly higher than in the negative control. Survival curves of males fed on *D. carota* and of both sexes fed on *R. officinalis*, *S. media*, *S. vulgaris*

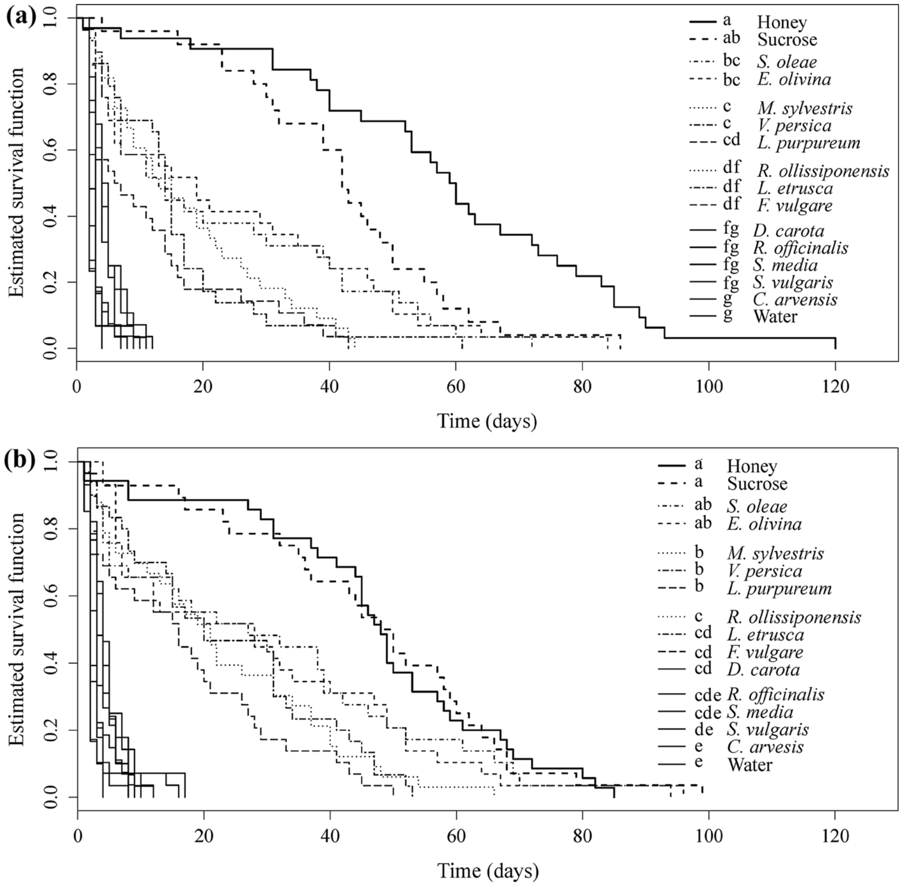

Fig. 1 Kaplan–Meier estimates of the survival functions for *Chrysoperla carnea* males (**a**) and females (**b**) reared on different non-prey foods. *Different letters* in the legend indicate significant differences among treatments after pairwise comparison of the survival curves. Hochberg correction was applied ($P < 0.05$)

and *C. arvensis* did not differ from the negative control (survival times of male and female adults of *C. carnea* fed on different non-prey food are shown in supplementary material, table S1).

Differences between survival curves of females and males were found on honey ($\chi^2 = 6.73$, $df = 1$, $P < 0.05$) and *V. persica* treatments ($\chi^2 = 3.97$, $df = 1$, $P < 0.05$), with better results of females on *V. persica* and of males on honey. On *D. carota*, the survival of females was higher than that of males with a marginal significance ($\chi^2 = 3.84$, $df = 1$, $P = 0.05$). The other treatments did not cause significant differences between female and male survival curves (*C. arvensis*: $\chi^2 = 0.06$, $df = 1$, $P = 0.810$; *F. vulgare*: $\chi^2 = 0.010, df = 1, P = 0.979$; *L. etrusca*: $\chi^2 = 0.08$, $df = 1$, $P = 0.778$; *L. purpureum*: $\chi^2 = 2.47$, $df = 1$, $P = 0.116$; *M. sylvestris*: $\chi^2 = 2.63$, $df = 1$, $P = 0.105$; *R. officinalis*: $\chi^2 = 0.430$, $df = 1$, $P = 0.513$; *R. ollisiponensis*: $\chi^2 = 0.37$, $df = 1$, $P = 0.545$; *S. media*: $\chi^2 = 0.87$, $df = 1$, $P = 0.352$; *S. vulgaris*: $\chi^2 = 0.49$, $df = 1$, $P = 0.483$; sucrose: $\chi^2 = 1.68$, $df = 1$, $P = 0.195$; *E. olivina*: $\chi^2 = 1.02$, $df = 1$, $P = 0.313$; *S. oleae*: $\chi^2 = 1.28$, $df = 1$, $P = 0.278$).

Reproduction

Only the positive controls (honey and sucrose), *L. purpureum*, *M. sylvestris* and *V. persica* generated eggs in more than three females per treatment. Due to the low number of fertile females fed on *E. olivina* (two fertile females) and *S. oleae* (one fertile female) honeydews, these and the other treatments that did not generate eggs were not included in the statistical analysis. GLMs showed that pre-oviposition and oviposition periods and lifetime fecundity were significantly different between treatments after Bonferroni correction (pre-oviposition period: $\chi^2 = 25.43$, $df = 4$, $P < 0.05/10$; oviposition period: $\chi^2 = 15.48$, $df = 4$, $P < 0.05/10$; lifetime fecundity: $\chi^2 = 12.01$; $df = 4$; $P < 0.05/10$). Females fed on *M. sylvestris* and *V. persica* had significantly longer pre-oviposition periods than those fed on honey and *L. purpureum* but they did not significantly differ from females fed on sucrose (Fig. 2a). Oviposition period (Fig. 2b) and lifetime fecundity (Fig. 2c) of females fed on *V. persica* were significantly higher than on *M. sylvestris*, but not significantly different from the other treatments.

GLMs showed that the lifetime fecundity significantly increased with the oviposition period in females fed on *M. sylvestris* and *V. persica* and no differences were obtained for the other treatments (Fig. 3a). No treatment yielded significant variation of the lifetime fecundity in function of the lifespan (Fig. 3b).

Offspring development time and survival

The total number of tested eggs in each treatment was 142 laid by females fed on honey solution, 37 on sucrose, 48 on *L. purpureum*, 51 on *M. sylvestris* and 206 on *V. persica*. GLMs indicated that the time spent from the mother emergence to the oviposition of each egg was significantly different among treatments ($\chi^2 = 617.73$, $df = 4$, $P < 0.05/10$), being significantly longer when females were fed on *V. persica*, followed by *M. sylvestris* and sucrose and significantly shorter on honey. Eggs hatched in four or five days and this development time was not significantly different among treatments ($\chi^2 = 5.40$, $df = 4$, $P = 0.249$). Both L1 and L2 stages took from three to four days for molting and treatments did not significantly differ (L1 for molting to L2: $\chi^2 = 0.40$, $df = 4$, $P = 0.982$; L2 for molting to L3: $\chi^2 = 4.88$, $df = 4$, $P = 0.3$). L3 took from eight to 16 days with significantly longer development times for offspring from females fed on *M. sylvestris* as compared with *V. persica* but no significant differences were found among the other treatments ($\chi^2 = 16.40$, $df = 4$, $P < 0.05/10$). Pupae took from 11 to 12 days to emerge and again no differences were found among treatments ($\chi^2 = 2.80$, $df = 4$, $P = 0.591$; supplementary material, tables S2).

The percentage of L1 larvae hatching from eggs and L2 developed from L1 larvae were significantly higher for females fed on *L. purpureum* than on *M. sylvestris* and *V. persica* (hatching percentage: $\chi^2 = 18.23$, $df = 4$, $P < 0.05/10$; molting from L1 to L2: $\chi^2 = 17.43$, $df = 4$, $P < 0.05/10$). The percentage of larvae that reached L3 instar was significantly higher for females fed on *L. purpureum* than on *M. sylvestris* but not significantly different from the

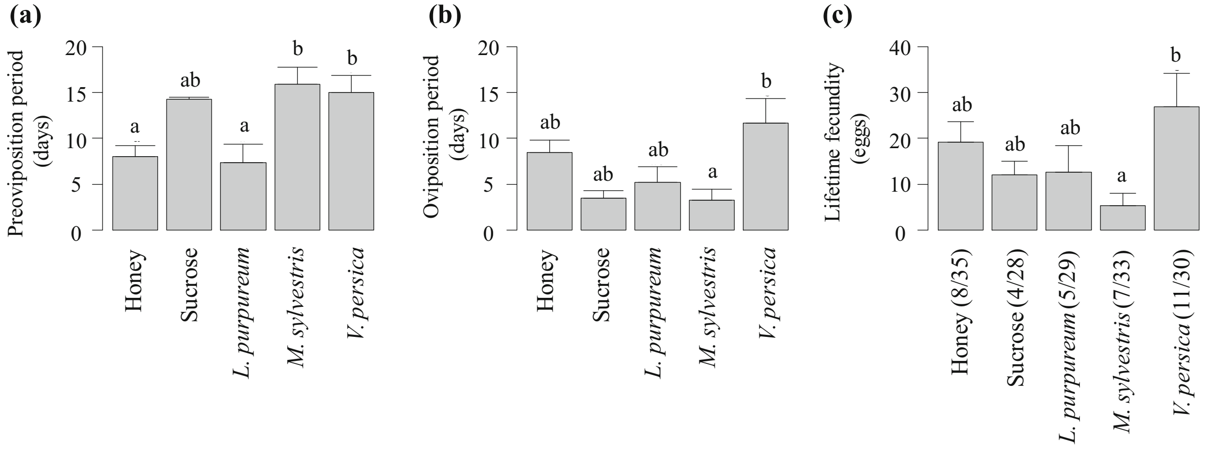

Fig. 2 Pre-oviposition (**a**) and oviposition (**b**) periods (days) and lifetime fecundity (eggs) (**c**) (mean + SE) of *Chrysoperla carnea* females reared on different non-prey foods. *Different letters* indicate significant differences among treatments at $P < 0.05/10$ with Bonferroni correction. In **c** the number of fertile females (*on the left of the bar*) and the total number of females (*on the right of the bar*) are given in *parentheses*

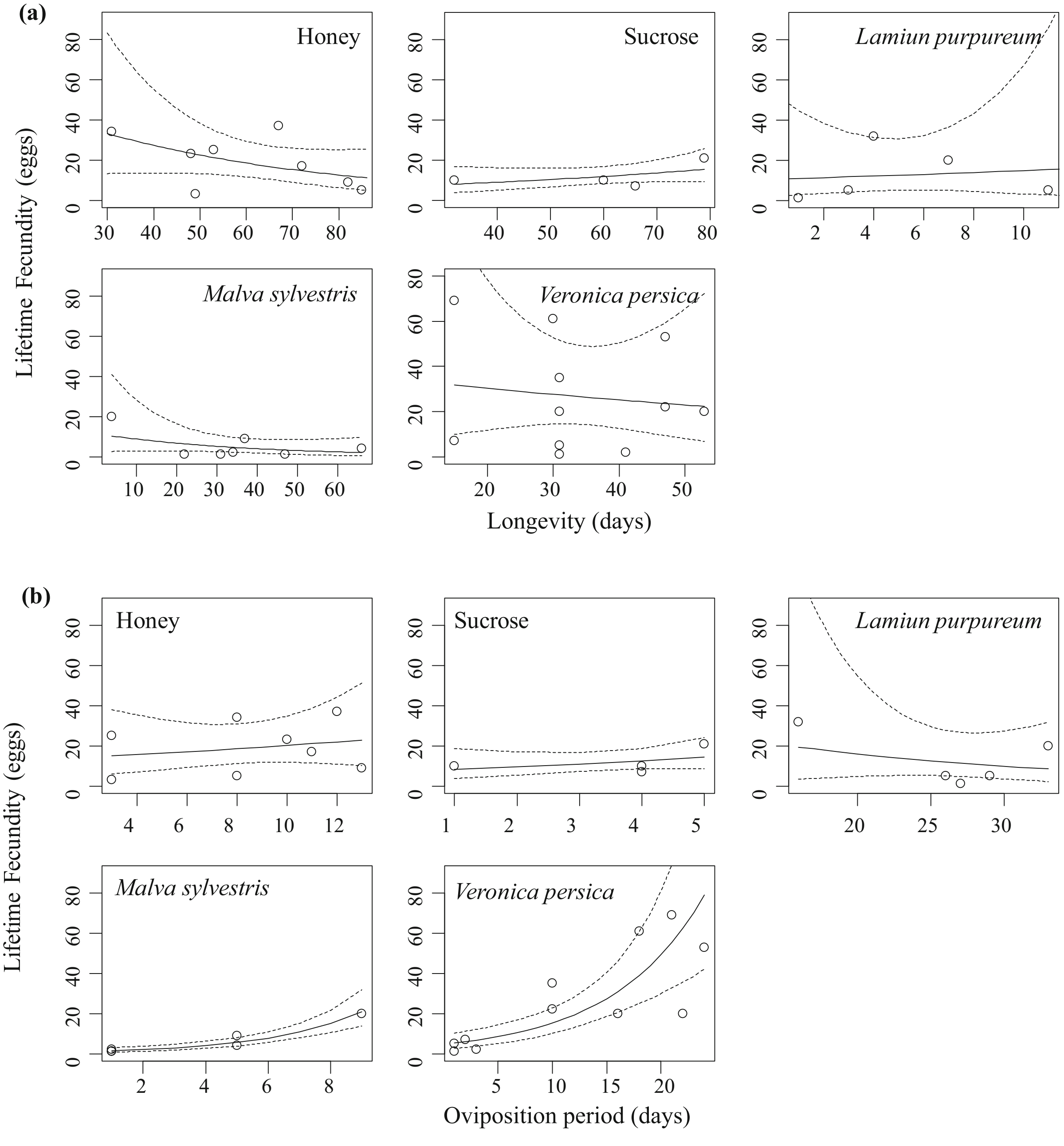

Fig. 3 Fitted curves (*solid lines*) obtained from generalized linear models for the lifetime fecundity variation as a function of the lifespan (**a**) and the oviposition period (**b**) in each treatment. *Dotted lines* represent the 95 % confidence intervals and points, the observed data

other treatments ($\chi^2 = 18.67$, $df = 4$, $P < 0.05/10$). The percentage of pupation was not significantly different among treatments ($\chi^2 = 12.18$, $df = 4$, $P = 0.016$). Finally, the GLM for adult emergence showed significant differences among treatments ($\chi^2 = 16.06$, $df = 4$, $P < 0.05/10$), but after pairwise comparison and Bonferroni correction no differences were found (supplementary material, table S3).

Discussion

In this study, several non-prey foods commonly found in olive groves were identified as potential resources for *C. carnea s.l.* adults. Honeydews of the secondary pests *S. oleae* and *E. olivina* and floral resources provided by *V. persica*, *L. purpureum*, *M. sylvestris* showed to be nutritionally suitable for *C. carnea s.l.* adults. The occurrence of suitable winter flowering plants in agroecosystems such as *V. persica* and *L. purpureum* is particularly relevant since they can enhance the nutritional status of *C. carnea s.l.*, which is essential for overwintering and reproduction at the end of winter (Sheldon 1975). Food of unsuitable quantity and quality maintains individuals in a quiescence state and slows down the reproductive activity in the beginning of spring (Principi 1991). These five non-prey foods improved survival of *C. carnea s.l.*. However, reproduction parameters were lower when compared with other studies (Sundby 1967; Krishnamoorthy 1984; Venzon et al. 2006). Chrysopids were found to lay none or only few eggs when fed on low proteinaceous diets (Sheldon and MacLeod 1971; McEwen et al. 1994; Gibson and Hunter 2005; Venzon et al. 2006). Also, a certain amount of carbohydrates is needed for egg laying (Sheldon and MacLeod 1971; Venzon et al. 2006). Insect honeydew and floral nectar are carbohydrate sources (while poor in proteins) that most probably enhanced lifespan of *C. carnea s.l.*. This finding is particularly relevant once flowers with accessible nectaries are less frequent than those with accessible pollen (van Rijn 2012). Low protein contents in the diet could have resulted in the low number of eggs laid by *C. carnea* and in the general lack of dependence found between lifetime fecundity and both lifespan and oviposition period. *V. persica* was the only plant species that improved reproduction of *C. carnea* females probably due to pollen consumption. Also, the honey solution caused better reproduction fitness, probably because of the higher content in proteins and amino acids in the honey solution, compared with the flowers with non-accessible pollen. However, this still needs further investigation.

Both *M. sylvestris* and *L. purpureum* improved adult survival but not reproduction which may suggest that *C. carnea s.l.* adults were not consuming sufficient amounts of pollen grains. In the case of *M. sylvestris*, reproduction did not improve in spite of its open corolla and the fact that it is an entomophilous plant (Comba et al. 1999). This plant has large and echinate (covered with spines) pollen grains (measuring >60 µm and sometimes >100 µm in diameter) (Moore et al. 1991). *M. sylvestris* and cotton (Malvaceae: *Gossypium hirsutum* L.) have similar pollen shapes and sizes and in a previous study Vaissière and Vinson (1994) found that bees, *Apis mellifera* L. (Hymenoptera: Apidae), did not consume cotton pollen. They suggested that the size of the pollen spines of cotton probably impeded the bees to groom and pack it. Also the big size of the cotton pollen grain may have influenced its consumption. In this work, *C. carnea s.l.* adults were observed to accumulate high quantities of *M. sylvestris* pollen grains on the surface of their bodies complicating the movement of insects (Villa, personal observation). This accumulation of pollen, together with the pollen size and the echinated ornamentation, probably prevented its consumption reducing protein contents in their diet and explaining the low reproduction performance. *L. purpureum* is also an entomophilous plant that produces large amounts of pollen that aggregate in clumps and has quite long flowers (17.09 ± 0.667 mm, mean ± SD) with petals that form a landing platform for insect visitors and a hooded part where anthers are located (Denisow and Bożek 2008). In our experiment, probably *L. purpureum* pollen was consumed in low quantities due to the difficult access or the incapability of *C. carnea s.l.* adults to consume pollen from the clumps, resulting in low reproduction performance. Additionally, the long corolla probably complicated the consumption of nectar from the landing platform. However, in this study, *C. carnea s.l.* adults were observed consuming nectar on the ovary or on the basis of the corolla when the flower detached from the ovary (M. Villa, personal communication).

Plants of *D. carota*, *F. vulgare*, *R. ollissiponensis* and *L. etrusca,* which slightly improved *C. carnea s.l.* survival but did not generate egg production, have well exposed nectaries, with the exception of *L. etrusca*. A lower nutritional quality or lower consumption of nectar could be responsible for the shorter lifespan of *C. carnea s.l.*. These results are in agreement with Gonzalez et al. (2016) who suggested that the reproductive success of *C. carnea s.l.* not only depends on food rich in proteins but also on food that provides energy to improve the lifespan and lifetime fecundity. In contrast with our results, van Rijn (2012) found that three Apiaceae species and two Asteraceae allowed *C. carnea s.l* adults to survive longer than 19 days and consistently produce eggs. Additionally, this author found that eight other Asteraceae yielded longer longevities than water only, but they did not give rise to reproduction. In this work none of the tested Asteraceae improved the survival of *C. carnea* adults.

Female survival curves were significantly higher on *V. persica* and honey than those of males on the same foods, indicating that probably pollen consumption benefited females more than males. Accordingly, Villenave et al. (2005) found females to consume more pollen grains than males.

Differences in offspring development time and survival were generally more noticeable in early stages. This could be related to differences in food quality and quantity consumed by adult females which could improve the nutrient content in the egg yolk and consequently the earliest stages of the offspring. However, this effect is expected to be diluted in later stages of the offspring.

In the field, *C. carnea s.l.* was found to consume pollen from several plant species (Villenave et al. 2005) and probably a mixture of plants would result in better performance by supplying different types of nutrients. Additionally, under laboratory conditions, factors such as weather conditions or intra- and inter-specific resource competition are not taken into account. Therefore, using a mixture of plants and field experiments would be needed to complement this experiment.

Finally, the effect of non-prey food in crops not always leads to pest reduction. For example, Wyss (1995) found a pest density reduction resulting from incorporating weed strips in apple orchards that increased aphidophagous predators. However, Markó et al. (2013) found that the habitat diversification through ground cover management benefited the abundance of *C. carnea s.l.* but the biological control of green apple aphids, *Aphis* spp. (Hemiptera: Aphididae), was not affected. Importantly, the tested honeydews produced by two secondary pests of the olive tree could have positive effects by feeding predators of olive pests. Therefore, the trophic relationships between non-prey food, predators and olive pests need to be thoroughly investigated in the olive grove agroecosystem.

In summary, in this study we identified for the first time several non-prey foods that occur throughout the year, influencing life-history parameters of *C. carnea s.l.*: three plant species (*M. sylvestris*, *V. persica* and *L. purpureum*) and two insect honeydews (*S. oleae* and *E. olivina*) highly enhanced *C. carnea* survival and four other plants also resulted in some survival improvement (*R. ollissiponensis*, *L. etrusca*, *F. vulgare* and *D. carota*). Additionally, foods rich in proteins together with foods rich in carbohydrates seem to be needed for reproduction. These findings will allow developing new approaches for conservation biological control strategies and the management of the olive grove agroecosystem.

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