


Intercropping impacts the host location behaviour and population growth of aphids

Agathe Mansion-Vaquié^{1*} , Aurélie Ferrer¹, Felipe Ramon-Portugal², Alexander Wezel¹ & Alexandra Magro^{2,3}

¹Research Unit Agroecology and Environment, ISARA-Lyon, 23 rue Jean Baldassini, Lyon 07 69364, France, ²University of Toulouse – ENSFEA, 2 rt de Narbonne, Castanet-Tolosan 31326, France, and ³UMR CNRS/UPS/IRD 5174 EDB (Laboratoire Evolution et Diversité Biologique), Toulouse F-31062, France

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Abstract

Increasing intrafield plant diversity has been shown to regulate pest populations in various agroecosystems. Among the suggested mechanisms for this bottom-up pest control, the disruptive crop hypothesis states that herbivores' abilities to locate and colonize their host plants are reduced by the presence of non-host plants. Under laboratory conditions, we evaluated how intercropping wheat and legumes modifies the behaviour of apterous cereal aphids, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae), in terms of host plant location and population growth. We compared two intercropping systems – soft winter wheat, *Triticum aestivum* L. (Poaceae), associated with winter pea, *Pisum sativum* L., or with white clover, *Trifolium repens* L. (both Fabaceae) – and sole stands of soft winter wheat. Aphids needed more time to locate their wheat host plant and then spent less time on wheat when it was intercropped with clover. At the population level, and accounting for host plant biomass, only intercropping wheat with clover significantly reduced aphid densities on wheat, as this was particularly disruptive to *S. avenae* behaviour and population growth. Our laboratory study points out that the species used as non-host plants and their density are important parameters that should be taken into account in field studies on intercropping systems.

Introduction

The link between biodiversity and ecosystem functioning has long been accepted (Naeem et al., 2002) and many experimental studies have shown that diversity is a key determinant of ecosystem processes, such as plant productivity (Tilman, 2015). Currently, a paradigm change is underway in agroecosystem management, with attempts to increase biodiversity at different spatio-temporal scales, from the crop field, to the farm, and finally across the landscape (Garibaldi et al., 2017). At the field level, increased diversity is mainly achieved by intercropping (Andow, 1991), defined as 'the cultivation of two or more species of crop in such a way that they interact agronomically' (Vandermeer, 1989). We may distinguish two types of intercropping: 'true intercropping' (Willey, 1979), that is the

simultaneous cultivation of two cash crops, and 'companion cropping', the cultivation of a cash crop and a beneficial non-crop (Ben-Issa et al., 2017; Verret et al., 2017). An increase in productivity in intercropping systems may be achieved through an improved yield per unit area, due to complementary use of resources, facilitation, and/or increased pest regulation (Brooker et al., 2015).

In this study, we are interested in the role of intercropping on the control of aphids (Hemiptera: Aphididae), which are among the main pests in temperate regions (Dedryver et al., 2010). We focus on cereals and legumes because cereals are important in most temperate farming systems and are dominant crops in terms of agricultural area, and the cultivation of legumes for animal feed, food, and other ecosystem services (e.g., supply of nitrogen) is increasing (Stagnari et al., 2017). Intercropping cereals and legumes has several agronomical and environmental advantages (Bedoussac & Justes, 2010a; Lithourgidis et al., 2011; Pelzer et al., 2012; Bedoussac et al., 2015), including the reduction in numbers of cereal and legume aphids (Ndzana et al., 2014; Lopes et al., 2015; Hatt et al., 2018;

*Correspondence: Agathe Mansion-Vaquié, Research Unit Agroecology and Environment, ISARA-Lyon, 23 rue Jean Baldassini, Lyon 07 69364, France. E-mail: agathe.mansion-vaquie@hotmail.fr

Xu et al., 2018). However, the ecological mechanisms at the origin of these changes in abundance are still under discussion.

Plant diversity may promote pest regulation either bottom-up, driven by lower trophic levels, or top-down, driven by higher trophic levels (Gurr et al., 2017). Aphid control seems to depend on both, with top-down effects of natural enemies (specialists or generalists) being modulated by interactions between aphids and their host plant (Diehl et al., 2013). Several hypotheses explain how increasing intrafield diversity may promote bottom-up control. The ‘disruptive crop’ hypothesis (Vandermeer, 1989) suggests that the presence of a non-host plant would lower a herbivore’s ability to locate and colonize its host plant, because of chemical and physical confusion imposed by a non-host species and/or because the herbivore is more likely to leave a patch following repeated encounters with a non-target plant. A non-host plant may interfere with the chemical cues used by a herbivore to locate its host plant by masking the host’s odours, altering the host’s chemical profile, or introducing repellent compounds (Randlkofer et al., 2010; Ben-Issa et al., 2017). Concerning physical interference, Perrin & Phillips (1978) refer to physical barriers, that is, the more arduous physical access of the herbivores to their host plants due to the increased distance between host plants or the presence of taller non-host plants. Additionally, camouflage in which host plants might be hidden by the presence of other green foliage or background (Smith, 1976; Francis, 1989) might also create physical disturbance. A more complex architecture of the vegetation would disrupt the herbivores’ behaviour and prevent them from easily reaching their host plant. One of the most important ways in which aphids find and select a host plant is by chemical cues (Webster, 2012; Döring, 2014), and chemical interference in aphid host plant location by non-host plants has been demonstrated (Xie et al., 2012; Ninkovic et al., 2013). However, less attention has been paid to the role of physical interference of non-host plants in the way aphids find their hosts.

Although aphids initially infest annual crops through the migration of flying morphs from surrounding habitats (Fievet et al., 2007; Irwin et al., 2007), later generations mainly comprise wingless individuals. In this work we are interested in this later phase of the infestation and on how intercropping might disrupt the behaviour and population growth of apterous aphids. Walking represents a frequent mode of dispersal during an aphid’s lifetime (Irwin et al., 2007): when the competition in a colony increases, local dispersion, such as intra- or inter-plant movements, acquires great importance and affects the aphids’ colonization process (Lombaert et al., 2006). For instance, in an alfalfa field experiment, the majority of apterous pea

aphids could walk up to 2 m within 4 h (Ben-Ari et al., 2015). Although walking plays a decisive role in the spread of the infestation at the field scale (Hodgson, 1991), on the individual aphid scale, leaving a plant to look for another is both risky and energy-expensive. Without a doubt, this carries fitness costs, and thus trade-offs between foraging and reproductive success are common (Stearns, 1992). In aphids the various dispersal strategies between alate and apterous individuals correspond to different sizes of gonads, with alate aphids being less fecund than apterous aphids (Dixon et al., 1993; Braendle et al., 2006). Moreover, apterous pea aphids that had dropped off or walked away from their host plant to avoid predators were shown to have reduced numbers of offspring, with consequences for population growth, compared to apterous aphids undisturbed by predators (Nelson et al., 2004).

In this study, we compared the host location behaviour and population growth of apterous individuals of the cereal aphid *Sitobion avenae* (Fabricius) in two intercropping and one mono-cropping systems: soft winter wheat, *Triticum aestivum* L. (Poaceae)–winter pea, *Pisum sativum* L. (Fabaceae), soft winter wheat–white clover, *Trifolium repens* L. (Fabaceae), and pure stands of soft winter wheat. First, we hypothesized that aphids’ host plant location is reduced in the presence of a non-host plant, and we conducted a short-term behavioural experiment to evaluate it. Second, in a long-term experiment we tested the hypothesis that aphid population growth is limited in the presence of a non-host plant. In this experiment, we also considered that intercropping may affect plant quality, and in turn may affect aphid performance, which is determined by plant sap quality, especially in terms of nitrogen content from free amino acids (Dinant et al., 2010). Finally, we compared winter pea and white clover, two structurally different non-host plants that could be used in wheat-based intercropping systems (Lopes et al., 2016; Vrignon-Brenas et al., 2018). Winter pea is characterized by an indeterminate growth (> 60 cm plant height) (Cousin, 1997; Bedoussac & Justes, 2010b) and often produces tendrils that enhance connectivity between plants, whereas white clover mostly grows horizontally and stays low (20–30 cm plant height) (Frame & Newbould, 1986; Frame, 2005).

Material and methods

Aphid stock cultures

A *S. avenae* aphid stock culture was initiated in the laboratory using individuals obtained from Koppert, Berkel en Rodenrijs, The Netherlands (Ervibank). The colony was maintained on winter wheat grown in 2-l pots in an insect-free chamber controlled for light and temperature (L16:D8, 21 °C).

Plants

Plants used in the experiments were grown from organic seeds (wheat: Moulin Marion, St Jean sur Veyle; clover: Jouffray Drillaud, Cisse; pea: Florimond Desprez, Cappel-en-Pevele, all in France), forming three experimental treatments: wheat monoculture (W), wheat–winter pea intercrop (WP), and wheat–white clover intercrop (WC) (Figure 1). For all experiments, winter wheat (*T. aestivum* cv. Renan) was grown in 4-l pots (18 cm diameter, 15 cm high) containing Jiffy soil substrate (NFU 44-551) in an insect-free chamber controlled for light and temperature (L16:D8, 21 °C) and watered with 500 ml at seeding and subsequently with 250 ml every 2 or 3 days. No fertilizer was applied to any treatment because intercropping is mainly used in low-N-input cropping systems and organic farming, in particular for cereals (Bedoussac & Justes, 2010b). In a regular pattern, 36 wheat seeds were sown per pot at 2 cm distance between seeds. Winter pea (*P. sativum* cv. Enduro) was sown with wheat in an additive design by inserting one pea seed every three wheat seeds for a total of 12 pea seeds per pot. White clover (*T. repens* cv. Rivendel) was homogeneously sown (70 mg, i.e., ca. 100 seeds per pot) in an additive design. All the seeds were free of pesticide treatments. Seed densities correspond to the ones used in the field (Ndzana et al., 2014; Vrignon-Brenas et al., 2018). In the few cases where some seeds of wheat or pea did not germinate after 5–6 days, they were replaced with plants grown under the same conditions in extra pots. We counted 85 grown clover plants on average per experimental pot. Plants used in the experiments were 7 days old.

Behavioural experiment

In order to investigate the behaviour of apterous aphids in the various treatments (W, WP, or WC), we ran an experiment with five sessions, with 10 pots of each treatment per session. Before starting, the vegetation cover was characterized for each pot. We measured the height of five plants of each species and the number of contacts among plants (i.e., among wheat plants and between wheat and associated plants). Additionally, we calculated the percentage of total ground area covered by vegetation: each experimental pot was photographed from above and the photographs were later analysed using ImageJ v.1.44o software (National Institute of Health, Bethesda, MD, USA). The experiment took place in an insect-free chamber controlled for light and temperature (L16:D8, 21 °C).

Experimental aphids were standardized for age prior to the experiments; adult aphids were individually placed in a glass tube (2 cm diameter, 15–20 cm high) on a 7-day-old wheat plant, previously transplanted from soil to a wet cotton wool substrate. After 48 h, the adult aphid was removed and the newborn nymphs were kept in the tube for 7–8 days until they became adults. They were then transferred to an insect-free chamber controlled for light and temperature (L16:D8, 21 °C), where the experiment took place.

Aphids were starved for 3 h before the beginning of the experiment to reduce the variability due to individual physiological conditions (Quiroz et al., 1992; Caillaud & Via, 2000). Then, using a fine brush, each aphid was carefully placed on the ground at the centre of a pot, and once the aphids started to move, the time spent on the wheat or

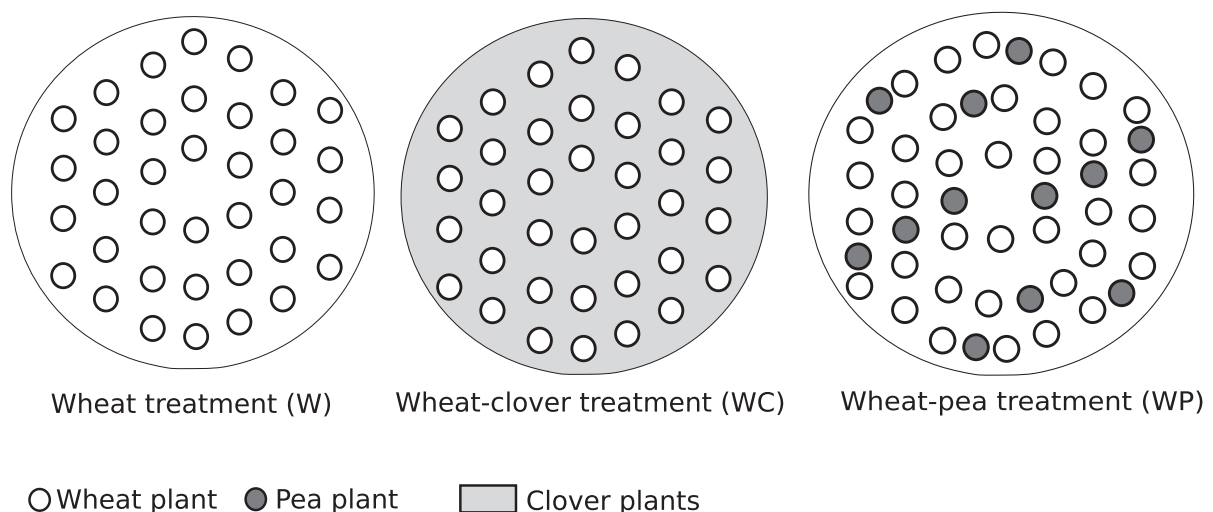


Figure 1 Schematic representation of the experimental design: seeds of wheat, pea, and clover were sown in 4-l pots in the laboratory, to form three treatments: wheat monoculture (W), wheat–clover intercrop (WC), or wheat–pea intercrop (WP).

the associated non-host plant or the ground was recorded continuously for 5 min. Additionally, the position of the aphid was recorded at 10, 20, 30, and 60 min after the beginning of the experiment. Due to the small size of the clover, it was difficult to follow the movement of the aphid from one individual clover plant to another without disturbing it; therefore we considered visits to the clover cover as a whole and recorded when it moved from the clover cover to the ground, or from clover to wheat plants and vice versa. Whenever the aphid reached the edge of the pot, the observation stopped and the aphid was considered to be escaping and so was removed from the experiment. In total 149 aphids were monitored.

Aphid population growth experiment

In order to investigate the aphid population growth in the various treatments, we used 66 experimental pots per treatment (i.e., 198 experimental pots in total). On the first day of the experiment and for each treatment, half of the experimental pots were inoculated each with 10 apterous aphids: five standardized adults (same protocol as above) and five randomly selected last instars from the stock cultures; the remaining pots were the non-infested controls. All pots were enclosed in a plastic frame (37 cm high, 20.5 cm diameter) covered with a tight meshed net, which prevented the aphids from either escaping from the infested pots or entering the control pots. Then, 7, 14, and 21 days after inoculation, 22 pots per treatment (11 infested and 11 control) were randomly removed from the experiment. In each infested pot, aphids were manually collected from the plant and placed in a Petri dish (55 mm diameter), which was then kept at -80°C . Later, the winged and wingless adults and nymphs were counted under a stereo microscope.

The wheat above-ground biomass was collected, dried at 60°C for 48 h, and weighed using a portable laboratory

scale PT210-000V1 balance (Sartorius, Göttingen, Germany), except 2.5 g of the fresh wheat biomass, that was kept aside at -20°C for chemical analyses in order to measure the concentration of free amino acids in the wheat leaves. These chemical analyses were performed as follows: the wheat leaves were dried in a vacuum oven (40°C and -1 bar) for 36 h and then shredded with a ball mill; 20 mg of this wheat leaf powder was diluted in 500 ml distilled water and heated at 70°C for 1 h. After cooling, the solution was centrifuged for 5 min at 10 000 rpm and the resulting supernatant was analysed with the K-PANOPA kit (Megazymes, Bray, Ireland), in accordance with the manufacturer's protocol.

Statistical analysis

Data of the behavioural experiment were analysed using mixed models (Table 1), with 'experimental session' as a random factor to take into account the dependency of observations of experimental pots made on the same date. Generalized linear mixed models (GLMMs) and linear mixed models (LMMs) were built with the `glmer` and `lmer` functions, respectively, from the `lme4` package (Bates et al., 2015).

The significance of fixed factors and their interaction was determined by an F-test with a Kenward-Roger correction for LMMs or likelihood ratio tests (LRT) for GLMMs, as implemented in the mixed function in the `afex` package (Singmann et al., 2018). If non-significant, the interaction was removed from the model and the significance of fixed factors was re-tested. The time that the aphids needed to reach their first wheat plant was compared among treatments with a survival time analysis using the log-rank test from the `survfit` function in the `survival` package (Therneau & Lumley, 2017).

Aphid numbers in the population growth experiment were compared with a quasiPoisson GLM to account for

Table 1 Statistical analysis for each variable in the behavioural experiment and the explanatory fixed factors and random factors, if applicable

Variables	Statistical test/model	Fixed factors	Random factors
% aphids leaving the first plant encountered	Binomial GLMM	Treatment, identity of first plant encountered	Experimental session
No. visits of plants or ground	Poisson GLMM	Treatment	Experimental session
No. escaped aphids	Poisson GLMM	Treatment	Experimental session
No. contacts among plants	Poisson GLMM	Treatment	Experimental session
Time spent on plants	LMM	Treatment	Experimental session
Vegetation cover	LMM	Treatment	Experimental session
Wheat height	LMM	Treatment	Experimental session
Time needed to reach the first wheat plant	Log-rank test	Treatment	NA
Probability to encounter wheat first	Exact binomial test	NA	NA

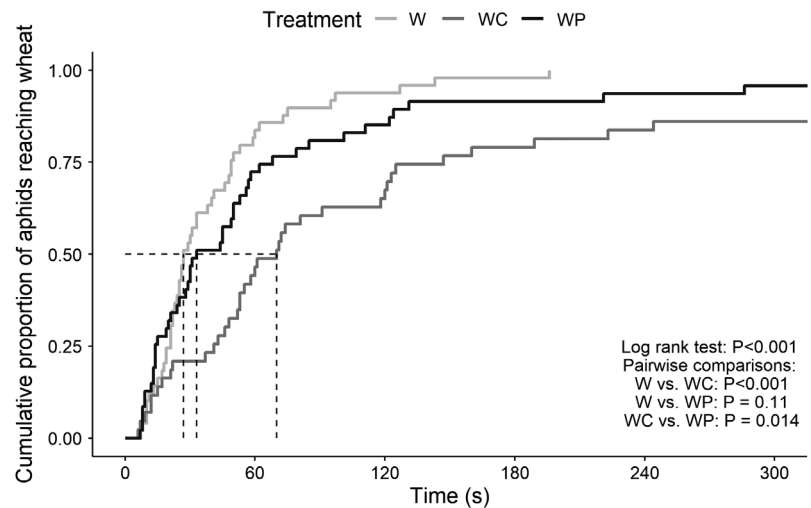
NA, not applicable.

Table 2 Statistical analysis for each variable in the aphid population growth experiment and the explanatory fixed factors

Variables	Statistical test/model	Fixed factors
Aphid abundance	quasiPoisson GLM	Treatment, days
Aphid densities	Quadratic (non-linear) regression	Treatment, days
Adult aphid densities	ANOVA	Treatment
Winged aphid densities	Kruskal–Wallis rank sum test	Treatment
Wheat dry biomass	ANOVA	Treatment, aphid (presence or absence), days
Free amino acid concentration	ANOVA	Treatment, days

Table 3 Aphid behaviour in each of three treatments [wheat monoculture (W), wheat intercropped with pea (WP), and wheat intercropped with clover (WC)]: the first plant encountered (wheat host or non-host) and whether the aphids leave (i.e., walk away from the first plant encountered) or stay for the duration of the assay (5 min)

	Treatment					
	W (n = 49)		WP (n = 50)		WC (n = 50)	
First plant visited						
Identity	Wheat	Wheat	Pea	Wheat	Clover	None
Rate (%)	100	66	34	28	70	2
Behaviour						
Leave (%)	57	34	26	16	56	–
Stay (%)	43	32	8	12	14	–

Figure 2 Kaplan–Meier plot of the time needed for aphids to reach a wheat plant during the first 5 min in each of three treatments: wheat monoculture (W), wheat intercropped with clover (WC), and wheat intercropped with pea (WP).

overdispersion (Table 2). When non-significant, the interaction was removed from the model and the significance of fixed factors was re-tested. Aphid densities (number of aphids over plant weight) were analysed with a non-linear quadratic regression. The demographic composition of the aphid populations at single dates was analysed with ANOVA, or with Kruskal–Wallis rank sum test if data were not normally distributed. For the ANOVA, all possible interactions were considered and non-significant interactions were removed using a stepwise model selection by Akaike's information criterion (AIC). For all models and when appropriate, pairwise comparisons were realized using Tukey-adjusted least-square means (LSMeans) with the emmeans package (Lenth, 2018). All statistical analyses were conducted in R v.3.4.3 software (R Core Team 2018).

Results

Behavioural experiment

Due to the difference in seeding densities, the probability that an aphid by chance first encountered a wheat plant was 0.75 for WP, 0.30 for WC, and obviously 1 for W. Our observations did not differ from this (Table 3): 66% of aphids first visited a wheat plant in WP (Exact binomial test: 95% confidence interval = 0.51–0.79, $P = 0.14$), whereas only 30% of aphids first visited a wheat plant in WC (Exact binomial test: 95% CI = 0.18–0.45, $P = 1.0$). All aphids reached a wheat plant in W. The time needed for aphids to reach a wheat plant differed among treatments (log-rank test: $\chi^2 = 17.9$, d.f. = 2, $P < 0.001$): it was longer in WC (70 s) than in WP (33 s) and W (27 s) (Figure 2).

All 149 aphids except one visited at least one plant during the first 5 min of the experiment, but more than 50%

of the aphids left the first plant that they visited during this period (Table 3). The probability of leaving the first plant visited was greater when the plant was a non-host plant (LRT on GLMM: $\chi^2 = 5.60$, d.f. = 1, $P = 0.02$) and this probability was similar across treatments ($\chi^2 = 0.37$, d.f. = 2, $P = 0.83$).

During the first 5 min, aphids came back to the ground more often in WC (1.3× on average) compared to the other treatments with less than one return on average (LRT on GLMM: $\chi^2 = 14.69$, d.f. = 2, $P < 0.001$). Aphids visited a similar number of wheat plants (1.5 on average) whatever the treatment ($\chi^2 = 3.43$, d.f. = 3, $P = 0.33$), but spent more time on wheat in the W (234 s) and WP (195 s) treatments than in the WC (139 s) treatment (F-test on LMM: $F_{2,140} = 14.5$, $P < 0.001$) (Figure 3). Concerning the non-host plants, aphids spent more time on clover (97 s) than on pea (49 s) (F-test on LMM: $F_{1,92} = 6.9$, $P = 0.01$).

During the 1-h experiment, 27% aphids left the experimental pot; the rate of escape was not different among the

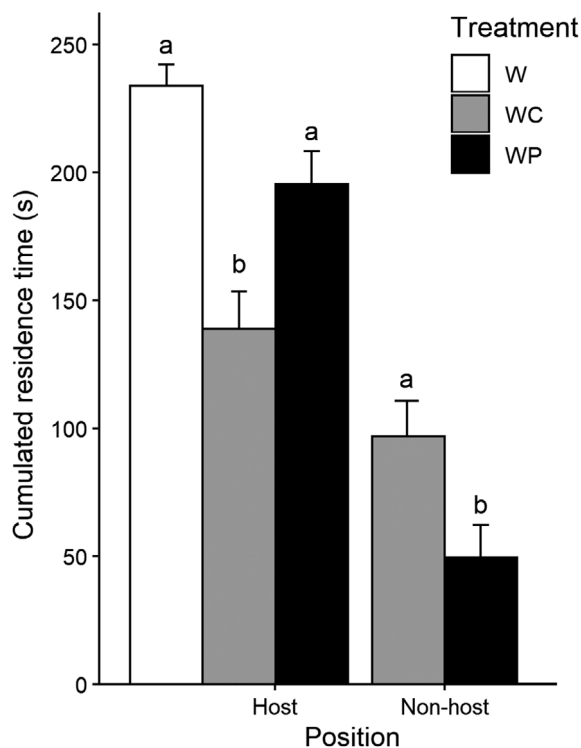


Figure 3 Mean (+ SEM) cumulated residence time (s) of aphids on wheat host plants and on clover or pea non-host plants during the first 5 min in each of three treatments: wheat monoculture (W), wheat intercropped with clover (WC), and wheat intercropped with pea (WP). Means within a plant type capped with different letters are significantly different (Tukey-adjusted pairwise LSM means comparisons: $P < 0.05$).

treatments (LRT on GLMM: $\chi^2 = 0.12$, d.f. = 2, $P = 0.94$). Of the remaining aphids, 94 and 88% visited at least one wheat plant in the WP and WC treatments, respectively ($\chi^2 = 0.49$, d.f. = 1, $P = 0.49$).

Differences in aphid behaviour among treatments may be related to contrasting vegetation cover (Table 4). As expected from seeding densities, ground cover differed among treatments (F-test on LMM: $F_{1,149} = 17.65$, $P < 0.001$) – it was largest for WC, intermediate for WP, and lowest for wheat in monoculture. Pea plants never touched each other, whereas clover plants formed a continuous cover such that it was difficult to distinguish every single contact among plants. Wheat plants were significantly more in contact with clover than with pea plants (Table 4).

Aphid population growth experiment

Aphid populations increased over the 3-week experiment (Figure 4) (GLM: $F_{1,91} = 223.97$, $P < 0.001$) and aphid number differed among treatments ($F_{2,92} = 5.68$, $P < 0.01$) – intercropping wheat with a legume reduced the number of aphids compared to solely wheat, but no difference between WC and WP was observed (Tukey-adjusted pairwise LSM means comparisons: $P < 0.05$). We did observe, however, differences among wheat biomasses from different treatments at 21 days of infestation (Figure 5) – the biomass of wheat associated with pea was significantly lower when compared to wheat in monoculture and to wheat associated with clover. Although there were differences among treatments, the biomasses of wheat

Table 4 Vegetation characteristics: mean (\pm SEM) ground cover (%), number of contacts between wheat plants and between wheat and non-host plants (clover and pea), and plant height (cm) in each of three treatments: wheat monoculture (W), wheat associated with clover (WC), and wheat associated with pea

	Treatments		
	W	WC	WP
Ground cover (%)	13.4 \pm 0.4a	16.5 \pm 0.4b	14.8 \pm 0.4c
No. contacts among wheat plants	6.0 \pm 0.5a	6.8 \pm 0.4a	4.4 \pm 0.4b
No. contacts between wheat and non-host plants	–	6.9 \pm 0.4a	2.3 \pm 0.2b
Wheat height (cm)	13.4 \pm 0.2a	13.6 \pm 0.2a	12.7 \pm 0.2b
Non-host plant height (cm)	–	1.4 \pm 0.0	4.3 \pm 0.1

Means within a row followed by different letters are significantly different (Tukey-adjusted pairwise LSM means comparisons: $P < 0.05$; applied on LMMs for vegetation cover and wheat height, or GLMMs for contacts among plants).

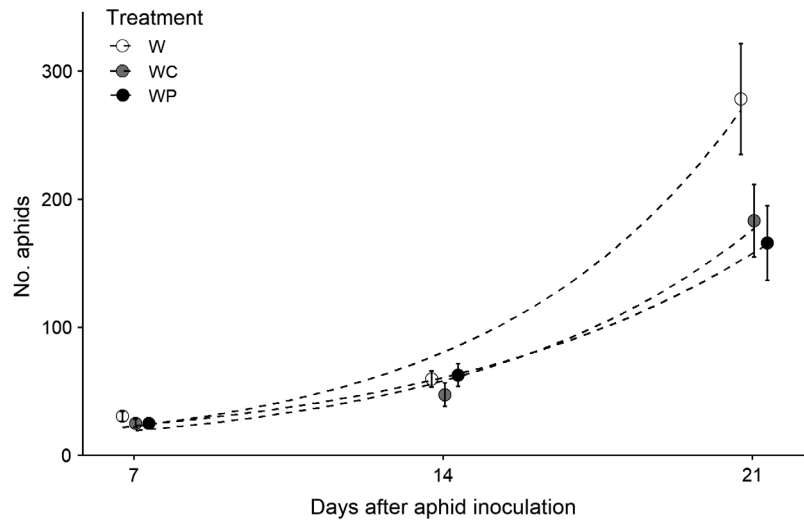


Figure 4 Mean (\pm SEM) number of aphids over 3 weeks after inoculation in each of three treatments: wheat monoculture (W), wheat intercropped with clover (WC) and wheat intercropped with pea (WP). Dashed lines result from the quasiPoisson GLM analysis that best described the data.

infested with aphids were significantly lower compared to the control plants. Therefore, taking into account aphid densities – number of aphids per g of dry wheat mass (Figure 6) – intercropping wheat with clover reduced aphid densities compared to sole wheat and to wheat intercropped with pea (Tukey-adjusted pairwise LSMeans comparisons: $P < 0.05$).

The demographic composition of the aphid populations indicated that aphid populations were dominated by apterous nymphs (Table 5). The density (number of aphids over wheat biomass) of winged adults and nymphs was negligible at the first two sampling dates and still represented less than 2% of the aphid population at 21 days. At this last sampling date, the mean density of winged aphids was not significantly different between treatments (Kruskal–Wallis rank sum test: $\chi^2 = 0.66$, d.f. = 2, $P = 0.72$). However, it should be noted that winged aphids were present in the WC treatment since the first sampling date, contrary to the other treatments where they only appeared in the 21-days sampling.

In order to explore a possible relationship between aphid population growth and plant sap quality when intercropped, we analysed the concentration of free amino acids in leaves of the non-infested wheat plants (i.e., controls) of the various treatments (Figure 7). Free amino acids decreased over time (ANOVA: $F_{2,87} = 63.07$, $P < 0.001$) and their concentration was not different among treatments ($F_{2,87} = 1.60$, $P = 0.57$). There was a significant interaction between time and treatment ($F_{4,87} = 7.56$, $P = 0.04$) due to the significant difference in free amino acid concentration in wheat plants from W and WP treatments 14 days after aphid infestation, but this difference disappeared later on.

Discussion

This laboratory study aimed at testing the hypotheses that combining wheat and legumes modifies the behaviour of the cereal aphid *S. avenae* in terms of host plant location and population growth. The aphids' residence time on

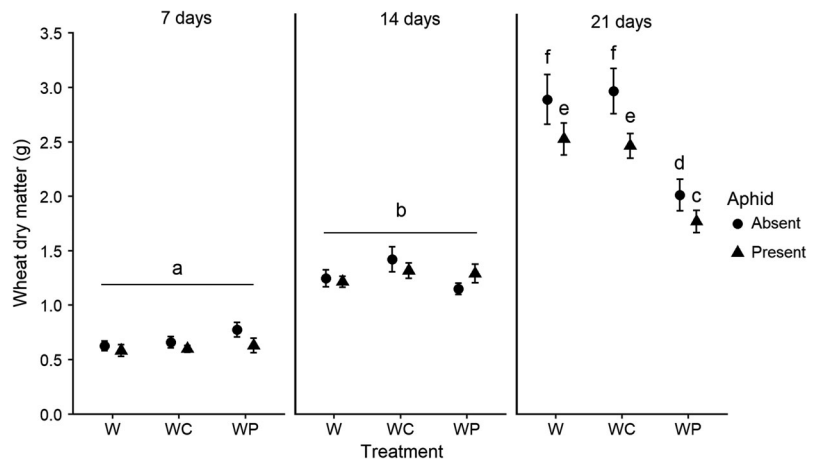


Figure 5 Mean (\pm SEM) wheat dry biomass (g) at 1, 2, and 3 weeks after aphid inoculation, in the presence or absence (control) of aphids, in each of three treatments: wheat monoculture (W), wheat intercropped with clover (WC), and wheat intercropped with pea (WP). Means capped with different letters are significantly different (Tukey-adjusted pairwise LSMeans comparisons: $P < 0.05$).

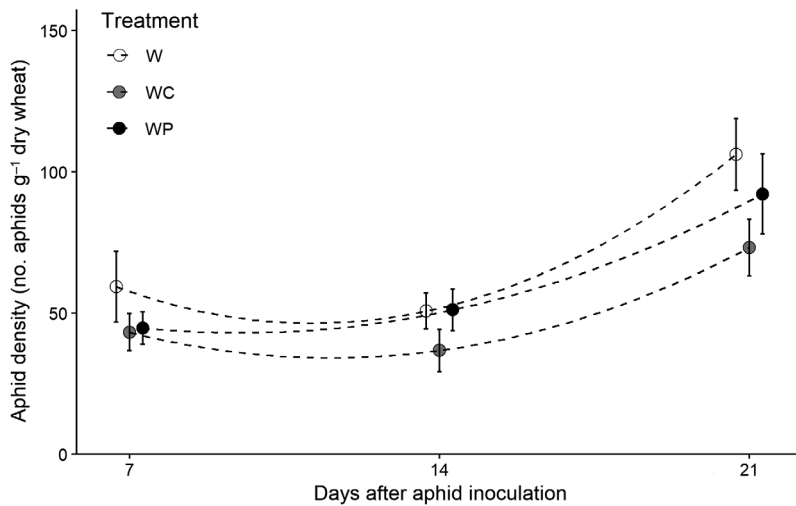


Figure 6 Mean (\pm SEM) aphid densities (no. aphids per g wheat dry biomass) over 3 weeks after aphid inoculation in each of three treatments: wheat monoculture (W), wheat intercropped with clover (WC), and wheat intercropped with pea (WP). Dashed lines result from the quadratic regression analysis that best described the data.

wheat decreased when this host plant was intercropped with clover. At the population level, wheat–legume intercrops reduced the number of aphids on wheat plants compared to sole crops of wheat, but if we account for plant

Table 5 Mean (\pm SEM) densities (number of aphids per g plant) of apterous and winged aphids according to their growth stage and the treatments: wheat monoculture (W), wheat associated with clover (WC), and wheat associated with pea (WP), at 7, 14, and 21 days after infestation

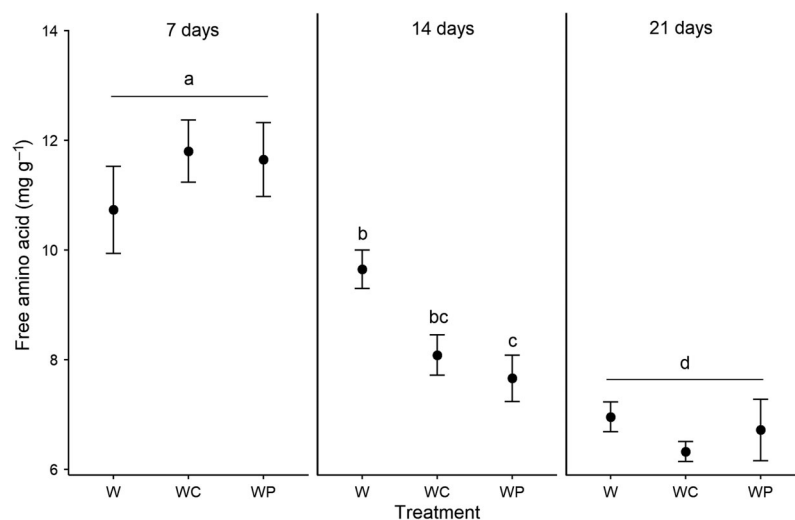
	Treatment		
	W	WC	WP
7 days			
Apterous			
Adults	2.0 \pm 0.5	1.2 \pm 0.5	1.4 \pm 0.4
Nymphs	57.4 \pm 12.3	41.8 \pm 6.3	43.3 \pm 5.7
Winged			
Adults	0	0.3 \pm 0.2	0
Nymphs	0	0	0
14 days			
Apterous			
Adults	7.3 \pm 1.3	5.1 \pm 0.9	9.8 \pm 1.3
Nymphs	43.3 \pm 5.6	31.6 \pm 6.7	41.3 \pm 6.1
Winged			
Adults	0	0.1 \pm 0.1	0
Nymphs	0	0	0
21 days			
Apterous			
Adults	14.0 \pm 1.8	10.8 \pm 1.6	12.1 \pm 2.2
Nymphs	90.7 \pm 10.9	61.1 \pm 8.3	79.1 \pm 12.2
Winged			
Adults	0.3 \pm 0.1	0.4 \pm 0.3	0.1 \pm 0.1
Nymphs	1.2 \pm 0.5	0.8 \pm 0.3	0.9 \pm 0.4

biomass, only intercropping clover with wheat significantly reduced aphid densities on wheat – intercropping clover with wheat was particularly disruptive to *S. avenae* behaviour.

Our observations support the first hypothesis that host location by apterous aphids is impeded by the presence of a non-host plant, corresponding in our experiment to an additive design simulating field seed densities of wheat–pea and wheat–clover intercrops. Although connectivity between plants was higher in the wheat–clover treatment, this did not help apterous aphids to more easily reach their host plant. Chemical and physical interference might explain this result, as they are both involved in host location (Gish & Inbar, 2006; Reeves, 2011; Webster, 2012).

Both alate and apterous aphids preferred the volatile organic compounds emitted by pure stands of wheat compared to those emitted by intercrops (Xie et al., 2012; Ninkovic et al., 2013). Odours therefore play a role as first attractors to host plants. However, the rejection of a non-host plant by insects occurs after reaching the plants, not before (Finch & Collier, 2012). Once on the plant, aphids initiate a second recognition process with their antennae by probing the plant to obtain physical and chemical information about its suitability (Caillaud & Via, 2000). This recognition stage takes around 60 s and is essential to avoid full stylet penetration on the wrong plant, which is time-consuming (45 min; Dixon 1998). Therefore, in the case of high non-host plant density, this second stage may have to be repeated for each encountered plant, increasing the time necessary to ultimately come upon the host plant. Indeed, in our experiment we observed that intercropping wheat with clover increased the aphids' movements between the ground and the clover cover and within the clover cover, significantly delaying the aphids' arrival to

Figure 7 Mean (\pm SEM) free amino acid content (mg g^{-1}) in leaves of non-infested wheat plants after 1, 2, and 3 weeks in each of three treatments: wheat monoculture (W), wheat intercropped with clover (WC), and wheat intercropped with pea (WP). Means capped with different letters are significantly different (Tukey-adjusted pairwise LSM means comparisons: $P < 0.05$).



the wheat. Although our experiments were not designed to test for the effect of plant density, these observations point out that the ratio of the two intercropped plants may be important for pest control.

Concerning the second hypothesis, the aphid population growth suggests bottom-up regulation through plant interspecific diversification. However, intercropping can reduce intercropped plant mass because of interspecific competition (Thorsted et al., 2006) and generate confounding effects that complicate the evaluation of herbivore responses to intercropping (Bukovinszky et al., 2004). Therefore, host plant biomass should be taken into account when considering intercrop infestation effects (Ndzana et al., 2014). This means that *S. avenae* infestation was only significantly lower for the wheat–clover intercrop treatment.

The lower number of aphids in wheat–clover intercrops may be explained by slower female development, decreased fecundity, increased mortality, and/or a higher level of emigration. Emigration can be discarded in our experimental setup because each pot was isolated under a net. However, in the wheat–clover treatment alate aphids appeared since the first week of infestation, indicating that migration was induced and thus could have strengthened the decrease in aphid numbers if winged aphids had been allowed to leave the experiment.

All these processes could be influenced indirectly by the effect of cover structure on aphids' behaviour and/or directly by plant quality. On the one hand, the increased walking activity in wheat–clover treatments, observed in the first experiment, could carry fitness costs which are known to trade-off with reproductive success (Stearns, 1992), with consequences for population growth (Hooks & Fereres, 2006). Moreover, repetitive probing of a non-

host plant associated with failure in host location is known to induce flight in aphids (Finch & Collier, 2000). On the other hand, cereals and legumes compete for soil nutrients at early growth stages (Corre-Hellou et al., 2006; Bedoussac et al., 2015). Such competition may impact plant quality and constrain the growth rate of aphids (Theunissen, 1994; Dixon, 1998) and induce the production of winged aphids to escape unsuitable conditions (Braendle et al., 2006; Awmack & Leather, 2007; Irwin et al., 2007). Nevertheless, in our experiment, we did not find a decrease in the biomass, nor differences in the concentration of free amino acids in the leaves of wheat intercropped with clover, compared to that from wheat monocultures. Unfortunately, our method does not distinguish essential from non-essential amino acids which, together with the ongoing debate about the pathways of uptake and utilization of plant nutrients by aphids, muddles the link between sap quality and aphid population growth (Vogel & Moran, 2011; Haribal & Jander, 2015).

Finally, the standardized design of our laboratory experiments assumed an equal inoculum of apterous aphids across treatments, but as intercropping might reduce the initial colonization of the crop by winged aphids (Finch & Collier, 2000; Döring, 2014), aphid population growth may differ under field conditions. Moreover, our results only concern the first 4 weeks of plant growth, whereas *S. avenae* mainly colonizes later wheat developmental stages.

Flight arrival of aphids on a crop has been shown to decrease with increased vegetation cover and diversity (Finch & Collier, 2000; Döring, 2014). Although it has been argued that increasing vegetation diversity may be more effective in reducing the winged aphid colonizers of wheat than in regulating the later spread of aphid colonies

(Costello & Altieri, 1995; Wratten et al., 2007), our study shows that vegetation diversity can also contribute to decreasing the growth of the later wingless aphid populations. Nevertheless, this depends on the species used as non-host plants and their density. Although additive vs. substitutive designs are often compared in studies of the effect of intercropping on pest control, little is reported about the influence of plant relative densities on pest dispersal (Lopes et al., 2016). Our study points out that these are important parameters that should be taken into account. In our experimental set up that copied additive designs used in real-farming conditions, intercropping clover with wheat was particularly disruptive to apterous *S. avenae* and may be an interesting strategy for its control. Nevertheless, our observations are valid only for aphid colonization of early plant growth stages. Later on, the vegetation structure as well as interspecific interactions – competition for nutrients – may change and influence aphid behaviour differently; this should be further investigated. Finally, our laboratory results need to be confirmed at a larger scale under actual field crop conditions, where other parameters such as the interaction with natural enemies might play an important part in the final aphid control (Vidal & Murphy, 2018).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Experimental set up of the behavioural experiment: (A) overall configuration and an experimental pot of the treatment: (B) wheat monoculture, (C) wheat intercropped with pea, and (D) wheat intercropped with clover.