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Herbivore-induced plant volatiles, not natural enemies, mediate a positive indirect interaction between insect herbivores

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Abstract

Many insect herbivores engage in apparent competition whereby two species interact through shared natural enemies. Upon insect attack, plants release volatile blends that attract natural enemies, but whether these volatiles mediate apparent competition between herbivores is not yet known. We investigate the role of volatiles that are emitted by bean plants upon infestation by *Acyrthosiphon pisum* aphids on the population dynamics and fitness of *Sitobion avenae* aphids, and on wheat phloem sap metabolites. In a field experiment, the dynamics of *S. avenae* aphids on wheat were studied by crossing two treatments: exposure of aphid colonies to *A. pisum*-induced bean volatiles and exclusion of natural enemies. Glasshouse experiments and analyses of primary metabolites in wheat phloem exudates were performed to better understand the results from the field experiment. In the field, bean volatiles did not affect *S. avenae* dynamics or survival when aphids were exposed to natural enemies. When protected from them, however, volatiles led to larger aphid colonies. In agreement with this observation, in glasshouse experiments, aphid-induced bean volatiles increased the survival of *S. avenae* aphids on wheat plants, but not on an artificial diet. This suggests that volatiles may benefit *S. avenae* colonies via metabolic changes in wheat plants, although we did not find any effect on wheat phloem exudate composition. We report a potential case of associational susceptibility whereby plant volatiles weaken the defences of receiving plants, thus leading to increased herbivore performance.

Keywords *Acyrthosiphon pisum* · Apparent competition · Aphids · Indirect interaction · Long-term dynamics · Phloem sap · *Sitobion avenae* · Volatile organic compound

Introduction

Plants form the base of most terrestrial ecosystems and harbour diverse communities of insect herbivores, which are in turn attacked by a wide variety of natural enemies, the majority of which are insect predators and parasitoids. These trophic webs are among the most complex on Earth and include direct as well as indirect interactions (Morin 2011). Indirect interactions occur when the presence of one species influences another through a third one. These interactions can be density- or trait-mediated, the former occurring when the effect is transmitted through changes in species densities. In arthropod communities, apparent competition is a common density-mediated indirect effect, which occurs when one herbivore has a negative effect on another, because its presence results in an increased abundance of a shared natural enemy (Morin 2011; van Veen et al. 2006). Trait-mediated indirect effects are transmitted through behavioural changes. A trait-mediated example may occur when the mere presence of a predator, detected by using visual or chemical cues, may lead a herbivore to be more wary and hide, resulting in lower rates of feeding that benefit its host plant (Schmitz et al. 2004; Thaler et al. 2012). Plants emit chemical cues that are involved in indirect effects. For example, most plant species respond to herbivore attack by releasing herbivore-induced volatiles, which can be used as cues by predators and parasitoids to locate

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their prey. These effects alter natural enemy behaviour, and they can thus be considered as trait-mediated (Turlings and Erb 2018). A density-mediated component, however, may also play a role if these volatiles attract natural enemies and increase their local densities.

The last decade has seen a growing appreciation of the role trait-mediated indirect effects may have in community ecology with particular attention paid to herbivore-induced plant volatiles (Ohgushi et al. 2012; Turlings and Erb 2018). Pioneering work in the 1990s showed that parasitoids and predators use these cues to locate their prey. Plants were shown to produce specific volatile blends in response to attack by different herbivore species, resulting in the attraction of natural enemies of the herbivores (Dicke and Sabelis 1987; Turlings et al. 1990). These volatiles are also used by herbivores to locate their host plants (Carrasco et al. 2015; Webster and Cardé 2017). Most early work was laboratory-based, but recent studies have explored the role of these volatiles in more natural ecological settings and have shown that they can influence the community of insects colonizing plants (Aartsma et al. 2017, 2020; Kaplan 2012). Despite this evidence, we still have limited knowledge on how herbivore-induced plant volatiles modulate interactions between herbivores that feed on different plant species and never directly compete for resources. These volatiles alter natural enemy behaviour, and they are therefore likely to modulate the trait-mediated component of apparent competition between herbivores, although experiments to test this idea have not been performed so far. Plant volatiles can also act as defensive products themselves. The effect most commonly observed is the repulsion of herbivores, as found in aphids, thrips, whiteflies, and lepidopterans (Maag et al. 2015), but increased susceptibility of herbivores to pathogens (Gasmi et al. 2019), or direct toxicity (Sugimoto et al. 2014) have also been reported. Plant volatiles can also modulate interactions between herbivores on different plants through indirect effects on plant physiology. Many reports have demonstrated that herbivore-induced plant volatiles may act as airborne cues that influence other plant species growing in the near vicinity, which respond to these chemicals by upregulating or priming their own defences in anticipation of possible herbivory (Engelberth et al. 2004; Erb et al. 2015; Karban et al. 2014; Ninkovic et al. 2020). The downstream physiological routes by which this occurs are complex and are both insect and plant-species dependent, but their expression commonly depends on more general cues that include plant phytohormones like ethylene, jasmonic and salicylic acid, and green leaf volatiles like aldehydes, alcohols, and terpenes (Karban 2015). There are also few studies, showing that this priming extends to the release of volatiles that are used to attract natural enemies: that is, primed plants recruit predators or parasitoids before they themselves are attacked by the herbivores (e.g., Choh et al. 2004). After more than 3 decades of research on plant defences mediated by volatiles, experiments aimed at pinpointing the potential mechanisms that volatiles have at the community level are still limited.

In this study, we explore how the volatiles that are emitted by plants upon infestation by an herbivore affect the population dynamics and fitness of another herbivore feeding on a different plant. We carried out field and laboratory experiments with two species of aphids that feed on different host plant species but which share natural enemies (Fig. 1). The short generation times of aphids make them good study systems to explore the multigenerational consequences of trait-mediated indirect interactions. As mentioned above, the presence of one herbivore species can influence the dynamics of the other through the shared natural enemies, through the response of one plant species to herbivore-induced plant volatiles released by the other, or through direct effects of the volatiles released by one plant on herbivores feeding on the other. Our experiments were designed to distinguish these effects. We studied the pea aphid (*Acyrthosiphon pisum*) and the English grain aphid (*Sitobion avenae*), which feed on broad bean (*Vicia faba)* and wheat (*Triticum aestivum*), respectively. These aphid species share a number of generalist aphid predators as well as several parasitoids, and are likely to interact indirectly through apparent competition (Van Veen et al. 2008). Previous work in the laboratory has shown that *V. faba* plants attacked by *A. pisum* release volatiles that attract the predator *Coccinella septempunctata* and the parasitoid *Aphidius ervi* (Du et al. 1996, 1998; Guerrieri et al. 1999; Powell et al. 1998; Takemoto and Takabayashi 2015). In replicated field plots, we explored the effect of these volatiles on the population dynamics of *S. avenae* aphids feeding on *T. aestivum* plants (Fig. 1A). We hypothesised that exposure to *A. pisum*-induced *V. faba* volatiles leads to lower *S. avenae* densities and reduced colony survival on wheat due to increased natural enemy recruitment. To test the effects of natural enemies, the treatment with volatiles was crossed with a natural enemy exclusion treatment using a factorial design. Contrary to our predictions, aphid-induced *V. faba* volatiles had a positive effect on *S. avenae* densities on wheat but only when natural enemies were excluded. This suggests that *V. faba* volatiles have a direct effect on *S. avenae* aphids, or an indirect effect through changes in the physiology of *T. aestivum* plants. To explore these two non-exclusive explanations, the role of aphid-induced *V. faba* volatiles on survival and reproductive potential of *S. avenae* aphids was tested in the laboratory using artificial diets (Fig. 1B) and plants (Fig. 1C). We also assessed the primary metabolite composition of phloem exudates of *T. aestivum* plants exposed or not exposed to *V. faba* volatiles to investigate whether this treatment affects plant physiology ultimately affecting *T. aestivum* development (Fig. 1D).

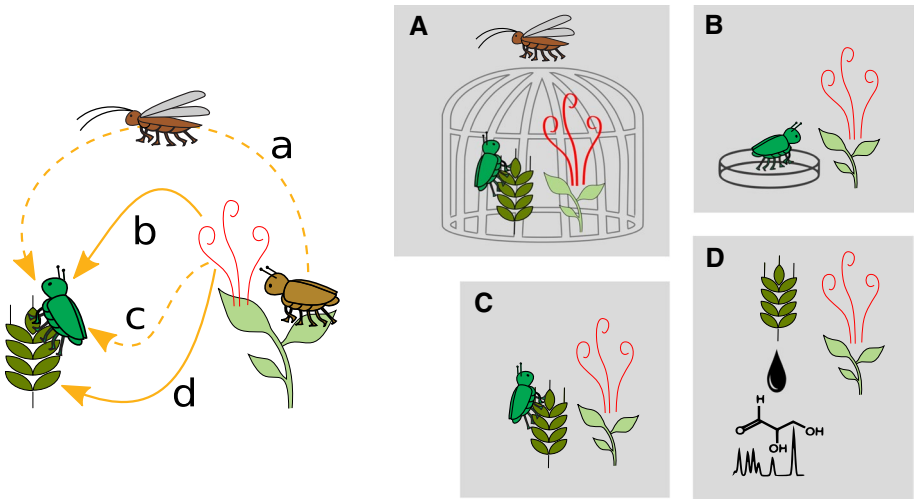

Fig. 1 Schematic representation of the different interactions tested, and the experiments performed. Herbivore-induced plant volatiles (represented by red lines) emitted by bean plants (*Vicia faba*) fed upon by pea aphids (*Acyrthosiphon pisum*, represented by a brown insect) may modulate the population dynamics of grain aphids (*Sitobion avenae*, represented by a green insect) feeding on wheat (*Triticum aestivum*) (**a**) indirectly through shared natural enemies (apparent competition), (**b**) through direct volatile effects on aphids, and (**c**, **d**) indirectly through volatile effects on wheat physiology. These interactions have been tested (**A**) in the field by assessing the population dynamics of *S. avenae* aphids and its natural enemies in an experiment that crossed volatile emissions and natural enemy exclusion treatments. Glasshouse experiments tested volatile effects on *S. avenae* aphids feeding on either (**B**) an artificial diet, or (**C**) on wheat plants. (**D**) Volatile effects on wheat physiology were explored by analysing wheat phloem exudate composition. Dashed lines represent indirect interactions

Materials and methods

Experimental organisms

A. pisum and *S. avenae* are common aphids in Europe, and are frequently found together in natural and semi-natural settings (Van Veen et al. 2008). *A. pisum* aphids used in the experiments were collected in Oxfordshire (England), and *S. avenae* was obtained from Koppert (Suffolk, UK). Like most aphids in temperate climates, both species reproduce parthenogenetically throughout spring and summer. Parthenogenetic females of both species have short generation times, and they undergo several generations in spring and summer with most clones producing sexual individuals in autumn in response to shortening photoperiods. Winters are spent as diapausing eggs. To ensure asexual reproduction, colonies were kept in the laboratory under conditions of 16:8 h light:dark (L:D) regime, 20 ± 1 °C and 70% relative humidity. Both aphid species were kept on non-flowering potted plants, *A. pisum* on the broad bean *V. faba* (cv. the Sutton) and *S. avenae* on wheat *T. aestivum* (Brow Farm Ltd., Ormskirk, UK) for at least 1 year before the experiments.

Effects of bean volatiles and natural enemies on grain aphid population dynamics in the field

The field experiment was performed between May and July 2011 at Wytham Woods near Oxford in southern England. There were two treatments crossed in a factorial design leading to a total of four plot types (Fig. 1A). The first treatment was the presence or absence of aphid-induced volatiles emitted by *V. faba,* which were manipulated by placing *A. pisum* on the plants of the induced volatile treatment, and then removing them before the experiment began. The second was the presence or absence of natural enemies, which we manipulated using mesh cages.

Colonies of *S. avenae* were first established in the laboratory by placing the aphids (13 3-to-4-day-old female nymphs and 10 wingless adult females) on pots with 6-day-old *T. aestivum* seedlings (about 5 cm high). Each pot was placed in a cylindrical two-litre plastic cage that had two lateral openings covered with a thin mesh for ventilation. Aphids were allowed to settle for 2 days prior to the start of the experiment. The *V. faba* plants used in the experiments were 3 weeks old. To prepare plants emitting aphid-induced volatiles, pots with 2-week-old seedlings were placed in groups of eight in $30 \times 30 \times 30$ cm ventilated Perspex cages under the laboratory conditions (see above) and 150 *A. pisum* aphids of mixed ages were introduced onto each pot. Six days later and one day before deployment in the field, insects were washed off the plants with pressurized water that did not damage the plants. The plants were checked again for aphids immediately before the experiment. Control plants were kept under the same conditions and were also washed with pressurized water to control for any plant defences triggered by mechanical damage.

In the field, a total of 40 plots were established in a grid design with five rows each containing eight plots. Each of the four plot types was replicated ten times, and the different treatments were allocated at random. Each plot consisted of a central two-litre pot with 30 T. *aestivum* plants infested with 23 *S. avenae* aphids, which was surrounded (i.e., direct contact among pots) by four one-litre pots each containing four *V. faba* plants. The soil used was John Innes no. 3 compost. The distance between plots was between 4.2 and 5 m. There is no precise information about the distance that *V. faba* volatiles may be able to disperse. In an open landscape like the one where the experiment was performed, however, we expect little between-plot interference, because volatiles disperse in the environment as plumes, and densities quickly drop due to dilution and degradation (Aartsma et al. 2017). During the experiment, *V. faba* plants were replaced weekly to ensure continuous release of aphid-induced volatiles (the duration of volatile emission after induction is not known in this system, though, in others, it may last for several weeks, Schoonhoven et al. (2005).

Exclusion of natural enemies was achieved by surrounding the five pots in each plot with 1-m-high, 50-cm-diameter cylindrical wire mesh (0.5-cm gauge) cages. The 0.5-cm gauge was used to avoid concentration of plant volatiles, but it required the wire to be coated with Tanglefoot (Forestry Suppliers, Jackson, MS, US), a viscous glue that does not dry out and acts as a sticky trap. This design has been used before to exclude aphid natural enemies, and was found to have only minor effects on temperature and moisture in the enclosure (Muller and Godfray 1999; Van Veen et al. 2009). The control treatments had no cages, but were protected from herbivorous mammals by a chicken wire fence and from slugs using salt granules. Twice a week, plants were watered, and *S. avenae* aphids and their associated natural enemies counted on wheat plants. Twice a week, bean plants were inspected, and any herbivore colonising them removed. Aphid predators found on the plants were counted in the field and some specimens were retained for identification. Aphid predators are very mobile, so that assessing their density in the field through visual inspection may underestimate their densities. To partially correct for this, we paid particular attention to sessile life stages like eggs and pupae. Mummified aphids containing parasitoids and hoverfly pupae were collected and reared in the laboratory, and the emerging adults were identified.

Effects of plant-volatile exposure on aphid reproduction and survival in a glasshouse

To explore the effect of aphid-induced bean volatiles on various life-history traits of *S. avenae* aphids feeding on *T. aestivum* plants, two experiments were conducted in a glasshouse in the Laboratory of Entomology at Wageningen University (The Netherlands). Insects were maintained at 25 ± 3 °C and $70 \pm 15\%$ RH under an L16:D8 light regime. Experiment 1 (Fig. 1B) was designed to assess the direct effect of *V. faba* volatiles on *S. avenae* survival and reproduction. In this experiment, aphids were fed an artificial diet (Febvay et al. 1988; Vogel and Moran 2011) to prevent any plant-mediated effect. Small amounts of the artificial diet were placed between two layers of Parafilm, which were stretched and attached to the base of a 4-cm-diameter Petri dish. To confine the aphids, these dishes were individually placed inside a larger 10 cm Petri dish. The larger Petri dish had a square (2×2 cm) hole in the lid that was covered with a thin gauze that allowed volatiles to diffuse in while preventing insects from escaping. A total of 10 1-day-old *S. avenae* nymphs were placed in each dish and their survival and the number of offspring produced were assessed twice a week for 15 days. Once a week, the diet was replaced and offspring removed from the dishes. Some individuals of the *S. avenae* clone used can become adults in 6 days, but young females were distinguished from those initially introduced in the Petri dish by their smaller size and paler green colour. The unit of replication was a Petri dish. For each dish, fecundity was determined as the total number of offspring produced by ten aphids and survival as the proportion of individuals (out of 10) that survived in each dish. The Petri dishes with *S. avenae* aphids were placed in mesh cages ($40 \times 40 \times 60$ cm) containing four pots each with four *V. faba* plants that had been stimulated to produce aphid-induced volatiles in the same way as in the field experiment. An equal number of controls with uninduced plants were prepared. Mesh cages were individually wrapped with plastic film to prevent volatiles from diffusing among them. Again, as in the field experiment, *V. faba* plants were replaced weekly. Three Petri dishes were placed in each cage, and three cages set up for both the treatment and control. There were thus 18 replicates in all ($n = 9$), with three blocks (cages) nested within the treatment and control.

In experiment 2 (Fig. 1C), we investigated plant-mediated effects of exposure to aphid-induced *V. faba* volatiles on *S. avenae* performance. The survival and fecundity of *S. avenae* were compared over a period of 20 days when they fed on wheat plants: (i) concurrently exposed to aphid-induced volatiles emitted by *V. faba*, (ii) previously exposed to these volatiles, and (iii) controls where the *V. faba* plants were undamaged. In this experiment, two 1-day-old aphids were placed inside 2-cm-diameter clip cages attached to different *T. aestivum* plants. Two *T. aestivum* seedlings (8 days old, about 5 cm high) were established in 50 ml Falcon tubes with soil. The unit of replication was a clip cage with two aphids. Similar to the first glasshouse experiment, *T. aestivum* seedlings were placed inside mesh cages wrapped with plastic film, and inside them, four pots with *V. faba* plants previously stimulated with *A. pisum* or not were also

introduced. In the pre-exposure treatment, before placing *S. avenae* aphids on the plants, 3-day-old *T. aestivum* plants were exposed to *V. faba* volatiles for 5 days in three different mesh cages containing four pots each with four *V. faba* plants previously infested with *A. pisum* aphids. Wheat plants that were not pre-exposed were grown similarly in cages with *V. faba* plants that were not previously stimulated by *A. pisum* feeding. Fecundity was estimated as the number of offspring produced by the two *S. avenae* aphids in the same clip cage, and their survival was determined as in the previous experiment. Four clip cages were placed in each cage with *V. faba* plants, and four cages set up for each treatment and control. There were thus 12 cages and 48 replicates in all ($n = 16$) with four blocks (cages) nested within each treatment and control.

Bean volatile effects on wheat phloem exudate composition

To assess whether *A. pisum*-induced bean volatiles affect wheat physiology (Fig. 1D), phloem exudates of 12-day-old wheat seedlings planted in 50 ml Falcon tubes filled with soil (5 plants per tube), exposed or not exposed to the volatiles were collected. Plants were exposed to volatiles when they were 5 days old (2–3 cm) and were exposed for 7 days. Exposure to the volatiles was performed as described in the second glasshouse experiment. There were $n = 12$ replicates for each treatment group, with three blocks (cages) nested within each treatment group. Six wheat leaf blades from each Falcon tube were pooled. Phloem exudates were collected in the early afternoon and analysed according to Jakobs et al. (2019). The cut ends of the leaf blades were incubated in 8 mM EDTA (pH 7) for 2 h, and exudates collected in Millipore-H2O for 2 h hereafter. Leaf blades were incubated at room temperature in the dark inside a 50 ml Falcon tube, which was covered with a similar tube, and both were sealed together with Parafilm. Blanks without plant material were included. Samples were frozen in liquid N2 and stored at − 80 °C, and two aliquots of each sample were lyophilised for chemical analyses The leaf blades from which the exudates had been collected were dried (40 °C) and weighed.

For analysis of carbohydrates and organic acids, aliquots were re-dissolved in 80% methanol (LC–MS grade, Th. Geyer, Höxter, Germany) at room temperature with ribitol (99%, Sigma-Aldrich, Steinheim, Germany) as internal standard. Samples were dried under N2, methoximated (using O-methylhydroxylamine HCl, ≥ 98%, Sigma-Aldrich; 20 mg mL^{-1} in pyridine), and silylated (using N-methyl-N-trimethylsilylfluoracetamide, ≥ 95%, Macherey–Nagel, Düren, Germany) at 37 °C for 90 and 30 min, respectively. Samples were analysed with gas chromatography–mass spectrometry (GC–MS; Focus GC-DSQII, Thermo Electron, Rodano, Italy). They were injected (1:10 split) at 225 °C and metabolites were separated on a VF-5 ms column (30 m × 0.25 mm i.d., 10 m guard column, Varian, Palo Alto, CA, USA) with a helium flow of 1.2 ml min^{-1} and a temperature gradient, starting at 80 °C for 3 min, followed by a ramp with 5 °C min^{-1} to 325 °C. The transfer line was operated at 250 °C. Electron impact ionisation at 70 eV was performed. Retention indices (RI; Kováts (1958)) were calculated based on *n*-alkanes (C8-C40, Sigma-Aldrich). Analytes were identified by comparing their RI and mass spectra with entries in the Golm database (Hummel et al. 2010; Kopka et al. 2005) and standards (Sigma-Aldrich; Merck, Darmstadt, Germany; Roth, Karlsruhe, Germany; Macherey–Nagel). Peaks were integrated in Xcalibur (1.4.SR1, Thermo Electron). Peak areas of analytes belonging to the same metabolite were added together and normalised via division by peak areas of ribitol and the dry weights of the leaf blades used.

For amino acid analysis, aliquots were re-dissolved in 80% methanol (LC–MS grade, Th. Geyer) containing the internal standards norvaline and sarcosine (Agilent Technologies, Waldbronn, Germany) at room temperature. Samples were analysed with ultra-high-performance liquid chromatography coupled to fluorescence detection (UHPLC–FLD; 1260/1290 Infinity, Agilent Technologies, Santa Clara, CA, USA). Pre-column derivatisation was done by incubating the samples with borate buffer, ortho-phthaldialdehyde (OPA; 10 mg mL^{-1} in 0.4 M borate buffer and 3-mercaptoproprionic acid, Agilent Technologies) and 9-fluorenylmethyl chloroformate (FMOC; 2.5 mg mL^{-1} in acetonitrile, Agilent Technologies), and diluting them [with 100 ml eluent A (see below) mixed with 0.4 ml 85% phosphoric acid (AppliChem, Darmstadt, Germany)] at 6 °C. Analytes were separated at 40 °C on a ZORBAX Eclipse Plus C18 column (250 mm × 4.6 mm, 5 µm particles, with guard column, Agilent Technologies) using a gradient of eluent A [1.4 g Na2HPO4 (> 99.5%, AppliChem), 3.8 g Na2B4O7 · 10 H2O (≥ 99.5%, Sigma-Aldrich), 32 mg NaN3 (≥ 98%, Roth) in 1 l Millipore-H2O, pH 8.2] and B [4.5:4.5:1 (v:v:v) mixture of methanol, acetonitrile (LC–MS grade, VWR International, Fontenay-sous-Bois, France), Millipore-H2O], going from 2 to 57% B within 43.4 min at a flow rate of 1.5 ml min^{-1}. The FLD excitation/emission wavelengths were 340/450 nm for OPA-derivatised primary amino acids and 260/325 nm for FMOC-derivatised secondary amino acids, respectively. Amino acids were identified by comparing retention times to those of standards (Agilent Technologies). Peaks were integrated in OpenLab ChemStation (C.01.06, Agilent Technologies) and peak areas divided by those of the internal standards (norvaline for primary amino acids and sarcosine for secondary ones) and the dry weight of the leaf blade. As arginine and alanine were chromatographically not fully separated, their peak areas were combined.

For metabolite data, the following components were compared between treatment groups: (i) overall metabolite composition, (ii) the ratio of essential to non-essential amino acids, and (iii) the ratio of sucrose to amino acids. According to Douglas (2006), the following amino acids were considered as essential: histidine, threonine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, and lysine. The ratios of essential to non-essential amino acids and of sucrose to amino acids were tested, because they are important indicators for the nutritional quality of phloem sap for aphids (Abisgold et al. 1994).

Statistical analyses

All data were analysed using the open source software R. 3.4.3 (R Development Core Team, 2020). In the field experiment, the impact of natural enemies, and *V. faba* volatiles on *S. avenae* population sizes were analysed both as aphid numbers through time (i.e., number of aphids at each time point, expressed as the square root of aphid numbers), and considering cumulative aphid numbers over time (i.e., the sum of all aphids across all time points, expressed as the logarithm of aphid numbers). Aphid numbers through time were analysed using a linear mixed-effects model using the function *lme* from the package *nlme* (Pinheiro et al. 2020). This model included as fixed factors, the two treatments mentioned above (natural enemy exclusion and *V. faba* volatile exposure) and time, while plot was included as a random factor. Pairwise interactions between the three fixed factors were also included and model simplification was carried out by sequentially removing non-significant interactions. Autocorrelograms revealed significant temporal autocorrelation in the residuals, which was corrected by including time (squared) as a covariate and a first-order autoregressive term. Post hoc tests among the four different plot types in the linear mixed-effects model were performed with Tukey test using the *glht* function in the *multcomp* package (Hothorn et al. 2008). Cumulative numbers were also analysed with a two-way ANOVA, including the factors enemy exclusion (yes/no) and *V. faba* volatile exposure (yes/no) as well as their interaction term. The effect of the treatments and their interaction on colony survival in the field experiment was assessed with a Mantel–Cox test using the *coxph* function in the *survival* package (Therneau 2015). In this experiment, plots in which aphids did not become extinct were treated as censored data. Post hoc tests on aphid colony survival were performed via pairwise comparisons among plot types and correcting *P* values using the Bonferroni approach. The effect of *V. faba* volatiles and the natural enemy exclusion treatment on the different natural enemies found in plots was tested on cumulative insect numbers on taxa in which at least 15 individuals were found using generalised linear models assuming a quasi-Poisson error distribution using the function *glm*. The effect of the natural enemy exclusion treatment was also assessed on parasitism rates, and on the proportion of adult natural enemies relative to aphids. These metrics were estimated, relatively, as the cumulative number of parasitised aphids relative to the cumulative number of aphids per plot, and as the cumulative abundance of adult natural enemies, relative to the cumulative number of aphids per plot. In the glasshouse experiments, differences in the number of offspring produced by female aphids as well as the proportion of surviving aphids relative to controls were tested using generalised linear mixed-effects models using the function *glmer* in the *lme4* package (Bates et al. 2015) assuming a Poisson and a binomial error distribution, respectively. The nested design was accounted for by building a random slopes model that included as a random factor block (i.e., cage) nested within the treatment, so that each cage is uniquely identified within each treatment (Arnqvist 2020). In these models, overdispersion was reduced to values that were always lower than 1 by adding an observational-level random factor. In mixed-effects models, model fit was checked by visual inspection of the residuals and the absence of data points (or groups of data nested within blocks) with high influence was checked by calculating Cook's distances (Cook 1979) with the function *CookD* from the *predictmeans* package (Luo et al. 2020).

To compare the relative compositions of primary metabolites between the treatment groups, normalised peak areas (see above) were transformed to percent data by relating them to the sum of the peak areas of all primary metabolites in the corresponding samples. Data were then analysed with Principal Least Squares Discriminant Analysis (PLS-DA) using the function *plsda* from the *mixOmics* package (Rohart et al. 2017). For this analysis, data were autoscaled, so that for each compound the mean was zero and the standard deviation was one. A permutation analysis (9999 repetitions) was used to test the significance of the differences using the *MVA.test* from the *RVAideMemoire* package (Hervé 2019). This same package was used to obtain variable importance in projection (VIP) scores using the *PLS-DA.VIP* function. VIP scores are useful to identify compounds that are important in treatment separation (Eriksson et al. 2006). Metabolites that had VIP values larger or equal to one were then compared between treatments using the Wilcoxon rank sum test, and *P* values were corrected for multiple comparisons using the false discovery rate technique using the *p.adjust* function. This method is less stringent than Bonferroni and is therefore more appropriate when a large number of comparisons are performed and some false positives are acceptable (Benjamini and Hochberg 1995). The ratio of essential to non-essential amino acids and the ratio of sucrose to amino acids were also compared between treatments with the Wilcoxon rank sum test.

Results

Field experiment

We explored the influence of exposure to aphid-induced *V. faba* volatiles and natural enemies on the population dynamics of *S. avenae* aphids feeding on *T. aestivum* plants in a field experiment (Fig. 2 and ESM-1). As expected, in plots where natural enemies were excluded, both parasitism rates by *Aphidius* wasps ($t_1 = -3.88$, $P < 0.001$), and the proportion of adult *Aphidius* per aphid were significantly lower ($t_1 = -2.86$, $P = 0.007$). The relative abundance of other natural enemy groups, however, was not significantly reduced (adult dermapteran *Forficula*, $t_1 = -1.04$, $P = 0.305$; hoverfly eggs, $t_1 = -0.01$, $P = 0.992$). In plots with natural enemies excluded, aphid abundance was significantly higher (mixed-effects model, $F_{1,36} = 37.59$, $P < 0.001$; Fig. 2), and colonies persisted longer (Mantel–Cox, $Z_1 = -3.29$, $P < 0.001$; Fig. 2). Even in the absence of natural enemies, aphid colonies declined by the end of the experiment, probably because plant growth was arrested due to the limited space plants had in two-litre pots, which likely triggered the dispersal of winged adults. We predicted that the presence of *V. faba* volatiles would attract generalist natural enemies that would attack *S. avenae* aphids feeding on nearby *T. aestivum* plants, leading to lower aphid densities and aphid colony persistence. This is not what we found. Overall, the volatile treatment did not affect aphid abundance (mixed-effects model, $F_{1,36} = 3.29$, $P = 0.078$). Unexpectedly, however, and as revealed by a significant interaction between the volatile treatment per natural enemy exclusion, when protected from natural enemies, aphids were more abundant when their host plants were adjacent to *V. faba* plants that had been previously fed on by *A. pisum* (mixed-effects model, $F_{1,36} = 4.21$, $P = 0.048$; post hoc test in Fig. 2). The volatile treatment did not affect aphid colony persistence (Mantel–Cox, $Z_1 = -0.82$, P = 0.412), and the effect of this treatment was not affected by whether natural enemies were excluded or not (volatile treatment per enemy exclusion interaction term: Mantel–Cox, $Z_1 = 0.31$, $P = 0.754$). The exclusion of natural enemies affected the time course of aphid numbers as revealed by a significant interaction between time and natural enemy exclusion (mixed-effects model, time effect: $F_{1,397} = 98.05$, $P < 0.001$; interaction term: $F_{1,397} = 14.33$, $P < 0.001$). The interaction between the treatment with plant volatiles and time was not significant, and was removed from the model. Similar results were obtained when the data were analysed using a two-way ANOVA. Mean cumulative *S. avenae* numbers over the 35 day period were larger (approximately twice as large, ESM-1) than in replicates where natural enemies were present (ANOVA, $F_{1,36} = 33.31$, $P < 0.001$). Overall, the volatile treatment did not affect *S. avenae* cumulative abundance (ANOVA, $F_{1,36} = 2.90$, $P = 0.097$), but aphid abundance was significantly higher in the volatile/no natural enemy treatment compared with the other three treatments (there was a significant interaction term; ANOVA, $F_{1,36} = 4.99$, $P = 0.032$; posthoc test in ESM-1). Exposure to aphid-induced bean volatiles in plots with natural enemies present did not affect the cumulative numbers of parasitoids and predators on wheat plants (adult parasitoid *Aphidius* in, $t_1 = -0.77$, $P = 0.440$; mummy parasitoid *Aphidius*, $t_1 = -0.71$, $P = 0.489$; adult dermapteran *Forficula*, $t_1 = -1.66$, $P = 0.114$; hoverfly egg, $t_1 = -0.91$, $P = 0.377$; mean cumulative numbers per treatment are presented in ESM-2).

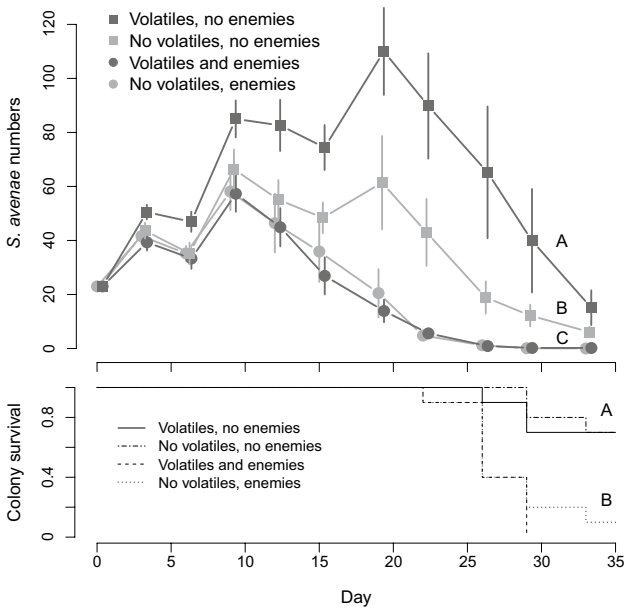

Fig. 2 Population dynamics (top) and colony survival (bottom) of *Sitobion avenae* aphids in the field experiment. The mean size of aphid colonies (±SE) and proportion of aphid colonies that survived ($n = 10$) over the course of the experiment are presented. Colonies of *S. avenae* aphids feeding on *Triticum aestivum* were surrounded by *Vicia faba* plants previously infested with *Acyrthosiphon pisum* aphids or not (volatile treatment). This treatment was crossed with natural enemy exclusion (enemy treatment) in a factorial design. For each panel, different letters at the end of the curves represent significant differences in aphid numbers or colony survival among plot types ($P < 0.05$ based on Tukey post hoc test, and pairwise comparisons between treatments on aphid numbers and colony survival, respectively). In the population dynamics plot, post hoc tests are based on mixed-effects models for aphid numbers through time. Mean cumulative aphid numbers per plot type are shown in Fig. ESM-1

Glasshouse experiments

In glasshouse experiment 1, we explored the direct effect of *V. faba* volatiles on *S. avenae* when the aphids fed on artificial diet. The presence of volatiles did not affect the

total number of offspring produced ($Z_1 = -0.36$, $P = 0.715$; Fig. 3A) or survival after 15 days ($Z_1 = 0.31$, $P = 0.759$; Fig. 3B). In glasshouse experiment 2, *S. avenae* aphids feed on live plants and might be influenced by both direct and indirect (via the food plant) effects of the presence of volatiles. The total number of offspring produced by two females inside a clip cage was not significantly affected by the treatment. Relative to controls, there was a tendency for *S. avenae* in the presence of *V. faba* plants emitting volatiles to produce more offspring, but this was not significant ($Z_2 = 1.86$, $P = 0.062$; Fig. 3C). Aphids on pre-exposed plants produced a similar number of offspring as aphids on controls ($Z_2 = 1.11$, $P = 0.267$; Fig. 3C). Relative to controls, *S. avenae* survival after 20 days was larger in the presence of *V. faba* plants emitting herbivore-induced volatiles ($Z_2 = -2.05$, $P = 0.039$; Fig. 3D), with survival of the pre-exposure treatment being intermediate and not significantly different from that of control aphids ($Z_2 = -1.15$, $P = 0.257$).

Bean volatile effects on wheat phloem exudate composition

Aphids are phloem feeders, and to test the potential role of aphid-induced *V. faba* volatiles on *S. avenae* aphids through changes in their host plant, *T. aestivum* phloem exudate composition was analysed. We identified a total of three carbohydrates, three organic acids, 18 primary amino acids (where arginine and alanine could not be separated and were pooled), and one secondary amino acid in phloem exudates (Table 1). The first two axes of the PLS-DA analysis explained 33% and 12% of the variance, respectively (Fig. 4), but there was no significant difference in the phloem exudate composition between plants exposed or not exposed to aphid-induced *V. faba* volatiles (permutation analysis: NMC statistic = 0.38, $n = 12$, $P = 0.1943$; Fig. 4). Concentrations of metabolites with VIP values equal or larger than 1, which are considered the most influential in separating treatments, were compared between the two groups of plants, but no significant differences between control and treatment samples were observed (Table 1). There were no significant differences between treatments in the ratio of essential to non-essential amino acids [0.266 ± 0.017 (mean ± SE of pooled plants); Wilcoxon rank sum test $W = 44$, $P = 0.113$], and in the ratio of sucrose to amino acids [0.043 ± 0.005 (mean ± SE of pooled plants); Wilcoxon rank sum test $W = 57$, $P = 0.410$].

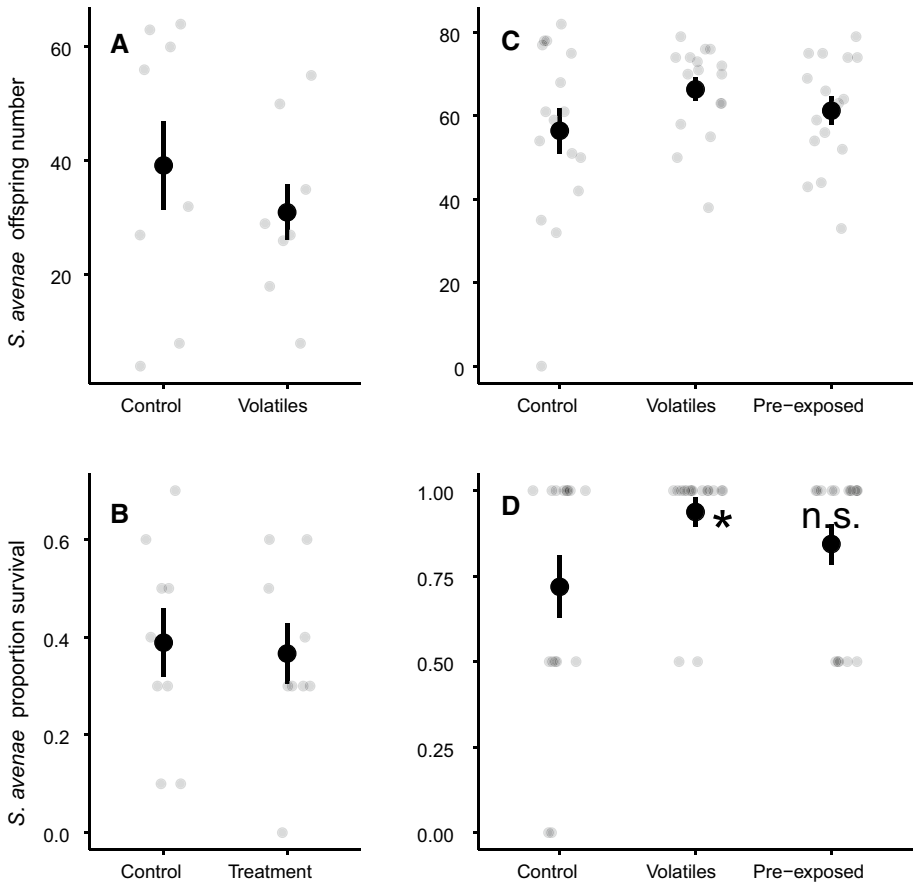

Fig. 3 Results of the glasshouse experiments on the effect of *Vicia faba* volatiles on offspring production (**A** and **C**) and proportion survival (**B** and **D**) of *Sitobion avenae* aphids. Mean (± SE) number of offspring ($n = 9$) produced by 10 *S. avenae* aphids (**A**), and survival (**B**) of aphids on artificial diet and exposed to *V. faba* plants previously infested with *Acyrthosiphon pisum* aphids (volatiles) or not (control). Mean (± SE) number of offspring ($n = 16$) produced by two *S. avenae* aphids (**C**), and survival (**D**) of aphids feeding on *Triticum aestivum* plants concurrently exposed to *V. faba* plants previously infested with *Acyrthosiphon pisum* aphids (volatiles), pre-exposed to *V. faba* plants infested with *Acyrthosiphon pisum* aphids (pre-exposed), or exposed to clean *V. faba* plants (control). In D, significant differences between control and the two treatments are shown (*$P < 0.05$; n.s.: non-significant). Light grey dots are observed values jittered along abscissa

Table 1 Metabolites identified in phloem exudates of *Triticum aestivum* plants exposed to *Vicia faba* plants previously infested with *Acyrthosiphon pisum* aphids (with volatiles) or not (no volatiles) ($n = 12$)

Metabolite	Abbreviation	Essential amino acid	Retention parameter	m/z	Identification		VIP score	Relative concentration (%)		Fold change	*P* value
					Golm	Standard		No volatiles	With volatiles		
Carbohydrates											
Fructose	Fruc	NA	1861 + 1871	217/277/364/335/307	x	x	0.90	4.22 ± 0.34	3.97 ± 0.37	0.94	NA
Glucose	Gluc	NA	1885 + 1903	319/229/343/305/160	x	x	0.87	4.48 ± 0.35	4.20 ± 0.35	0.94	NA
Sucrose	Sucr	NA	2615	451/361/319/157/437	x	x	0.86	3.42 ± 0.63	3.90 ± 0.60	1.14	NA
Organic acids											
Succinic acid	Succ	NA	1312	172/147/262/129/247	x	x	0.83	0.11 ± 0.01	0.09 ± 0.01	0.82	NA
Malic acid	Mali	NA	1485	245/335/307/217/233	x	x	0.63	0.26 ± 0.04	0.21 ± 0.02	0.81	NA
Quinic acid	Quin	NA	1847	345/334/537/419/255	x		0.70	0.11 ± 0.01	0.11 ± 0.02	1.00	NA
Amino acids											
Aspartic acid	ASP	No	2.97			x	1.05	16.27 ± 0.85	15.36 ± 0.80	0.94	0.569
Glutamic acid	GLU	No	4.63			x	1.43	17.46 ± 0.72	17.50 ± 1.37	1.00	0.932
Asparagine	ASN	No	7.99			x	1.31	9.51 ± 1.20	6.77 ± 1.08	0.71	0.275
Serine	SER	No	8.50			x	1.00	8.33 ± 0.32	8.46 ± 1.03	1.02	0.429
Glutamine	GLN	No	9.72			x	1.42	5.69 ± 0.67	5.10 ± 0.40	0.90	0.784
Histidine	HIS	Yes	10.11			x	1.59	0.63 ± 0.05	1.11 ± 0.30	1.76	0.275
Glycine	GLY	No	10.71			x	1.05	0.36 ± 0.05	1.00 ± 0.58	2.78	0.784
Threonine	THR	Yes	10.99			x	0.99	2.73 ± 0.13	3.11 ± 0.16	1.14	NA
Arginine + alanine	ARG + ALA	No	13.36			x	1.06	7.40 ± 0.33	8.12 ± 0.29	1.10	0.429
γ-Aminobutyric acid	GABA	No	13.92			x	0.52	4.36 ± 0.40	4.19 ± 0.27	0.96	NA
Tyrosine	TYR	No	15.82			x	0.88	0.97 ± 0.05	1.10 ± 0.09	1.13	NA
Valine	VAL	Yes	19.13			x	0.94	3.61 ± 0.26	4.04 ± 0.29	1.12	NA
Methionine	MET	Yes	19.60			x	0.81	0.72 ± 0.04	0.82 ± 0.08	1.14	NA
Tryptophan	TRP	Yes	21.20			x	0.97	0.50 ± 0.05	0.54 ± 0.07	1.08	NA
Phenylalanine	PHE	Yes	21.90			x	0.92	1.50 ± 0.14	1.81 ± 0.16	1.21	NA
Isoleucine	ILE	Yes	22.16			x	0.92	3.08 ± 0.24	3.45 ± 0.31	1.12	NA
Leucine	LEU	Yes	23.40			x	0.91	2.80 ± 0.31	3.35 ± 0.30	1.20	NA
Lysine	LYS	Yes	24.10			x	0.73	1.12 ± 0.07	1.21 ± 0.10	1.08	NA
Proline	PRO	No	31.01			x	0.98	0.36 ± 0.03	0.50 ± 0.14	1.39	NA

Carbohydrates and organic acids were analysed with gas chromatography–mass spectrometry (GC–MS); Kováts retention indices (for all analytes belonging to the same metabolite), and characteristic mass-to-charge ratios (m/z) are given. Amino acids were quantified via ultra-high performance liquid chromatography coupled to fluorescence detection; retention times (minutes) are shown. It is indicated whether metabolite identifications were based on the Golm metabolome database (GC–MS only) and/or standards. This table also shows which amino acids were considered as essential, VIP scores of the PLS-DA analysis (Fig. 3), relative concentrations (%) in treatment and control plants (mean ± SE), mean fold changes, and *P* values of the Wilcoxon rank sum tests adjusted after the false discovery rate procedure (these latter values only for metabolites with VIP scores larger or equal to one)

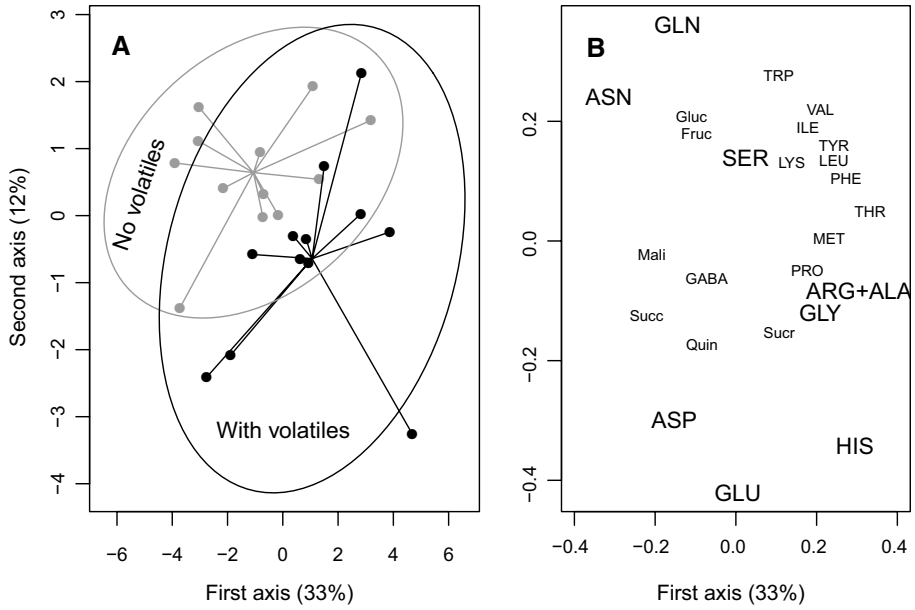

Fig. 4 PLS-DA plot on the relative concentrations of metabolites in phloem exudates of *Triticum aestivum* plants exposed to *Vicia faba* plants previously infested with *Acyrthosiphon pisum* aphids (with volatiles) or not (no volatiles) ($n = 12$). (A) PLS-DA score plot with the first two principal components represented; each dot represents a sample, the centre of the star is the multivariate centroid, and the ellipse the 95% confidence interval. **B** PLS-DA loadings plot with all metabolites depicted with respect to the first two principal components. Metabolites depicted in a larger font are those with a VIP score larger or equal to one (Table 1). Abbreviations can be found in Table 1. Some labels have been slightly displaced to increase clarity

Discussion

Our study was designed to test the hypothesis that herbivore-induced changes in plant physiology are able to increase local densities of insect predators and parasitoids leading to increased mortality of insects feeding on nearby plants. We worked with the grain aphid, *S. avenae,* and the pea aphid, *A. pisum,* two species that do not compete for the same plant resource, because they feed on members of the Poaceae and Fabaceae, respectively. These two insect species can, however, interact indirectly through apparent competition, because they share several natural enemies (Van Veen et al. 2008). *V. faba* fed upon by *A. pisum* releases plant volatiles that attract natural enemies (Du et al. 1996, 1998; Guerrieri et al. 1999; Takemoto and Takabayashi 2015), and we hypothesised that in the field, these volatiles would attract natural enemies that would increase the rates of predation and parasitism on nearby *S. avenae* colonies. Our experimental design crossed volatile induction with natural enemy exclusion. To test these two effects, colonies of *S. avenae* aphids feeding on *T. aestivum* plants were surrounded by *V. faba* plants previously infested with *A. pisum* aphids. To test the role of natural enemies in this interaction, the volatile treatment was fully crossed with natural enemy exclusion. Contrary to our prediction, in the presence of natural enemies, there were no differences in *S. avenae* density or colony survival, nor in natural enemy density, between field plots with or without exposure to aphid-induced volatiles. In plots where natural enemies were excluded, however, exposure to aphid-induced *V. faba* volatiles had a positive effect on *S. avenae* colony dynamics. This result was confirmed in the laboratory and suggests that exposure to aphid-induced *V. faba* volatiles promotes performance of *S. avenae* aphids directly or indirectly through changes in the host plant. Laboratory experiments with artificial diet ruled out a direct effect of aphid-induced *V. faba* volatiles on *S. avenae* aphid colony development, thus suggesting that the effects were mediated by volatile-induced changes in the focal plants. Metabolomic analyses of wheat phloem exudates, however, did not reveal any volatile effect on the composition of selected carbohydrates, organic acids, and amino acids.

Apparent competition has been experimentally demonstrated several times for aphids, including for the two species studied here (Frago and Godfray 2014), where the presence of one aphid species attracted natural enemies that also attack the other species. Under the experimental conditions in the present study, we found no evidence that aphid-induced volatiles released by nearby bean plants increased the attraction of natural enemies, and more strongly reduced *S. avenae* colony development on wheat compared to the situation where bean plants had not been infested by *A. pisum* aphids before. In our study, we sampled several guilds of natural enemies, but it is possible that other predatory guilds that we were unable to sample (like highly mobile mirid

predators) were more abundant in plots where bean plants were emitting aphid-induced volatiles. These predators may have played a role in apparent competition between the two aphids studied, but the effect may have been offset by the positive impact that bean volatiles had on *S. avenae* aphids. Further studies to better understand this result would be thus welcome, especially in seasons or locations where generalist predators are less abundant and where colonies survive for a longer period allowing more subtle effects to be observed.

Unexpectedly, in the field experiment, *S. avenae* densities were higher in the presence of *A. pisum*-induced bean volatiles when natural enemies were excluded. Similarly, we found in glasshouse experiments that *S. avenae* survival on wheat was higher in the presence of aphid-induced volatiles emitted by bean plants than in the presence of volatiles emitted by non-induced bean plants. Survival of aphids on artificial diets was not influenced by these volatiles, which suggests that the effect was mediated by the host plant's response to the volatiles rather than any direct effect on the aphid. One caveat to this conclusion is that survival in the artificial diet experiment was low and this might have masked a response. In an additional experiment, we tried to elucidate possible changes in wheat phloem sap quality induced by exposure to aphid-induced *V. faba* volatiles. However, the profiles of primary metabolites of wheat phloem exudates did not differ between plants exposed and not exposed to aphid-induced *V. faba* volatiles. As the EDTA-based collection of phloem exudates only allows the determination of relative but not absolute concentrations of compounds, we cannot rule out that the total concentrations of the primary metabolites were affected. Moreover, plant phloem sap is a complex fluid that may contain metabolites and proteins that were not measured here (Carella et al. 2016). In wheat, these metabolites may include benzoxazinoids, which are defensive metabolites often present in the phloem (Li et al. 2018). It is therefore possible that volatile exposure led to changes in the concentrations of compounds that we did not measure. In addition, relative to other aphids like pea aphids, the metabolism of aphids that feed on grasses (but see Sandström et al. 2000)], and the nutritional services that the obligatory symbiont *Buchnera* may provide (Douglas 2006), are little understood. The positive indirect effect found here may be due to *S. avenae* benefiting from phytohormonal responses or induction of defensive compounds in wheat after exposure to aphid-induced *V. faba* volatiles. This has been previously found in other specialist aphids that benefit from phytohormonal responses or plant defensive metabolites (Züst and Agrawal 2016). Plant responses to volatiles produced by other plant species have been documented in a number of other systems (Engelberth et al. 2004; Erb et al. 2015; Karban et al. 2014; Ninkovic et al. in press), though we are not aware of studies involving wheat. Insect responses to plant volatiles emitted by non-host plants are also well documented (Turlings and Erb 2018), but long-term multigenerational experiments exploring this question are scarce. In our study, the magnitude of the volatile effect was larger after three aphid generations (20 days), which reveals the importance of performing studies over multiple insect generations.

In most examples of plant species responding to herbivore-induced volatiles, the response has been increased investment in defence against herbivory, which would tend to depress herbivore numbers [i.e., defence induction, e.g., Erb et al. (2015) and Timilsena et al. (2020)]. This may lead to plant associational resistance whereby a given plant individual shows increased resistance against herbivores due to the presence of plants infested with the same insect species nearby. A study with the moth *Spodoptera littoralis* revealed that oviposition on cotton, *Gossypium hirsutum*, was reduced if these plants were exposed to volatiles of plants of the same species that had suffered previous damage by this pest (Zakir et al. 2013). Another study with the wild plant *Baccharis salicifolia* and the aphids *Uroleucon macolai* and *Aphis gossypii* revealed that volatile-mediated defence priming is highly specific, because resistance was only induced against the same aphid species used to induce plant volatiles (Moreira et al. 2018). These effects can also extend to microbial pathogens as revealed by a study in which fungus-induced volatiles of a common bean *Phaseolus vulgaris* variety that is resistant to the fungus *Colletotrichum lindemuthianum*-induced resistance in conspecific plants of a susceptible variety (Quintana-Rodriguez et al. 2015). Ecologists are increasingly aware that volatile-mediated associational resistance is common and highly specific (Moreira and Abdala-Roberts 2019), although evidences of plant volatiles rendering neighbouring plants more susceptible (the result that we found) are less often reported. This effect can be referred to as associational susceptibility and has recently been reported between tomato plants of the same variety attacked by the whitefly *Bemisia tabaci* (Zhang et al. 2019). The Zhang et al.'s (2019) study and ours thus suggest that associational susceptibility deserves further attention as it may be more common than currently appreciated, particularly between different plant species. This study and most of the examples given above used crop plant species, which have been selected to enhance/reduce expression of specific traits, and it is thus possible that the results observed are a maladaptive consequence of selection. It would be interesting to explore how often associational susceptibility in response to exposure to herbivore-induced plant volatiles occurs in wild progenitors of these crop plant species, or among plants in natural communities.

In the field experiment, in the absence of natural enemies, we did not find any differences in *S. avenae* colony survival but larger population sizes on wheat plants exposed to volatiles from *A. pisum*-induced bean plants. Aphid populations are exposed to several mortality factors

including natural enemies, abiotic stressors, or plant toxic compounds (Dixon 1977), but aphid densities on a given plant can also decline if adult aphids disperse away from it. A possible adaptive explanation for our findings is therefore that the aphids directly or indirectly detected the presence of volatiles and increased their investment in current colony growth and winged female dispersal at the expense of long-term colony persistence (in spring aphids are clonal and hence colony rather than individual fitness is maximized, Dixon 1977). Finally, although we tried to control for extraneous differences between induced and non-induced *V. faba* plants, it is possible that some remained. For example, plant biomass (and therefore shading) may be reduced in *V. faba* plants attacked by *A. pisum* with potential impacts on the photosynthesis of nearby *T. aestivum* plants. Experiments to rule this explanation out may include exposing *S. avenae* aphids placed on wheat to *V. faba* volatiles using two-chamber cage experiments as in Ninkovic (2003) and Ninkovic et al. (2013).

Insect herbivores live embedded in complex communities, and the last decade has shown remarkable progress in understanding the structure of trophic webs, at both the theoretical and the experimental level. Despite these advances, our knowledge of how behavioural changes modulate trophic web interactions is still very limited. Given their omnipresence and their known influence on insect behaviour, plant volatiles are likely to play an important role in this context. Studies of these interactions may also be of applied importance. In agricultural contexts, interactions between insects that share natural enemies or which interact through plant volatiles should be taken into consideration. For example, the grass *Melinis minutiflora* is often planted in maize fields in sub-Saharan Africa, because it constitutively emits volatile compounds that repel stemborer pests, but which attract their natural enemies (Khan et al. 2010). Our study provides evidence that herbivore-induced volatiles from one plant species have a positive influence on insect herbivores feeding another plant species, a finding that is not commonly reported (Zhang et al. 2019). Elucidating which plant combinations lead to positive or negative consequences for pest densities will help to design ecological crop protection strategies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00442-021-05097-1.

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Author contribution statement EF conceived and designed the research; EF performed the field experiment; RG, and EF performed the glasshouse experiments; RS and CM collected, quantified, and identified phloem exudate metabolites; EF analysed data; HCJG and EF wrote the manuscript, and all authors contributed with revisions.

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Data availability The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interests.

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