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Perennial flower strips for pest control in organic apple orchards - A pan-European study



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ABSTRACT

In many crops, the intensive use of pesticides causes major problems both for the environment and for natural ecosystem services. Apple is Europe's most frequently produced orchard fruit, requiring high pesticide input to combat the most important apple insect pests, Dysaphis plantaginea (Passerini) and Cydia pomonella (L.). Here we sought to control these pests by promoting natural enemies using sown perennial flower strips. We trialled these in the alleyways of organic apple orchards in seven European countries. Visual assessments over two years revealed a higher number of natural enemies on plant parts, and specifically in D. plantaginea colonies on trees, in flower strip plots than on trees in control plots with standard orchard vegetation. Trees in the flower strip plots recorded a slower D. plantaginea population increase as compared with control plots, resulting in reduced fruit damage after the second fruit drop. Likewise, from 2016-2017, the number of preadult codling moths decreased more in the flower strip plots as compared to the control plots resulting in reduced fruit damage. Our study shows on a wide continental scale that the implementation of perennial flower strips in the alleyways between apple tree rows boosts natural enemies and reduces key apple pests and the associated fruit damage. This supports the role of functional agrobiodiversity as a way to potentially reduce insecticide use in orchards and thus further promote conservation of agrobiodiversity. We also provide suggested plant composition for flower strips adapted to different European countries and recommendations for implementation and management in practice.

1. Introduction

Phytophagous insect species have several natural enemies, ranging from generalist to specialist predators and parasitoids (Hawkins et al., 1997; Memmott et al., 2000). These natural enemies can suppress and regulate phytophagous insects in both natural and cultivated systems (Bianchi et al., 2006). The widespread use of synthetic pesticides and agricultural intensification, including also the disturbance and simplification of the surrounding environment, has led to significant losses in abundance and diversity of natural enemies and thereby impaired ecosystem services (Krauss et al., 2011; Bommarco et al., 2013; Uyttenbroeck et al., 2016). Despite the use of synthetic pesticides, the severity and frequency of pest outbreaks and the resulting yield loss are not always under the control of the grower (Oerke, 2005), and have even increased in unbalanced agricultural systems (Begg et al., 2017). In order to regain ecosystem services, European agricultural policy encourages farmers to reduce the use of pesticides and to increase and restore biodiversity within the agricultural landscape via the use of subsidies (European Commission, 2005; European Parliament, 2009; Regulation (EU) No 1307/2013 et al., 2013). Since broad-spectrum pesticides as used in conventional farming harm populations of beneficial arthropods (Krauss et al., 2011; Bommarco et al., 2013;

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Fig. 1. Map showing the distribution of the nine investigated organic orchards in Europe. The number of assessed blocks in seven European countries is indicated by different symbols (circles = 2 blocks, diamonds = 3 blocks, squares = 4 blocks).

Uyttenbroeck et al., 2016), organic production systems may be highly favorable for the implementation of conservation biological control (Porcel et al., 2018; Samnegård et al., 2018). However, conservation biological control may also be implemented in integrated pest management systems, assuming application of target-specific pesticides with fewer side effects carefully timed.

Many studies have shown that natural and semi-natural habitats at landscape and local scale can enhance populations of natural enemies and reduce agricultural pests (Bianchi et al., 2006; Chaplin-Kramer et al., 2011; Veres et al., 2013; Uyttenbroeck et al., 2016). Although recorded positive effects prevail over neutral or negative effects, they are not always guaranteed and the reported effect sizes are sometimes limited (Andow, 1983; Begg et al., 2017; Karp et al., 2018; Porcel et al., 2018). Appropriate measures are required to promote the correct species of natural enemy, in sufficient abundance and in alignment with pest and crop development stages to increase the likelihood of successful pest control (Pfiffner and Wyss, 2004; Dib et al., 2012; Tscharntke et al., 2016; van Rijn and Wäckers, 2016; Begg et al., 2017). For instance, adjacent *Fagopyrum esculentum* flower strips increased the abundance of Coccinellidae in the edges of soybean fields, but not in the fields themselves (Woltz et al., 2012). Thus, aphid control by coccinellids in fields with flower strips did not increase.

Table 1

Dicotyledon species	and grass species sown	in the flower strip	os in the seven European countrie
Taxonomic group	Species		Country

		CH	IT	BE	DE	PL	DK	SE
Dicotyledon species	Achillea millefolium L.							
	Ajuga reptans L.							
	Bellis perennis L.							
	Campanula rotundifolia L.							
	Cardamine pratensis L.							
	Carum carvi L.							
	Centaurea jacea L.							
	Crepis capillaris (L.) Wallr.							
	Daucus carota L.							
	Galium mollugo L.							
	Geranium pyrenaicum Burm. fil.							
	Hieracium aurantiacum L.							
	Hieracium lactucella Wallr.							
	Hieracium pilosella L.							
	Hypochaeris radicata L.							
	Lathyrus pratensis L.							
	Leontodon autumnalis L.							
	Leontodon hispidus L.							
	Leontodon saxatilis Lam.							
	Leucanthemum vulgare Lam.							
	Lotus corniculatus L.							
	Medicago lupulina L.							
	Myosotis scorpioides L.							
	Primula elatior (L.) Hill							
	Prunella vulgaris L.							
	Silene dioica (L.) Clairv.							
	Silene flos-cuculi (L.) Greuter & Burdet							
	Trifolium pratense L.							
	Veronica chamaedrys L.							
	Vicia sepium L.							
Grass species	Anthoxanthum odoratum L.							
	Cynosurus cristatus L.							
	Festuca guestfalica Boenning. ex Rchb.							
	Festuca rubra rubra Mit.							
	Lolium perenne L.							
	Poa nemoralis L.							
	Poa pratensis L.							
	Poa trivialis L.							
Additional species	Cichorium intybus L.							
*	Gallium album Mill.							
	Plantago lancelota L.							
	Plantago media L.							
	Sanguisorba minor Scop.							
	Festuca nigrescens Lam.							
Total number of								
species		38	30	39	33	34	33	33

The Swiss mixture was the reference. Grey squares = species included in the seed mixture, open squares = not included in the seed mixture.

Promoting natural enemies with a view to controlling pest insects is promising in orchards because their complex multi-strata structure offers diverse niches to enhance natural enemy populations. In addition, since orchards are perennial, a certain stability and resilience enabling to build-up of beneficial arthropod populations can be assumed (Pfiffner and Wyss, 2004; Nilsson et al., 2016; Demestihas et al., 2017; Daniel et al., 2018). Natural elements, such as hedges and ground cover plants, adjacent to and within the orchard, can increase nectar and pollen availability. Furthermore, these elements can enhance alternative prey in times of low pest abundance, and offer shelter and overwintering sites (Pfiffner and Wyss, 2004; Gurr et al., 2017; Daniel et al., 2018). Indeed, the potential of ecosystem services provided by natural enemies has been highlighted in orchards (Simon et al., 2010). Conservation biological control is influenced by the proximity of the crops to natural elements such as flower strips, the size of these elements in relation to the crops (source size) and the time needed to build up the natural enemy populations (Bostanian et al., 2004; Blaauw and Isaacs, 2012; Sigsgaard, 2014). In contrast to flower strips at the edge of orchards, perennial flower strips in the alleyways allow intensive interactions between the flower strips and the crop. However, it is challenging to establish perennial, species-rich flower strips and furthermore to maintain them under subsequent years (De Cauwer et al., 2005; Uehlinger et al., 2005; Pfiffner et al., 2018). A suitable seed mixture is essential alongside the correct management of the flower strip according to its stage of development, soil conditions, and the life cycle of pests and natural enemies. Consequently, Nilsson et al. (2016) and Uyttenbroeck et al. (2016) point out that there are few field studies in orchards that actually demonstrate pest control by flower strips as compared to a control, such as Wyss (1995); Irvin et al. (2006) and Gontijo et al. (2015). This gap applies particularly to field studies on a large scale and in different environments.

Apples are the most frequently produced fruit in orchards worldwide, with 89 million tons harvested in 2016 (Food and Agricultural Organization, 2016). The use of pesticides in apple production is high for several reasons: resistant or less susceptible varieties are rarely used, there are 'zero default fruit' market standards (Simon et al., 2010) and a lack of alternatives to pesticides to achieve these standards (Demestihas et al., 2017). Additionally, pest populations can build-up over time in perennial crops regularly treated with insecticides that reduce the natural enemy abundance (Bostanian et al., 2004).

In the present pan-European study, we investigated whether sown perennial flower strips can (I) promote natural enemies, (II) decrease pest insects, and (III) reduce crop damage as compared to control plots without flower strips. We designed perennial flower strips that exploit the stability and resilience of perennial apple orchards to promote natural enemies and boost conservation biological control. In order to gain general knowledge about the use of flower strips for pest control in apple orchards, within a latitudinal gradient and across a variable range of cropping conditions, the same experimental protocol was simultaneously implemented in nine orchards in seven European countries (CoreOrganic plus project EcoOrchard), from the Italian South Tyrol to Skåne in Southern Sweden.

2. Material and methods

Mean values \pm standard errors are presented throughout the manuscript.

2.1. Study orchards and flower strips

Twenty-three experimental blocks were established in nine organic apple orchards (for commercial production and at research stations) in seven European countries (Fig. 1, Table A.1). Each block consisted of seven or eight tree rows with flower strips sown in the alleyways of one part of the block (mean length of flower strips: 39.11 \pm 1.79 m, minimum length: 27.00 m, maximum length: 50.00 m) (Fig. A.1). An assessment plot (3 \times 10 trees in the three central rows) was placed in the center of the part with flower strips and in the control part (intensely mulched). The plots were separated by a mean distance of 41.46 \pm 6.97 m, a minimum distance of 18.00 m and a maximum distance of 154.50 m. The use of insecticides was generally prohibited but regarded as an option in agreement with the producer in exceptional cases of uncontrolled pest outbreaks in commercial orchards. The use of fungicides was permitted according to the standard organic farming practices in each country. Pruning and fertilization of the trees was performed according to regional management practices.

In most cases ecotypes (wild forms) of dicotyledon and grass species were selected for the flower strips (Pfiffner et al., in press) (Table 1). Dicotyledon species that (I) meet the specific requirements of natural enemies and pollinators in apple orchards and (II) tolerate mulching (small rosettes) were selected. Additionally, grasses that (I) stabilize the plant community and (II) improve resistance to machinery were added to the flower mixture. Due to differences in seed availability, seed mixtures varied slightly between countries (Table 1). For the establishment of the flower strips, after spading or the use of rotocultivator, the soil in the alleyways was harrowed several times to initiate the germination of the spontaneous orchard vegetation. The spontaneous vegetation was then removed in successive steps. The seed mixture (4 g

per m², in a weight ratio of 18 : 82% forbs to grasses) was sown in spring to early summer 2015, four weeks after the soil treatment. In order to guarantee an even distribution of the seeds a 1:1 vermiculite, barley or soy grist mixture was used. During the first year of establishment, flower strips were mulched (8 cm cutting height) four times at approximate intervals of eight weeks according to their vegetation development. All flower strips became established, save for two blocks in Germany, where the flower strips were therefore re-sown in spring 2016. In consecutive years, flower strips were mulched (8-12 cm cutting height) three to four times per year (Pfiffner et al., 2018) at the following stages: (I) during pre-flowering of apple trees (possibly omitted in northern countries to assure flowering at fruit tree bloom). (II) 1–6 weeks after beginning of flower strip bloom (BBCH 67–72) (Biologische Bundesanstalt für Land und Forstwirtschaft, 2010) when all apple flower petals had fallen or small fruits are visible, (III) after the summer break (July/August) and (IV) if necessary at the end of the season (October). Mulching is necessary to ensure proper light conditions to facilitate the growth of subsequent species and to guarantee a succession in flower resources throughout the season. The flower strips were not fertilized. In order to keep the spontaneous vegetation in the control plots at a low level, as is common practice in commercial orchards, the alleyways without flower strips were mulched more intensively (up to six times a year). The vegetation in drive alleys next to the flower strips was also mulched intensively. Mechanical weed control within the tree rows was mostly carried out three to four times a year.

2.2. Assessments

The number of plant species and percentage ground cover were assessed in spring (April/May), summer (June) and autumn (August) of 2016 and 2017 in subplots (2mx0.6m) in three alleyways of each assessment plot. Apple trees in the assessment plots were assessed four times per year: during pre-flowering BBCH 59 (PF), after flowering BBCH 69-70 (AF), after the second fruit drop (FD), and at harvest (H). Six branches were randomly selected per tree situated in all cardinal directions and in the lower half of the canopy. The flower cluster (PF), fruitlet cluster with leaves (AF) or long shoot (FD; freshly grown shoots) closest to the tree trunk of the selected branches was visually assessed for natural enemies and pests. The number of Forficulidae, generalist predators (spiders, Miridae, Anthocoridae) and specialist aphid predators Coccinellidae (eggs, larvae, pupae and adults), Syrphidae (eggs, larvae and pupae) and Chrysopidae (eggs and larvae) were counted. The number of Lepidoptera larvae and the number of plant parts infested with Dysaphis plantaginea (Passerini) (Homoptera: Aphididae), Dysaphis devecta (Walker) (Homoptera: Aphididae) and Rhopalosiphum insertum (Walker) (Homoptera: Aphididae) were also counted. After the second fruit drop, the incidence of fruit damage by D. plantaginea (malformation and/or reduction in fruit size as compared to undamaged fruits) and codling moth Cydia pomonella (L.) (Lepidoptera: Tortricidae) (entry holes in apples) were assessed on 60 apples per row in both the flower strip plots and the control plots. Additionally, apples of the 30 trees in both the flower strip plots and the control plots were assessed for specific D. plantaginea and codling moth damage at harvest.

Thirty (10 per row) fruitlet clusters (AF) or long shoots (FD) in both the flower strip plots and the control plots with *D. plantaginea* symptoms (curled leaves) were assessed in more detail to link natural enemy abundance directly with the presence of *D. plantaginea* colonies. The presence or absence of living colonies was recorded and the number of natural enemies counted.

In order to record the number of codling moth larvae, cocoons and pupae (Maalouly et al., 2015), corrugated cardboard bands (10 cm wide and 80 cm long) were wrapped around 15 trunks in the flower strip plots and 15 trunks in the control. Codling moth larvae use cardboard bands as a hiding place. These cardboard bands were collected in 2016 and 2017 in Belgium (after 8 weeks), Denmark (after 10 weeks),

Germany (after 4–8 weeks), Italy (after 4 weeks) and Poland (after 10 weeks), when the larvae of the first generation were ready to pupate (southern countries) or formed cocoons to overwinter (northern countries).

White cardboards $(2 \times 2 \text{ cm})$, with 15–25 *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs per card (glued using egg white), were used as sentinel-prey egg cards to measure predation. In 2016 and 2017 during the three visual assessment periods AF, FD and H, 15 sentinel-prey cards in the flower strip plots and 15 in the control were installed for 24 h on the underside of apple tree leaves. In order to estimate the activity of natural enemies, the number of unimpaired eggs before and after exposure in the orchard was counted under a stereomicroscope.

2.3. Statistical analysis

The data was analyzed with mixed effect models. All models included the fixed effects treatment, latitude and year and the random effects block, country, and cultivar (except the models analyzing the botanical data). The models analyzing the number of plant species, ground cover by plants, natural enemies in curled leaves and plant parts infested with D. plantaginea colonies included also the fixed effect season. The models analyzing the number of natural enemies additionally used the number of plant parts infested by D. plantaginea as a covariate. The In-transformed number of living D. plantaginea colonies was used as a covariate for the number of natural enemies in curled leaves. The explanatory variables latitude, the number of plant parts infested by D. plantaginea and the number of living D. plantaginea colonies were scaled by dividing the variables by their standard deviation. All models (except the models analyzing the botanical data) included the In-transformed number of assessed plant parts as an offset, to account for deviant numbers of assessed flower clusters, fruitlet clusters, long shoots or fruits. Non-significant quadratic terms and interactions were stepwise removed (Zuur et al., 2009). Individual smoothing curves for the two treatments were included in the saturated generalized additive mixed models (GAMM) and simplified to one smoothing curve for both treatments if necessary (based on the AIC). Additive modelling allows for the implementation of non-linear relationships between the response variable and one or more predictors of the model (Wood, 2006). A difference of 2 and more between the AICs was considered significant (Zuur et al., 2009).

Linear mixed models (LMM) were used to analyze ground cover by plants, the ln-transformed number of Syrphidae, Chrysopidae, Coccinellidae, Forficulidae, generalist predators (spiders, Miridae and Anthocoridae), natural enemies in curled leaves, preadult codling moths and fruit damage by D. plantaginea at harvest. In order to assess effects of flower strips on each specific group of natural enemies, separate LMMs were used. Due to the small number of specific taxa, the numbers of natural enemies from the three assessment dates per taxa were pooled. GAMMs were used to analyze the non-linear development of the number of plant species (Poisson-distributed errors corrected for overdispersion), the proportion of attacked eggs (sentinel prey, binomial-distributed errors corrected for overdispersion) and the number of plant parts infested by D. plantaginea (negative binomial distributed errors) over the season. Generalized linear mixed models (GLMM) with Poisson-distributed errors were used to analyze fruit damage after the second fruit drop and fruit damage by codling moth at harvest. Since few apples had damage from D. plantaginea and codling moth at the same time, fruit damage by D. plantaginea and codling moth were analyzed separately. Because the removal of damaged fruits in orchards for commercial production could have affected the proportion of damaged fruits at harvest as compared to after the second fruit drop, fruit damage of the two assessments was analyzed separately.

Because of temporal autocorrelation, an auto-regressive moving average (ARMA) correlation structure was included in the models analyzing ground cover by plants, the number of plant species, the

proportion of attacked eggs and the number of plant parts infested by D. plantaginea. Except the models analyzing the number of Coccinellidae and plant parts infested with D. plantaginea and the proportion of attacked eggs, all models used different slopes for the two years for the random effect country. Except the models analyzing the number of Coccinellidae and fruit damage by D. plantaginea after the second fruit drop, all models used only one slope for the random effect *block*. Except the models analyzing the number of Coccinellidae and fruit damage by D. plantaginea after the second fruit drop and fruit damage by codling moth at harvest, all models used only one slope for the random effect cultivar. For better convergence, the models analyzing fruit damage after the second fruit drop used the optimizer "bobyga" instead of "Nelder-Mead" for the second phase. To fit a statistical model to fruit damage by codling moth at harvest, data from Poland had to be excluded because infestation in Poland was particularly high (mean fruit damage: 44.43 \pm 15.32%), with a high variance.

Visual inspection of residual plots was used to test model assumptions and temporal autocorrelation. The generalized linear mixed models were checked for overdispersion. *P*-values of LMMs were obtained with the Anova function, with type = 3 for models including interactions. We used R 3.3.1 (R Development Core Team, 2016) and the R-packages lme4 (Bates et al., 2012), mgcv (Wood, 2006) car (Fox and Weisberg, 2019) and nlme (Pinheiro et al., 2018).

3. Results and discussion

3.1. Flower strips promote natural enemies

The number of plant species and percentage ground cover were significantly higher in the flower strip plots than in the control plots with standard orchard vegetation (Tables 2 and 3). Flower strips significantly increased the number of Syrphidae, Chrysopidae and generalist predators on trees in the flower strip plots as compared to the control (Tables 2 & 3). This suggests that the specifically designed flower mixtures boosted natural enemies. This is an important condition in order to promote biological pest control throughout the season in perennial orchards (Campbell et al., 2017; Gurr et al., 2017; Daniel et al., 2018). Flowers can attract (Piekarska-Boniecka et al., 2017;

Table 2

Mean values \pm standard error (SE) of the orchard vegetation, natural enemies and pests on trees and fruit damage in flower strip plots and control plots.

Group of variables	Variable	Flowe	r strip	Control		
		Mean	SE	Mean	SE	
Flower strip vegetation Number of plant species		15.24	0.32	6.88	0.15	
	Percentage ground cover	59.82	1.19	42.62	1.68	
Natural enemy abundance	Syrphidae*	2.83	0.84	2.71	1.06	
	Chrysopidae*	1.24	0.39	0.96	0.38	
	Coccinellidae*	3.16	0.61	4.18	1.10	
	Forficulidae*	1.55	0.48	1.04	0.38	
	Generalist predators*	7.03	1.13	5.60	0.91	
	Natural enemies [†]	1.09	0.16	0.91	0.15	
	Percentage attacked	46.79	2.37	44.94	2.32	
	eggs					
Pest abundance	Colonies of <i>D</i> . plantaginea*	12.21	1.36	12.95	1.50	
	Preadult codling moth [§]	0.96	0.14	1.00	0.12	
% fruit damage	D. plantaginea (FD)	7.69	1.87	10.07	2.40	
	D. plantaginea (H)	3.06	0.94	2.75	0.79	
	Codling moth (FD)	13.54	2.38	14.22	2.57	
	Codling moth (H)	9.01	2.4	10.36	3.12	

FD = after the second fruit drop, H = at harvest, * in 100 plant parts, † in curled leaves of ten *D. plantaginea* colonies, § per tree trunk. The percentage of attacked eggs was assessed with sentinel-prey cards.

Table 3

Effects of different variables on vegetation, natural enemies and pests on trees and fruit damage.

Group of variables	Dependent variable	Model type, distribution	Effects	t	z	Р	SD	Effect direction
Flower strip vegetation	Percentage ground cover	LMM, Gaussian	Treatment	9.53		< 0.001		FS > C
			Latitude	1.45		0.149		
			Year	-0.56		0.575		
			Year × treatment	2.63		0.009		Difference between FS vs. C smaller in 2017 than 2016
			Season	-0.82		0.414		
			Country in 2016				18.57	
			Country in 2017				8.22	
			Block				6.09	
	Number of plant species	GAMM, Poisson ⁸	Treatment	34.33		< 0.001		FS > C
			Latitude	-1.85		0.065		
			Latitude ²	-2.55		0.011		Highest number mid-latitude
			Year	0.15		0.882		
			Smoother for season (df $=$	2.78		< 0.001		Slightly weaker increase towards
			1.46)					autumn
			Country in 2016				0.18	
			Country in 2017				0.24	
Natural anomy	Sumbidoo	IMM Coursian	BIOCK	0.11		0.025	0.06	$\mathbf{F}\mathbf{C} > \mathbf{C}$
abundance	Syrphidae	Linivi, Gaussian	Heatment	2.11		0.035		r3 > C
			Latitude	-0.14		0.890		
			Year	-0.81		0.418		
			D. plantaginea*	2.49		0.013		See Fig. 2
			Country in 2016				1.19	
			Country in 2017				1.76	
			Block				0.00	
	Characacida a	IMM Coursian	Cultivar	0.05		0.010	0.39	EC > C
	Chrysopidae	LIMIN, Gaussian	Ireatment	2.35		0.019		FS > C
			Vear	2.20		0.024		+ 37.00 % per degree fattude
			D plantaginea*	2.00		0.045		See Fig 2
			Country in 2016	2.00		0.000	1.03	000 118. 2
			Country in 2017				1.13	
			Block				0.00	
			Cultivar				0.00	
	Coccinellidae	LMM, Gaussian	Treatment	0.55		0.580		
			Latitude	-0.42		0.674		
			Year	-0.87		0.387		
			D. plantaginea*	3.62		< 0.001		See Fig. 2
			Country				1.08	
			Block in 2016				0.75	
			Block in 2017				0.53	
			Cultivar in 2016				0.85	
	Forficulidae	IMM Gaussian	Treatment	1 5 2		0 1 2 0	0.84	
	Forneulidae	Livilvi, Gaussiali	Latitude	-0.42		0.129		
			Year	-1.28		0.202		
			D. plantaginea*	2.21		0.027		See Fig. 2
			Country in 2016				1.09	0
			Country in 2017				0.45	
			Block				0.13	
			Cultivar				0.16	
	Generalist predators	LMM, Gaussian	Treatment	3.04		0.002		FS > C
			Latitude	1.59		0.112		
			Year	0.35		0.723		
			Year \times treatment	-2.28		0.022		Difference between FS vs. C smaller in 2017 than 2016
			D. plantaginea*	1.13		0.260		
			Country in 2016				0.93	
			Country in 2017				1.10	
			Block				0.22	
	Notice 1 and 1		Cultivar	0.00		0.005	0.24	
	Natural enemies in curled leaves	LIVIIVI, Gaussian	reatment	2.80		0.005		FS > C
			Latitude	-0.38		0.705		
			Year	-0.91		0.362		
			Living colonies [†]	4.13		< 0.001		+6.16 % per additional living colony
			Season	3.45		< 0.001		
			Country in 2016				1.31	
			Country in 2017				0.99	
			Block				0.00	
			Cultivar				0.39	

(continued on next page)

Table 3 (continued)

Group of variables	Dependent variable	Model type, distribution	Effects	t	Z	Р	SD	Effect direction
	Proportion of attacked	GAMM, Binomial [§]	Treatment	1.96		0.051		
	-000		Latitude Year	2.51 0.37		0.013 0.714		+4.10 % per degree latitude
			Smoother for season (df = 1.95)	6.56		< 0.001		Highest activity FD
			Country Block				0.56 0.00	
D (1 1		CHIRCH IN 1	Cultivar	0.50		0.000	0.00	
Pest adundance	Colonies of D. plantaginea	binomial	Ireatment	0.52		0.606		
			Year	-1.19		0.338		
			Smoother for season in FC $(df = 0.67)$	1.08		0.051		See Fig. 3A
			Smoother for season in C $(df = 0.96)$	1.95		0.002		See Fig. 3A
			Country				0.61	
			Block				0.00	
	Preadult codling moth	LMM, Gaussian	Treatment	0.69		0.493	0.00	
	Ū	,	Latitude	4.87		< 0.001		+64.12 % per degree latitude
			Year	-1.39		0.165		
			Year \times treatment	-2.07		0.038	0.25	See Fig. 3B
			Country in 2016				0.35 1.24	
			Block				0.25	
			Cultivar				0.34	
Fruit damage	D. plantaginea (FD)	GLMM, Poisson	Treatment		- 9.67	< 0.001		FS < C
			Year		-0.01	0.993		
			Country in 2016				1.41	
			Country in 2017				2.66	
			Block in 2016 Block in 2017				0.28	
			Cultivar in 2016				0.38	
			Cultivar in 2017				0.72	
	D. plantaginea (H)	LMM, Gaussian	Treatment	0.55		0.581		
			Latitude	-0.54		0.592		
			Country in 2016	0.72		0.471	1.43	
			Country in 2017				1.52	
			Block				0.21	
	Codling moth (FD)	GIMM Poisson	Cultivar Treatment		1 04	0.300	0.53	
		Gillini, i olision	Latitude		2.01	0.045		+68.58 % per degree latitude
			Latitude2		-2.91	0.004		Highest fruit damage mid- latitude
			Year		-1.72	0.086	0.07	
			Country in 2016				0.87	
			Block in 2016				0.66	
			Block in 2017				0.73	
	0 11: d (ID	01101 D :	Cultivar		0.15	0.001	0.43	
	Codling moth (H)	GLMM, Poisson	Latitude		2.15	0.031		FS > C
			Latitude ²		- 3.57	< 0.001		Highest fruit damage mid- latitude
			Year		2.23	0.026		
			Year \times treatment		-4.14	< 0.001	1.00	See Fig. 3C
			Country in 2016				1.09	
			Block in 2016				0.46	
			Block in 2017				0.57	
			Cultivar in 2016				0.08	
			Cultivar in 2017				1.11	

The proportion of attacked eggs was assessed with sentinel-prey cards. GAMM = generalized additive mixed model, LMM = linear mixed model, GLMM = generalized linear mixed model, § corrected for overdispersion, SD = standard deviation, FD = after the second fruit drop, H = at harvest, treatment = flower strip (FS) versus control (C), year = 2016 versus 2017, * plant parts infested with *D. plantaginea*, † number of living *D. plantaginea* colonies. *P*-values and *t* or *z*-values of the fixed effects and SD of the random effects are presented.

Porcel et al., 2017) and promote the nectar and pollen-feeding adults of Syrphidae and Chrysopidae that oviposit their aphidophagous offspring in aphid colonies (Wyss, 1995; Miñarro and Dapena, 2000 2005; Dib et al., 2016, 2017). In Central and Southern Europe, the syrphid Episyrphus balteatus (De Geer) usually occurs in spring (Miñarro et al., 2005; Martínez-Uña et al., 2013) and its larvae feed on aphid fundatrices before they can build up large colonies (Villiger, 1998; Wyss et al., 1999). Accordingly, E. balteatus is often recognized as one of the most important aphid predators (Wyss et al., 1999; Miñarro et al., 2005). In contrast to Syrphidae and Chrysopidae, the number of Coccinellidae, whose larvae and adults are also important predators of aphids (Wyss, 1995; Dib et al., 2010), did not differ significantly between the treatments (Tables 2 & 3). Only eggs, aphidophagous larvae and pupae of Chrysopidae and Syrphidae could be recorded on apple trees, but not the highly mobile adults foraging for pollen and nectar. In Coccinellidae, however, all developmental stages were well monitored on the trees, but mainly adults were found. Because of the high mobility of adult Coccinellidae and the possibly too short distances between the treatments (Table A.1), we cannot exclude possible interference between flower strips and control plots. There is also the possibility that the flower strips attracted the natural enemies from the rest of the orchard and thus reduced their density in the control plots, which ultimately does not lead to a net increase in natural enemies throughout the orchard. We recommend that in subsequent studies, flower strips and control plots should be placed at a higher separation, for example in different orchards with similar microclimates. However, placing both treatments in the same orchard has the advantage of providing the most similar environmental and agronomic conditions (soil, surrounding landscape, apple variety, machinery for flower strip management etc.) for both treatments. The number of Forficulidae did not differ significantly between the treatments (Tables 2 & 3). Flower strips may not provide these omnivorous insects with additional food sources. Nevertheless, the higher abundance of different natural enemies in the flower strip as compared to the control plots indicates the potential of flower strips to promote natural enemies for biological pest control.

Irrespective of the treatment effect, the number of Syrphidae, Chrysopidae and Coccinellidae on trees was positively correlated with the number of plant parts infested by *D. plantaginea*, and natural



Proportion of plant parts infested with D. plantaginea

Fig. 2. Natural enemies in relation to the presence of *Dysaphis plantaginea* colonies. Ln-transformed number of Syrphidae (open circles, solid line), Chrysopidae (closed circles, dashed line), Coccinellidae (open squares, dotted line) and Forficulidae (closed squares, dashed and dotted line) in relation to the proportion of plant parts infested with *D. plantaginea*. In both treatments 540 plant parts per plot were assessed.

enemies were more abundant in curled leaves when living *D. plantaginea* colonies were present (Fig. 2, Tables 2 & 3). This suggests that the aphidophagous natural enemies are attracted by *D. plantaginea* infestations. In contrast to aphid specialists, the relationship between the abundance of *D. plantaginea* and omnivorous Forficulidae was weaker (Fig. 2) and generalist predators (including spiders, Anthocoridae and Miridae) were not attracted (Table 3). The fact that the presence of some important natural enemies of the rosy apple aphid such as Anthocoridae (Sigsgaard, 2010; Porcel et al., 2018) and spiders (Cahenzli et al., 2017), was not dependent on the density of aphids but enhanced by flower strips makes them ideal targets for habitat manipulation and conservation strategies. Because such natural enemies may be attracted to the orchard independently from aphid infestation level, they may considerably improve the resilience of the crop due to early predation upon arrival of the pest (Sigsgaard, 2010; Porcel et al., 2018).

The abundance of natural enemies varied not only between countries, but Chrysopidae varied with latitude and between years, and the effect of flower strips on generalist predators was stronger in 2016 than 2017, for example (Table 3). To mitigate such variations, it is important to provide resilient flower strips that continuously supply flowers throughout the crop season and over years (Bostanian et al., 2004; Sigsgaard, 2010; Campbell et al., 2017). Despite the slightly higher plant diversity in mid-latitudes, the flower strips, initially developed for Swiss climate, became well established in all countries. While in the control the percentage ground cover varied between the two assessment years (2016: 40.02 ± 2.34; 2017: 45.13 ± 2.39), it was relatively consistent in the flower strips (2016: 60.78 ± 1.40 ; 2017: 58.89 \pm 1.90; Table 3). Additionally, plant diversity increased from spring to autumn (Table 3), providing a succession of different flowering plant species throughout the season. Our results clearly support the integration of perennial flower strips in organic apple orchards in order to promote natural enemies.

3.2. Flower strips and pest control

Flower strips did not only increase the number of natural enemies on apple trees, but specifically in D. plantaginea colonies (Tables 2 & 3). Accordingly, the population increase of D. plantaginea was significantly slower on trees in the flower strip plots than in the control plots (Fig. 3A, Table 3) and fruit damage caused by D. plantaginea after the second fruit drop was significantly reduced (Tables 2 & 3). Although the ultimate goal of implementing flower strips for pest control is to reduce crop damage or increase yield gain, most studies only show how natural enemies can be promoted (Bianchi et al., 2006; Letourneau and Bothwell, 2008; Simon et al., 2010; Chaplin-Kramer et al., 2011; Schellhorn et al., 2015). Studies that prove the effect of enhanced natural enemies upon the populations of pest insects or even crop damage or yield are scarce (Uyttenbroeck et al., 2016; Begg et al., 2017). Similarly, in our trials there was no significant difference in fruit damage by D. plantaginea at harvest (Table 2). Of note, the removal of damaged fruits in orchards for commercial production could have masked significant differences between the treatments and might have equalized the differences observed earlier. Furthermore, various other effects may have contributed to the observed insignificant differences in fruit damage at harvest between the treatments, such as asynchrony between the flower strips, natural enemies, aphids and tree phenology, aphid-promoting ants, intraguild predation or the high dispersal of aphidophagous natural enemies between treatments (Dib et al., 2016; Nilsson et al., 2016; Rousselin et al., 2017). A conclusion on the infestation with aphids other than D. plantaginea was also not possible, since they only occurred in small numbers and were unevenly distributed between orchards and countries. In apple, flower strips can promote aphidophagous natural enemies and decrease D. plantaginea (Wyss, 1995), but general aphid control through enhanced natural enemies is not guaranteed (Haley and Hogue, 1990; Bianchi et al., 2006; Markó et al., 2013; Campbell et al., 2017). Cahenzli et al. (2017)



Fig. 3. Pest abundance and fruit damage by codling moth over time. (A) Estimated smoothing curve for the ln-transformed number of plant parts infested with *Dysaphis plantaginea* on trees in the flower strip plots (solid line) and control plots (dashed line) at different assessment dates: flower clusters (PF: pre flowering), fruitlet clusters (AF: after flowering) or long shoots (FD: after the second fruit drop). In both treatments 180 plant parts per plot were assessed. (B) Interaction plot: From 2016–2017, the number of preadult codling moths *Cydia pomonella* (mean \pm SE) in the flower strip plots (closed circles, solid line), indicated by a significant interaction (t = -2.07, P = 0.038). (C) Interaction plot: From 2016–2017, the increase in the percentage of apples damaged by *C. pomonella* (mean \pm SE) in the flower strip plots (closed circles, solid line) was less pronounced than in the control plots (open circles, dashed line), indicated by a significant interaction (Z = -4.14, P < 0.001).

promoting elements, while other aphidophagous natural enemies had no indirect effect on fruit damage. This finding is in contrast to the results in this study, in which aphidophagous natural enemies likely had an effect on the rosy apple aphid and thus reduced fruit damage. The promotion therefore, of not only individual taxa, but rather the whole complex of aphidophagous and generalist predators, appears to be more promising in order to achieve a sustainable aphid control throughout the season and subsequent years (Rousselin et al., 2017).

Incidence of codling moth varied considerably between countries and over the two assessment years (Table 3). The number of preadult codling moths was higher in northern countries as compared to southern countries (Table 3). In contrast to northern countries, where C. pomonella has one generation per year, the single assessment per season may have underestimated the population in southern countries, where more generations per year can occur (Riedl, 1983), as a single assessment cannot comprise multiple generations. In addition, pest control with insecticides is less intensive in Northern Europe, possibly allowing for larger populations of codling moth at a landscape scale (Porcel et al., 2015). Fewer generations per vear in Northern Europe and higher pest control in Southern Europe could explain highest fruit damage in mid-latitudes (Table 3). The number of preadult codling moths dropped more from 2016 to 2017 in the flower strip plots as compared to the control plots (Fig. 3B, Table 3), indicating a positive control effect of the flower strips. The relative fruit damage caused by codling moth in 2017 was higher than in 2016 due to the low number of available apples caused by frost in April 2017. However, although there was no direct treatment effect (FD, H), the increase in relative fruit damage (H) from 2016 to 2017 was lower in the flower strip plots as compared to the control plots (Fig. 3C, Table 3). While predators can control eggs and young larvae (Glen, 1977), other larval stages of codling moth are more susceptible to parasitism (Sigsgaard, 2014). A disadvantage of our study is that parasitoids were not included in the assessment of natural enemies. Leius (1967) found, for example, that the presence of wildflowers in apple orchards increased the parasitism of codling moth larvae fivefold, while other studies did not find such a difference (Dib et al., 2012; Sigsgaard, 2014). Furthermore, Markó et al. (2012) did not find any reduction in the codling moth damage due to sown flower strips in an orchard with integrated pest management. Further research is thus needed to quantify predation as well as parasitism of codling moth and a potential effect of flower strips.

Although flower strips significantly increased the abundance of natural enemies along with a reduction of pests and fruit damage in our study, sentinel-prey cards showed no significant increase in the activity of natural enemies in the flower strip plots as compared to the control plots (Tables 2 & 3). Sentinel-prey card usage is itself not without controversy (Lovei and Ferrante, 2017). Notably this method rarely takes the entire predation pressure into account, since the size of the prey and the exposure time are not adjusted to all natural enemy groups (Lovei and Ferrante, 2017) and predators attacking sentinel-prey cards do not necessarily also attack the investigated pests (Campbell et al., 2017). Sentinel-prey card usage may therefore not adequately reflect

the actual activity of natural enemies in the field against the pests of interest. Nevertheless, the proportion of attacked eggs increased from after flowering to after the second fruit drop and with increasing northern latitude (Table 3). The egg cards were therefore not completely indifferent to a certain predator activity, but could reflect mainly the activity of Forficulidae that were not affected by flower strips. Therefore, it is difficult to draw exact conclusions.

3.3. Conclusions

In the present study, we show that perennial flower strips sown in the alleyways of apple orchards increase the number of natural enemies on plant parts, and specifically in *D. plantaginea* colonies on trees, in different climatic regions across Europe. At the same time, a reduction of key pests such as the rosy apple aphid as well as fruit damage before fruit pruning was observed. Furthermore, the increasing control of codling moth from 2016 to 2017 implies that natural enemies and the associated conservation biological control may increase with subsequent years. Indeed, the benefits of an established perennial flower strip may be best observed over a more prolonged period. The observed reduction in fruit damage may not support the use of this conservation biological control strategy as a stand-alone practice, but our study supports the role of functional agrobiodiversity as a way to potentially reduce insecticide use in orchards.

Author contributions

All coauthors conceived and designed the study. CD, FC, LP, LS drafted the protocols for arthropod sampling. LP developed the flower strip and its management strategy. AH, LP, MT organized and coordinated additional financing. FC, LP, LJ, LS coordinated and supervised the study and AH, DK, FC, LJ, LP, LS, MK, MT, WS, MP coordinated the study within specific countries. CD, FC, JT, LJ, MP, MS, MT, SKJ, SM, WS performed the experiments. FC analyzed the data. FC wrote the draft manuscript, supplemented by contributions from AH, CD, LJ, LP, LS, MP, MT, SKJ, SM WS. All authors read and approved the manuscript.

Declarations of interest

None.

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Appendix A. Supplementary data

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