Effect of flower traits and hosts on the abundance of parasitoids in perennial multiple species wildflower strips sown within oilseed rape (*Brassica napus*) crops

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ABSTRACT

Reducing the use of insecticides is an important issue for agriculture today. Sowing wildflower strips along field margins or within crops represents a promising tool to support natural enemy populations in agricultural landscapes and, thus, enhance conservation biological control. However, it is important to sow appropriate flower species that attract natural enemies efficiently. The presence of prey and hosts may also guide natural enemies to wildflower strips, potentially preventing them from migrating into adjacent crops. Here, we assessed how seven flower traits, along with the abundance of pollen beetles (*Meligethes* spp., Coleoptera: Nitidulidae) and true weevils (*Ceutorhynchus* spp., Coleoptera: Curculionidae), affect the density of parasitoids of these two coleopterans in wildflower strips sown in an oilseed rape field in Gembloux (Belgium). Only flower traits, not host (i.e. pollen beetles and true weevils) abundance, significantly affected the density of parasitoids. Flower colour, ultra-violet reflectance and nectar availability were the main drivers affecting parasitoids. These results demonstrate how parasitoids of oilseed rape pests react to flower cues under field conditions. Similar analyses on the pests and natural enemies of other crops are expected to help to develop perennial flower mixtures able to enhance biological control throughout a rotation system.

INTRODUCTION

Conserving natural enemies in farming landscapes is a primary challenge for increasing the sustainability of agriculture. Indeed, natural enemies may help to biologically control pests and, thus, reduce the use of insecticides, which irrational uses have led to environmental degradation (Krebs et al. 1999; Gibbons et al. 2015) and may be harmful to human health (Baldi et al. 2013). Conservation biological control is defined as "the manipulation of the environment (i.e., the habitat) of natural enemies so as to enhance their survival, and/or physiological and behavioral performance, and resulting in enhanced effectiveness [in terms of predation and parasitism]" (Barbosa 1998). These habitats include woodlots, hedgerows, and wildflower or grassy strips introduced to fields, farms and landscapes. Because these habitats are less disturbed compared to annual crop fields, they provide a range of resources for natural enemies, including food, alternative prey or hosts, shelters and overwintering sites (Landis et al. 2000).

There is clear evidence supporting that agricultural practices (i.e. mowing, pesticide use, harvesting) negatively affect natural enemy populations in fields (Colignon et al. 2001; Horton et al. 2003; Hanson et al. 2015) and, thus, the potential for biological control (Geiger et al. 2010). In most cases, wildflower strips (WFS) sown at field margins support a higher abundance and diversity of insects compared to adjacent fields (Haaland et al. 2011). Some of these insects are natural enemies of pests that are able to migrate to adjacent crops to control them, which reduces damage and potentially enhances yield and crop quality (Büchi 2002; Balzan and Moonen 2014; Tschumi et al. 2016). However, these beneficial effects may not always occur (Uyttenbroeck et al. 2016). One reason is the potential incompatibility between the natural enemies and the floral resource provided (Lundgren 2009; Tscharntke et al. 2016). As simply sowing flowers may not necessarily support the targeted natural enemies, it is

important to improve our understanding about how flowers affect their behaviour to enhance the efficiency of WFS to support these species.

Flowers may be described through their traits, which are defined as "any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organisation" (Violle et al. 2007). Once a plant is considered in the context of the environment in which it grows, its traits may affect ecological processes. Consequently, these traits are termed as *functional* (Díaz and Cabido 2001; Lavorel and Garnier 2002), even though debate remains on the use of this term (Violle et al. 2007). Díaz and Cabido (2001) showed that the range and values of functional traits carried by plants are strong drivers of ecological processes. Furthermore, different insect taxa may respond differently to a specific flower trait. For example, Campbell et al. (2012) showed that for the flower trait 'corolla length', long corolla flowers were visited by bumblebees *Bombus* spp. Latreille (Hymenoptera: Apidae), while flowers with short corollas were visited by hoverflies (Diptera: Syrphidae) and hymenopteran parasitoids. Many studies have explored the effect of one or two traits on insect behaviour in relatively controlled experiments (Patt et al. 1997; Vattala et al. 2006; Döring et al. 2012; Cook et al. 2013; Van Rijn and Wäckers 2016), or through modelling (Bianchi and Wäckers 2008). In field conditions, the effect of multiple flower traits was tested by developing small monospecific plots (Fiedler and Landis 2007a; Sivinski et al. 2011). However, no studies investigated how traits of flowers incorporated in multiple species mixtures affect natural enemies whereas such mixtures are bought and sown by farmers.

The attractiveness of WFS for natural enemies may also arise from the presence of prey and host species. They may help support natural enemy populations at field margins (Landis et al. 2000), potentially retaining them there if they are more abundant than in the adjacent field (Rand et al. 2006). Thus, it is important to assess whether the presence of prey and hosts are

significant drivers of natural enemy behaviour in WFS, or whether flower traits alone are important.

Oilseed rape (OSR) *Brassica napus* is an important crop in Western Europe. Pollen beetles *Meligethes* spp. (Coleoptera: Nitidulidae) and true weevils *Ceutorhynchus* spp. (Coleoptera: Curculionidae) are its main pests in spring (Williams 2010). Their natural enemies are mainly parasitoids – some being more specialists than others –, parasitising pest larvae (Nilsson 2003; Ulber 2003; Williams 2003). With levels of parasitism which can exceed 50% (Ulber et al. 2010), finding ways to support their presence at OSR field margins may allow enhancing biological control. In the present study, we analysed how WFS sown within OSR crops affected the parasitoids of these two coleopteran pests by answering the following questions: (i) what factors affect parasitoid abundance in WFS (i.e. flower traits or hosts); and (ii) which flower traits are drivers? The results are expected to provide information on potential perennial flower mixtures that would enhance biological pest control in crop rotation systems.

MATERIALS AND METHODS

Field set up

This study was conducted at the experimental farm of Gembloux Agro-Bio Tech (University of Liège), Namur Province, Belgium (50° 34′ 03″ N; 4° 42′ 27″ E). In a field of about 9 ha, five replicated WFS (125 m × 8 m) were sown, separated by 27 m (Fig. 1). Each WFS was divided into five equally sized plots (25 m × 8 m). In each plot, a different flower mixture was sown (mixtures A to E, Table 1). The layout resulted in a Latin square design with 25 plots. However, in the present study, only three strips (thus totally 15 plots, named 1 to 15, Fig. 1) were used. Four out of the five mixtures (i.e., mixtures A to D) sown in each strip were composed of seven flower species and three grass species (*Festuca rubra*, *Agrostis capillaris*

and *Poa pratensis*), while the remaining mixture (i.e., mixture E) only contained the three grass species (Table 1). The five mixtures were originally chosen regarding their value of functional diversity using the Rao quadratic entropy index described by Botta-Dukát (2005) (mixture A had the highest value while mixture E the lowest one, see Uyttenbroeck et al., 2015 for more details). However the present study focuses on how flowers affect insect behaviour through their traits rather than through mixture functional diversity. All flowering plants were native perennial species commonly found in Belgian grasslands (benefits of such species, compared to exotic and/or annual ones were reviewed by Fiedler and Landis 2007b), used in Agri-Environmental Schemes (AES) in Wallonia, Belgium, and available on the market (seeds were obtained from ECOSEM, Belgium). Each species was described based on seven traits (Table S1). Visual traits were (i) flower colour (i.e. yellow, white, violet), (ii) the ultra-violet (UV) reflectance of the peripheral part of the flower (numerical value indicated as 'UV periphery') and (iii) whether the UV reflectance of the internal flower part differed to that of the external flower part (i.e. yes or no, indicated as 'UV pattern'). Phenological traits were (iv) the month of the onset of blooming (i.e. numerical value from 1 to 12 with '1' being January) and (v) the number of blooming months (numerical value). (vi) Height (numerical value) was chosen based on its effect on insect flight (Wratten et al. 2003) and (vii) flower class was delineated after Müller (1881) (indicated as 'Flower type') because it notably gives the availability of nectar for insects that visit flowers (i.e. bee flowers, hymenoptera flowers, flowers with open nectaries, flowers with partly hidden nectar, flowers with totally hidden nectar, flower associations with totally hidden nectar). For each plant species, the values on the phenological traits and plant height were obtained from Lambinon et al. (2008), while those on the visual and nectar availability traits were retrieved from the TRY database (Kattge et al. 2011). The flower mixtures were sown on 6 June 2013 and the OSR was sown on 10

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September 2013. During the experimental period, the WFS were mown twice a year, at the end of June and September.

Table 1 Composition of the flower mixtures sown, record of the ones that bloomed in May and June and mean cover (% ± standard error) of each species through the different plots. Among those that bloomed, *A. cynapium, C. bursa-pastoris, M. recutita, S. alba* and *T. repens* were not sown.

Family	Species	Mixtures					Blooming		Cover (%)
		A	B	C	D	E	May	June	Mean (±SE)
	Flowering species								
Apiaceae	*Aethusa cynapium*						x	x	0.02 (± 0.09)
Apiaceae	*Anthriscus sylvestris*	x		x	x				
Apiaceae	*Heracleum sphondylium*	x							
Asteraceae	*Achillea millefolium*	x	x	x	x		x	x	6.56 (± 5.36)
Asteraceae	*Crepis biennis*		x				x	x	0.78 (± 1.74)
Asteraceae	*Hypochaeris radicata*		x				x	x	3.02 (± 6.33)
Asteraceae	*Leontodon hispidus*		x	x			x	x	0.18 (± 0.49)
Asteraceae	*Leucanthemum vulgare*	x		x			x	x	18.09 (± 25.42)
Asteraceae	*Matricaria recutita*						x	x	0.49 (± 0.79)
Brassicaceae	*Capsella bursa-pastoris*						x		0.02 (± 0.09)
Brassicaceae	*Sinapis alba*						x	x	0.51 (± 1.33)
Dipsacaceae	*Knautia arvensis*	x	x				x	x	0.07 (± 0.14)
Fabaceae	*Lotus corniculatus*				x		x	x	2.35 (± 8.57)
Fabaceae	*Medicago lupulina*				x		x		0.31 (± 0.69)
Fabaceae	*Trifolium pratense*	x							
Fabaceae	*Trifolium repens*						x	x	0.02 (± 0.09)
Geraniaceae	*Geranium pyrenaicum*			x			x	x	0.40 (± 1.20)
Lamiaceae	*Origanum vulgare*			x					
Lamiaceae	*Prunella vulgaris*			x	x				
Lythraceae	*Lythrum salicaria*		x		x				
Malvaceae	*Malva moschata*				x			x	0.64 (± 1.24)
Rubiaceae	*Galium verum*	x	x					x	0.91 (± 1.22)
	Grass species								
Poaceae	*Agrostis capillaris*	x	x	x	x	x			
Poaceae	*Festuca rubra*	x	x	x	x	x			
Poaceae	*Poa pratensis*	x	x	x	x	x			

Flower species monitoring

Flower species and their relative cover were monitored on 17 and 18 June 2014. In brief, three 1 m² permanent quadrats were laid at a distance of 6 m from one another in each plot (Fig. 1). Every flower species present in the quadrats was listed and its cover assessed. Plant nomenclature of Lambinon et al. (2004) was followed. Every month, the species that bloomed were recorded by visual observations following a 20 m × 2 m transect in each plot. To address

the effect of flower traits on parasitoids fully, only the flower species that were blooming

when wasp abundance peaked were used for the analyses.

Fig. 1 Field experimental design. The composition of the wildflower mixtures originally sown (A to E) is detailed in Table 1. Each plot (1 to 15) was then considered independent in the statistical analyses.
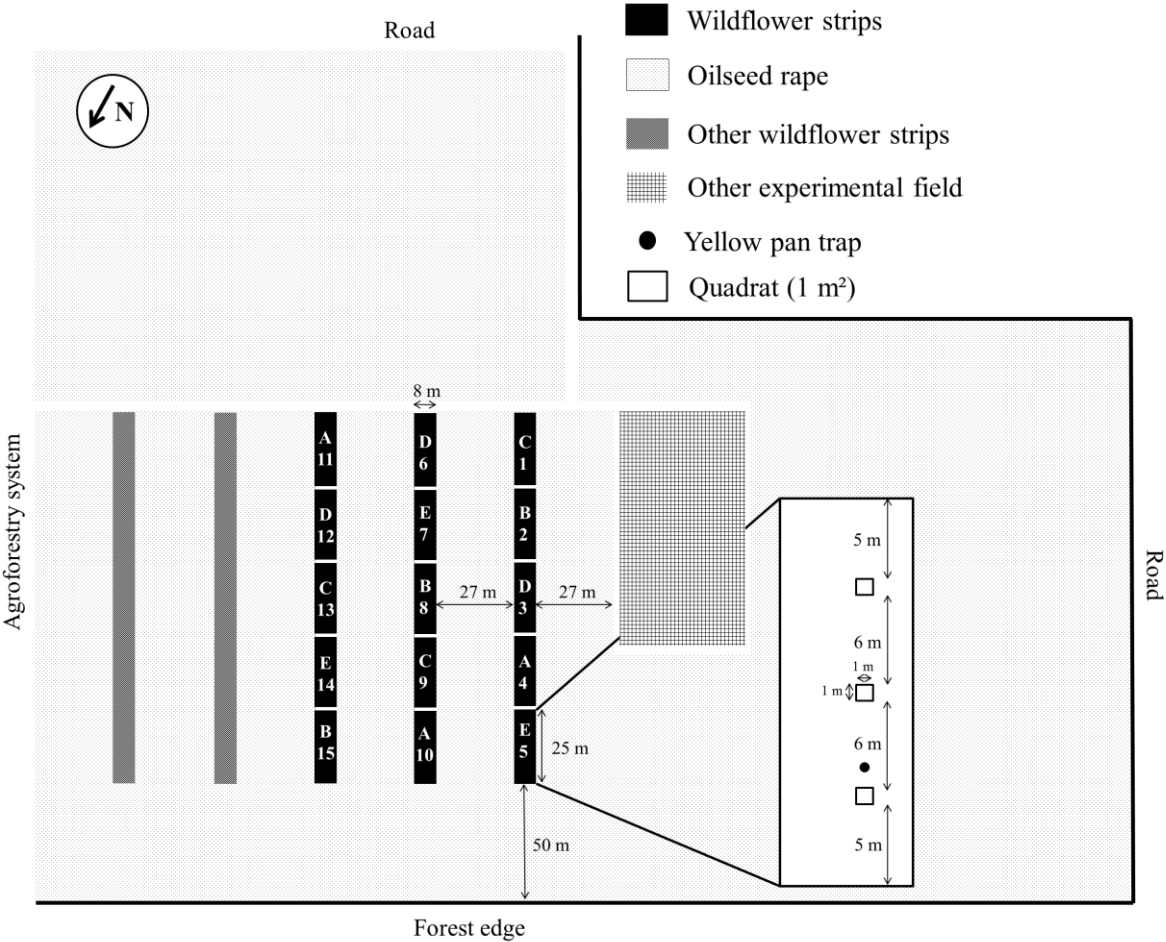
Insect species monitoring

OSR pests and their associated parasitoids were monitored for 11 weeks from 2 April to 25

June 2014. In brief, a yellow pan trap (Flora®, 27 cm diameter and 10 cm depth) was installed

on a fibreglass stick in each plot. Traps were positioned at vegetation height, and were filled

with water containing a few drops of detergent (dish-washing liquid) to reduce the surface

tension of the water. Their position was adjusted during the growing season to follow plant

growth. The traps were emptied and refilled every seven days during the survey period, and the trapped insects were conserved in 70% ethanol. Pollen beetles and true weevils were identified to the genus level following Kirk-Spriggs (1996) and Morris (2008), respectively. Parasitoids were identified to the species level following Ferguson et al. (2010).

Statistical analysis

For the data analysis (performed with R Core Team 2013), each plot (i.e., plots 1 to 15) was considered unique. We assumed that the sown replicated mixtures (i.e., mixtures A to E) could have developed to form different vegetation compositions. Therefore, 15 flower plots, thus 15 flower mixtures, were considered. For each plot, the average cover of each species found (i.e., both sown and spontaneous ones) was calculated from the three quadrats (the average cover of each blooming flowering species in each plot is given in Table S2). Then, for each plot, the Community Weight Mean (CWM, Lavorel et al. 2008; Laliberté and Legendre 2010) value was calculated for each trait based on the trait values obtained for each flowering species and their average cover in the plot (R function 'dbFD', package 'FD', Laliberté et al. 2014). For numerical trait values, a single CWM value was obtained per trait for each plot. For class trait values, a single value for each class was obtained per plot. Both CWM values and the abundance of *Meligethes* spp. and *Ceutorhynchus* spp. were used as explanatory variables (details about CWM values in each plot are given in Table S3).

The interactions between parasitoids and explanatory variables were analysed by considering only data when the populations of parasitoids reached their abundance peak in order to limit the random dilution effect of parasitoids in the different plots. Indeed, it was hypothesised that the effect of explanatory variables would be clearer on when parasitoid population was the highest. To do so, a forward selection of the significant explanatory variables was firstly performed, and secondly a redundancy analysis (RDA) was used. This method combines

multivariate multiple linear regression and principal component analysis (Borcard et al. 2011). The matrix of the CWM values and host abundance was the 'matrix of explanatory variables', while the 'matrix of centred response' was the $\log_{10}(x+1)$ transformation of parasitoid abundance in each plot (respectively the matrices X and Y in Borcard et al. 2011). Through the forward selection process (function 'ordistep', package 'vegan', Oksanen et al. 2015), the significant variables ($P < 0.05$) affecting parasitoid abundance were identified and those with the lowest Akaike Information Criterion (AIC) at each step were selected for inclusion in the RDA model. Based on the selected model, the constrained ordination between parasitoid abundance and the selected variables was obtained using Bray-Curtis distances (function 'capscale', package 'vegan', Oksanen et al. 2015) and tested with a permutation test (n = 1000, P = 0.05). Finally, correlation circles of significant explanatory variables were plotted to visualise how they were correlated with the parasitoid species.

Fig. 2 Mean number (± SE) per trap in WFS and OSR of (a) *Meligethes* spp. and their parasitoids and (b) *Ceutorhynchus* spp. and their parasitoids along the trapping period. For *Meligethes* spp. and their parasitoids, the three last trapping weeks (from 11 to 25 June) are not included in the graph to facilitate the reading. Indeed, in the end of June, abundance of *Meligethes* spp. increased to reach, on 18 June, 1902 and 2444 individuals on average per trap in WFS and OSR, respectively, while abundance of parasitoids decreased. However at that time, crops were not sensitive to *Meligethes* spp. anymore. Details of abundance means for all trapping dates are given in Table S4.
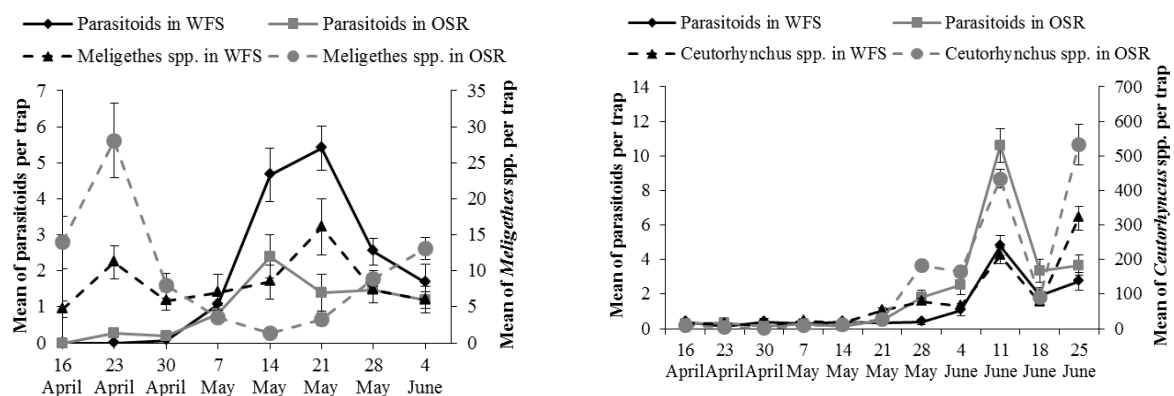
Table 2 Diversity and abundance of parasitoid species trapped during the whole season and at respective peaks (i.e. *Meligethes* spp. parasitoids: 14 and 21 May; *Ceutorhynchus* spp. parasitoids: 11 June). The proportion (%) of each species among those having the same host is given.

Host	Family, Species	Abundance (total)	%	Abundance (at peak)	%
Meligethes spp.	**Braconidae**				
	Blacus nigricornis Haeselbarth	230	90.5	136	90.1
	Diosphilus capito (Nees)	1	0.4	0	0.0
	Ichneumonidae				
	Phradis interstitialis (Thomson)	14	5.5	10	6.6
	Tersilochus heterocerus Thomson	6	2.4	4	2.6
	Proctotrupidae				
	Brachyserphus parvulus (Nees)	3	1.2	1	0.7
	Total	254		151	
Ceutorhynchus spp.	**Ichneumonidae**				
	Tersilochus fulvipes (Gravenhorst)	1	0.5	0	0.0
	Tersilochus obscurator Aubert	1	0.5	0	0.0
	Pteromalidae				
	Mesopolobus morys (Walker)	50	25.9	15	20.8
	Stenomalina gracilis (Walker)	51	26.4	15	20.8
	Trichomalus lucidus (Walker)	5	2.6	4	5.6
	Trichomalus perfectus (Walker)	85	44.1	38	52.8
	Total	193		72	

RESULTS

Flowers

Fourteen flowering species bloomed during insect peaks, which occurred in May and June. *Leucanthemum vulgare* and *Achillea millefolium* were the most abundant species found in the different plots. *Capsella bursa-pastoris* and *Medicago lupulina* only bloomed in May, while *Galium verum* and *Malva moschata* only flowered in June. *Aethusa cynapium*, *C. bursa-pastoris*, *Matricaria recutita*, *Sinapis alba* and *Trifolium repens* were not sown, but grew spontaneously in the quadrats (Table 1, see also Table S2 for details of each plot).

Table 3 Permutation test (n=1000) of the forward selection of the explanatory variables affecting *Meligethes* spp. parasitoids in May, and *Ceutorhynchus* spp. parasitoids in June. When the value of a trait is a class (i.e Colour, Flower type, UV pattern), each class is considered as an explanatory variable. When it is numerical (i.e. Blooming duration, Blooming start, Height, UV periphery), each trait is an explanatory variable. For the first two steps, the Akaike Information Criterion (AIC), F-value and P-value (*: P< 0.05; **: P < 0.01) are given.

| Explanatory variables		Step 1			Step 2		
Trait	Value	AIC	F	P(>F)	AIC	F	P(>F)
***Meligethes* spp. parasitoids**							
Flower type	Totally hidden nectar	8.512	1.76	0.010 **			
Colour	Yellow	8.025	2.24	0.020 *	8.076	2.12	0.060
Colour	Violet	8.527	1.74	0.045 *	9.690	0.68	0.745
Colour	White	8.417	1.85	0.060	8.078	2.11	0.030 *
UV Pattern	No	8.608	1.66	0.085	8.084	2.11	0.070
Flower type	Open nectaries	8.631	1.64	0.090	8.696	1.54	0.160
UV Pattern	Yes	8.608	1.66	0.115	8.084	2.11	0.040 *
Meligethes spp.	Numerical	8.730	1.55	0.150	8.521	1.70	0.095
UV Periphery	Numerical	8.909	1.37	0.210	7.914	2.27	0.025 *
Flower type	Associations with totally hidden nectar	9.259	1.04	0.400	9.043	1.23	0.295
Blooming duration	Numerical	9.427	0.88	0.505	9.447	0.88	0.530
Height	Numerical	9.507	0.81	0.595	9.386	0.94	0.460
Blooming start	Numerical	9.548	0.77	0.605	9.544	0.80	0.565
Flower type	Bee flowers	9.585	0.74	0.735	9.530	0.81	0.690
Flower type	Hymenoptera flowers	9.622	0.70	0.740	9.485	0.85	0.635
Flower type	Partly hidden nectar	9.622	0.71	0.775	9.688	0.68	0.735
***Ceutorhynchus* spp. parasitoids**							
Flower type	Totally hidden nectar	29.502	3.32	0.010 **			
Colour	Violet	29.555	3.26	0.020 *	30.506	0.82	0.550
Blooming duration	Numerical	31.194	1.58	0.130	29.647	1.58	0.165
Flower type	Associations with totally hidden nectar	31.604	1.19	0.290	30.740	0.63	0.795
Ceutorhynchus spp.	Numerical	31.774	1.03	0.360	30.074	1.20	0.245
UV Periphery	Numerical	31.806	1.00	0.400	30.341	0.97	0.405
Colour	Yellow	31.861	0.95	0.510	30.809	0.57	0.835
UV Pattern	No	32.092	0.73	0.625	30.320	0.98	0.395
Flower type	Bee flowers	32.055	0.77	0.640	30.424	0.89	0.505
UV Pattern	Yes	32.092	0.73	0.700	30.320	0.98	0.405
Height	Numerical	32.195	0.64	0.720	30.596	0.75	0.625
Blooming start	Numerical	32.205	0.63	0.755	30.544	0.79	0.630
Flower type	Open nectaries	32.304	0.54	0.835	30.647	0.70	0.660
Colour	White	32.433	0.42	0.950	30.804	0.57	0.845

Parasitoids and hosts

Five parasitoid species of *Meligethes* spp. and six parasitoid species of *Ceutorhynchus* spp. were trapped, representing 254 and 193 individuals, respectively. The diversity and relative abundance of the parasitoid species are presented in Table 2. In WFS, population peaks of *Meligethes* spp. and their parasitoids in WFS occurred simultaneously on 14 and 21 May (Fig. 2a). The populations of *Ceutorhynchus* spp. and their parasitoids peaked on 11 June (Fig. 2b). On these dates, four parasitoid species of both *Meligethes* spp. and *Ceutorhychus* spp. were identified in WFS (Table 2). For each host, a second peak occurred at end of June. Despite parasitoids and their hosts occurred simultaneously in WFS, neither the abundance of *Meligethes* spp. nor *Ceutorhynchus* spp. affected the abundance of their related parasitoid species (Table 3, see also Fig. S1 for the abundance of hosts and parasitoids in each plot at their peaks).

Parasitoid–flower trait interactions

Instead, flower traits were the main drivers of parasitoid density in WFS. Indeed, flower colour, UV reflectance (i.e. both 'UV Pattern' and 'UV Periphery') and the availability of nectar (i.e. 'Flower type') were the traits that significantly affected parasitic wasps (Table 3). *Meligethes* spp. parasitoids *Blacus nigricornis* Haeselbarth (Hymenoptera: Braconidae), *Brachyserphus parvulus* (Nees) (Hymenoptera: Proctotrupidae) and *Phradis interstitialis* (Thomson) (Hymenoptera: Ichneumonidae) were more abundant in mixtures dominated by yellow flowers, rather than mixtures dominated by white and violet flowers. Flowers with high UV reflectance for both internal and external flower parts were also more attractive to these parasitoids. Moreover, parasitoids were less abundant in mixtures containing flowers with totally hidden nectar (Figs 3a-b, Table 3). *Tersilochus heterocerus* Thomson (Hymenoptera: Ichneumonidae) generally responded to the opposite flower cues compared to

the other species (Fig. 3a). *Ceutorhynchus* spp. parasitoids *Mesopolobus morys* (Walker) (Hymenoptera: Pteromalidae), *Trichomalus lucidus* (Walker) (Hymenoptera: Pteromalidae) and *Trichomalus perfectus* (Walker) (Hymenoptera: Pteromalidae), but not *Stenomalina gracilis* (Walker) (Hymenoptera: Pteromalidae), were significantly less abundant in mixtures containing violet flowers and flowers with totally hidden nectar (Figs 3c-d, Table 3).

Fig. 3 Factorial map of the redundancy analysis (RDA) carried out on (a) *Meligethes* spp. parasitoids and (c) *Ceutorhynchus* spp. parasitoids. The empty triangles represent the flower mixtures. The variance explained by each axis is given, as well as the effect of the selected factors (i.e. those with the lowest AIC – see Table 3) on them (Permutation test: n=1000; *P < 0.05; ***P < 0.001). (b) and (d) are the respective correlation circles of the significant factors affecting the parasitoids (the significant factors are detailed in Table 3).
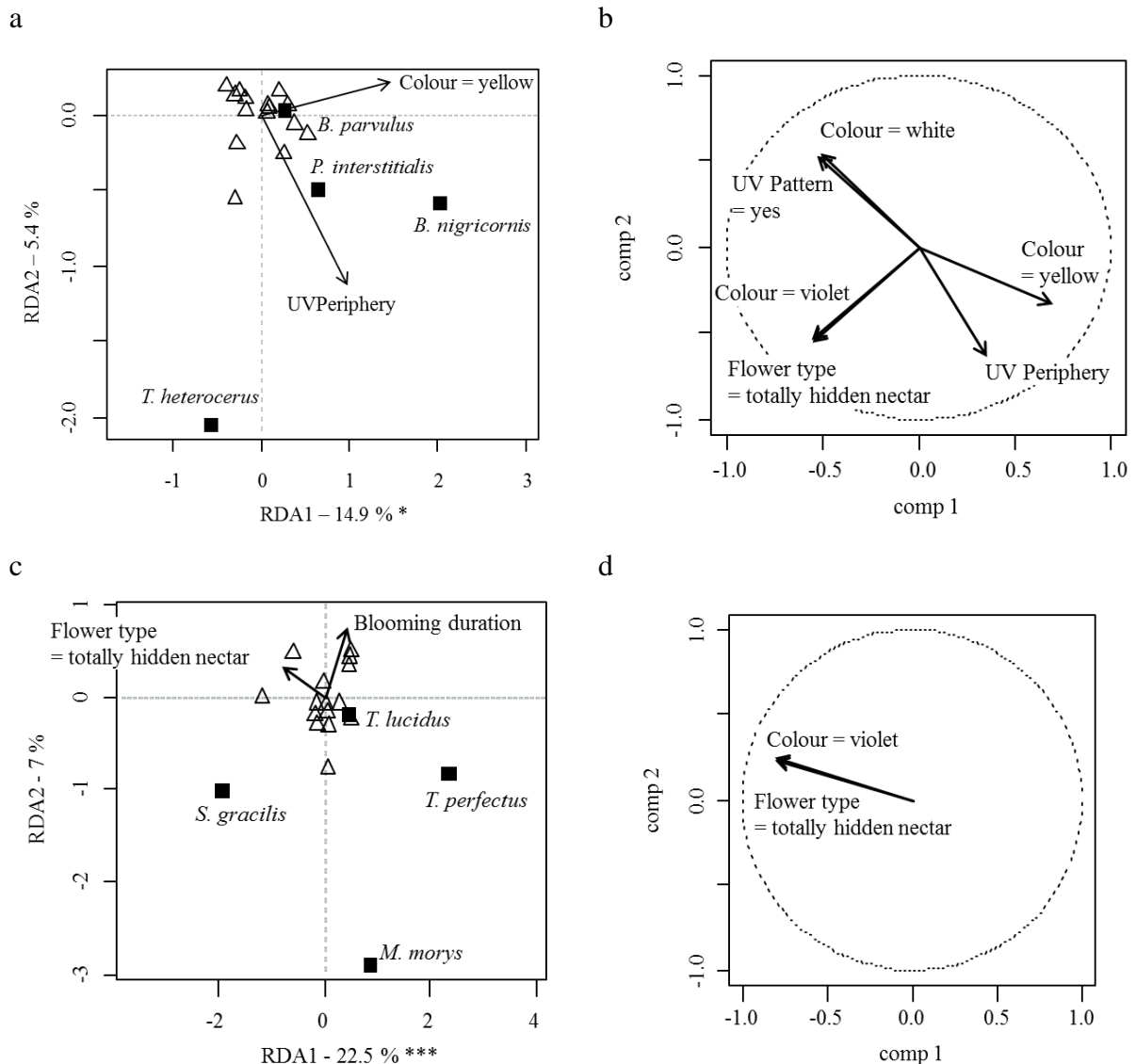
DISCUSSION

Meligethes spp. and *Ceutorhychus* spp. parasitoids and their respective hosts reached their abundance peaks in WFS simultaneously in May and June, respectively. Pests, but not their parasitoids, showed a second peak at the end of June. However, at that time, harvesting of OSR was imminent and crops were not sensitive to the pests anymore.

Effect of hosts on parasitoids

Despite the simultaneity of their abundance peak, the density of *Meligethes* spp. and *Ceutorhynchus* spp. in the WFS did not affect the one of parasitoids. Instead, several flower traits had a significant role. Previous studies have warned that the presence of crop pests in WFS could affect natural enemy behaviour (Rand et al. 2006; Carrié et al. 2012). However, this study is the first to assess pest abundance in WFS as a potential explanatory variable for natural enemy density. It matters as *Meligethes* spp. are polyphagous feeders (Free and Williams 1978), thus may have settled in the WFS. *Ceutorhynchus* spp., however, are oligophagous on Brassicaceae and none of them were sown. Only *S. alba* was found flowering in June, certainly as a regrowth from previously cultivated crop. Nevertheless, even if OSR pests were trapped in the WFS, we previously indicated that they were always more attracted by the adjacent crops at their abundance peak, which shows that OSR pests did not prefer wildflower species (Hatt et al. 2015). Our results demonstrate that flower traits are stronger drivers at attracting parasitoids in WFS compared to hosts. An explanation is that parasitoids, at early stage of their adult life and before ovipositing, need energy they can find through flower nectar, which also increases their reproduction capacity (Lundgren 2009). The presence of attractive and suitable flowers may orient their flight when they start colonising

WFS. This confirms the importance of understanding which flower traits affect parasitoid behaviour in open fields and how.

Traits affecting parasitoid abundance in WFS

The abundance of *Meligethes* spp. parasitoids was significantly affected by flower colour and UV reflectance in the WFS. Visual cues have an important role in insect–plant interactions (Wäckers 1994; Begum et al. 2004). In the present study, yellow coloured flowers attracted more parasitic wasps compared to white and violet flowers. This result is consistent with that obtained by Jönsson (2005), who also found that yellow is a strong visual cue for pollen beetle parasitoids. The attraction to yellow is caused by the positive input from green receptors coupled with a negative input from blue receptors in the insect eye, termed the 'green–blue colour opponent mechanism' (Döring et al. 2009; Döring et al. 2012). In contrast to *Meligethes* spp. parasitoids, *Ceutorhynchus* spp. parasitoids were less sensitive to flower colour. Only violet had a significant effect, repellent to most wasps. Yellow also attracts *Meligethes* spp. (Döring et al. 2012), which partly explains why it is one of the main pest of OSR (Williams 2010). Their parasitoids, also attracted by yellow coloured flowers, are more likely to find their hosts on such flowers. Thus, we hypothesise that flower colour is a main driver for pollen beetle parasitoids to locate their hosts. This phenomenon is not the case for *Ceutorhynchus* spp. parasitoids. Indeed, the majority of these species were not positively affected by any colour, with Williams and Cook (2010) also reporting that their parasitism rate is not affected by flower colour.

In addition to colour, flowering species with high UV reflectance for both internal and external flower parts attracted *Meligethes* spp. parasitoids. Indeed, the UV reflectance of flowers affects insect behaviour (Chittka et al. 1994; Tansey et al. 2010). Yet, it did not affect *Ceutorhynchus* spp. parasitoids. This study is the first to assess the attraction of OSR pest

parasitoids to UV. However, pollen beetles have been shown to be attracted to flowers with higher UV reflectance (Döring et al. 2012), such as OSR (Ômura et al. 1999). Like the colour yellow, the attractiveness of flowers with high UV reflectance to *Meligethes* spp. parasitoids may help them to locate their hosts.

After visual cues, nectar availability, which is linked to flower morphology, also influenced insect attraction. In particular, parasitoids search for sugar resources to increase their longevity and help to increase oviposition rate of females by accelerating egg maturation (Lundgren 2009). In the present study, the parasitoids of both *Meligethes* spp. and *Ceutorhynchus* spp. were negatively affected by flowers with totally hidden nectar. This result is consistent with all previous studies, which showed that hymenopteran parasitoids preferentially feed on flowers that offer accessible nectar, notably those with open nectaries (Idris and Grafius 1995; Patt et al. 1997; Vattala et al. 2006; Bianchi and Wäckers 2008). Jervis et al. (1993) reported that Ichneumonidae and some Braconidae species are generally too large and have mouth parts that are too short, which prevents them from exploiting the nectar of flowers with narrow and tubular corolla. In contrast, Proctotrupoidea species may feed on such flowers; however, in the present study, very few individuals of this superfamily were trapped (Table 2). There is a lack of information on the interactions between Pteromalidae species and flower corolla. In the present study, they were mainly negatively affected by flowers with totally hidden nectar, as were the majority of the studied parasitoids.

Practical implications and further research

Parasitoids were trapped at their adult stage. As mentioned here-above, flower nectar is an essential resource for parasitoid longevity and reproduction capacity, thus its provision may favour their ability of controlling pests. Nectar from OSR flowers may be exploited, however, the resource must be available at the latest when pests flight on crops in order to increase

parasitoid chance to find and parasite their host at the early stage of infestation. Because pollen beetles damage crops from their green bud stage, other source of nectar than those from OSR flowers must be available earlier. In the present study, OSR was even not blooming anymore when *Meligethes* spp. parasitoids occurred (i.e. in mid-May, simultaneously in WFS and OSR, Fig. 2a). The present results on the effects of flower morphology, colour and UV may be used in order to select flower species – among those blooming earlier than OSR – able to attract and support *Meligethes* spp. parasitoids at field margins before OSR flourishes and pollen beetles occur on them. In the present study, the first *Meligethes* spp. individuals were trapped on 23 April in OSR, three weeks before parasitoids peaked (Fig. 2a). As for *Ceutorhynchus* spp. parasitoids, they appeared in the field along with weevil adults (i.e. mid-June, simultaneously in WFS and OSR, Fig. 2b). The presence of blooming flowers in WFS from which they may have benefited could have increased their longevity and foraging capacity in order to parasite weevil larvae in the following days in adjacent crops. Their simultaneous occurrence in both WFS and adjacent OSR may have favoured parasitism, thus pest control.

By focusing on the effect of flower traits on natural enemies in cropping conditions, the present study may allow improving the constitution of mixtures sown at field margins or within fields. The sowing of WFS is subsidised by several European countries through the AES policy. The AES were firstly introduced to "reduce environmental risks associated with modern farming on the one hand, and preserve nature and cultivated landscapes on the other hand" (European Commission 2005). Today, there is a need to optimise AES to benefit from important ecosystem services, such as biological control (Haaland et al. 2011; Ekroos et al. 2014). The results of the current study indicate that yellow flowers with high UV reflectance should be sown – if only they can bloom early in spring – while those with totally hidden nectar should be excluded to attract the parasitoids of OSR pests when OSR is cultivated.

However, in cropping systems following rotations (which is actually a practice in itself to control pests as it disrupts the presence of pest host plants through time, Oerke 2006), the challenge remains to develop flower mixtures that are able to support a wide diversity of natural enemies associated with the different pests of crops that are sown adjacent to perennial WFS. It could be proposed to sow annual flowering plants adapted to each crop each year, as some previously studied (Balzan and Wäckers 2013; Tschumi et al. 2015). Nevertheless, perennial WFS present the advantage of preserving permanent vegetation in annual cropping systems, providing an overwintering site and resources for natural enemies, thus, reducing ecological disturbance in agricultural landscapes and potentially favouring the early colonisation of crops (Rusch et al. 2013). Previous studies have shown that increasing the range of values taken by various traits within mixtures may optimise the conservation of several arthropod functional groups that provide multiple ecosystem services (Campbell et al. 2012; Balzan et al. 2014). However, research is needed to determine whether flower mixtures with higher functional diversity support a wide range of natural enemies that are able to control different crop pests. Better knowledge on how flower cues affect different predators and parasitoids may help with the selection of appropriate perennial species for inclusion in mixtures. Similar analyses to those presented in this study, but on crops other than OSR, could provide such information. The present study provides first results, as well as a methodology, towards long term research on the development of perennial flower mixtures that are able to enhance biological control throughout a whole rotation period.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

Baldi I, Cordier S, Coumoul X, et al (2013) Pesticides: effets sur la santé. INSERM, Institut national de la santé et de la recherche médicale, Paris

Balzan MV, Bocci G, Moonen A-C (2014) Augmenting flower trait diversity in wildflower strips to optimise the conservation of arthropod functional groups for multiple agroecosystem services. J Insect Conserv 18:713–728. doi: 10.1007/s10841-014-9680-2

Balzan MV, Moonen A-C (2014) Field margin vegetation enhances biological control and crop damage suppression from multiple pests in organic tomato fields. Entomol Exp Appl 150:45–65. doi: 10.1111/eea.12142

Balzan MV, Wäckers FL (2013) Flowers to selectively enhance the fitness of a host-feeding parasitoid: adult feeding by Tuta absoluta and its parasitoid Necremnus artynes. Biol Control 67:21–31. doi: 10.1016/j.biocontrol.2013.06.006

Barbosa PA (1998) Conservation Biological Control. Academic Press, San Diego, USA

Begum M, Gurr GM, Wratten SD, Nicol HI (2004) Flower color affects tri-trophic-level biocontrol interactions. Biol Control 30:584–590. doi: 10.1016/j.biocontrol.2004.03.005

Bianchi FJJA, Wäckers F (2008) Effects of flower attractiveness and nectar availability in field margins on biological control by parasitoids. Biol Control 46:400–408. doi: 10.1016/j.biocontrol.2008.04.010

Borcard D, Gillet F, Legendre P (2011) Numerical ecology with R. Springer, New York, USA

Botta-Dukát Z (2005) Rao's quadratic entropy as a measure of functional diversity based on multiple traits. J Veg Sci 16:533–540. doi: 10.1111/j.1654-1103.2005.tb02393.x

Büchi R (2002) Mortality of pollen beetle (Meligethes spp.) larvae due to predators and parasitoids in rape fields and the effect of conservation strips. Agric Ecosyst Environ 90:255–263. doi: 10.1016/S0167-8809(01)00213-4

Campbell AJ, Biesmeijer JC, Varma V, Wäckers FL (2012) Realising multiple ecosystem services based on the response of three beneficial insect groups to floral traits and trait diversity. Basic Appl Ecol 13:363–370. doi: 10.1016/j.baae.2012.04.003

Carrié RJG, George DR, Wäckers FL (2012) Selection of floral resources to optimise conservation of agriculturally-functional insect groups. J Insect Conserv 16:635–640. doi: 10.1007/s10841-012-9508-x

Chittka L, Shmida A, Troje N, Menzel R (1994) Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. Vision Res 34:1489–1508. doi: 10.1016/0042-6989(94)90151-1

Colignon P, Hastir P, Gaspar C, Francis F (2001) Effects of insecticide treatments on insect density and diversity in vegetable open fields. Meded Van Fac Landbouwkd En Toegepaste Biol Wet Rijksuniv Te Gent 66:403–411.

Cook SM, Skellern MP, Döring TF, Pickett JA (2013) Red oilseed rape? The potential for manipulation of petal colour in control strategies for the pollen beetle (Meligethes aeneus). Arthropod-Plant Interact 7:249–258. doi: 10.1007/s11829-013-9252-5

Díaz S, Cabido M (2001) Vive la différence: plant functional diversity matters to ecosystem processes. Trends Ecol Evol 16:646–655. doi: 10.1016/S0169-5347(01)02283-2

Döring TF, Archetti M, Hardie J (2009) Autumn leaves seen through herbivore eyes. Proc R Soc Lond B Biol Sci 276:121–127. doi: 10.1098/rspb.2008.0858

Döring TF, Skellern M, Watts N, Cook SM (2012) Colour choice behaviour in the pollen beetle Meligethes aeneus (Coleoptera: Nitidulidae). Physiol Entomol 37:360–368. doi: 10.1111/j.1365-3032.2012.00850.x

Ekroos J, Olsson O, Rundlöf M, et al (2014) Optimizing agri-environment schemes for biodiversity, ecosystem services or both? Biol Conserv 172:65–71. doi: 10.1016/j.biocon.2014.02.013

European Commission (2005) Agri-environment measures. Overview on general principles, types of measures and application. European Commission, Directorate General for Agriculture and Rural Development.

Ferguson AW, Williams IH, Castle LM, Skellern MP (2010) Key parasitoids of the pests of oilseed rape in Europe: A guide to their identification. In: Williams IH (ed) Biocontrol-Based Integrated Management of Oilseed Rape Pests. Springer, The Netherlands, pp 77–114

Fiedler AK, Landis DA (2007a) Plant Characteristics Associated with Natural Enemy Abundance at Michigan Native Plants. Environ Entomol 36:878–886. doi: 10.1093/ee/36.4.878

Fiedler AK, Landis DA (2007b) Attractiveness of Michigan native plants to arthropod natural enemies and herbivores. Environ Entomol 36:751–765. doi: 10.1093/ee/36.4.751

Free JB, Williams IH (1978) The responses of the pollen beetle, Meligethes aeneus, and the seed weevil, Ceuthorhynchus assimilis, to oil-seed rape, Brassica napus, and other plants. J Appl Ecol 15:761–774. doi: 10.2307/2402773

Geiger F, Bengtsson J, Berendse F, et al (2010) Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. Basic Appl Ecol 11:97–105. doi: 10.1016/j.baae.2009.12.001

Gibbons D, Morrissey C, Mineau P (2015) A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environ Sci Pollut Res 22:103–118. doi: 10.1007/s11356-014-3180-5

Haaland C, Naisbit RE, Bersier L-F (2011) Sown wildflower strips for insect conservation: a review. Insect Conserv Divers 4:60–80. doi: 10.1111/j.1752-4598.2010.00098.x

Hanson HI, Smith HG, Hedlund K (2015) Agricultural management reduces emergence of pollen beetle parasitoids. Agric Ecosyst Environ 205:9–14. doi: 10.1016/j.agee.2015.03.001

Hatt S, Uyttenbroeck R, Lopes T, et al (2015) Do wildflower strips favor insect pest populations at field margins? Agric Agric Sci Procedia 6:30–37. doi: 10.1016/j.aaspro.2015.08.034

Horton DR, Broers DA, Lewis RR, et al (2003) Effects of mowing frequency on densities of natural enemies in three Pacific Northwest pear orchards. Entomol Exp Appl 106:135–145. doi: 10.1046/j.1570-7458.2003.00018.x

Idris AB, Grafius E (1995) Wildflowers as nectar sources for Diadegma insulare (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Yponomeutidae). Environ Entomol 24:1726–1735. doi: 10.1093/ee/24.6.1726

Jervis MA, Kidd NAC, Fitton MG, et al (1993) Flower-visiting by hymenopteran parasitoids. J Nat Hist 27:67–105. doi: 10.1080/00222939300770051

Jönsson M (2005) Responses to oilseed rape and cotton volatiles in insect herbivores and parasitoids. Doctoral thesis, Swedish University of Agricultural Sciences

Kattge J, Díaz S, Lavorel S, et al (2011) TRY – a global database of plant traits. Glob Change Biol 17:2905–2935. doi: 10.1111/j.1365-2486.2011.02451.x

Kirk-Spriggs AH (1996) Pollen beetles: Coleoptera: Kateretidae and Nitidulidae: Meligethinae. Royal Entomological Society, London, UK

Krebs JR, Wilson JD, Bradbury RB, Siriwardena GM (1999) The second Silent Spring? Nature 400:611–612. doi: 10.1038/23127

Laliberté E, Legendre P (2010) A distance-based framework for measuring functional diversity from multiple traits. Ecology 91:299–305. doi: 10.1890/08-2244.1

Laliberté E, Legendre P, Shipley B (2014) FD: measuring functional diversity from multiple traits, and other tools for functional ecology. R package.

Lambinon J, De Langhe J-E, Delvosalle L, Duvigneaud J (2008) Flora van België, het Groothertogdom Luxemburg, Noord-Frankrijk en de aangrenzende gebieden. Nationale Plantentuin van België, Meise

Lambinon J, Delvosalle L, Duvigneaud J (2004) Nouvelle flore de Belgique, du Grand-Duché de Luxembourg, du Nord de la France et des régions voisines., 5th edn. Jardin Botanique National de Belgique, Meise

Landis DA, Wratten SD, Gurr GM (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu Rev Entomol 45:175–201. doi: 10.1146/annurev.ento.45.1.175

Lavorel S, Garnier E (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Funct Ecol 16:545–556. doi: 10.1046/j.1365-2435.2002.00664.x

Lavorel S, Grigulis K, McIntyre S, et al (2008) Assessing functional diversity in the field– methodology matters! Funct Ecol 22:134–147. doi: 10.1111/j.1365-2435.2007.01339.x

Lundgren JG (2009) Relationships of natural enemies and non-prey foods, Progress in Biological Control. Springer, The Netherlands

Morris MG (2008) True Weevils (Part II): (Coleoptera: Curculionidae, Ceutorhynchinae). Royal Entomological Society, London, UK

Müller H (1881) Alpenblumen, ihre Befruchtung durch Insekten und ihre Anpassungen an dieselben. Leipzig

Nilsson C (2003) Parasitoids of the pollen beetles. In: Alford DV (ed) Biocontrol of oilseed rape pests. Blackwell, Oxford, UK, pp 73–86

Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31–43. doi: 10.1017/S0021859605005708

Oksanen J, Guillaume Blanchet F, Kindt R, et al (2015) Vegan: Community Ecology Package. R package.

Ômura H, Honda K, Hayashi N (1999) Chemical and chromatic bases for preferential visiting by the cabbage butterfly, Pieris rapae, to rape flowers. J Chem Ecol 25:1895–1906. doi: 10.1023/A:1020990018111

Patt JM, Hamilton GC, Lashomb JH (1997) Foraging success of parasitoid wasps on flowers: interplay of insect morphology, floral architecture and searching behavior. Entomol Exp Appl 83:21–30. doi: 10.1046/j.1570-7458.1997.00153.x

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rand TA, Tylianakis JM, Tscharntke T (2006) Spillover edge effects: the dispersal of agriculturally subsidized insect natural enemies into adjacent natural habitats. Ecol Lett 9:603–614. doi: 10.1111/j.1461-0248.2006.00911.x

Rusch A, Bommarco R, Jonsson M, et al (2013) Flow and stability of natural pest control services depend on complexity and crop rotation at the landscape scale. J Appl Ecol 50:345–354. doi: 10.1111/1365-2664.12055

Sivinski J, Wahl D, Holler T, et al (2011) Conserving natural enemies with flowering plants: Estimating floral attractiveness to parasitic Hymenoptera and attraction's relationship to flower and plant morphology. Biol Control 58:208–214. doi: 10.1016/j.biocontrol.2011.05.002

Tansey JA, Dosdall LM, Keddie BA, Noble SD (2010) Contributions of visual cues to cabbage seedpod weevil, Ceutorhynchus obstrictus (Marsham) (Coleoptera: Curculionidae), resistance in novel host genotypes. Crop Prot 29:476–481. doi: 10.1016/j.cropro.2009.11.005

Tscharntke T, Karp DS, Chaplin-Kramer R, et al (2016) When natural habitat fails to enhance biological pest control – Five hypotheses. Biol Conserv In press. doi: 10.1016/j.biocon.2016.10.001

Tschumi M, Albrecht M, Bärtschi C, et al (2016) Perennial, species-rich wildflower strips enhance pest control and crop yield. Agric Ecosyst Environ 220:97–103. doi: 10.1016/j.agee.2016.01.001

Tschumi M, Albrecht M, Entling MH, Jacot K (2015) High effectiveness of tailored flower strips in reducing pests and crop plant damage. Proc R Soc B 282:20151369. doi: 10.1098/rspb.2015.1369

Ulber B (2003) Parasitoids of Ceutorhynchid stem weevils. In: Alford DV (ed) Biocontrol of oilseed rape pests. Blackwell, Oxford, UK, pp 87–96

Ulber B, Williams IH, Luik A, et al (2010) Parasitoids of oilseed rape pests in Europe: Key species for conservation biocontrol. In: Williams IH (ed) Biocontrol-Based Integrated Management of Oilseed Rape Pests. Springer, The Netherlands, pp 45–76

Uyttenbroeck R, Hatt S, Paul A, et al (2016) Pros and cons of flowers strips for farmers: a review. Biotechnol Agron Soc Environ 20:225–235.

Uyttenbroeck R, Hatt S, Piqueray J, et al (2015) Creating perennial flower strips: think functional! Agric Agric Sci Procedia 6:95–101. doi: 10.1016/j.aaspro.2015.08.044

Van Rijn PCJ, Wäckers FL (2016) Nectar accessibility determines fitness, flower choice and abundance of hoverflies that provide natural pest control. J Appl Ecol 53:925–933. doi: 10.1111/1365-2664.12605

Vattala HD, Wratten SD, Vattala CB, et al (2006) The influence of flower morphology and nectar quality on the longevity of a parasitoid biological control agent. Biol Control 39:179–185. doi: 10.1016/j.biocontrol.2006.06.003

Violle C, Navas M-L, Vile D, et al (2007) Let the concept of trait be functional! Oikos 116:882–892. doi: 10.1111/j.0030-1299.2007.15559.x

Wäckers FL (1994) The effect of food deprivation on the innate visual and olfactory preferences in the parasitoid Cotesia rubecula. J Insect Physiol 40:641–649. doi: 10.1016/0022-1910(94)90091-4

Williams IH (2010) The major insect pests of oilseed rape in Europe and their management: an overview. In: Williams IH (ed) Biocontrol-Based Integrated Management of Oilseed Rape Pests. Springer, The Netherlands, pp 1–43

Williams IH (2003) Parasitoids of the cabbage seed weevil. In: Alford DV (ed) Biocontrol of Oilseed Rape Pests. Blackwell, Oxford, UK, pp 97–112

Williams IH, Cook SM (2010) Crop location by oilseed rape pests and host location by their parasitoids. In: Williams IH (ed) Biocontrol-Based Integrated Management of Oilseed Rape Pests. Springer, The Netherlands, pp 215–244

Wratten SD, Bowie MH, Hickman JM, et al (2003) Field boundaries as barriers to movement of hover flies (Diptera: Syrphidae) in cultivated land. Oecologia 134:605–611. doi: 10.1007/s00442-002-1128-9

Supplementary materials

Table S1 Theoretical trait values for each flower species. The values on blooming start, blooming duration and plant height were obtained from Lambinon et al. (2008), while those on colour, UV periphery, UV pattern, and the flower type were retrieved from the TRY database (Kattge et al., 2011).

Table S2 Mean cover (%), calculated from the three quadrats in each plot, of each blooming flower species found. Sum of percentage may be less than 100 as grass cover and bare soil was also considered when assessing flower cover.

Table S3 Community Weight Mean (CWM) calculated for each plot based on the average cover of each flower species found in the quadrats and blooming in May and June (see Table S2), and the traits of each species (see Table S1). CWMs of May was put into relation with *Meligethes* spp. parasitoids, while CWMs of June was linked with *Ceutorhynchus* spp. parasitoids, as these two parasitoids groups did not occurred at the same time in the wildflower strips.

Table S4 Mean per trap of pests and their related parasitoids in wildflower strips (WFS) and oilseed rape (OSR)

Figure S1. Total number of a) *Meligethes* spp. and b) their parasitoids trapped at peak (14 and 21 May), as well as of c*) Ceutorhynchus* spp. and d) their parasitoids at peak (11 June) in each plot.