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Influence of the margin vegetation on the conservation of aphid biological control in apple orchards

Luan Alberto Odorizzi Santos^{1,3} · Marlice Botelho Costa^{2,3} · Claire Lavigne³ · Odair Aparecido Fernandes¹ · Armin Bischoff⁴ · Pierre Franck³ 

Abstract

The influence of three margin strip treatments (wildflower strips, grass strips and spontaneous vegetation) adjacent to apple orchards on the biological control of *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) was compared during two consecutive years. The wildflower strips provided the highest amount of floral resources. Within the margin strips, hoverflies responded positively to higher resource provisioning whereas ladybird abundance did not differ between strip treatments. Within the orchards, the presence of parasitoids, hoverflies, and ladybirds in aphid colonies and the predation of sentinel aphids were not significantly affected by the adjacent strip treatments. The number of natural enemies observed in aphid colonies was mainly driven by aphid number. Aphid numbers were higher close to the margin strips suggesting that aphid colonization from orchard edges may counteract the positive effect of wildflower strips on natural enemy abundance and on a reduction of aphid infestation. The results confirm the positive influence of floral resource provisioning by wildflower strips on the conservation of aphid natural enemies, but also suggest that effects of wildflower strips on aphid regulation inside orchards are not very strong compared with spontaneous vegetation naturally occurring in the margins.

Keywords Hemiptera · Aphididae · Aphidophagous predators · *Dysaphis plantaginea* · Conservation biological control · Floral resources · Apple orchard

Introduction

The management of non-crop habitats in agricultural landscapes has received a considerable interest during the last decades as a way to conserve biodiversity and agricultural sustainability (e.g. Scherr and McNeely 2008). The semi-natural vegetation in the farmland support shelter and resource provision for many arthropods and it can be manipulated to reinforce pest biological control (e.g. Landis et al. 2000; Gurr et al. 2017). The implementation of wildflower strips optimizing floral resource provisioning has been suggested to improve conservation biological control (Griffiths et al. 2008; Dib et al. 2012; Tschumi et al. 2015). Such wildflower strips have positive effects on natural enemy abundance and associated ecosystem services (Wyss and Pfiffner 2008; Haaland et al. 2011; Balzan et al. 2014). Sugar resources from floral or extrafloral nectars significantly increase the fitness of many parasitoids and hoverfly species and, usually, their efficiency to control insect pests (Wäckers 2004; Winkler et al. 2006; Laubertie et al. 2012). The choice of plant species for wildflower strips needs to

take into account the availability of pollen and nectar, the floral morphology, and the feeding preference of natural enemies (Sivinski et al. 2011; Campbell et al. 2012; Wäckers and van Rijn 2012). Plants in field margins may also provide resources for insect pests and increase crop damage (Koji et al. 2007). Thus, plant species in wildflower strips need to be properly selected to favour natural enemies without increasing pest abundance (Lee and Heimpel 2005; Lavantero et al. 2006).

The rosy apple aphid (RAA), *Dysaphis plantaginea* Pas-serini (Hemiptera: Aphididae), is a major insect pest in apple orchards causing considerable damages, in particular fruit deformation (Blommers et al. 2004). Due to economic losses and socio-economic pressure to reduce pesticide applications, an increasing number of studies analyzed the efficiency of naturally occurring parasitoids and predators in controlling RAA populations (e.g. Albert et al. 2017). RAA is attacked by numerous generalist predator species such as spiders and earwigs (Dib et al. 2016; Lefebvre et al. 2017), and aphidophagous specialists such as hoverflies, ladybirds, and hymenopteran parasitoids (Wyss 1995; Dib et al. 2010; Cahenzli et al. 2017). Among these RAA natural enemies, the most common species observed in south-eastern France were the hoverfly *Episyrphus balteatus* (De Geer) (Syrphidae), the ladybug *Adalia bipunctata* L. (Coccinellidae), the parasitoid *Ephedrus persicae* Froggatt (Braconidae) and the earwig *Forficula auricularia* L. (Forficulidae). No evidence of intra- or inter-specific competition on RAA prey was observed so far within these RAA natural enemy species (Wyss et al. 1999; Dib et al. 2016). Hoverflies and parasitoids depend on nectar and pollen and it was hypothesized that vegetation providing these resources may improve spring regulation of aphids in apple orchards (Markó et al. 2013; Miñarro and Prida 2013).

We designed a wildflower strip mixture comprising flowering species with different functional traits and life cycles (early to late flowering, annual to perennial, and various floral morphologies) in order to optimize floral resource provisioning. The effects of this wildflower strip mixture were compared with two different controls, spontaneous vegetation (SV) and grass strips. The effect of wildflower strips on the dynamics of the arthropod communities has usually been tested against the complete absence of non-crop vegetation (Pfiffner et al. 2009; Haaland et al. 2011; Tschumi et al. 2016). However, SV may be as efficient as flower strips in attracting natural enemies of insect pests (Denys and Tschartke 2002) without producing costs for seed material and strip management. Sowing of grass strips may be a cost-efficient alternative providing habitat functions for natural enemies (Collins et al. 2002; Al Hassan et al. 2013). They provide less floral resources but generalist predators such as spiders, ground and rove beetles may benefit from their shelter function (Wyss 1995; Collins et al.

2002). An efficient control of RAA requires a natural enemy movement from the field margins into the apple orchard. Most studies on wildflower strips are limited to observations of natural enemy diversity and abundance within the strips (Haaland et al. 2011; Blaauw and Isaacs 2014). The studies that analyzed the presence of natural enemies both in wildflower strips and within the crops usually confirmed the attraction of natural enemies by flowering plants but not always a corresponding reduction of insect pests (Pfiffner and Wyss 2004; Haaland et al. 2011). The few studies demonstrating a significant contribution of wildflower strips to pest control, showed that their contribution to pest control decreased with the distance to field margins (Collins et al. 2002; Tylianakis et al. 2004; Tschumi et al. 2015; Albert et al. 2017). A better understanding of the spatial dynamics of natural enemies is needed to evaluate the efficiency of wildflower strips and to improve regulation of aphids in orchards.

In this study, we tested the influence of three margin strips (wildflower strips, grass strips, and SV) on the abundance of RAA and its natural enemies from the margin into apple orchard during two consecutive years in order to answer several unsolved questions: (I) Do wildflower strips optimized for nectar and pollen production increase natural enemy abundance in orchard margins in comparison with grass and SV strips? (II) Does the attraction of natural enemies by the flowering plants in the margins also increase their abundance inside the orchards? (III) Is the decrease in RAA abundance close to orchard margins presenting floral resources due to higher natural enemy density? (IV) Do orchard margin effects on natural enemy abundances and aphid control decrease with distance to the edge?

Materials and methods

Study sites and design

The experiment was set up in the three mono-cultivar apple orchards located at INRA Saint-Paul, Montfavet, France (43°54'51.57"N, 4°52'56.15"E). The two first orchards were contiguous, each comprising five rows of 24 apple trees planted in 1996 (cv Royal Gala or Granny). The third orchard, distant from the two first ones by 250–290 m, comprised six rows of 48 apple trees planted in 2004 (cv Ariane). A hedgerow parallel to the apple rows bordered the northern side of two orchards (cv Royal Gala and Ariane). A permanent grass sward was regularly mown largely limiting the presence of floral resources within each orchard. No pesticides were applied in any orchard during the study period that ran from March 2014 to June 2015. In March 2014, a 2.5 m wide and 40–80 m long margin was ploughed and then harrowed to remove weed seedlings emerging after

ploughing. In each orchard, this margin was subdivided into three strips of equal size to establish the following three treatments (Fig. 1): (a) a wildflower strip mixture comprising 30 vascular plant species (WS; Table S1) optimized for a high and long lasting production of floral resources, (b) a grass strip (GS) including two perennial grasses, *Lolium perenne* L. (28%) and *Festuca arundinacea* Schreb (72%), used in France to sow buffer zones between field and water bodies to limit water pollution by fertilizer and pesticide drift, (c) an unsown strip allowing the natural development of SV. The minimum distance between the margin strips among the orchards was 40 m. The position of the strip treatments was randomized within each orchard margin. Thus, each orchard was considered to represent a replicate block. The approach to set up all treatments within the same orchard is relatively conservative since mobile arthropods may move between different strip types and the adjacent orchard, which may reduce the probability to find significant treatment differences. However, this design largely reduced confusion of the treatment effects with the influence of cultivar, plantation date or other environmental factors compared with designs that would apply each strip treatment in a different orchard.

Observations within margin strips

Plant species composition and provision of floral resources were evaluated in spring 2014 and 2015. First, plant richness was estimated based on the number of vascular plant species observed in each margin strip. Second, we calculated the percentage cover of flowering entomophilous plants as a proxy of floral resource provisioning. Percentage cover of all vascular plant species and proportion of vegetative, flowering and fruiting individuals of each plant species were recorded in each strip treatment at each orchard. We considered a plant species as entomophilous if indicated as insect-pollinated in the BioFlor database (Kühn et al. 2004). Percentage of each entomophilous plant was multiplied by the estimated proportion of flowering individuals at the respective survey date, which were added for all the entomophilous species to estimate the total percentage cover of flowering

entomophilous plants in each margin strip. Survey dates were the 6 June 2014 and the 22 May 2015 corresponding to peaks of RAA abundance.

At the same dates, aphidophagous predators that were visible from outside the strip were counted during 10 min walking along each margin strip to evaluate the abundance of hoverfly and ladybug predators per margin strip. The entomological observations were limited to a period between 10:00 and 17:00 under favorable weather conditions (sunny, no rain, low wind speed).

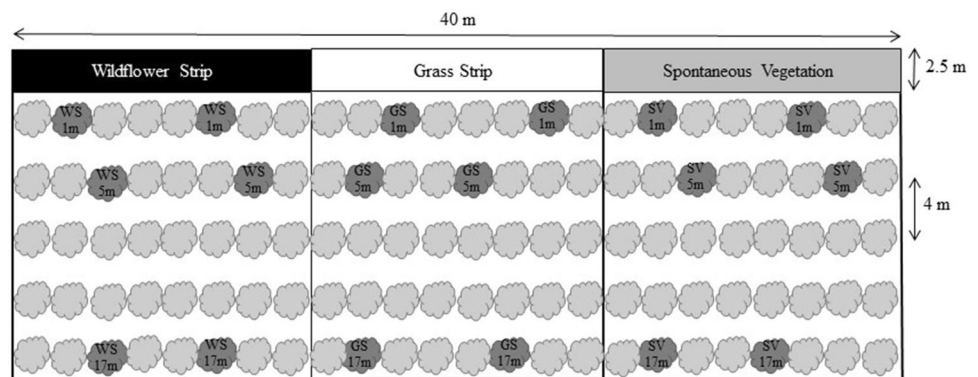
Observations within apple orchards

Depending on orchard size, observations were performed on two or three labeled trees per treatment and per distance resulting in a total of 18 or 27 analyzed trees per orchard (Fig. 1). According to the position of the apple tree rows, the distances of the observed trees were 1, 5, and 17 m from the margin strips that are the main distances at which spillover from the margins occur (Brown and Lightner 1997). The same trees were observed twice a year at the end of April or the beginning of May (session 1) and 4 weeks later (session 2). Observations were performed at the tree and the RAA colony levels to calculate estimators of aphid infestation, change in aphid infestation between sessions, numbers of RAA natural enemies, and predation of sentinel aphids within the apple orchards.

First, the numbers of terminal growing shoots and of RAA colonies were counted in the canopy of each labeled tree. The terminal growing shoots were counted to take into account the apple tree vigor because mainly those shoots are attacked by RAA (Simon et al. 2012; Rousselin et al. 2018). Aphid colony infestation per apple tree was calculated as the proportion of terminal growing shoots with a *D. plantaginea* colony. Aphid colony increment per apple tree was defined as the occurrence of trees with higher number of RAA colonies at session 2 than at session 1.

Second, three RAA colonies were randomly chosen at the northern and at the southern sides of each labeled tree (up to six colonies per tree). The number of aphids, mummies

Fig. 1 Experimental design. Shaded symbols indicate observation trees at three distance classes (1, 5 and 17 m) from the orchard margin treatments



(dead bodies of aphids parasitized by braconid wasp) and aphid predators (mainly hoverflies, ladybugs, and earwigs) were recorded in each colony. Parasitism rate in the RAA colony was estimated as the ratio of the number of mummies on the total number of aphids assuming that parasitized and non-parasitized aphid were eaten at the same rates by the RAA predators. Occurrences per apple tree of each RAA natural enemy (aphid mummy or RAA predators) were estimated as their occurrences in at least one of the six colonies observed per tree.

Finally, sentinel aphids glued to sandpaper cards were used to evaluate potential aphid predation at tree level (Östman 2004). Three living aphids were attached to each 5 cm x 5 cm semi-rigid sandpaper card. The cards were fixed at the abaxial leaf surface of the observation trees to avoid sun exposition (1.5 m above the ground) and were removed the following day. Aphid predation was estimated as the proportion of sentinel aphids per card attacked during 24 h.

Statistical analysis

We used generalized linear models (*glmer* function, lme4 package, R version 3.2.4) to test: (1) the effect of the strip treatment on the number of vascular plant species, on the cover of flowering entomophilous plants, and on the abundance of aphid enemies in the orchard margins; (2) the effects of the strip treatment, of the distance to the orchard margin and the treatment x distance interaction on aphid infestation, aphid colony increment between session, occurrences of RAA natural enemies, and predation of sentinel aphids at apple tree level; (3) correlations between the number of aphids and the number of natural enemies at the RAA colony level. To take into account temporal variation, year and session (when relevant) were fitted as additional factors.

The plant species richness (number of vascular plant species) and the cover of flowering entomophilous plants (arcsin square root transformation of the percentage cover) were fitted using a Gaussian error distribution with identity link function. The abundance of natural enemies within margin strips was analyzed using a Poisson error distribution with log link function. Finally, the binary data of aphid infestation, aphid colony increment, predation of sentinel aphids and occurrence of mummies and RAA predators per apple tree were analyzed using a binomial error distribution with a logit function. Linear regressions were calculated to analyze relationships between the number of aphids (ln-transformed) and the total number of natural enemies (ln-transformed) within the RAA colonies.

Strip treatment (WS, GS, SV), distance to the margin strips (1, 5, and 17 m), year (2014 and 2015), observation date within year (session 1 and 2) and treatment x distance interaction were fitted as fixed factors. Orchard was included as a random factor in each model. In case of over-dispersion,

an additional random factor (corresponding to the observation tree label) was included in the model (Harrison 2014). A reduced model only comprising strip treatment and year was applied to test observations within the margins.

The *Anova* and *glht* functions (car and multcomp R libraries, respectively) were used to test factors and the treatment x distance interaction (Wald's sequential probability likelihood ratio tests) and to compare the effects of a particular strip treatment and distance classes within each significant factor (Z-tests).

Results

Observations within margin strips

As expected, the cover of flowering entomophilous species and the number of vascular species significantly differed among the margin strips, but differences between years were not significant (Table 2). The cover of flowering entomophilous species was 2.5 fold higher on average in wildflower strips than in grass strips and in SV (Fig. 2A). No significant difference occurred between the SV and the grass strips. The number of vascular species per strip was also significantly higher in wildflower strips than in SV and grassy strips (Fig. 2B). On average, the wildflower strips presented ten more plant species than the other treatments.

Hoverflies and ladybugs, notably *E. balteatus* and *A. bipunctata*, were the most abundant aphid predators recorded in the margin strips. Hoverfly abundance differed significantly between years and strip treatments (Table 2). Hoverfly abundance increased from three individuals per strip on average in 2014 to 12 individuals per strip on average in 2015. The significant strip treatment effect was explained by a higher hoverfly abundance in the wildflower strips compared with grass and the SV strips (Fig. 3A, B). Ladybug abundance within margin strips (mean = $3.4 \pm SD 4.4$) did neither differ between years nor between treatments (Table 2).

Observations within the orchard on the apple tree

Inside orchards, ladybugs, hoverflies, and earwigs were the most frequently observed predators within the RAA colonies (Table 1). Lacewings, predatory bugs and spiders were only observed in a few colonies each year. Aphid mummies were only observed in the second spring session. The occurrence of all these RAA natural enemies per apple tree differed significantly between years and between spring sessions, but not among margin strip treatments, or among distance classes to the margin strip (Table 2). The occurrence of hoverfly larvae doubled between 2014 and 2015 (25–54% of the apple trees), but did not significantly differ among the strip

Fig. 2 Mean (\pm SE) cover of entomophilous flowering plants (A, B) and number of plant species (C, D) in 2014 (A, C) and in 2015 (B, D) in each orchard margin treatment; WS wildflower strip, SV spontaneous vegetation strips, GS grass strip. Different lowercase letters above columns indicate significant differences at $p < 0.05$

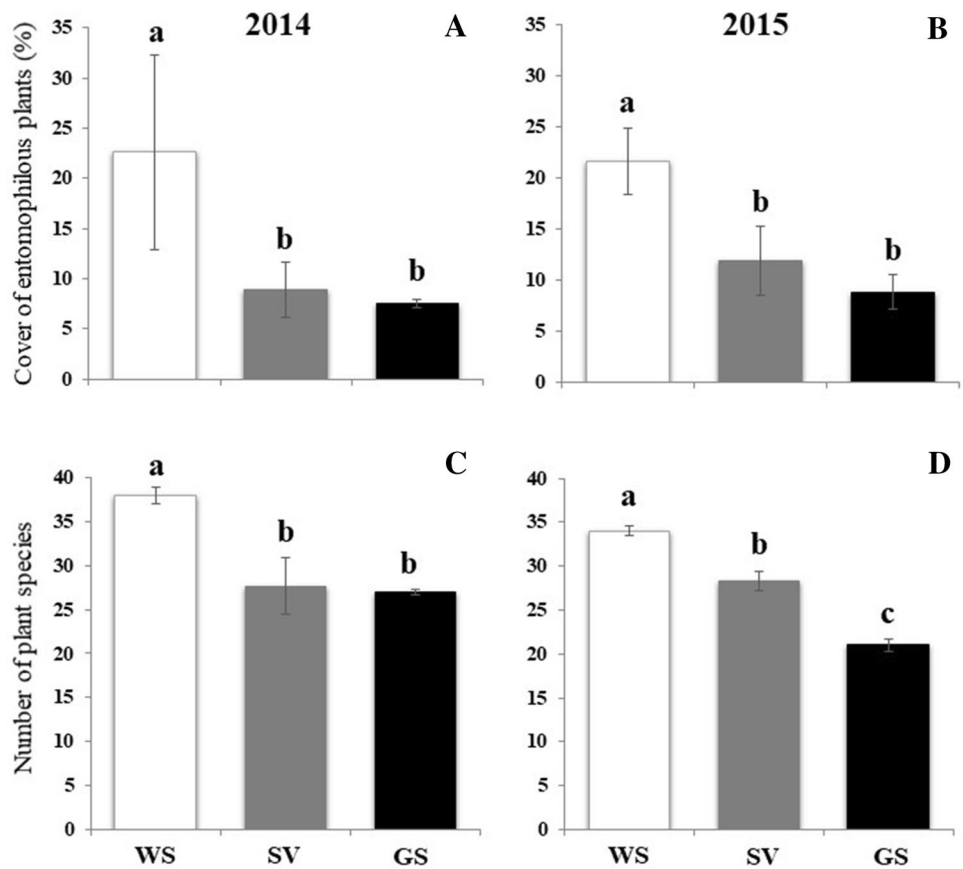


Fig. 3 Mean (\pm SE) number of hoverflies observed in margin strips (A, B) and in *D. plantaginea* colonies inside the orchards (C, D) in 2014 (A, C) and in 2015 (B, D); WS wildflower strip, SV spontaneous vegetation strips, GS grass strip. Different lowercase letters above columns indicate significant differences at $P < 0.05$

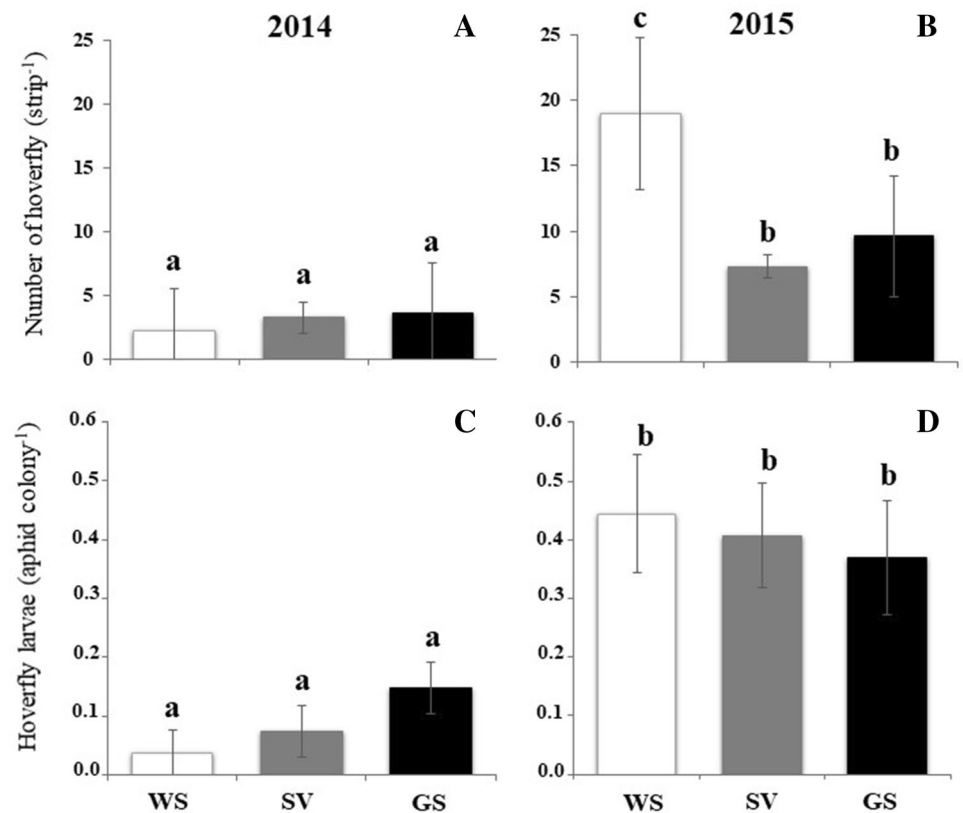


Table 1 Mean number of *D. plantaginea* colonies (aphid colonies) and mean occurrence of RAA predators (hoverflies, ladybirds and earwigs) or aphid mummies (parasitized aphids) per apple tree in 2014 and in 2015

	2014		2015	
	6 May	3 June	24 April	19 May
Aphid colonies	19.9 [15.3–24.6]	41.9 [31.8–56.0]	1.5 [1.5–1.6]	22.3 [11.3–27.4]
Hoverflies	0.3 [0.2–0.5]	0.1 [0.0–0.4]	0.0 [0.0–0.0]	0.7 [0.4–0.9]
Ladybirds	0.1 [0.0–0.2]	0.6 [0.5–0.9]	0.0 [0.0–0.0]	0.5 [0.2–0.7]
Earwigs	0.1 [0.1–0.2]	0.6 [0.5–0.9]	0.0 [0.0–0.0]	0.2 [0.0–0.8]
Parasitized aphids	0.0 [0.0–0.0]	0.7 [0.5–1.0]	0.0 [0.0–0.0]	0.6 [0.3–0.7]

Minimum and maximum values per orchard are indicated in brackets

Table 2 Temporal and spatial variation at the apple tree level of aphid infestation (proportion of terminal growing shoots with at least one *D. plantaginea* colony), aphid colony increment (occurrence of a higher number of *D. plantaginea* colonies at session 2 than session

1), number of growing shoots, sentinel aphid predation and natural enemy occurrences. Temporal and spatial variation in orchard margins of plant species richness, cover of flowering entomophilous plants and hoverfly and ladybird abundances

Response variables	df	Year	Session	Strip treatment	Distance to margin	Spatial interaction
		1	1	2	2	4
Plant species richness in margins	χ^2	1.5	–	15.1	–	–
	P-value	0.223	–	5.2×10^{-4}	–	–
Cover of flowering entomophilous plants in margins	χ^2	0.3	–	14.4	–	–
	P-value	0.598	–	7.4×10^{-4}	–	–
Hoverfly abundance in margins	χ^2	50.2	–	11.8	–	–
	P-value	1.3×10^{-12}	–	0.003	–	–
Ladybug abundance in margins	χ^2	0.8	–	0.7	–	–
	P-value	0.366	–	0.696	–	–
Number of growing shoots	χ^2	18.3	52.1	5.5	1.3	4.0
	P-value	1.8×10^{-5}	5.1×10^{-13}	0.064	0.510	0.406
Aphid infestation	χ^2	120.7	81.9	0.5	7.1	6.7
	P-value	$< 2.2 \times 10^{-16}$	$< 2.2 \times 10^{-16}$	0.758	0.028	0.149
Colony increment	χ^2	0.4	–	0.7	5.1	5.1
	P-value	0.548	–	0.704	0.079	0.279
Predation of sentinel aphids	χ^2	0.5	0.3	1.1	0.4	2.6
	P-value	0.466	0.560	0.572	0.810	0.625
Total natural enemy occurrence	χ^2	10.9	32.8	1.5	0.6	1.4
	P-value	9.4×10^{-4}	9.8×10^{-9}	0.380	0.970	0.831
Ladybug occurrence	χ^2	6.1	41.8	0.7	4.1	2.7
	P-value	0.014	1.0×10^{-10}	0.690	0.127	0.611
Hoverfly occurrence	χ^2	13.0	0.5	0.9	2.5	1.8
	P-value	3.0×10^{-4}	0.465	0.646	0.292	0.781
Earwing occurrence	χ^2	26.2	27.1	2.0	0.6	7.4
	P-value	3.1×10^{-7}	1.9×10^{-7}	0.368	0.757	0.118
Mummy occurrence	χ^2	3.4	–	2.9	3.6	3.7
	P-value	0.065	–	0.237	0.167	0.449

Spatial interaction refers to margin strip treatment \times distance interaction. Significant *P*-values were highlighted in bold

treatments (Table 2; Fig. 3C, D). The occurrence of earwigs dramatically decreased between 2014 and 2015 (42–2% of the apple trees; Table 2). The occurrence of aphid mummies were slightly higher in 2014 than in 2015 (75 and 56% of the apple trees, respectively; Table 2).

On average, 18 RAA colonies were observed per tree and per date (Table 1). Aphid infestation was estimated as the proportion of terminal growing shoots carrying a RAA colony. The number of growing shoots per tree varied between years and between sessions, and marginally differed among

the strip treatments (Table 2). Infestation by RAA was significantly different between years, sessions within years and distances from the margin strips (Table 2). Infestation by RAA was three times lower in 2015 than in 2014 (16 and 55% of the growing shoots, respectively). Infestation by RAA was significantly lower at 17 m than at 5 or 1 m from the margin strips (33, 37 and 36% of the growing shoots at 17, 5 and 1 m distances, respectively).

The increment of RAA colonies was measured to evaluate variation of aphid infestation between sessions at the tree level. The proportion of apple trees with more RAA colonies at session 2 than at session 1 was marginally higher at the closest distances to the orchard margin strips (71, 74 and 62% of the apple trees at 1, 5 and 17 m distances respectively; Table 2).

On average, 25% of the sentinel aphids were preyed, but their predation was neither spatially structured within the orchards between distance classes, nor between margin strip treatments, nor temporally structured between sessions or between years (Table 2).

Observations within rosy apple aphid colonies

Very few aphids within RAA colonies were parasitized at the second session, but parasitism rate differed among the classes of distance ($\chi^2 = 20.2$, $df = 2$, $P = 4.0 \times 10^{-5}$). It was significantly lower at the closest distances to the orchard margin strips than at the 17 m distance (in average, 1.3 ± 0.7 and $3.2 \pm 1.8\%$, respectively). Linear regressions between the number of aphids and the total number of their natural enemies in RAA colonies were used to test density-dependence effects of margin strip treatments. Numbers of natural enemies per RAA colony were significantly correlated to numbers of aphids (one natural enemy for 34 aphids on average; slope: $\chi^2 = 14.93$, $P = 1.1 \times 10^{-4}$; intercept: $\chi^2 = 5.04$, $P = 0.025$). Separate linear regressions for each margin strip treatment were significant for the SV treatment (slope: $\chi^2 = 14.90$, $P = 1.1 \times 10^{-4}$) but not for the wildflower and grass strip treatments (Fig. 4). The intercept was only significantly different from 0 in the wildflower strip treatment (intercept: $\chi^2 = 4.28$, $P = 0.039$; Fig. 4) indicating a higher availability of natural enemies in the absence of aphids.

Discussion

Our results showed that the selected wildflower strip mixture optimized for quantity and duration of nectar production was successful in increasing floral resource provisioning. The cover of flowering entomophilous plants species as a proxy for nectar and pollen production was significantly higher in wildflower strips than in grass and SV strips. Plant species richness was also higher although not all sown species

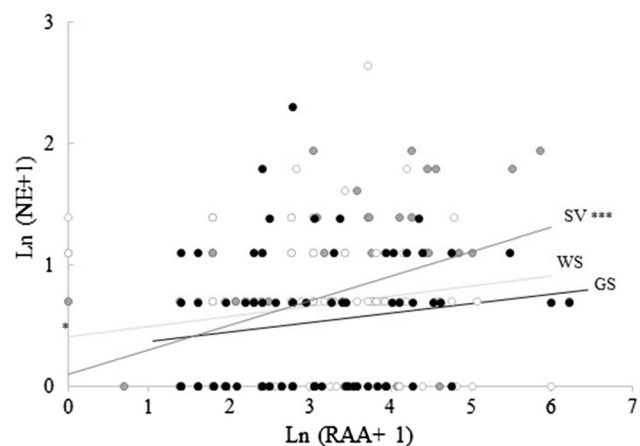


Fig. 4 Relationship between the number of aphids (RAA) and the number of natural enemies (NE) per aphid colony at the second 2015 session (ln-transformed values). Regression lines are presented to illustrate slope and intercept differences between WS (wildflower strip, white circles), SV (spontaneous vegetation strips, grey circles), and GS (grass strip, black circles) treatments. Stars indicate slopes or intercepts that significantly differ from zero ($*P < 0.05$; $***P < 0.001$)

developed in the wildflower strips. Only small differences occurred between grassy and SV strips but plant species richness in the grass strips was lower confirming the suppressive effect of competitive grass mixtures on spontaneously emerging species (Cordeau et al. 2012). SV emerging from the soil seed bank considerably contributed to both plant diversity and floral resources in both sown strips reducing the magnitude of treatment effects compared with other studies (Bischoff et al. 2016). Annual plant species were dominant during the first year but were replaced by biennials and perennials in the course of plant succession.

Among the two major aphid predators observed in the strips, only hoverflies responded to the strip treatment. Hoverfly densities were higher in the wildflower strips than in the other strips. Adult hoverflies rely on nectar resources (Wäckers 2004; Fiedler et al. 2008). Laboratory experiments on the aphidophagous hoverfly *E. balteatus* showed that adult fitness depends on floral resource provisioning (Laubertie et al. 2012). Markó et al. (2013) and Miñarro and Prida (2013) also found a positive effect of the surrounding vegetation on hoverfly abundance in orchards. Ladybirds did not respond to the strip treatments. Although occasionally feeding on pollen and nectar (Triltsch 1999), their performance depends much less on floral resources because larvae and adults are predominantly predators (Ricci et al. 2005). Nevertheless, several other studies found a positive effect of floral resources on ladybird abundance (Tschumi et al. 2015; Bischoff et al. 2016).

The effect of strip treatments on natural enemies observed in aphid colonies inside orchards was not significant. The energetic costs and the relative attractiveness of margin

strips may have reduced natural enemy movements into the orchard (Heimpel and Jervis 2005; Wanner et al. 2006). However, hoverflies that strongly responded to the strip treatments are highly mobile insects. Hoverfly movements between the different treatment blocks may have reduced the probability to find significant treatment effects. Additionally, a time lag has to be considered between the measures of hoverfly abundance in wildflower strips and the occurrence of larvae in the orchards. Effects of resource provisioning on adult oviposition occur with a delay since larvae hatch after a couple of days and larvae feed for about three weeks on aphids (Laubertie et al. 2012; van Rijn and Wäckers 2016).

A combination of generalist predators, including some non-observed predators in the margin strips may contribute to a reduction of aphid infestation close to the wildflower strips (Balzan et al. 2014; Tschumi et al. 2015). However, similarly to the natural enemies observed in the aphid colonies, the predation of sentinel aphids did not differ among the strip treatments neither among the classes of distance. Furthermore, the overall treatment effect on the aphid infestation was not significant. The lack of statistical power of our experiment because of a low number of replicated orchards and observations within each orchard treatment is a limitation to interpret such non-significant results. It seems that wildflower strips do not enhance aphid regulation to reduce spring RAA damages compared with less diversified plant strips, which is in agreement with previous observations in apple orchards on other aphid pests (Markó et al. 2013).

Aphid infestation and aphid colony increment were higher on apple trees close to the margin strips than on those that were inside the orchards. Inversely, the highest RAA parasitism rates were observed at the larger distances from the orchard margin. RAA secondary host during summer, *Plantago lanceolata* L. (Blommers et al. 2004), is a common species in the study region and was similarly abundant in all the strip treatments (6% of the total plant cover on average, Table S2). Although we did not observe any correlation between *P. lanceolata* cover and aphid infestation in margin strips (Table S2), it is possible that field margins represent a source of RAA infestation counteracting potential positive effects of strip treatments mediated by higher natural enemy densities.

In both years, a rapid and significant increase of aphid infestation was observed resulting in a high increment of aphid colonies and suggesting a relatively low natural regulation potential during early spring. Such a strong and rapid increase of aphid infestation was also observed in a previous study of RAA dynamics in orchards of the same region (Dib et al. 2010). An efficient biological control would require the presence of a sufficient number of natural enemies in March and April when the number of aphids per RAA colony is still low (Lefebvre et al. 2017). Most wildflower strip species started to flower later than April. In

order to provide floral resources in time, a higher proportion of early flowering plant species would be necessary for controlling the exponential development of the RAA colonies. Early flowering species such as the arable annuals *Veronica hederifolia*, *Sinapis arvensis* and *Capsella bursa-pastoris* and the perennials *Ajuga reptans* and *Bellis perennis* were included in our wildflower strip mixture but their cover was low (Table S1). Similarly, late flowering species in autumn may increase the number of web spiders in the apple orchard (Wyss et al. 1995), which may contribute to reduce aphid damage by catching RAA elates when they migrate back to their primary apple host (Cahenzli et al. 2017). Such autumnal biocontrol may partially explain the reduction of RAA infestation between the two studied years.

We found a positive relationship between aphid colony size and natural enemy density indicating that natural enemies within orchards mainly responded to the available amount of prey or hosts. However, natural enemy numbers did not significantly depend on aphid numbers in the wildflower strip treatment. In absence of aphids, the regression model predicted higher natural enemy densities for the wildflower strip treatment than for the other treatments (regression intercepts), which may be the result of a higher spill-over of natural enemies from the wildflower strip. A relatively high mobility of natural enemies attacking aphid colonies may compensate for a lower spill-over of natural enemies in the other strip treatments.

In conclusion, we found positive effects of wildflower strips on floral resource provisioning increasing the abundance of hoverflies as important natural enemies of RAA in apple orchards. However, the effect of wildflower strips on aphid control was less strong than in studies using annual crops as model system (Pfiffner et al. 2009; Tschumi et al. 2015). Such differences may be partially due to differences in the experimental design. Most of the studies performed in annual crops have used less attractive controls than we did to evaluate wildflower strip effects (absence of vegetation, crop strips), increasing the probability to obtain significantly positive results (Haaland et al. 2011; Tschumi et al. 2015). However, differences in insect abundances between wildflower strips and other margin types also depend on the age of the wildflower strips and the time of the year (Haaland et al. 2011). However, we are convinced that a successful conservation biological control approach needs to provide a significantly better control service than spontaneously occurring vegetation to justify higher sowing and management costs. In order to improve pest regulation by wildflower strips, sowing inside orchards should be considered in order to reduce the distance of movements required to reach the target pest species (Albert et al. 2017). Our study also demonstrated the importance of temporal dynamics and the need to provide floral resources at the beginning of the season for an early control of the RAA colonies. Future studies should

focus on natural enemy and aphid movements including their temporal dynamics to improve our understanding of interactions between natural enemies and RAAs and to evaluate the efficiency of wildflower planting in orchard margins.

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Compliance with ethical standards

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

Research involving human and animal participants This work does not include experiment with any animal or human subjects performed by any of the authors.

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