Pollen feeding habits of *Chrysoperla carnea s.l.* adults in the olive grove agroecosystem

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ABSTRACT

Chrysoperla carnea s.l. (Stephens) larvae are important natural enemies in agroecosystems. However, adults feed on honeydew excreted by hemipterans, and pollen and nectar from flowering plants. Pollen is essential for egg production, but to our knowledge, the pollen diet of *C. carnea* in perennial crops has never been addressed. In this work, the objective was to study the diversity and potential selection of pollen types consumed by *C. carnea* in the olive grove agroecosystem. For this study, *C. carnea* adults were captured from April to December of 2012 and 2013, and simultaneously, inventories of the plant diversity were obtained in olive orchards and adjacent scrubland and herbaceous patches. The pollen types contained in *C. carnea* guts were identified by microscopy and compared with the pollen types in the environment using Jacobs`s second selection index. The results indicated that (i) *C. carnea* females and males captured in the olive tree canopy visited scrub and herbaceous vegetation patches; (ii) they fed on different anemophilous and entomophilous pollen types from tree and scrub (*Olea europaea*, Fabaceae, Pinaceae, Cistaceae or Ericaceae) and herbaceous (Asteraceae, Apicaceae, Brassicaceae, Poaceae, *Rumex* type or *Plantago* type) strata; and (iii) adults fed not only on flowers but also on pollen settled on vegetation surfaces. Here, we demonstrated that *C. carnea* could benefit not only from the diversity of entomophilous pollen during the plant flowering periods but also from other pollens that could remain in the environment in different periods. This has important implications for the management of the floral diversity adjacent to the crop.

1. Introduction

Conservation biological control seeks to maintain or enhance resources in the crop environment to improve the action of natural biological control agents (Landis et al., 2000). Therefore, understanding how and which resources natural enemies use, as well as the relevance of those resources, for the success of control agents becomes a milestone in biological control. It is assumed that a species will select resources that better satisfy its living requirements and that the quantity of a resource utilized by an animal may change with the quantity available to the animal (Manly et al., 2002). Flowering plants are important resources for many entomophagous insects because they rely on floral food during some stages of their life cycle (Wäckers and van Rijn, 2012). Thus, a lower plant biodiversity in the environment could lead

to a poor diet for natural enemies. However, only increasing the botanical diversity is not enough to enhance the abundance of natural enemies: the right flowering plants must be selected (Wäckers and van Rijn, 2012).

Chrysopidae (Neuroptera) is a family of common natural enemies in many crop agroecosystems; *Chrysoperla carnea s.l.* (Stephens) is one of the most important species. Their larvae are generalist predators, while the adults feed on sugary liquids, such as honeydew, produced by hemipteran insects and nectar and pollen from flowers (Canard, 2001). *Chrysoperla carnea* presents an important ecological versatility that facilitates the colonization of multiple habitats (Monserrat and Marín, 1994), and previous work suggested that chrysopids are attracted by large patches of flowering plants (Villenave et al., 2006). Moreover, food of unsuitable quantity and quality restricts individuals in a

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quiescence state and retards reproductive activity in the beginning of spring (Principi, 1991), and different flowering plants cause different reproductive and survival performances in *C. carnea* (van Rijn et al., 2012; Villa et al., 2016a; Resende et al., 2017). Proteinaceous diets are essential for egg production in chrysopids (Villa et al., 2016a), and proteins in the field are likely provided by pollen from flowers. Thus, an increase in floral resources may have a positive impact on chrysopid populations by increasing the survival and reproduction possibilities.

Analysis of pollen grains within the gut of insects can reveal the habitats and/or plants that they visit, their movements, what they eat, and their dispersal patterns throughout the cropping system (Jones, 2014). Combinations of pollen analyses with inventories of richness and abundant flowering plants can address the importance of plant species for insects (Villa et al., 2016b). Few studies have addressed the consumption of pollen by chrysopids by the analysis of pollen grains within guts (Villenave et al., 2005, 2006; Medeiros et al., 2010; Nunes Morgado et al., 2014), but to our knowledge, no studies have been carried out in a perennial crop. Villenave et al. (2006) evaluated the selection of plants surrounding the crop and concluded that pollen was consumed according to its availability. Medeiros et al. (2010), in vegetable crops (onion, carrot, broccolis, cucumber and garlic) surrounded by Asteraceae, Poaceae and a riparian forest, found that the pollen consumed by *C. externa* belonged mainly to the Poaceae family. In a greenhouse, Nunes Morgado et al. (2014) compared pollen in the guts of *Chrysoperla agilis* Henry with the concurrently flowering plants and found that 14 out of 26 species were consumed, and the Plantaginaceae pollen type dominated, followed by Asteraceae and Poaceae pollen types. Both studies performed by Medeiros et al. (2010) and Nunes Morgado et al. (2014) addressed the consumption of pollen grains but did not analyse the selection of plants.

The olive tree (*Olea europaea* L.) is a perennial crop typically cultivated in Mediterranean regions; several pests can cause important economic damage to the crop, such as the olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae), which can be found in Europe, northern Africa and Asia (CAB International). Chrysopid larvae are considered important natural enemies of this pest (Arambourg, 1984; Neuenschwander and Michelakis, 1980; Porcel, 2012; Paredes et al., 2015), and a synchrony between *C. carnea* and *P. oleae* populations was observed by Bento (1999). Moreover, chrysopid larvae can prey on immature stages of the secondary pests of the olive tree, *Saissetia oleae* (Hemiptera: Coccidae) (Olivier) (Arambourg, 1984) and *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae) (Pantaleoni et al., 2001). Despite its importance, feeding habits of adult chrysopids in the olive grove agroecosystem are poorly known. Porcel et al. (2013) found that *C. carnea* adults were positively influenced by the presence of weed cover, and McEwen and Ruiz (1994) found an association between non-crop vegetation and chrysopid eggs in the olive grove agroecosystem.

In this context, the objective of this work was to identify the plant species exploited by *C. carnea* adults throughout the year. Through the observation of pollen in guts, we analysed whether *C. carnea* preferentially consumed herbaceous or woody plants and whether they exploited several patches in surrounding agricultural areas to determine the importance of botanical diversity in supplying food resources for *C. carnea*.

2. Materials and Methods

2.1. Study areas

Field studies were conducted in the Mirandela municipality, northeastern Portugal, during 2012 and 2013 in three olive groves (1: 41°29′16″N, -7°07′34″W; 2: 41°32′8″N, -7°07′29″W; 3: 41°34′12″N, -7°09′59″W) and two surrounding field areas (an herbaceous vegetation patch and a scrubland) next to each olive grove. Selection of the study sites was described by Villa et al. (2016b).

Additionally, to estimate the percentage of area covered by olive

crops, herbaceous vegetation and scrubland in the *C. carnea* habitat, nine olive groves located in Mirandela Municipality were selected (41°33′34.39″N, 7°05′37.17″W; 41°34′12.25″N, 7°09′59.01″W; 41°25′44.91″N, 7°12′20.71″W; 41°33′00.58″N, 7°08′39.92″W; 41°32′17.31″N, 7°05′43.89″W; 41°29′16.86″N, 7°07′34.02″W; 41°33′01.46″N, 7°6′31.57″W; 41°26′38.09″N, 7°13′28.69″W; 41°29′30.02″N, 7°15′28.72″W).

2.2. Availability of pollen types in the environment

First, flowering plant inventories were carried out in each patch every week from the last week of March through June and every other week during July and August in 2012 and 2013 following the methodology described in Villa et al. (2016b): five flowering plant inventories were carried out in circular plots of 25 m² (olive groves and herbaceous patches) and three were in circular plots of 100 m² (scrubland patches). Due to the larger size of the plants (trees and shrubs) in the scrublands, larger plots were required to record all of the species. Hence, there was a total of 39 plant inventories in each sampling date for characterizing the plant community of the olive grove agroecosystem. The percentage ground cover for each flowering plant species was recorded following the Daubenmire cover scale modified by Bailey (Mueller-Dombois and Ellenberg, 1974), i.e., class 1 for 0–1% cover; class 2 for 1–5% cover; class 3 for 5–25% cover; class 4 for 25–50% cover; class 5 for 50–75% cover; class 6 for 75–95% cover; and class 7 for 95–100% cover. Data presented in Villa et al. (2016b) were used for flowering plant inventories from September to December. Plant species were grouped by pollen type for further analysis. Grouping was based on similarities related to the morphology of the pollen grains: family, genus, species, or type (grouping several species or genus).

After all of the vegetation areas had been individually assessed, the percentage of area covered by olive crops, herbaceous vegetation and scrubland in the *C. carnea* habitat, which typically flight through many kilometres (Duelli, 1984), was estimated. This estimation was made considering several areas across the region (see Section 2.1). A circular area with a diameter of 1000 m was nested around each olive grove. Land-use classes occurring across study sites were obtained from "Carta de Uso e Ocupação do Solo de Portugal Continental para 2007" (COS, 2007), and the percentage of olive crops, scrublands and herbaceous vegetation patches was calculated using the software Patch Analyst for ArcGIS, version 9.3.1 (ESRI, Redlands, California).

The availability of each pollen type in the environment was estimated as $m_i = \sum m_{ij}$ where i is the category of pollen type, j each habitat (herbaceous scrubland and olive grove), and m results from multiplying the percentage of ground covered by the pollen type i in each habitat registered in the inventories and the estimated percentage of area of the same habitat for the region.

2.3. Pollen consumed by C. carnea

From the last week of March until the middle of December 2012 and 2013, chrysopids were captured on a weekly basis. Olive tree branches were shaken, and flying chrysopids were captured using a sweep net. The period spent collecting chrysopids was 30 min per grove. Collected specimens were identified at the species level, and *C. carnea* specimens were sexed and frozen at 18°C.

The frozen *C. carnea* were thawed at room temperature, washed with 96% alcohol and vortexed for two minutes to eliminate external pollen. Chrysopids have a strong muscular diverticulum at the distal part of the oesophagus where the pollen undergoes the first breakdown being partially digested (Canard, 2001). However, for a more accurate pollen identification, an additional artificial acid digestion (with acetic anhydride and sulfuric acid) in a process known as acetolysis is recommended (see Jones, 2014) to digest proteins, lipids and insect and pollen grain debris.

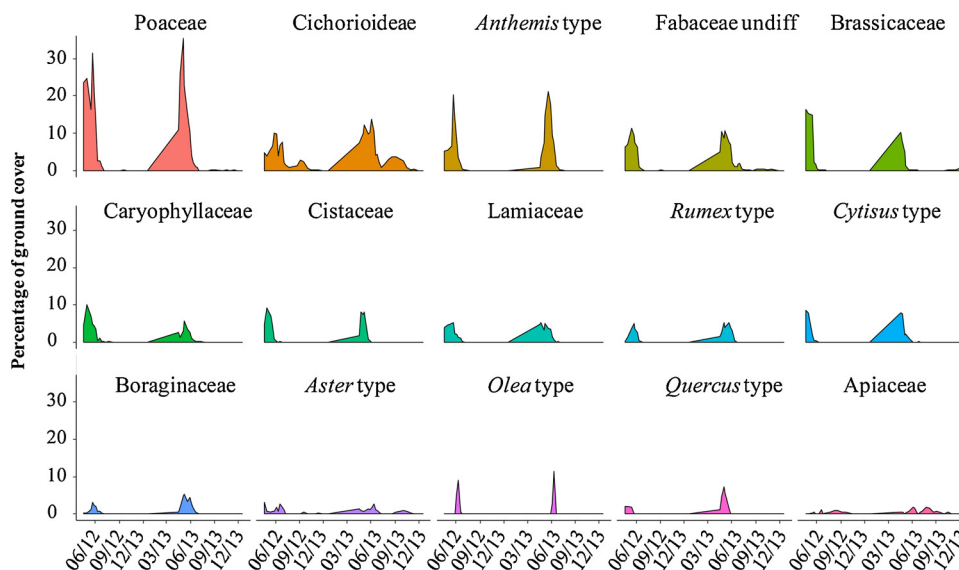The acetolysis was accomplished as follows: (i) each insect was

Fig. 1. Percentage of available pollen types in the olive grove agroecosystem. Blooming plants were identified in olive groves and surrounding scrublands and herbaceous patches from April to December of 2012 and 2013, and an estimation considering the percentage of land cover by each patch type (48% of olive grove, 26% of scrubland, 15% of herbaceous patches) is shown. The most abundant pollen types are shown.

transferred individually into a 1.5 mL microcentrifuge tube with 0.5 mL of glacial acetic acid for 5 min to eliminate any residual water from the sample and avoid reactions with the sulfuric acid used thereafter. Then, the glacial acetic acid was discarded; (ii) the insect was smashed using a clean toothpick and, to accomplish the artificial acid digestion, 0.5 mL of acetolysis mixture (9:1 ratio of acetic anhydride and sulfuric acid) was added; (iii) the tubes were heated (100°C) in a dry heater for 5 min; (iv) after that, 0.5 mL of glacial acetic acid was added to stop the acetolysis process; (v) the samples were centrifuged for 3 min at 5000 rpm, and the supernatant was discarded; (vi) to clean the remaining reagents, the samples were washed three times by the addition of 0.5 mL of distilled water and stirring in a vortex; and (vii) then, the samples were centrifuged again (for 3 min at 5000 rpm), and the supernatant was discarded.

All reactive processes were conducted in a fume hood. Extreme caution was taken in all the processes, particularly while decanting the supernatant (see Jones, 2014) to avoid loss of pollen. Two drops of glycerin jelly:water (1:1) were added; microscopic slides were prepared with the solution, and an optical microscope was used to count and identify the pollen grains. When there were more than 5000 pollen grains, half of the slide was counted, and when there were more than 15 000, a quarter of the slide was counted; the total number of grains was estimated thereafter. 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. Pollen grains were grouped using the same criteria used for grouping the available pollen in the environment i.e., similarities related to the morphology of the pollen grains: family, genus, species, or type (grouping several species or genus).

2.4. Selection index calculation

Jacobs`s second selection index ($D_i$) (Manly et al., 2002) was used to compare the consumption frequency of a species to its availability. A positive Jacobs`s index value indicates selection of the resource and a negative avoidance. This index is defined as:

$$ Di = \frac{O_i - \hat{\pi}_i}{O_i - \hat{\pi}_i - 2O_i\,\hat{\pi}_i} $$

where O_i is the proportion of used units (number of pollen grains) in category i (pollen type) calculated as $Oi = U_i/U_+$, (U_i: number of consumed pollen grains of pollen type i by a specimen; U_+: total number of consumed pollen grains by a specimen), and $\hat{\pi}_i$ is the sample of the proportion of available units (percentage of ground covered) that are in

pollen type i calculated as $\hat{\pi}_i = m_i/m_+$ (m_i: availability of pollen type i, previously estimated; m_+: percentage of total available pollen, i.e., sum of the percentages of all available pollen types for each date).

Thus, D_i was calculated for each specimen using the corresponding $\hat{\pi}_i$ registered at the date (a total of 77 dates) of its capture. Then, the means of D_i and O_i for both males and females captured during the spring, the summer and the autumn of 2012 and 2013 were calculated.

2.5. Amount of pollen in male and female guts

To analyse the amount of pollen in male and female guts, a generalized additive model (gam) with a negative binomial distribution and logarithmic link function was fitted. The number of pollen grains contained in the gut was the response variable. The sex and a smooth of Julian Day by year (to capture the non-linear relationship between pollen consumption and the sampling date) were the explanatory variables. A plot of the residuals versus each covariate was used to investigate model misfit (Zuur, 2012). The gam function from the "mgcv" package (Wood, 2011) in R (R Core Team, 2016) was used.

3. Results and discussion

3.1. Flowering plant inventories and pollen types identified in the C. carnea gut

Considering the flowering plant inventories, a total of 257 plant species (172 in herbaceous patches, 169 in olive groves and 180 in scrublands) belonging to 47 families were identified. The total number of plant species was grouped by pollen types resulting in 65 groups and was used to compare them with the pollen found in the C. carnea guts (the most abundant pollen types in the environment are shown in Fig. 1).

In this study, a total of 290 specimens of C. carnea were captured in 2012 and 2013. In the gut of 190 specimens, 44 pollen types were identified (with a mean of 4.42 pollen types per adult), and the total pollen grains were between 0 and 62,533. Although the number of pollen grains consumed in the spring was higher than that in the summer and autumn of both years (Fig. 2), the majority of C. carnea adults contained pollen in their guts in all seasons.

Of the 145 females analysed, 93.79% consumed pollen, and of the 145 males analysed, 95.52% had pollen grains in their guts. The richness of pollen types consumed by females and males was similar, but in general, the number of pollen grains consumed by females was significantly higher than the number of pollen grains consumed by males
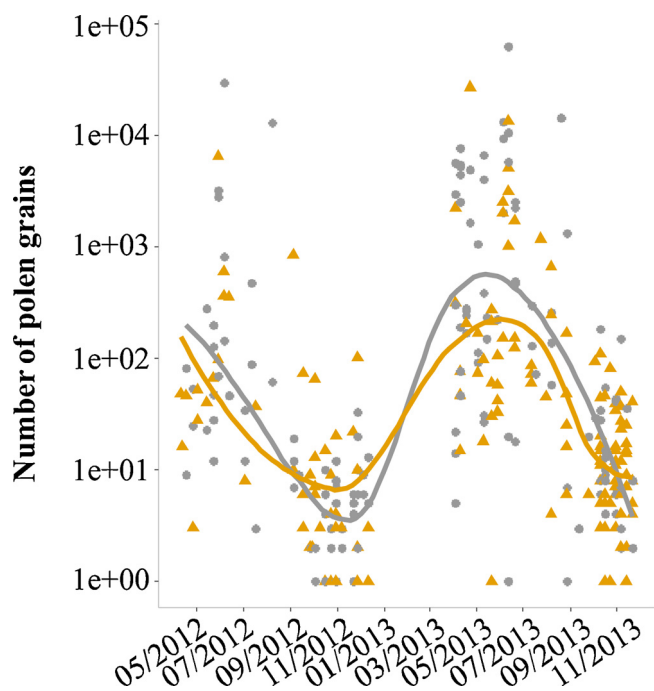
Fig. 2. Number of pollen grains consumed by females (■) and males (▲) of *Chrysoperla carnea sl* in the olive grove agroecosystem from April to December of 2012 and 2013. Points represent individual observations, and lines represent smooth curves (local polynomial regression method – span = 0.6) of the number of pollen grains consumed during the year.

(Table 1) (estimate = -0.490; SE = 0.19; Z = −2.567; p-value = 0.01) with the smoothed functions for Julian Days by year highly significant (2012: edf = 6.963; χ^2 = 416.0; p-value < 2e-16; 2013: edf = 3.188; χ^2 = 181.5; p-value < 2e-16). These results differed from those obtained by Villenave et al. (2005) and Villenave et al. (2006), who observed a lower number of pollen grains consumed by *Chrysoperla* spp. in all seasons in a cabbage crop. Additionally, different methods used for pollen extraction (not acetolyzed in Villenave et al., 2005 and 2006) could have influenced the differences found in the results.

3.2. Pollen selection

In this study, several pollen types (such as Cichorioideae or *Olea*) resulted in a positive Jacobs`s index selection, D_i and/or a high proportion of consumed pollen grains, O_i (the most abundant pollen types in guts are shown in Table 2). When the selection is positive, the adults could (i) have actively searched for flowers or (ii) licked the pollen from vegetation surfaces. When the selection index is negative, this could be due to (i) the plant species being unattractive; (ii) the impossibility of finding those plant species because they are too rare (a low π_i), i.e., percentage of ground cover by that plant that would make it

undetectable by *C. carnea;* (iii) the flowers can be closed at dusk (Villenave et al., 2006); or (iv) the percentage of ground cover by that plant species could exceed the adult needs (a high π_i).

3.2.1. Anemophilous pollen

Several anemophilous plants were highly consumed by *C. carnea* adults, namely, *Olea*, Pinaceae and *Betula,* from the tree stratum and Poaceae, Chenopodiaceae. *Plantago* type and *Rumex* from the herbaceous stratum (Table 2, Fig. 3).

3.2.1.1. Tree stratum. From the tree stratum, the most consumed pollen type in all seasons was *Olea,* ranging from 11.6 to 52.7% of the consumed pollen grains among all specimens, and although the flowering period lasted only a few days, D_i was 1 or close to 1 for both females and males. This result indicates that *C. carnea* fed not only on pollen from flowers but also on settled pollen. The consumption of *Olea* pollen could be related to the fact that lacewings hide during the day in olive agroecosystems. In this agroecosystem, *Olea* seem to be an interesting food resource for *C. carnea*, and it could be concluded that the pollen from this tree is sufficient to feed *C. carnea* adults. However, a poor diet can negatively influence the survival and reproduction of chrysopids (Principi, 1991). Therefore, the other pollen types consumed probably had an essential role in the performance of *C. carnea*. The nutrient composition of the different pollen types can be an important aspect. For example, Liolios et al. (2016) found that *Olea* pollen contained 17.26% of proteins, while other species belonging to Polygonaceae, Fabaceae, Asteraceae or Brassicaceae, also consumed by *C. carnea* in the present study, presented more proteins in their composition than *Olea* pollen. Additionally, since the olive tree flowering period takes a few days, in an olive grove agroecosystem poor in flowering plants, the adults would rely only on settled *Olea* pollen for most of the season, and therefore, the insect reliance on this pollen could be deleteriously affected by strong rains or other unfavourable conditions that could remove the settled pollen. Villenave et al. (2006) also observed the consumption of crop plants (Brassicaceae, Apiaceae, Fabaceae), but in their study, the pollen consumption of crop plants occurred only during the flowering periods. In the present study, the anemophilous pollen of Pinaceae had a positive index of selection in almost all the seasons of both years for females and in the summer and the autumn of 2013 for males. This was because the Pinaceae flowering period occurred only in the spring, and π_i in the summer and autumn was zero; however, the higher consumption occurred in the spring. *Betula* was consumed by several females and males, although it was not present in the study patches (making D_i equal to 1), representing less than 1% of the consumed pollen in guts. Other works found Pinaceae and Betulaceae to be important for *Chrysoperla* (Villenave et al., 2005; Monserrat and Díaz-Aranda, 1989).

3.2.1.2. Herbaceous stratum. From the anemophilous pollen in the herbaceous stratum, the Poaceae was among the most consumed and

Table 1
Number of individuals of *Chrysoperla carnea* females and males with and without pollen in in their guts and total number of individuals analyzed in the Spring (*Spr*), Summer (*Sum*) and Autumn (*Aut*) of 2012 and 2013. Richness of consumed pollen types - *R*, median, maximum and minimum of consumed pollen types - *Mdn (Max-Min)* and mean of consumed pollen grains $\pm$ standard error - *M $\pm$ SE*, are shown.

Sex	*Chrysoperla carnea*	2012			2013			Total	R	Mdn (Max-Min)	M $\pm$ SE
		Spr	Sum	Aut	Spr	Sum	Aut				
Females	Individuals with pollen	18	8	31	43	10	26	136	37	4 (1–11)	1686 $\pm$ 541
	Individuals without	0	0	8	0	1	0	9			
	Total analyszed females	18	8	39	43	11	26	145			
Males	Individuals with pollen	13	4	25	30	13	53	138	39	5 (1–13)	546 $\pm$ 233
	Individuals without	0	0	5	0	0	2	7			
	Total analyzed males	13	4	30	30	13	55	145			

Table 2

Number of specimens (females and males of *Chrysoperla carnea*) with pollen type i–n. mean (minimum and maximum) - Mean (Min-Max) of pollen grains consumed by each specimen. Jacobs`s second selection index - D$_i$. and p ercentage of consumed pollen grains of pollen type i- Oi % for females and males during the spring (Spr). summer (Sum) and autumn (Aut) of 2012 and 2013. The main type of pollination for the plant families (anemophilous – A or entomophilous – E) is indicated. From the 44 pollen types found, the most consumed types (n = 17) are shown.

Sex	Family	Pollination	Pollen type	n	M (Min-Max)	Di						Oi%					
						2012			2013			2012			2013		
						Spr	Sum	Aut	Spr	Sum	Aut	Spr	Sum	Aut	Spr	Sum	Aut
Females	Apiaceae	E	Apiaceae	23	836 (1–12,800)	−0.9	−0.5	−0.7	−0.7	−0.7	−0.6	0.3	17.0	4.9	2.7	10.0	4.3
	Asteraceae	E	*Anthemis* type	29	257 (1–6000)	−0.8	−1.0	−0.9	−0.6	−0.4	−0.8	2.6	0.0	0.5	6.4	0.9	3.4
			Aster type	31	27 (1–486)	−0.7	−1.0	−0.9	−0.4	−0.8	−0.6	0.5	0.0	2.0	4.4	0.6	2.0
			Cichorioideae	38	131 (1–4104)	−1.0	−0.7	−0.9	−0.8	−0.2	−0.4	0.1	6.3	1.3	1.7	23.8	10.4
	Betulaceae	A	*Betula*	13	7 (1–44)	1.0	_	_	1.0	1.0	_	0.0	0.0	0.0	0.2	0.9	0.0
	Boraginaceae	E	Boraginaceae	16	9 (1–50)	−1.0	−1.0	−0.5	−0.8	−1.0	−0.9	0.0	0.0	0.4	0.5	0.0	0.5
	Brassicaceae	E	Brassicaceae	17	547 (1–5150)	−1.0	−1.0	−0.5	−0.6	−0.8	−0.8	0.0	0.0	9.5	8.3	1.7	5.8
	Chenopodiaceae	A	Chenopodiaceae	15	13 (1–137)	1.0	−0.1	1.0	1.0	0.0	−0.8	0.0	2.4	3.4	0.0	0.8	4.5
	Cistaceae	E	Cistaceae	29	97 (1–1588)	−0.9	−0.3	1.0	−0.4	1.0	1.0	2.6	0.1	4.7	8.4	0.2	1.5
	Ericaceae	E	Ericaceae	31	4 (1–40)	1.0	1.0	1.0	1.0	1.0	1.0	0.6	1.1	1.2	0.5	0.2	0.7
	Fabacea	E	*Cytisus/Genista* type	51	129 (1–4276)	−0.2	1.0	1.0	0.1	1.0	1.0	9.6	16.4	17.5	7.2	0.8	5.9
			Fabaceae undiff.	14	6 (1–43)	−0.9	−0.1	−0.8	−1.0	−1.0	−1.0	2.8	10.1	0.6	0.3	0.0	0.0
	Oleaeceae	A	*Olea*	104	1609 (1–62,520)	1.0	1.0	1.0	1.0	1.0	1.0	41.8	24.1	40.2	40.3	11.6	46.1
	Pinaceae	A	Pinaceae	49	14 (1–108)	1.0	_	1.0	0.6	1.0	1.0	6.9	0.0	1.2	7.6	9.6	1.4
	Plantaginaceae	A	*Plantago* type	27	375 (1–10,012)	−0.4	−0.5	−1.0	−0.7	0.1	−0.9	1.6	0.0	0.0	0.8	13.0	1.9
	Poaceae	A	Poaceae	54	17 (1–312)	−0.8	0.7	0.1	−0.6	−0.6	−0.4	3.3	11.8	2.2	2.3	0.2	4.4
	Poligonaceae	A	*Rumex*	27	42 (1–225)	−0.5	1.0	1.0	−0.6	1.0	_	10.2	1.1	0.4	4.0	11.3	0.0
Males	Apiaceae	E	Apiaceae	23	45 (1–755)	−0.7	0.0	−0.8	−0.7	−0.5	−0.8	1.0	23.9	3.0	0.8	2.8	2.0
	Asteraceae	E	*Anthemis* type	28	21 (1–299)	−0.8	−1.0	−1.0	−0.4	−0.6	−0.8	1.5	0.0	0.0	10.0	0.4	2.0
			Aster type	21	6 (1–37)	−0.8	−1.0	−0.9	−0.6	−0.7	−0.9	0.3	0.0	0.2	0.9	1.8	0.3
			Cichorioideae	47	6 (1–76)	−0.9	−1.0	−0.8	−0.8	−0.3	−0.4	0.9	0.0	0.9	1.3	5.9	8.5
	Betulaceae	A	*Betula*	10	4 (1–10)	_	_	1.0	1.0	1.0	_	0.0	0.0	0.0	0.8	0.6	0.0
	Boraginaceae	E	Boraginaceae	12	14 (1–80)	−1.0	−1.0	−1.0	−0.8	−1.0	−1.0	0.0	0.0	0.0	2.5	0.0	0.0
	Brassicaceae	E	Brassicaceae	7	3796 (1–26,528)	−1.0	−1.0	−0.9	−0.8	−1.0	−0.9	0.0	0.0	1.3	4.3	0.0	2.6
	Chenopodiaceae	A	Chenopodiaceae	20	35 (1–600)	_	−1.0	1.0	1.0	0.7	−0.6	0.0	0.0	0.8	0.1	10.3	6.4
	Cistaceae	E	Cistaceae	20	16 (1–182)	−0.8	−1.0	1.0	−0.7	_	1.0	5.3	0.0	0.5	6.6	0.0	2.7
	Ericaceae	E	Ericaceae	27	2 (1 -8)	1.0	_	1.0	1.0	1.0	1.0	1.3	0.0	4.8	1.2	0.1	1.1
	Fabaceae	E	*Cytisus/Genista* type	44	16 (1–259)	0.2	1.0	1.0	0.1	1.0	1.0	11.1	2.8	7.0	7.0	6.3	2.7
			Fabaceae undiff	17	8 (1–57)	−0.9	0.0	−0.5	−1.0	−0.8	−0.9	0.5	2.3	2.9	0.2	0.2	0.2
	Oleaeceae	A	*Olea*	114	350 (1–13,456)	1.0	1.0	1.0	1.0	1.0	1.0	39.2	52.7	56.0	41.2	37.0	45.2
	Pinaceae	A	Pinaceae	43	7 (1–52)	−1.0	_	_	−0.4	1.0	1.0	13.6	0.0	0.0	3.6	2.6	0.8
	Plantaginaceae	A	*Plantago* type	24	27 (1–279)	−0.8	−1.0	−0.1	−0.6	−0.3	−0.8	0.1	0.0	4.8	0.8	13.9	1.8
	Poaceae	A	Poaceae	47	15 (1–392)	−0.9	0.4	−0.5	−0.4	−0.2	−0.7	2.1	8.6	2.6	4.6	3.2	3.0
	Poligonaceae	A	*Rumex*	27	16 (1–91)	−0.3	−1.0	1.0	−0.5	−0.3	1.0	7.2	0.0	0.6	6.2	5.0	1.3

presented a positive selection index during the summer and autumn of 2012 for females and the summer of 2012 for males. This family was found to be important for chrysopids in other works (Medeiros et al., 2010; Villenave et al., 2005 and 2006). Chenopodiaceae was selected by females and males during autumn 2012 and spring and summer 2013, with O_i % always lower than 10.3%. *Plantago* type (Plantaginaceae) and *Rumex* (Polygonaceae) were consumed and generally underselected, except when they did not occur in the inventoried patches. The maximum consumption of *Plantago* type occurred in summer 2013 and *Rumex* in spring 2012 and autumn 2013.

In other studies, Chenopodiaceae were important along with Plantaginaceae, while Polygonaceae were consumed at a low percentage (Villenave et al., 2005 and 2006). Pollen grains of all these anemophilous plants, particularly when they were consumed out of their flowering periods, could have remained on vegetation surfaces where *C. carnea* could have licked them, as suggested by Wäckers and van Rijn (2012).

3.2.2. Entomophilous pollen

Several entomophilous plants were also highly consumed by *C. carnea* adults, namely, Cistaceae, the Fabaceae *Cytisus/Genista* type and Ericaceae from the bush stratum and Apiaceae, Asteraceae, Brassicaceae, Boraginaceae, and undifferentiated (undiff.) Fabaceae from the herbaceous stratum (Table 2, Fig. 3).

3.2.2.1. Bush stratum. From the bushes, Cistaceae was mainly

consumed by females and males in the spring of both years, although the selection index was only positive during the autumn of both years and summer of 2013. Despite the papilionaceus shape of the flowers of *Cytisus/Genista* (shrub Fabaceae), their pollen type was consumed by a considerable number of males and females. This pollen type was generally selected and consumed in a high percentage in almost all sessions (Table 2). To our knowledge, this is the first record of *C. carnea* feeding on both Cistaceae and shrub Fabaceae. Ericaceae pollen (except *Arbutus unedo* L.) was found in adult guts in the spring and autumn of both years. Villenave et al. (2006) also found Ericaceae pollen in *Chrysoperla* adults.

3.2.2.2. Herbaceous stratum. Regarding the entomophilous pollen in the herbaceous stratum, the proportion of pollen of Apiaceae consumed showed more importance during the summer and in the autumn to a certain degree for both males and females. However, the selection index was always negative. Similarly, Villenave et al. (2006) found *Daucus carota* L. (Apiaceae) consumption with negative selection, while in laboratory experiments, *D. carota* and *Foeniculum vulgare* L. (Apiaceae) improved the survival and/or reproduction of *C. carnea* (Villa et al., 2016a; van Rijn et al., 2012). Therefore, a negative index selection may not imply unimportance for some pollen types, such as Apicaceae. Several Asteraceae pollen types (*Anthemis* type, *Aster* type and Cichorioideae) were well represented in the adult guts, particularly Cichorioideae during summer and autumn 2013. The selection index was always negative, indicating the generally high cover of
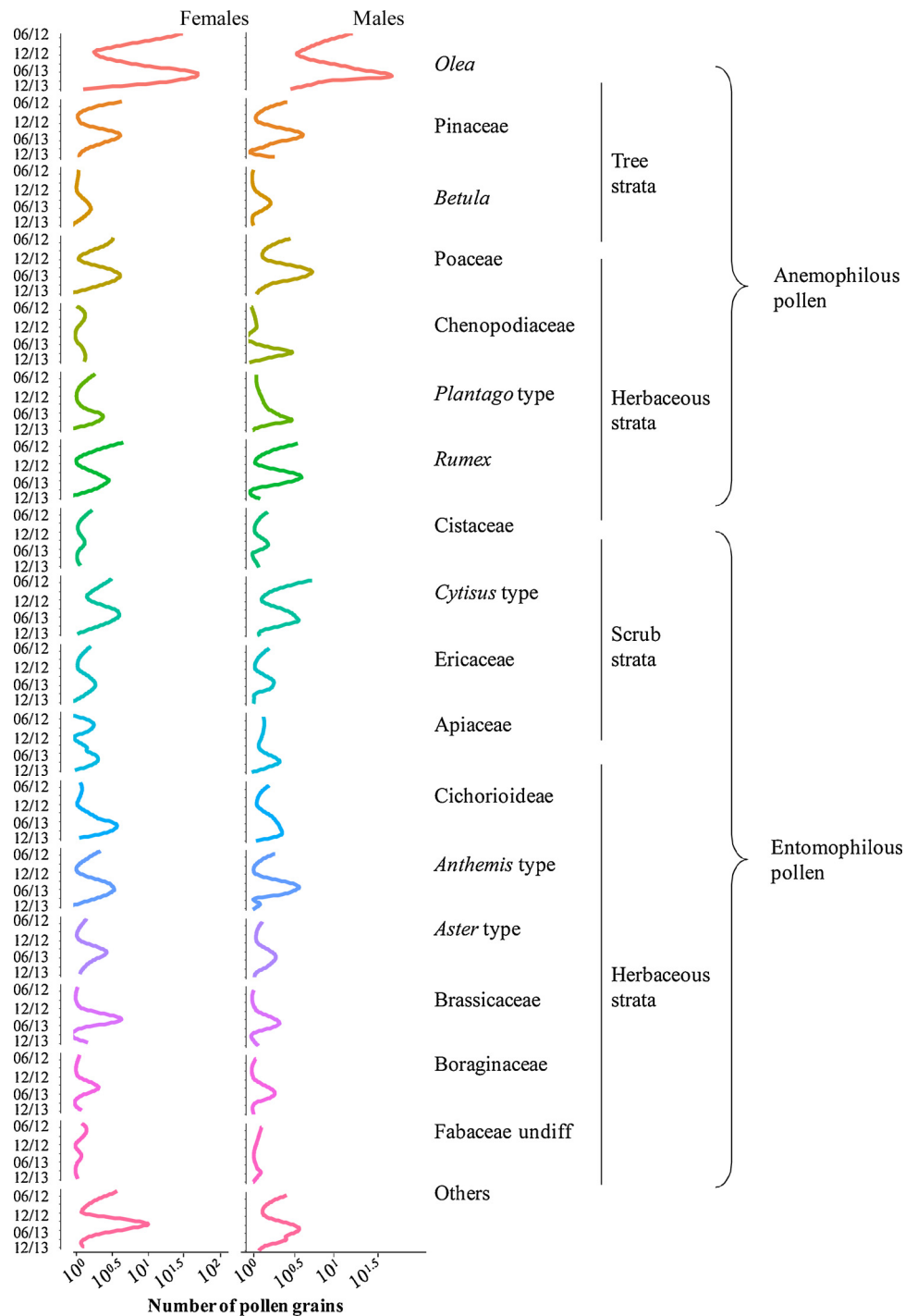
Fig. 3. Number of pollen grains discriminated by type consumed by females and males of *Chrysoperla carnea s.l.* in the olive grove agroecosystem from April to December 2012 and 2013. Lines represent smooth curves (local polynomial regression method – span = 0.6) of the number of pollen grains consumed during the year.

Cichorioideae in the herbaceous stratum. This result is in accordance with those of Villenave et al. (2005). Moreover, van Rijn et al. (2012) found that *C. carnea* feeding on Asteraceae with accessible extrafloral nectar lived longer and produced eggs; however, Asteraceae without the nectar gave rise to shorter longevities, albeit still longer than with water only. In contrast, none of the Asteraceae tested by Villa et al. (2016a) improved the survival of *C. carnea* adults. This finding suggests that most Asteraceae species may be very important as pollen providers but probably not as nectar providers due to their production of insufficient or inaccessible nectar for many short-tongued insects.

Brassicaceae were moderately consumed in spring 2013 and autumn 2012 and 2013, but the selection index was always negative (Table 2). Boraginaceae were consumed by several individuals, but in a low percentage, and the selection index was always negative. Fabaceae undiff (herbaceous Fabaceae) was moderately consumed in the summer of 2012 but showed a negative index selection in all seasons. This pollen type was previously found in chrysopid guts by Villenave et al. (2005 and 2006).

3.2.3. Pollen rarely consumed

In this study, some pollen types were rarely consumed. In those cases, adults could have inadvertently consumed pollen stuck in nectar or honeydew (Wäckers and van Rijn, 2012) or on other vegetation surfaces. Some of those pollen types were not present in the inventoried patches, but they occur in the region (Flora-on, 2017). In all those cases the percentage of consumed pollen grains was low (O_i % < 7.3%), however the proportion of available pollen of some types ($\hat{\pi}_i$) was 0 (not present in the inventoried patches), and the resulting D_i was 1 (maximum selection index). These pollen types were *Acacia dealbata*, *Alnus* type, *Castanea sativa*, Cucurbitaceae, *Scabiosa* type, *Ephedra fragilis* type, *Helianthemum* type, *Quercus suber*, *Juniperus*, *Polygonum persicaria* type, *Frangula alnus* type, *Salix* and *Urtica dioica* type.

Other pollen types were rarely consumed or not consumed at all (the percentage of consumed pollen grains was low with O_i % < 4% in all cases), although they were available in the environment (high $\hat{\pi}_i$). In this case, the resulting selection index, D_i, was generally close to -1 (minimum selection index). These pollen types were *Arbutus unedo*, *Campanula* type, Caryophyllaceae, Convolvulaceae, *Daphne gnidium* type, *Geranium*, Liliaceae undiff, Lamiaceae, *Malva sylvestris*, *Quercus* type, Scrophulariaceae, Cardueae type, *Euphorbia* type, *Jasione* type, *Hypericum*, Orobanchaceae, Rubiaceae, Caprifoliaceae, Ranunculaceae, and *Crassula* type.

Our results agreed with Villenave et al. (2006), who did not find pollen consumption of Convolvulaceae, Geraniaceae, Cucurbitaceae, Malvaceae or Scrophulariaceae in the *Chrysoperla* specimens analysed. They pointed out that flowers of these families were closed at dusk, and therefore, their pollen would not be available during that period of activity of *Chrysoperla* adults. Villenave et al. (2006) also did not find Fagaceae pollen type in the adult guts, which was attributed to the fact that few *C. carnea* were actually captured during the flowering time of Fagaceae (autumn for the area of study). Other pollen types, such as Caprifoliaceae, represented by *Lonicera periclymenum* L. subsp. *hispanica* (Boiss. & Reut.) Nyman, and Ranunculaceae, represented by *Ranunculus ollissiponensis* Pers. and *Ranunculus trilobus* Desf. were not consumed in the study areas. Further studies are needed to determine if mechanisms, such as competitive interactions (Wäckers and van Rijn, 2012) with other pollinators or the low availability of some of these pollen types could limit chrysopids from feeding on them, or if they are actually avoided.

Although some pollen types were not consumed, those plant species could offer other resources, such as extrafloral nectar. Proteins supplied by pollen are essential for egg production, but carbohydrate sources are also needed for both egg production and survival (Villa et al., 2016a). For example, *Lamium purpureum* L. (Lamiaceae) resulted in low egg production but improved the survival of *C. carnea*, probably because pollen was not accessible, but insects could feed on its nectar (Villa et al., 2016a).

4. Conclusion

The results of this study indicate that *C. carnea* females and males captured in the olive tree canopy visited scrub vegetation patches to feed on bush and tree pollen belonging to Fabaceae, Pinaceae, Cistaceae or Ericaceae and herbaceous vegetation to feed on Asteraceae, Apicaceae, Brassicaceae, Poaceae, *Rumex* type or *Plantago* type. These results are in agreement with Villenave et al. (2005), who found *Chrysoperla* spp. feeding on both arborescent and herbaceous strata. A high plant diversity in the crop could increase the pollen diversity consumed by *C. carnea*. Interestingly, the results indicated that *C. carnea* fed on a high amount of *Olea* pollen and raised the question about the potential nutritional value of this pollen for adults. In the present study, *C. carnea* fed on anemophilous and entomophilous pollen not only during the flowering period but also when flowers were not available, suggesting that they can feed on pollen from vegetation surfaces. Additionally, they did not feed on some abundant plant

species. Different mechanisms might be involved, such as unattractive characteristics, competitive exclusion by other pollinators or closed flowers at twilight. Further studies should also address the nutritional composition of the most abundant pollen types found in *C. carnea* guts. Finally, few studies have addressed the effect of olive grove agroecosystem vegetation on olive tree pests (Villa et al., 2016c). Further studies should address how changes in flowering plant biodiversity affect pest control accomplished by *C. carnea*.

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