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Selection of insectary plants for the conservation of biological control agents of aphids and thrips in fruit orchards

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Abstract

This study evaluated the potential of flowering plant species naturally occurring to promote the conservation and early establishment of key natural enemies of aphids and thrips in apple and peach orchards. Flowering plants present in the North East of Spain, a main fruit production area in Europe, were sampled to determine their flowering period and to identify potential natural enemies present on each plant species. Thirty-six plant species were found blooming from early March to late May and provided an array of flowers that might ensure food resources for natural enemies. Among them, six species – *Eruca vesicaria* (L.) Cav., *Cardaria draba* (L.) Desv., *Euphorbia serrata* (L.) S.G. Gmel., *Malva sylvestris* L., *Anacyclus clavatus* (Desf.) Pers. and *Diplotaxis erucoides* (L.) DC. – hosted a high diversity of potential natural enemies of aphids and thrips. Their blooming started early in the season and lasted for several sampling weeks and they were widely distributed. Moreover, they had available nectar even in those species with protected nectaries. Therefore, these plant species can be considered as promising candidates for inclusion in the ecological infrastructure designed for fruit orchards in the study area to promote the conservation of the biological control agents of aphids and thrips.

Introduction

Spain is the primary producer of stone and pip fruits (EUROSTAT, 2019) in the European Union, and the production of peaches and nectarines (*Prunus persica* L. Batsch) and apples (*Malus domestica* Borkh) are concentrated in the North East (MAPA, 2020). Fruit production can be affected by aphids, which are considered a significant pest of peach, nectarine and apple orchards under temperate and Mediterranean climates (Barbagallo *et al*., 2017), whereas thrips inflict damage to nectarines (González *et al*., 1994). *Myzus persicae* Sulzer and *Hyalopterus* spp. in peach and *Eriosoma lanigerum* Hausmann and *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) in apple are the most common aphids that attack stone and pome fruit trees (Barbagallo *et al*., 2017). *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is the main thrips species of nectarines in Spain and other Mediterranean countries, where it causes feeding damage to flowers and ripe fruits (Teulon *et al*., 2018). Aphids and thrips are present in the field early in the season. *M. persicae*, *Hyalopterus* spp. and *D. plantaginea* overwinter as eggs on trees (Barbagallo *et al*., 2017). Conversely, *E. lanigerum* overwinters as adults either on the roots or within the canopy of apple trees (Lordan *et al*., 2014). Thrips hibernate in the weed flowers that are present around or within the fruit orchards (Trdan *et al*., 2005), and they fly to the flowers of the nectarine trees during blooming.

To date, aphids and thrips in fruit orchards are mostly managed with insecticides (Penvern *et al*., 2010). The social concern for healthier food provision and more sustainable agriculture has led to the search for healthy and environmentally friendly tools for pest management. The intensification of agriculture has promoted the simplification of agroecosystems, and the subsequent removal of non-crop habitats has caused a decline in biodiversity (Gurr *et al*., 2004). Hence, there has been an increasing interest in restoring biodiversity and in conservation biological control (CBC) by modifying the environment or existing practices to protect and enhance specific natural enemies to reduce the effect of pests (Eilenberg *et al*., 2001). Dedryver *et al*. (2010) suggested that CBC was the best option for biological control of aphids in open field crops. That is why it is crucial to determine with confidence which natural enemies to promote. The studies by Rodríguez-Gasol *et al*. (2019) and Aparicio *et al*. (2019) reported on several species of Braconidae and one of Aphelinidae that parasitized several aphid pests in fruit orchards in the same area as the current study, and on hyperparasitoids from the Pteromalidae, Encyrtidae and Figitidae families. By contrast, only one species, *Ceranisus menes* (Walker) (Hymenoptera: Eulophidae), parasitizes *F. occidentalis* in

Mediterranean agroecosystems, although this species only plays a minor role in thrips control (Loomans, 2006). In Spain, several predatory groups (Coccinellidae, Chrysopidae, Anthocoridae, Syrphidae and Aeolothripidae) have also been recorded from peach and apple orchards (Miñarro *et al.*, 2005; Davidson *et al.*, 2014; Rodríguez-Gasol *et al.*, 2019; Aparicio *et al.*, 2021).

One of the most commonly adopted measures to enhance the presence of natural enemies close to crops is the increase of plant biodiversity in flower strips, ground covers and field edges, among others. Plants can provide various food sources for adult parasitoids and insect predators, including floral nectar, extrafloral nectar, honeydew, pollen and seeds (Wäckers, 2005; Araj and Wratten, 2015), and they can also provide suitable habitat for alternative hosts and prey. Wäckers (2005) reviewed the effect of nectar on parasitoids and predators and discussed its role as a survival food when the host or prey is not available and its role in increasing fitness when they are available. Several studies have addressed the selection and field testing of companion plants to enhance biological control in orchards. For example, in apples, Gontijo *et al.* (2013) demonstrated the efficacy of *Lobularia maritima* L. (Brassicaceae) at increasing populations of generalist predators and at reducing attacks from *D. plantaginea*. Cahenzli *et al.* (2019) in field experiments conducted in seven European countries demonstrated the positive effect of sown perennial flower strips with selected dicotyledon and grass species compared to spontaneous vegetation in the control of aphids in apple orchards. Fitzgerald and Solomon (2004) and Winkler *et al.* (2007) observed that the presence of flowers increased the densities of anthocorids and contributed to the control of *Cacopsylla pyri* L. (Hemiptera: Psyllidae). In Chinese peach orchards, Wan *et al.* (2014*a*, 2014*b*) demonstrated that a ground cover of *Trifolium repens* L. (Fabaceae) enhanced the diversity of generalist predators in tree canopies and decreased the incidence of aphids and *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae).

The selection of appropriate plant species for target natural enemies is a crucial issue to enhance their populations effectively. Shanker *et al.* (2013) argued that the selection of plants from their own agroecological system increased the potential for establishment of natural enemies. Similarly, several studies have screened other plants such as weeds that are not conventionally used as insectary plants (Wäckers, 2004; Araj and Wratten, 2015; Jado *et al.*, 2018; Araj *et al.*, 2019). Another selection criterion is the bloom period to ensure the presence of flower-food resources before the pest population starts to build up. However, food availability is not only a question of timing but also one of attractiveness and flower architecture, which might constrain nectar accessibility (Wäckers, 2005). Moreover, the selection of candidate plants must take into account their role as a potential reservoir of pests or diseases detrimental to the crop (Bugg and Waddington, 1994).

Considering this background, our study aimed to identify candidate plant species to be included in ecological infrastructure tailored to promote aphid and thrips CBC in fruit orchards in the study area early in the season when these pests are most damaging. To achieve that we (1) determined the flowering period of the most common herbaceous plants spontaneously present near fruit orchards in the North East of Spain, (2) identified the predominant functional groups of natural enemies present on these plant species and (3) evaluated the nectar availability of the different plant species in terms of floral architecture and natural enemy morphology.

Materials and methods

Survey of flowering plants and natural enemies

The survey was conducted from early March (week 11) to the third week of May 2017 (week 21) at 20 sampling sites in the Segrià, Pla d'Urgell and La Litera counties (North East of Spain), which has an area of approximately 20,000 ha of apple and peach orchards (DARP, 2020; Gobierno de Aragón, 2020). The sites were selected to be representative of the orchard vegetation and were within an area of approximately 400 km^2 (fig. 1). All sites were visited fortnightly, and plant species in full-bloom were recorded. At each sampling site and date, one sample was taken. It consisted of beating separately three bunches of flowers of each plant species in bloom on a 30×17 cm^2 white plastic tray. The number of hymenopteran parasitoids, Coccinellidae, Chrysopidae, Anthocoridae, Aeolothripidae, aphids and phytophagous thrips (hereafter thrips) in the tray were recorded. The average number of individuals of the different functional groups per tray was calculated for each sampling site, date and flower species. Hymenopteran parasitoids and Anthocoridae and Aeolothripidae specimens were collected with an aspirator and kept in 70% alcohol for identification. Parasitoids were identified when possible at the family level using the taxonomic keys of Grissell and Schauff (1990) and Hanson and Gauld (2006). Parasitoids that could not be identified were grouped as *Other Parasitica*. Braconidae were identified at the species level by Aparicio. Anthocoridae were identified using Péricart (1972) and Aeolothripidae with the taxonomy keys of Alavi and Minaei (2018). The number of aphids and other thrips per tray was also recorded (but were not identified at the species level).

Accessibility to nectar

Flowers of the different species were collected and placed in an ice chest cooler and transported to the laboratory, where they were inspected for the presence of nectaries. Plants were classified as harboring extrafloral or floral nectaries (unprotected or protected). Of the flowers with protected nectaries, nectar presentation was observed and classified as fully exposed or protected inside the flower. For species with nectar protected inside the flower, 20 fully open flowers of each plant species were photographed twice: one for width and one for depth measurements of the corolla under a Stereo Microscope Carl Zeiss stemi 2000C. Measurements were made with the use of ImageJ software (Rueden *et al.*, 2017).

Similarly, measurements were made on the width of the head and the thorax of several natural enemies of aphids and thrips already sighted in the study area (Aparicio *et al.*, 2019, 2021; Rodríguez-Gasol *et al.*, 2019), including: *Aphidius matricariae* Haliday, *Aphidius ervi* Haliday, *Lysiphlebus testaceipes* Cresson, (Hymenoptera: Braconidae), *Aphelinus abdominalis* Dalman, *Aphelinus mali* Haldemann, (Hymenoptera: Aphelinidae), *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), *Orius majusculus* Reuter (Hemiptera: Anthocoridae) and *Aeolothrips intermedius* Bagnall (Thysanoptera: Aeolothripidae). *O. majusculus* were obtained from the colony kept in the IRTA laboratory. *A. mali* and *A. intermedius* were collected in the field, and the other species were purchased from AgroBio S.L. (Almería, Spain). Ten females and ten males randomly selected from each species were used.

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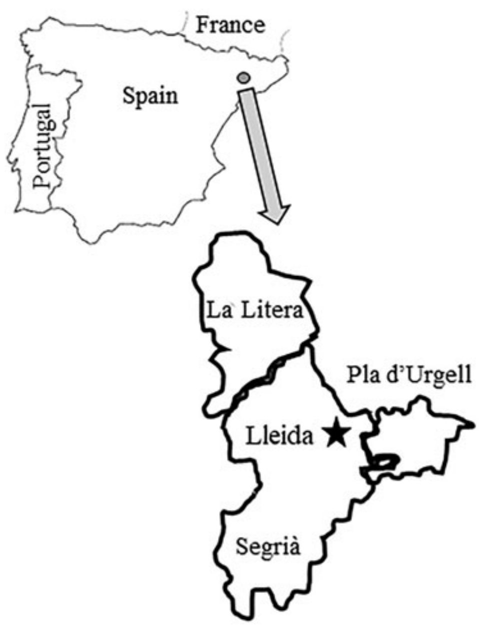

Sampling point	Latitude	Longitude
TS1	41.632972	0.562974
TS2	41.634872	0.560331
Ai1	41.479526	0.467340
Ai2	41.461788	0.484210
Ai3	41.464492	0.482224
Ai4	41.462820	0.483507
Ai5	41.459068	0.485522
Ai6	41.454841	0.508229
Ai7	41.454952	0.511832
Be1	41.639610	0.776872
Be2	41.637870	0.765899
Be3	41.641124	0.755552
Al1	41.807879	0.584663
Al2	41.814188	0.579569
Al3	41.824310	0.575448
Tl1	41.834708	0.545427
Tl2	41.811098	0.525626
Tl4	41.812927	0.527237
Tl5	41.776572	0.507117
Tl7	41.741330	0.454324

Figure 1. Coordinates of the 20 sampling points of the study located in Segrià, Pla d'Urgell and La Litera (North East of Spain). For reference, coordinates of the city of Lleida are 41.62026 and 0.61976.

Data analysis

The mean number of individuals from each parasitoid and predator family for all the sampling dates and sites was used to calculate the Shannon's diversity index (H') for each plant species: $H' = \sum_{i=1}^{S} -(P_i \times \ln P_i)$, where P_i is the proportion of the mean number of individuals of family i vs. the mean number of individuals of all the natural enemies recorded in this plant species, and S is the number of families encountered. This index was calculated using the Paleontological Statistics Software Package for Education and Data Analysis (PAST) (Hammer *et al.*, 2001). For males and females of the selected natural enemies, the Student's t test ($P < 0.05$) was used to test whether the thorax was wider than the head.

Results

Survey of flowering plants and natural enemies

A total of 36 spontaneous growing herbaceous species belonging to 17 families were found to be blooming during the sampling period in the close surroundings of the fruit tree orchards in Lleida (table 1). Many blooming plants belonged to Brassicaceae and Asteraceae (ten and eight species, respectively), whereas Fabaceae, Euphorbiaceae and Lamiaceae had only two species each in bloom. The remaining 12 families included only one species. Of these plants, 25 were early flowering plants (weeks 11–15) and 11 species started to bloom later (weeks 17–21). Among the early flowering plants, five of them were already in bloom in week 11 (early March) when the sampling started. Of these, *Eruca vesicaria* (L.) Cav., *Diplotaxis erucoides* (L.) DC and *Moricandia arvensis* (L.) DC were the most widely distributed as can be inferred by the higher numbers of sampling sites where they were found. Additionally, *E. vesicaria* and *M. arvensis* had an extended flowering period that lasted until weeks 19 and 21, respectively. *Cardaria draba* (L.) Desv, *Euphorbia serrata* (L.) S.G. Gmel., *Crepis* sp. L. and *Sisymbrium irio* L. extended their flowering period from week 13 to week 19. Of those plant species that started to bloom later, *Anacyclus clavatus* (Desf.) Pers. and *Malva sylvestris* L. bloomed from week 15 to week 21 and were present in many sampling sites. Of the plants that bloomed by week 17, *Beta maritima* L., *Galium aparine* L., *Papaver rhoeas* L. and *Rumex crispus* L. were the most prevalent.

Natural enemies were collected from 30 plant species and accounted for 145 parasitoid and 285 predator individuals (table 2). No natural enemies were recruited from six plant species: namely, *Fumaria officinalis* L., *Thymus vulgaris* L., *Erodium ciconium* (L. et Juslin) L'Hér., *Scandix pecten-veneris* L., *Erucastrum* sp. (DC.) C. Presl and *Silene vulgaris* (Moench) Garcke, and were therefore not included in table 2 or further analysis. No parasitoids were found in association with *M. arvensis*, *Calendula arvensis* L., *Capsella bursa-pastoris* (L.) Medik., *Chrysanthemum segetum* L., *Plantago* sp. L. and *Pallenis spinosa* (L.) Cass. On the other hand, no predators were recruited from *Lamium* sp. L., *Diplotaxis virgata* (Cav.) DC. and *Rapistrum rugosum* (L.) All. The Shannon biodiversity indexes were higher than 1.5 for the following five species – *Carduus pycnocephalus* L., *R. crispus*, *E. vesicaria*, *C. draba* and *G. aparine* – with values reaching up to 1.87.

Table 3 depicts the number of samples in which families of natural enemies known to be associated with aphids or thrips were found. The number of plant species where the presence of Braconidae and Aphelinidae families were recorded increased from three to nine from the first sampling period (weeks 11–15) to the second sampling period (weeks 17–21), as did the number of samples with at least one individual (from 4 to 21). Of the 30 recruited parasitoids that belonged to the abovementioned families, 28 were identified as Braconidae and two as Aphelinidae. Among the Braconidae, 24 individuals were classified as belonging to the Aphidiinae subfamily: ten *A. matricariae*, five *Binodoxys angelicae* Haliday (Hymenoptera: Braconidae),

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Table 1. Number of sample sites where each of the plant species was recorded in full bloom during the sampling weeks

	Early period weeks			Late period weeks		
Plant species (Family)	11	13	15	17	19	21
Moricandia arvensis (Brassicaceae)	3	2	3	2	1	1
Eruca vesicaria (Brassicaceae)	4	5	7	4	2	
Medicago sativa (Fabaceae)	1	1			2	
Calendula arvensis (Asteraceae)	1	1	3	1		
Diplotaxis erucoides (Brassicaceae)	2	5	6	1		
Crepis sp. (Asteraceae)		3	3	4	1	
Cardaria draba (Brassicaceae)		1	6	8	3	
Euphorbia serrata (Euphorbiaceae)		1	5	6	2	
Sisymbrium irio (Brassicaceae)		1	4	1	1	
Euphorbia helioscopia (Euphorbiaceae)		1	1	1		
Fumaria officinalis (Fumariaceae)		1	1	1		
Thymus vulgaris (Lamiaceae)		1	1	2		
Brassica napus (Brassicaceae)		1	3			
Capsella bursa-pastoris (Brassicaceae)		2	2			
Erodium ciconium (Geraniaceae)		1	2			
Lamium sp. (Lamiaceae)		1	1			
Scandix pecten-veneris (Apiaceae)		1	1			
Taraxacum officinale (Asteraceae)		1				
Anacyclus clavatus (Asteraceae)			4	11	12	8
Malva sylvestris (Malvaceae)			1	10	10	8
Sonchus sp. (Asteraceae)			4	1	4	1
Asphodelus fistulosus (Xanthorrhoeaceae)			5	5	1	
Chrysanthemum segetum (Asteraceae)			1	1	1	
Plantago sp. (Plantaginaceae)			3	1	3	
Diplotaxis virgata (Brassicaceae)			3			
Beta maritima (Amaranthaceae)				4	7	5
Galium aparine (Rubiaceae)				3	6	3
Papaver rhoeas (Papaveraceae)				4	4	5
Rumex crispus (Polygonaceae)				3	4	5
Carduus pycnocephalus (Asteraceae)				3	3	1
Reseda lutea (Resedaceae)				1	1	
Erucastrum sp. (Brassicaceae)				1		
Rapistrum rugosum (Brassicaceae)				2		
Silene vulgaris (Caryophyllaceae)				1		
Dorycnium pentaphyllum (Fabaceae)					5	2
Pallenis spinosa (Asteraceae)					3	3

Twenty sampling sites were visited on each sampling date. Plant species are ordered from early to late and from longest to shortest flowering period.

four *Aphidius* sp., three *A. ervi* and two *Aphidius colemani* Dalman (Hymenoptera: Braconidae). Moreover, three Figitidae and one Pteromalidae, known as hyperparasitoids of aphids, were recruited during the sampling. Aeolothripidae were the most prevalent predators in both sampling periods. They were reported from 12 and 20 plant species and in 17 and 35% of the samples, in the first and second sampling periods, respectively. Out of the 205 Aeolothripidae individuals collected in the samples, 88 were identified at the species level. Half of them corresponded to *A. intermedius*, and the other half to *Aeolothrips tenuicornis* Bagnall (Thysanoptera: Aeolothripidae). Other predators were much less widespread, making up less than 10% of

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Table 2. Abundance of natural enemies (mean number of individuals over all sampling sites and dates) and value of Shannon's diversity index for each plant species

Plant species	Braconidae	Ichneumonidae	Aphelinidae	Eurytomidae	Eulophidae	Platygastridae	Mymaridae	Perilampidae	Megaspilidae	Figitidae	Pteromalidae	Other Parasitica	Coccinellidae	Chrysopidae	Anthocoridae	Aeolothripidae	Shannon index
C. pycnocephalus	0	0	0	0	0	0.07	0.14	0.05	0	0	0	0.12	0.14	0	0.1	0.21	1.87
R. crispus	0.06	0	0	0	0.15	0	0	0	0.01	0	0	0.21	0.18	0	0.08	0.14	1.76
E. vesicaria	0.02	0.05	0	0.02	0.02	0	0	0.19	0	0	0	0.01	0	0.01	0.07	0.07	1.71
C. draba	0.04	0	0	0	0.02	0.02	0	0	0	0.02	0	0.13	0.02	0	0.02	0.11	1.71
G. aparine	0.08	0	0	0.03	0	0.06	0	0	0	0	0	0.01	0.11	0	0	0.04	1.60
B. maritima	0.02	0	0.02	0	0	0	0	0	0	0.01	0	0.05	0.04	0.04	0	0.31	1.27
E. serrata	0.01	0	0	0	0.02	0	0	0	0	0	0	0.06	0	0	0.02	0.13	1.23
A. clavatus	0	0	0	0	0.05	0.02	0	0	0	0	0	0.01	0.01	0	0.04	0.20	1.23
Crepis sp.	0.12	0	0	0	0	0	0	0	0	0	0	0.03	0.03	0	0	0.05	1.20
S. irio	0.05	0	0	0	0	0	0	0	0	0.05	0	0.05	0	0	0.05	0.36	1.12
D. pentaphyllum	0.19	0	0	0	0.10	0	0	0	0	0	0	0	0.14	0	0	0.57	1.14
D. erucoides	0	0.07	0	0	0.02	0	0	0	0	0	0	0.05	0.02	0	0.45	0.05	1.11
M. sylvestris	0.01	0	0	0	0	0	0	0	0	0	0.01	0.02	0.02	0.01	0.02	0.23	1.08
M. sativa	0	0	0	0	0	0	0	0	0	0	0	0.08	0.08	0	0	0.17	1.03
Sonchus sp.	0	0	0	0	0	0.07	0	0	0	0	0	0.03	0	0	0	0.13	0.95
E. helioscopia	0	0.08	0	0	0	0	0	0	0	0	0	0	0.08	0	0	0	0.69
B. napus	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0.42	0.69
Plantago sp.	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0.05	0.69
R. lutea	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	1.33	0.49
A. fistulosus	0	0	0	0.27	0	0	0	0	0	0	0	0	0	0.03	0	0.03	0.60
P. rhoeas	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0.03	0	0.27	0.55
T. officinale	0	0	0	0	0	0	0	0	0	0	0	0.16	0	0	0	0.83	0.45
P. spinosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.06	0.56	0.30
M. arvensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.82	0
C. arvensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.11	0
C. bursa-pastoris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.17	0
Lamium sp.	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. segetum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0
D. virgata	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R. rugosum	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0

Plant species are ordered from higher to lower Shannon index.

Table 3. Total number of samples (#) and samples with presence of target families that include important natural enemies of aphids and thrips during the early and late flowering periods

	Early flowering period (weeks 11–15)									Late flowering period (weeks 17–21)								
		Parasitoid families				Predator families					Parasitoid families				Predator families			
Plant species	#	Brac	Aphel	Pter	Figit	Cocc	Chry	Anth	Aeol	#	Brac	Aphel	Pter	Figit	Cocc	Chry	Anth	Aeol
M. arvensis	8	.	.	.	.	.	.	.	2	4	.	.	.	.	.	.	.	4
E. vesicaria	16	1	.	.	.	.	1	4	1	6	.	.	.	.	.	.	.	1
M. sativa	2	.	.	.	.	1	.	.	.	2	.	.	.	.	.	.	.	1
C. arvensis	5	.	.	.	.	.	.	.	2	1	.	.	.	.	.	.	.	.
D. erucoides	13	.	.	.	.	1	.	3	1	1	.	.	.	.	.	.	.	1
Crepis sp.	6	.	.	.	.	1	.	.	1	5	2	.	.	.	.	.	.	.
C. draba	7	.	.	.	.	.	.	.	2	11	1	.	.	1	1	.	1	4
E. serrata	6	.	.	.	.	.	.	.	1	8	1	.	.	.	.	.	1	3
S. irio	5	1	.	.	1	.	.	.	1	2	.	.	.	.	.	.	1	2
E. helioscopia	2	.	.	.	.	1	.	.	.	1	.	.	.	.	.	.	.	.
B. napus	4	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
C. bursa-pastoris	4	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.
Lamium sp.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
T. officinale	1	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
A. clavatus	4	.	.	.	.	.	.	.	.	31	.	.	.	.	1	.	5	9
M. sylvestris	1	.	.	.	.	.	.	.	.	28	2	.	1	.	3	1	2	9
Sonchus sp.	4	.	.	.	.	.	.	.	.	6	.	.	.	.	.	.	.	2
A. fistulosus	5	.	.	.	.	.	.	.	1	6	.	.	.	.	.	1	.	.
C. segetum	1	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	2
Plantago sp.	3	.	.	.	.	1	.	.	.	4	.	.	.	.	.	.	.	1
D. virgata	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
B. maritima	.	.	.	.	.	.	.	.	.	16	1	2	.	1	1	2	.	7
G. aparine	.	.	.	.	.	.	.	.	.	12	3	.	.	.	3	.	.	2
P. rhoeas	.	.	.	.	.	.	.	.	.	13	1	.	.	.	.	1	.	6
R. crispus	.	.	.	.	.	.	.	.	.	12	3	.	.	.	4	.	1	4
C. pycnocephalus	.	.	.	.	.	.	.	.	.	7	.	.	.	.	2	.	1	3
R. lutea	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	2
R. rugosum	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.
D. pentaphyllum	.	.	.	.	.	.	.	.	.	7	2	.	.	.	1	.	.	2
P. spinosa	.	.	.	.	.	.	.	.	.	6	.	.	.	.	.	.	1	3

Parasitoids: Braconidae (Brac), Aphelinidae (Aphel), Pteromalidae (Pter) and Figitidae (Figit). Predators: Coccinellidae (Cocc), Chrysopidae (Chry), Anthocoridae (Anth) and Aeolothripidae (Aeol). For an easier table reading, zeros have been replaced by points. Plant species are ordered from early to late and from the longest to shortest flowering period.

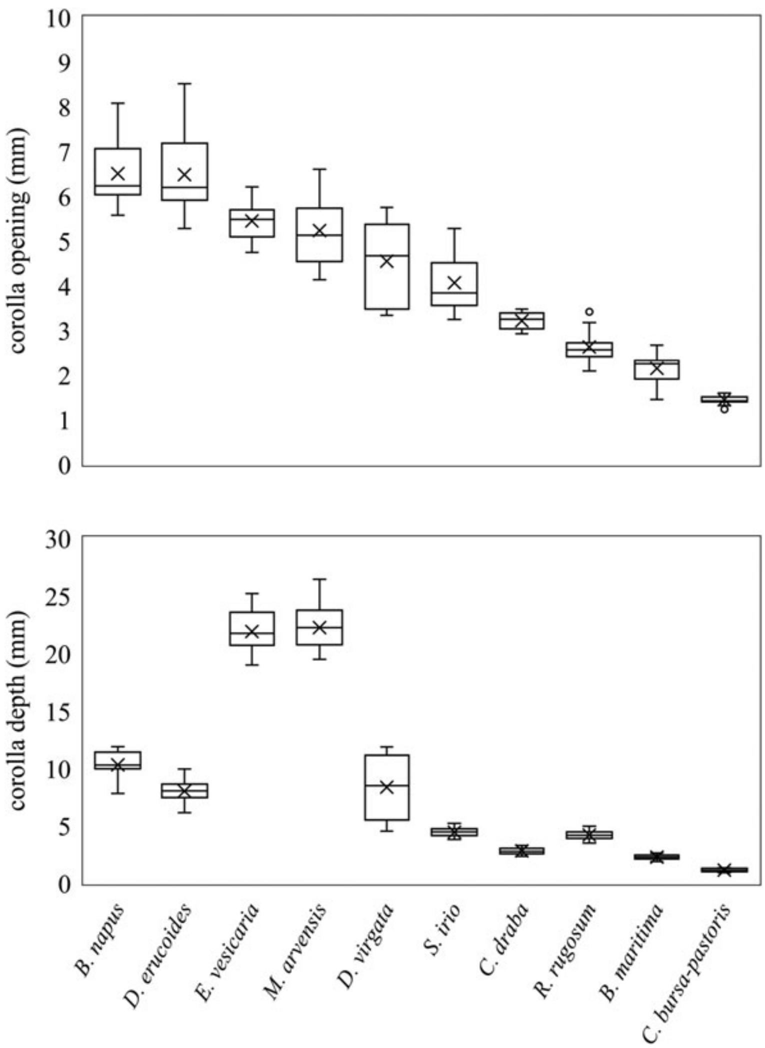

Figure 2. Box plot of flower corolla opening and depth measures of the ten plant species that have their nectaries partially protected. In the *X*-axis, plant species are ordered from widest to narrowest corolla opening.

the samples. Concerning the 41 individuals belonging to Anthocoridae, *Orius* spp. was the most abundant genus. A sample of 26 individuals was identified at the species level: 20 *O. majusculus* and six *Orius laevigatus* Fieber. Additionally, 33 ladybirds and six lacewings were recruited. During the samplings, aphids or phytophagous thrips were found in all the flowering plants with potential natural enemies, except in *Lamium* sp. For all plant species, the average values of aphids and thrips was highly variable depending on the sampling sites and dates. Pooling together all sampling sites and dates, *Medicago sativa* L. hosted the highest number of aphids (11.2 ± 10.3) and *Brassica napus* L. the highest number of thrips (10.2 ± 1.9).

Accessibility to nectar

No nectaries were observed in three out of the 36 plant species sampled (*P. rhoeas*, *Plantago* sp. and *R. crispus*), and four species presented extrafloral nectaries (*Dorycnium pentaphyllum* Scop., *M. sativa*, *Euphorbia helioscopia* L. and *E. serrata*). Unprotected floral nectaries were only recorded in *G. aparine*, whereas all the remaining plants had more or less protected nectaries. Additionally, nectar was observed on the outer surface of the flower as an exudate in *M. sylvestris*, *Asphodelus fistulosus* L. and *Lamium* sp., although nectaries were classified as partially protected. Similarly, nectar exudates were also present outside the florets of some Asteraceae with protected nectaries (*A. clavatus*, *Crepis* sp., *C. pycnocephalus*, *Taraxacum officinale* (L.) Wiggers and *Sonchus* sp. L.). For the Asteraceae species (*C. arvensis*, *C. segetum* and *P. spinosa*) and for the Resedaceae species (*Reseda lutea* L.), nectar exudate was not observed. In the other ten species belonging to Brassicaceae and Amaranthaceae, nectaries were protected or partially protected, and nectar was not observed on the surface of the flower, and the width and depth of their corolla were measured (fig. 2). The narrowest corolla opening was measured in *C. bursa-pastoris* (1.22–1.59 mm), whereas *B. napus* (5.56–8.07 mm) and *D. erucoides* (5.27–8.51) had the widest corolla opening. *C. bursa-pastoris* also had the shallowest corolla (with a mean of 1.11 mm), and *M. arvensis* and *E. vesicaria* presented the deepest (with means of 22.23 and 21.89 mm, respectively).

Table 4 depicts the values of head and thorax width for female and male parasitoids and predators, which in all cases were less than 1.22 mm (the narrowest corolla opening). For the three measured predators, the thorax was always significantly wider than the head. For the parasitoids, the thorax of the female was not significantly wider than the head. By contrast, the thorax of males was significantly wider than their head for *A. ervi*, *L. testaceipes* and *A. matricariae*.

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Table 4. Mean (±SE) and maximum (Max.) size (mm) of thorax and head width of selected insect species ($n = 10$)

		Thorax		Head		Statistical analysis	
Insect species	Sex	Mean	Max.	Mean	Max.	*t*	*P*
Aphelinus abdominalis	♀	0.55 ± 0.01	1.07	0.54 ± 0.00	0.78	−0.009	0.182
	♂	0.50 ± 0.01	0.93	0.49 ± 0.01	0.69	−0.023	0.255
Aphidius ervi	♀	0.57 ± 0.01	1.07	0.54 ± 0.01	0.78	−0.009	0.059
	♂	0.54 ± 0.01	0.93	0.50 ± 0.01	0.69	0.011	**<0.01**
Aphidius mali	♀	0.72 ± 0.02	1.07	0.69 ± 0.02	0.78	−0.02	0.117
	♂	0.64 ± 0.02	0.93	0.61 ± 0.02	0.69	−0.031	0.132
Aphidius matricariae	♀	0.42 ± 0.01	1.07	0.41 ± 0.01	0.78	−0.015	0.188
	♂	0.42 ± 0.01	0.93	0.38 ± 0.01	0.69	0.015	**<0.01**
Lysiphlebus testaceipes	♀	0.42 ± 0.02	1.07	0.42 ± 0.02	0.78	−0.048	0.427
	♂	0.49 ± 0.01	0.93	0.44 ± 0.01	0.69	0.018	**<0.01**
Aeolothrips intermedius	♀	0.41 ± 0.02	1.07	0.24 ± 0.01	0.78	0.129	**<0.001**
	♂	0.27 ± 0.00	0.93	0.17 ± 0.00	0.69	0.094	**<0.001**
Aphidoletes aphidimyza	♀	0.44 ± 0.02	1.07	0.33 ± 0.01	0.78	0.055	**<0.001**
	♂	0.39 ± 0.02	0.93	0.32 ± 0.01	0.69	0.019	**<0.01**
Orius majusculus	♀	1.00 ± 0.01	1.07	0.47 ± 0.00	0.78	0.509	**<0.001**
	♂	0.89 ± 0.01	0.93	0.43 ± 0.01	0.69	0.429	**<0.001**

Bold values indicate significant differences.

Discussion and conclusions

In our study, 36 plant species were found blooming during the sampling period, providing a continuous flowering period that might ensure food resources for natural enemies from early March to late May . Target pests in our study were aphids and thrips that start inflicting damage from early spring. Therefore, plants flowering in late winter and early spring are needed. An early establishment of wildflowers on crop margins will provide benefits to various groups of insects as a significant number of natural enemies disperse outside the refuge and colonize adjacent crops before and during the initial accumulation of the pest population (Corbett and Rosenheim, 1996). Many of the early flowering plants close to fruit orchards belonged to Brassicaceae and Asteraceae families, which was in agreement with data reported by Alins *et al.* (2019) from the same area. In fact, from the five species that were found in bloom at the beginning of the sampling, three were Brassicaceae (*M. arvensis*, *E. vesicaria* and *D. erucoides*) and one was Asteraceae (*C. arvensis*). These species bloom early when temperatures are still low and can keep on flowering up to the first summer months (Alins *et al.*, 2019). Species of Brassicaceae and Asteraceae have also been included in several seed mixtures used either in flower margins or ground covers in orchards (e.g., Pfiffner *et al.*, 2019).

Only five plant species had Shannon's diversity index values between 1.5 and 3.5, which comprise the common values of this index (Magurran, 2004), and another ten had values slightly above or equal to 1. Therefore, diversity of target natural enemies, collected during the samplings of the flowering plants can be considered in general low. Values were probably influenced either by the sampling period (March–May) when temperatures are still low in the area, a condition that reduces insect activity, and by the method used (beating), which only allows the evaluation of the insects present at a given time. It can be assumed that greater diversity of natural enemies in naturally occurring plants close to the crop may play a crucial role in maintaining ecosystem services and would lead to better pest control (Bàrberi *et al.*, 2010; Balzan *et al.*, 2014). Therefore, these 15 plants with Shannon indexes higher than 1 can become functional allies to attract beneficial species to the orchards.

Records of natural enemies on plant species can be used as a proxy for plant attraction (Thomson *et al.*, 2007) and enables comparisons among them to select candidates to congregate and provide resources to the natural enemies of interest. Target natural enemies that can be useful to control aphids and thrips were found in a large number of the sampled plant species, which could indicate their potential to contribute to the establishment of these natural enemies in fruit orchards. Regarding parasitoids, Braconidae was the earliest in the season and the most widely distributed (found on more plant species and more samples), with *A. matricariae* being the most abundant. This is a positive result since this species is by far the main parasitoid species attacking *M. persicae* and *D. plantaginea* in the surveyed area (Aparicio *et al.*, 2019; Rodríguez-Gasol *et al.*, 2019). Other aphid parasitoids mentioned in these two studies (*A. colemani* and *A. ervi*) were also found during the present samplings visiting flowers at the border of orchards. Finally, *B. angelicae* has also been reported to parasitize *D. plantaginea* and *M. persicae* (Kavallieratos *et al.*, 2004; Dassonville *et al.*, 2013). By contrast, individuals from the Aphelinidae family were detected only in two samples of *B. maritima*. It is worth noting that *A. mali*, the main parasitoid of *E. lanigerum* in the area sampled (Lordan *et al.*, 2014; Rodríguez-Gasol *et al.*, 2019), belongs to this family.

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Table 5. Summary of criteria used to select flowering species from those present in sampled area

Plant species	Shannon index[a]	Flowering earliness[b]	Blooming span[c]	Target parasitoids[d]	Target predators[e]	# sample sites[f]
E. vesicaria	++	Early	5	+	+	22
C. draba	++	Early	4	+	+	18
M. sylvestris	+	Early	4	+	+	29
E. serrata	+	Early	4	+	+	14
A. clavatus	+	Early	4	0	+	35
D. erucoides	+	Early	4	0	+	14
B. maritima	+	Late	3	+	+	16
R. crispus	++	Late	3	+	+	12
G. aparine	++	Late	3	+	+	12
C. pycnocephalus	++	Late	3	0	+	7
Crepis sp.	+	Early	4	+	+	11
S. irio	+	Early	4	+	+	7
Sonchus sp.	+	Early	4	0	+	10
M. sativa	+	Early	3	0	+	4
D. pentaphyllum	+	Late	2	+	+	7

(a) Only flowering plants with Shannon index higher or equal to 1 are listed. Two categories of the index were defined: $H \geq 1.5$ (++), $1.5 > H \geq 1$(+). (b) Flowering earliness refers to the period when blooming started: early (weeks 11–15) and late (weeks 17–21). (c) Blooming span stands for the number of sampling weeks when the plant was found in bloom. (d & e) The presence of target parasitoids belonging to Braconidae and Aphelinidae families and predators are identified with +. (f) # sample sites indicate the total number of sites across the whole sampling where the plant was recorded in bloom.

Predatory, Aeolothripidae were recruited from more plant species and a higher number of samples. The high abundance of Aeolothripidae may be biased by the sampling method used since predatory thrips spend most of their life cycle in flowers, feeding on prey and pollen (Bournier *et al*., 1978). Pizzol *et al*. (2017) reported the presence of several species of *Aeolothrips* in many naturally occurring plants, including many of the ones sampled in the current study. Other predators reported in our survey (i.e., Coccinellidae, Chrysopidae and Anthocoridae) were by far much less abundant and widespread but also present in the early flowering period. They are frequent visitors of flowers when searching for pollen and nectar to complement their diets, especially when prey is scarce (Wäckers, 2005).

The criteria considered to select appropriate plant species to enhance target natural enemies are summarized in table 5. Four plant species arose as the most promising candidates (i.e., *E. vesicaria*, *C. draba*, *E. serrata* and *M. sylvestris*). They had a high diversity index, and their blooming started early in the season and lasted for several sampling weeks. Furthermore, they attracted the target natural enemies of aphids and thrips and were widely distributed. Additionally, *A. clavatus* and *D. erucoides* demonstrated similar characteristics although parasitoids were not recruited from them. Out of these species, three of them belonged to Brassicaceae. Numerous studies demonstrate the benefits of the Brassicaceae for natural enemies (Araj *et al*., 2019; Badenes-Pérez, 2019). Their nectar favored the longevity and fertility of parasitoids, such as *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae), and *Cotesia marginiventris* Cresson and *Diaeretiella rapae* Mcintosh (Hymenoptera: Braconidae) (Idris and Grafius, 1997; Johanowicz and Mitchell, 2000; Araj and Wratten, 2015).

According to our results, the six selected plant species (*E. vesicaria*, *C. draba*, *M. sylvestris*, *E. serrata*, *A. clavatus* and *D. erucoides*) have nectar available to natural enemies. Comparison of the measures of flowers on the first three mentioned species (Brassicaceae) with measures of insects proved that their floral architecture should not be an impediment for tested target natural enemies to access nectar. For *E. serrata*, Papp (2004) already mentioned the presence of extrafloral nectaries, and an open corolla was reported by Comba *et al*. (1999) for *M. sylvestris*. Finally, in the current study, nectar exudates were observed outside the florets for *A. clavatus*.

Measurements of the flower and the width of insect heads and thorax have been used on numerous occasions to evaluate the accessibility of flower nectar to insects (e.g., Patt *et al*., 1997; Nave *et al*., 2016; Villa *et al*., 2017). However, all sampled nectar-producing plants during the study had nectar easily available for all tested natural enemies, suggesting that comparison of measures of insects and flowers would not be a useful criterion for the selection of plants able to promote natural enemy populations. Additionally, for some insects, neither the thorax nor the head would be valid measures to evaluate the capability of an insect to penetrate the flower. Adults of the predator *A. aphidimyza* cannot access the nectaries at the bottom of the open flowers of *L. maritima* not due to their head or thorax width but due to their wide leg span (Aparicio *et al*., 2018). Winkler *et al*. (2009) also stated that the ability to feed does not only depend on floral architecture and insect size, but also on other factors, such as searching behavior. Furthermore, the availability of nectar does not guarantee that the insects feed on nectar. Other factors, such as the morphology of insect mouthparts, gustatory response to these sugar and capacity to digest and metabolize them, could affect the exploitation of nectar (Wäckers, 2004, 2005).

In conclusion, 36 plant species were found blooming during the sampling period (from early March to late May), which provided an array of flowers that attracted several families of natural

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enemies and which might ensure food resources for them. Among them, six species arose as candidates to enhance a complex of predators and parasitoids targeting aphids and thrips: *E. vesicaria*, *C. draba*, *M. sylvestris*, *E. serrata*, *A. clavatus* and *D. erucoides*. It is worth to note that, according to our results, these six species are not important refugee of aphids and thrips, and to our knowledge, or of other key pests in orchards such as Tortricidae. This selection does not exclude other potential candidates being included in ecological infrastructure for specific needs. For example, *B. maritima* could be of special interest in apple orchards since it was the only species recruited from Aphelinidae. Little is reported in the literature regarding the effects of such plant species on the biology of natural enemies. *D. erucoides* increases the longevity and parasitism rate of *A. colemani* on *M. persicae* (Jado *et al.*, 2018), and it also increases the longevity, egg load, fecundity and the parasitism rate of *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) on *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and of *D. rapae* on *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) (Araj and Wratten, 2015; Araj *et al.*, 2019). *M. sylvestris* increases the survival of females of *Elasmus flabellatus* Fonscolombe (Hymenoptera: Eulophidae), a major parasitoid of *Prays oleae* Bernard (Lepidoptera: Praydidae), compared to other candidate flowers (Villa *et al.*, 2017), and of *Episyrphus balteatus* De Geer (Diptera: Syrphidae) (Pinheiro *et al.*, 2013), an important aphid predator widely present in apple and peach orchards in the studied area (Rodríguez-Gasol *et al.*, 2019). Therefore, further studies are needed to determine the benefits of such flower rewards on several fitness parameters before verifying their contribution to the biological control of aphids and thrips in fruit orchards.

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