Journal of Pest Science (2023) 96:1049–1059
https://doi.org/10.1007/s10340-023-01592-x

ORIGINAL PAPER

Volatile interactions between specific undamaged barley cultivars affect aphid feeding behavior and performance

Sokha Kheam[1,2] · Dimitrije Markovic[3,4] · Diana Rubene[3] · Saveng Ith[2] · Velemir Ninkovic[1]

Received: 1 August 2022 / Revised: 2 December 2022 / Accepted: 6 January 2023 / Published online: 12 January 2023

Abstract

Recent studies have demonstrated that cultivar mixtures can reduce aphid plant acceptance and population development. It is still unknown as to which underlying mechanisms may contribute to this phenomenon. We investigated the effects of volatile interactions between undamaged barley cultivars on aphid feeding behavior and performance in the laboratory. Spring barley (*Hordeum vulgare* L.) cultivar Salome was exposed to volatiles from Fairytale (SeF), Anakin (SeA), or clean air (Se0). We used an electrical penetration graph to test the effect of exposure to neighbor volatiles on the feeding behavior and performance of bird cherry-oat aphids (*Rhopalosiphum padi* L.). We also assessed aphid relative growth rate, intrinsic rate of increase, and development time on exposed and unexposed Salome plants. Aphids spent significantly longer time on epidermis and mesophyll plant tissues on SeF than Se0, and no difference was observed between SeA and Se0. Significant decreases in the duration of phloem ingestion and phloem sustained ingestion were recorded in SeF showing that volatile-induced effects cause difficulty for aphids to feed. However, no differences in these variables were detected between SeA and Se0. We also observed reduced aphid relative growth rate and intrinsic rate of increase on SeF compared to Se0 and SeA. Our study demonstrated that, in a specific combination, exposure of one barley cultivar to volatiles from another one can change aphid feeding behavior and performance, probably due to changes in host plant properties/quality. Our results provide an insightful explanation of mechanisms responsible for the reduced aphid population development previously observed in the field.

Keywords Variety mixture · Plant–plant interactions · Plant–insect interactions · Plant protection · Induced defense · EPG

Key messages

- Volatile interactions between undamaged barley cultivars disrupt aphid feeding behavior and reduce aphid relative growth rate and intrinsic rate.
- Volatile-induced responses in plants, which affect aphid feeding and performance, depend on the genotype of the neighboring cultivar.
- Volatile interactions between specific cultivars could be the underlying mechanism, which reduces aphid population development in cultivar mixtures in the field.

Communicated by Orlando Campolo.

✉ Velemir Ninkovic
velemir.ninkovic@slu.se

[1] Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

[2] Department of Biology, Royal University of Phnom Penh, Phnom Penh, Cambodia

[3] Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

[4] Faculty of Agriculture, University of Banja Luka, Banja Luka, Bosnia and Herzegovina

Introduction

Plant diversity contributes to ecosystem stability (Prieto et al. 2015; Isbell et al. 2017), while in agroecosystems, botanical diversity can reduce damage by insect pests, improve biological pest control and increase food production (Ratnadass et al. 2012). Combining different cultivars in mixtures to increase within field diversity has been suggested as a promising strategy to reduce pest pressure (Tooker and Frank 2012; Koricheva and Hayes 2018; Snyder et al. 2020). The quality of evidence for pest suppression in

cultivar mixtures is varied, as some studies show that cultivar mixtures can reduce aphid population sizes (Shoffner and Tooker 2013; Snyder et al. 2020), while others report the lack of effects on aphids (Mansion-Vaquié et al. 2019; Grettenberger and Tooker 2020). Understanding these inconsistent effects of cultivar mixtures on aphids is important from both an ecological and practical perspective and could be achieved by clarifying the underlying mechanisms responsible for reduced aphid abundance.

Plant species diversity or genotypic diversity could affect pest insects via several mechanisms including dilution effect, abundance of natural enemies, and associational resistance. The dilution effect leads to reduced pest population spread via increased host plant finding time (Malézieux et al. 2009; Hambäck et al. 2014). The natural enemy hypothesis predicts that diverse plant communities host a higher abundance of natural enemies, which could suppress herbivorous pests (Cook-Patton et al. 2011). The cultivar mixtures can attract more natural enemies than pure stands (Ninkovic et al. 2011). Associational resistances involve specific plant associations that provide physical and chemical barriers that suppress insect pests (Malézieux et al. 2009; Dahlin et al. 2018). Volatile interactions between undamaged neighboring plants via changes in receiving plant physiology can potentially present one of the underlying mechanisms for reduced aphid performance (Ninkovic et al. 2016).

Volatile organic compounds (VOCs) play a major role as cues and signals in trophic interactions (Ninkovic et al. 2020), and can stimulate or prime defense responses in neighboring plants (Heil and Karban 2010; Brilli et al. 2019). Herbivore-induced plant volatiles (HIPVs) induce changes in the neighboring plants directly and indirectly, which can both lead to pest suppression and attraction of their natural enemies (Ninkovic et al. 2001; Dicke and Baldwin 2010; War et al. 2011; Karban et al. 2014). Volatile interactions between undamaged plants can also change the physiology of the receiving plants with subsequent influence on organisms through higher trophic levels (Ninkovic et al. 2006). This phenomenon, known as allelobiosis (Pettersson et al. 2003), could potentially be responsible for aphid suppression in cultivar mixtures. For instance, Dahlin et al. (2018) reported that mixing specific barley cultivars significantly reduced aphid population development in a field trial, while volatile interactions in the same cultivar combinations reduced aphid plant acceptance in a laboratory test. This clearly indicates that volatile interactions between specific cultivars can affect the early stages of aphid establishment in plants.

We hypothesized that the genetic identity of emitting cultivar expressed through their specific volatile profile plays an important role in the induction of defense responses in receiving cultivar and affect aphid performance. The primary aim of this study was to investigate the effect of volatile interactions between specific barley cultivars on (i) aphid feeding behavior, (ii) aphid relative growth rate, intrinsic rate, and development time on receiving cultivar after exposure to volatiles from another cultivar, and (iii) test whether aphid responses are dependent on specific cultivar used as an emitter.

Materials and methods

Plants and insects

Spring barley (*Hordeum vulgare* L.) cultivars Salome (Nordaat Saatzucht GmbH, Germany), Fairytale and Anakin (Sejet Plant Breeding, Denmark) were used in this study, because some of these cultivars in combination can reduce aphid plant acceptance in the laboratory and lower population size in the field (Dahlin et al. 2018). The pedigrees of the cultivars were Auriga × (Publican × Beatrix) for Salome, Colston × (Receipt × Power) for Fairytale and Tumbler × Response for Anakin. These cultivars were obtained from Scandinavian Seed AB, Linköping, Sweden. Before sowing, seeds were germinated in Petri dishes between two filter papers for 24 h at room temperature. One seed was sown per pot (9 × 9 × 7 cm), filled with P-soil (Hasselfors, Sweden), and kept in the growing chamber for 9 days at 18–22 °C, 50–60% relative humidity, and L16:D8 h photoperiod.

As a model insect, we used the bird cherry-oat aphid (*Rhopalosiphum padi* L.), which is one of the most important pests in cereals. The aphids used in this experiment were reared on oat (*Avena sativa* L.) cultivar Belinda in separate growing chambers under the same growing conditions as for the plants.

Plant volatile exposure

To study the effects of volatile interactions between different barley cultivars on aphid feeding behavior and performance, we used twin Perspex cages (Ninkovic 2003). These cages are divided into two chambers–inducing and responding (each 10 × 10 × 40 cm), connected by a circular opening (7 cm diameter) in the middle wall. Air entered into the system through the chamber with an emitter plant and passed through the hole in the middle wall into the chamber with a receiver plant, before being vented outside the room. Airflow in the system was 1.2 L/min. Each individual potted plant was placed in a Petri dish to avoid the potential interactions between plants by root exudates. Plants were watered by an automated drop system (DGT Volmatic) for 2 min every day without adding extra fertilizers.

The plants were placed in an exposing system at the one-leaf stage (7 days old). The exposure time was 5 days. The receiving cultivar Salome was exposed to: volatiles from

Fairytale (SeF), volatiles from Anakin (SeA), and clean air (Se0).

Electrical penetration graph recording setup

Aphid stylet activities are commonly monitored by an electrical penetration graph (EPG) device to determine different plant tissues where resistance can occur (Tjallingii 2006). We used an EPG system to determine whether the volatile interactions between barley cultivars induce plant resistance affecting aphid feeding behavior. The experimental setup was placed in a Faraday cage for electrical noise isolation. After a 5-day exposure to either Fairytale (SeF), Anakin (SeA), or clean air (Se0), receiving cultivar (Salome) plants were moved from exposing the system to a Faraday cage. The second leaf of the plants was fixed with transparent nylon strips with tape at the ends. Because aphids prefer to settle on the abaxial leaf side, this side was faced up (Pettersson et al. 2017). Adult apterous aphids of *R. padi* were collected from colonies using a marten-hair brush (size 0) and starved in a Petri dish for a period of 30 min. A vacuum on a small hole in a pipet tip was deployed to fix aphids (Schliephake et al. 2013). Water-soluble silver glue was placed on the middle-back of the aphid dorsum to attach a thin gold wire of about 1.5–2.5 cm in length to the aphid (18 microns in diameter). The other end of the gold wire was connected to a copper wire electrode soldered to a brass nail that functioned as an aphid electrode and was inserted into the input of the EPG headstage amplifier. Another electrode with a length of 8 cm was placed in the soil.

An eight-channel Giga-8 DC EPG system was employed. Devices with 1G Ohm were used to monitor the probing and feeding behavior of aphids on the different treatments evaluated (EPG Systems, Wageningen, The Netherlands). A USB analog/digital converter card (DI 710-UL) was used to transfer the EPG signals to a PC computer. The duration of the recording was eight hours. To ensure the proper data remained in limited output signals, the adjustments of voltages on the EPG system were manually attempted for later analysis. The aphids that produced nymphs, died, or left the plant were discarded from the analysis. EPG signals were acquired and analyzed using Stylet + software for Windows (EPG systems).

We split the EPG data collection into two experiments, where the first one compared aphid feeding on SeF versus Se0 and the second one compared aphid feeding on SeA versus Se0. In each experiment, aphids were run on the EPG device for six trials, where in each trial we tested four aphids on treatment and four aphids on control. We tested one trial (8 plants) per day until we obtained 20 replicates for SeF and 19 replicates for Se0 (experiment 1) and 22 replicates for SeA and 23 replicates for Se0 (experiment 2).

Due to limited space (an 8-channel EPG device) for simultaneous observations, we run only one treatment against control at a time, in order to accumulate a sufficient sample size for each comparison in as a short time as possible, which ensures similar conditions for the aphids and the plants used. This sets a major limitation on our study, as we cannot directly compare data for SeF with SeA, but need to imply differences between them via relative comparisons with the control treatment.

Electrical penetration graph waveforms and variables

The "Stylet + a" software (EPG Systems) was used to analyze the data from the Stylet + d program (Tjallingii and Esch 1993). This software defines clear waveform patterns to determine different phases of stylet performance during aphid penetration and feeding. We used online EPG-Calc 6.1.7 software to calculate different EPG variables (Giordanengo 2014). Consequently, waveform data were calculated based on the several sequential and non-sequential variables of standardized EPG-variable listed on epgsystems.eu. Twenty-nine different EPG variables were used to assess the aphid feeding behavior on different treatments in both experiments. Waveforms in certain phases of aphid feeding behavior were selected for analyses, including: none probing (NP), probing (Pr), pathway (C), potential drops (Pd), sieve element salivation (E1), phloem sap ingestion (E2), stylet penetration difficulties (F), and xylem phase (G) (Tjallingii 1990). Waveform "NP" refers to none probing behavior, which is described as no contact or penetration between stylet and plant tissues (studied variables: number of non-probing (n_NP), average of non-probing (a_NP), and duration of non-probing (s_NP)). Waveform "Pr" refers to the general probing activity, during which the stylet penetrates the plant tissues (studied variables: number of probing (n_Pr), average of probing (a_Pr), total duration of probing (s_Pr), number of first brief probes (n_bPr), and time to first probe (t > 1Pr)). Waveform "C" refers to the pathway phase, described as intercellular penetration movements of the stylet (studied variables: number of C (n_C), average of C (a_C), and total duration of C (s_C)). Waveform potential drops "Pd" describe brief intracellular stylet punctures in the stylet pathway (studied variables: number of potential drop (Pd), and total duration of potential drop (s_Pd). Phloem activity consists of two waveforms: E1 and E2. Waveform "E1" refers to sieve element salivation at the beginning of the phloem phase (studied variables: time to first E (t > 1E), number of single E1 (n_sgE1), number of E1 (n_E1), and total duration of E1 (s_E1)). Waveform "E2" refers to phloem sap ingestion with concurrent salivation (studied variables: time to first E2 (t > 1E2), number of E2 (n_E2), total duration of E2 (s_E2), time to first sustained E2

(t > 1sE2), number of sustained E2 (n_sE2), total duration of sustained E2 (s_sE2), number of E12 (n_E12), and total duration of E12 (s_E12)). Waveform "G" refers to active xylem sap/water ingestion activity (studied variables: number of G (n_G), and total duration of G (s_G)). Waveform "F" is the derailed stylet mechanics, indicating stylet penetration difficulties (studied variables: number of times stylet derailed (n_F), and total duration of stylet derailed (s_F).

Aphid relative growth rate

To test aphid growth, 24-h-old aphids were introduced to receiving cultivar (Salome) after the plants had been exposed for 5 days to volatiles of Fairytale, Anakin, or clean air. The observations were carried out in the exposing system where the receivers with aphids were exposed to volatiles from emitters until the end of the experiment. To produce first-instar nymphs, adult apterae of *R. padi* were randomly selected from aphid culture and placed on eight-day-old oat plants for a period of 24 h. The first instars (24-h-old nymphs) were weighted by using the microbalance (Mettler Toledo, USA). One 24-h-old nymph was placed on each receiving plant (Salome). After 5 days, each nymph was re-weighed and the procedure was repeated in several trials, resulting in 16 replicates for the SeF, 18 replicates for SeA, and 22 replicates for Se0. We used aphid weights to calculate the mean relative growth rate based on the equation suggested by Radford (1967):

$$\text{MRGR } (\mu g/\mu g/\text{day}) = \left(\log W_2 - \log W_1\right)/t_2 - t_1$$

where MRGR = mean relative growth rate, W_1 = weight at the first weighing, W_2 = weight at the second weighing, and t_2–t_1 = the time (days) between first (t_1) and second (t_2) weighing.

Aphid development time

The 24-h-old nymphs of *R. padi* were placed between the first leaf and stem of a single Salome plant in the receiving cages. If the nymph disappeared (e.g., due to unsuccessful establishment or mortality), a new 24-h nymph was released the next day. The nymphs were monitored until they produced the first offspring. The day of introducing 24-h-old nymphs on the plant was counted as day 1. The aphid development time was calculated from day 1 to the day of producing the first offspring. The experiment was repeated in several trials until there were 20 replicates for SeF, 18 for SeA, and 19 for Se0. The observations of aphid development and intrinsic rate of increase were carried out in the same way as for the aphid relative growth rate.

Aphid intrinsic rate of increase

After development time observations, we started recording the intrinsic rate of increase (r_m). The day of producing the first offspring was recorded as day 1, and the total number of nymphs produced on each plant was counted after the following 5 days. We obtained 20 replicates for SeF, 18 for SeA, and 19 for Se0. The fecundity of an individual aphid to the intrinsic rate of increase (r_m) was calculated based on Wyatt and White (1977):

$$r_m = \left(\ln M_d x\, c\right)/d$$

where M_d is the number of nymphs produced by the adult in the first d days of reproduction after the adult molt. The constant ($c = 0.738$) is an approximation of the proportion of the total fecundity produced in the first days of reproduction.

Statistical analyses

The statistical analyses were carried out with the R statistical software (R Core Team 2021). Due to the non-normal distributions of most EPG data, the Wilcoxon rank-sum test (unpaired test) was used for the majority of the variables. For the variables that met the assumptions of parametric tests, general linear models (GLM) were employed (package lme4). We used GLM with the Poisson family to analyze count data (e.g., number of probing) and Gamma family to analyze the continuous variables (e.g., time to first sustained E2). The models were validated by graphic examination of residual plots (Zuur et al. 2010) and overdispersion tests in the DHARMa package. The $\alpha = 0.05$ significance level was applied to test the differences between treatments. Twenty-nine variables were analyzed and compared for each experiment (Table 1).

Generalized linear models (GLMER) were used to analyze response variables aphid development time, intrinsic rate of increase, and relative growth rate, by using the Gamma family with a log link. As a fixed factor, we used the cultivar combination with the categories of SeF, SeA, and Se0, and as a random effect we used trial. The control treatment was used as a reference category in the models, but in order to obtain estimates, errors and p values for pairwise comparisons between SeA and SeF, we rerun the models with SeA as the reference category.

Results

Aphid feeding behavior

The different variables used to analyze aphid *R. padi* feeding behavior at different phases are summarized in Table 1. A significant increase in the number of aphid non-probing (Wilcoxon, $p = 0.02$) and probing (Wilcoxon, $p = 0.01$) was recorded in SeF compared to Se0. It took approximately twice as long for aphids to probe on SeF compared to Se0. The average duration of aphid probing was significantly lower in SeF than in Se0 (GLM, Estimate = −0.51, SE = 0.24, $t = -2.06$, $p = 0.04$). No significant differences between SeA and Se0 were detected in non-probing and probing phases (Table 1).

Table 1 Comparisons of feeding behavior variables (means ± SEM) of *Rhopalosiphum padi* on cultivar Salome exposed to Fairytale (SeF) and Salome exposed to clean air (Se0) for experiment 1, and Salome exposed to Anakin (SeA) and Salome exposed to clean air (Se0) for experiment 2. Most of the variables were analyzed by Wilcoxon rank-sum test and the variables with (a) in the table were analyzed by using GLM models to compare the differences between treatments in each experiment with $p \leq 0.05$. Statistical differences between treatments are highlighted in bold and with asterisks

Variables	Experiment 1			Experiment 2		
	SeF	Se0	*P*	SeA	Se0	*P*
Number of replicates	20	19		22	23	
None-probing and probing (tissue penetration)						
1. Number of none probing (n_NP)	**6.4 ± 1.03**	**3.42 ± 0.66**	**0.02***	6.77 ± 1.46	6.83 ± 0.92	0.76(a)
2. Average of none probing (a_NP) (min)	10.4 ± 4.66	19.51 ± 9.45	0.71	11.08 ± 2.73	9.34 ± 1.86	0.91
3. Total duration of none probing (s_NP) (min)	54.77 ± 14.21	52.21 ± 22.22	0.47	71.53 ± 17.52	69.44 ± 13.18	0.61
4. Number of probing (n_Pr)	**6.35 ± 1.02**	**3.35 ± 0.64**	**0.01***	6.55 ± 1.47	6.7 ± 0.92	0.68(a)
5. Average of probing (a_Pr) (min)	**134.07 ± 30.17**	**234.31 ± 38.74**	**0.03*(a)**	183.32 ± 39.38	122.44 ± 27.57	0.38(a)
6. Total duration of probing (s_Pr) (min)	425.02 ± 12.22	427.6 ± 22.21	0.47	408.34 ± 17.52	410.44 ± 13.18	0.61
7. Number of first brief probe (< 3 min) (n_bPr)	0.75 ± 0.28	0.79 ± 0.27	0.75	0.59 ± 0.2	0.48 ± 0.19	0.60(a)
8. Time to first probing (t > 1Pr)	15.98 ± 9.42	10.55 ± 4.36	0.98	3.87 ± 1.55	11.96 ± 4.64	0.53
Pathway phase						
9. Number of C	**13.35 ± 1.57**	**7.42 ± 1.21**	**0.007***	13.45 ± 2.28	16.74 ± 1.7	0.12
10. Average of C (min)	7.36 ± 0.46	9.58 ± 1.51	0.54	6.84 ± 0.53	6.61 ± 0.58	0.53
11. Total duration of C (min)	**99.72 ± 12.93**	**56.45 ± 8.15**	**0.03***	86.6 ± 14.33	104.14 ± 11.29	0.22
12. Number of potential drop (Pd)	**73.35 ± 10.75**	**37.11 ± 4.91**	**0.01***	69.05 ± 14	86.83 ± 12.03	0.13
13. Total duration of potential drop (s_Pd) (min)	**5.25 ± 0.73**	**2.7 ± 0.36**	**0.01***	4.97 ± 1.03	6.3 ± 0.83	0.6
Phloem phase						
14. Time to first E (t > 1E)	96.35 ± 15.85	73.27 ± 16.52	0.29(a)	89.79 ± 22.94	108.35 ± 21.2	0.36
15. Number of single E1 (n_sgE1)	0.95 ± 0.36	0.53 ± 0.3	0.15	1.77 ± 0.35	1.83 ± 0.43	0.79
16. Number of E1 (n_E1)	**4.4 ± 0.61**	**3 ± 0.71**	**0.05***	4.18 ± 0.66	4.57 ± 0.66	0.71
17. Total duration of E1 (s_E1) (min)	**3.56 ± 1.49**	**2.1 ± 0.91**	**0.03***	7.14 ± 2.1	11.89 ± 3.1	0.38
18. Time to first E2 (t > 1E2)	125.39 ± 20.14	76.54 ± 16.3	0.09	113.55 ± 28.19	156.89 ± 24.48	0.09
19. Number of E2 (n_E2)	3.45 ± 0.55	2.47 ± 0.6	0.11	2.23 ± 0.34	2.48 ± 0.35	0.62
20. Total duration of E2 (s_E2) (min)	**202.11 ± 35.98**	**314.93 ± 32.32**	**0.05***	227.56 ± 33.64	148.6 ± 25.91	0.09
21. Time to first sustained E2 (t > 1sE2) (min)	**215.04 ± 33.62**	**90.15 ± 17.08**	**0.008***	180.17 ± 36.68	241.6 ± 34.81	0.19
22. Number of sustained E2 (n_sE2)	1.15 ± 0.2	1.21 ± 0.12	0.56	1.41 ± 0.25	1.13 ± 0.16	0.40(a)
23. Total duration of sustained E2 (s_sE2) (min)	**197.01 ± 36.3**	**312.8 ± 32.69**	**0.03***	226.01 ± 33.71	144.87 ± 25.83	0.09
24. Number of E12	3.45 ± 0.55	2.42 ± 0.58	0.09	2.18 ± 0.34	2.39 ± 0.34	0.65
25. Total duration of E12 (min)	**204.25 ± 35.9**	**316.42 ± 32.43**	**0.05***	233.34 ± 34.06	157.66 ± 26.63	0.1
Xylem phase						
26. Number of G (n_G)	**1.55 ± 0.4**	**0.47 ± 0.14**	**0.001*(a)**	**1.14 ± 0.18**	**1.83 ± 0.15**	**0.004***
27. Total duration of G (s_G) (min)	**44.87 ± 11.99**	**11.42 ± 5.49**	**0.01***	**67.03 ± 15.54**	**101.97 ± 12.08**	**0.01***
Stylet penetration difficulty						
28. Number of Stylet derailed (n_F)	1.7 ± 0.3	1.32 ± 0.36	0.33(a)	**1.09 ± 0.35**	**2.17 ± 0.43**	**0.04***
29. Total duration of Stylet derailed (s_F) (min)	74.63 ± 23.31	42.64 ± 19.29	0.09	19.78 ± 6.11	43.42 ± 12.4	0.12

In SeF, the number of aphid attempts in C phase (pathway phase) was significantly higher (Wilcoxon, $p=0.006$) and aphids spent considerably more time in C phase (Wilcoxon, $p=0.03$) than in Se0. There was also a significant increase in the number of potential drops (Pd) and total duration of potential drops (Wilcoxon, $p=0.01$, $p=0.01$, respectively) in SeF compared to Se0. In contrast, no differences in these variables were detected between SeA and Se0 (Table 1).

In the case of the phloem phases, aphids had a slightly higher number of attempts to salivation phase (E1) (Wilcoxon, $p=0.05$) with significantly longer duration (E1) in SeF compared to Se0 (Wilcoxon, $p=0.03$). The total duration of the phloem ingestion phase (E2) was slightly shorter (Wilcoxon, $p=0.05$), and the total duration of sustained phloem ingestion (sE2) was significantly reduced (Wilcoxon, $p=0.03$) in SeF compared to Se0. Time to the first sustained phloem ingestion ($t>1$sE2) was significantly longer in SeF than Se0 (Wilcoxon, $p=0.008$). On the other hand, there were no differences in these variables between SeA and Se0 (Table 1).

The number of attempts and the total duration of xylem ingestion (G) were higher in SeF than in Se0 (GLM, Estimate = 1.18, SE = 0.37, $t=3.13$, $p=0.001$; Wilcoxon, $p=0.01$, respectively). Interestingly, these two variables were lower in SeA than Se0 (Wilcoxon, $p=0.004$, $p=0.01$, respectively) (Table 1). There was a significantly lower number of stylet derailment (F) (Wilcoxon, $p=0.04$) but there was no difference in the total time of stylet derailment between SeA and Se0 (Wilcoxon, $p=0.12$). No differences in these variables were detected between SeF and Se0.

Overall, in experiment 1, SeF aphids spent 48% of the time in non-phloem ingestion phases vs. 32% in Se0, 43% vs. 66% in phloem ingestion and 9% vs. 2% in xylem ingestion, whereas in experiment 2, SeA aphids spent 37% of time in non-phloem ingestion vs. 45% in Se0, 49% vs. 33% in phloem ingestion, and 14% vs. 21% in xylem ingestion.

Aphid growth and development

The relative growth rate of individual *R. padi* nymphs after 5 days ranged from 0.29 to 0.66 µg/day. The GLM analysis showed that the relative growth rate of aphids was significantly reduced in SeF compared to Se0 (GLM, Estimate = − 0.12, SE = 0.05, $t=-2.26$, $p=0.02$) and SeA (GLM, Estimate = − 0.14, SE = 0.05, $t=-2.45$, $p=0.01$). However, no significant differences between SeA and Se0 (GLM, Estimate = 0.01, SE = 0.05, $t=0.30$, $p=0.76$) were detected (Fig. 1).

The development time of aphids ranged from 5 to 8 days to reach the adult stage and produce the first batch of new offspring. A significant increase was detected in SeF compared to SeA (GLM, Estimate = 0.1, SE = 0.03, $t=2.81$, $p=0.004$), while no difference was observed between

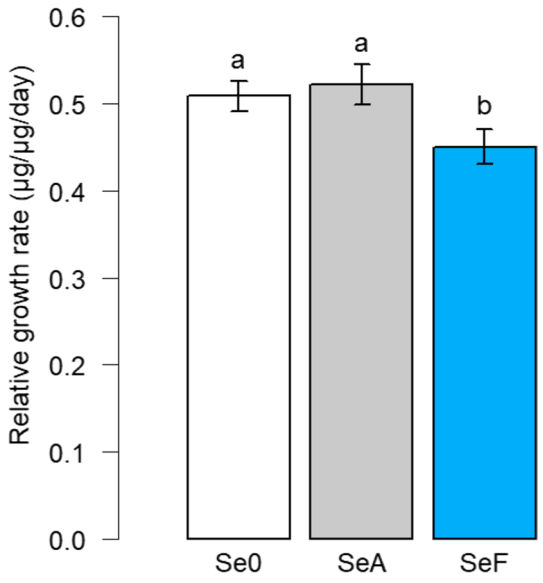

Fig. 1 Mean relative growth rate of *Rhopalosiphum padi* on Salome exposed to Fairytale (SeF), Salome exposed to Anakin (SeA), and Salome exposed to clean air (Se0). Error lines represent standard error of mean (SEM). Letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses

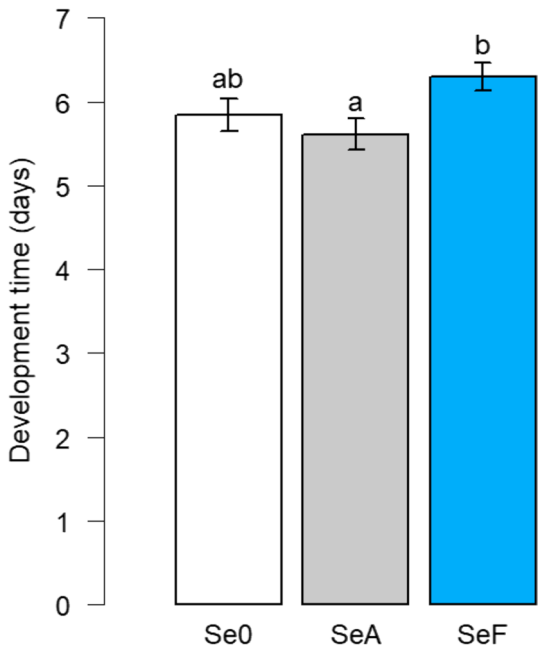

Fig. 2 Mean development time of *Rhopalosiphum padi* on Salome exposed to Fairytale (SeF), Salome exposed to Anakin (SeA), and Salome exposed to clean air (Se0). Error lines represent standard error of mean (SEM). Letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses

SeF and Se0 (GLM, Estimate = 0.05, SE = 0.04, $t = 1.46$, $p = 0.14$) (Fig. 2).

The potential of aphids to produce new nymphs ranged from 0.47 to 0.64 per day. We observed that aphids on SeF had a significantly lower intrinsic rate compared to on Se0 (GLM, Estimate = − 0.07, SE = 0.02, $t = -2.61$, $p = 0.008$) and SeA (GLM, Estimate = − 0.08, SE = 0.02, $t = -2.99$, $p = 0.002$), respectively. In contrast, aphid intrinsic rate did not significantly differ on SeA and Se0 (GLM, Estimate = 0.007, SE = 0.02, $t = 0.26$, $p = 0.79$) (Fig. 3).

Discussion

This study revealed that volatile interactions between certain undamaged barley cultivars lead to significant ecological effects by interrupting aphid feeding behavior and reducing performance on exposed plants. We have shown that aphid feeding behavior, growth rate, and intrinsic rate were significantly reduced on Salome after exposure to Fairytale, but no differences were found after exposure to Anakin. Our results confirmed the hypothesis that the genetic identity of emitter cultivar expressed through their specific volatile profile can induce resistance factors in the receiving cultivar, which affect aphid performance. A recent field study shows that aphid populations decreased most in the mixture of Salome and Fairytale compared to their pure stands,

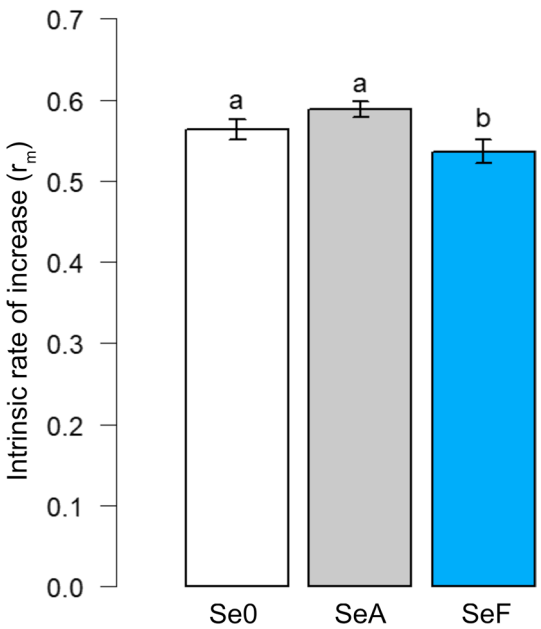

Fig. 3 Mean intrinsic rate of increase (r_m) of *Rhopalosiphum padi* on Salome exposed to Fairytale (SeF), Salome exposed to Anakin (SeA), and Salome exposed to clean air (Se0). Error lines represent standard error of mean (SEM). Letters above the bars represent statistical significance at $p \leq 0.05$ using GLM analyses

but not in Salome and Anakin mixture (Dahlin et al. 2018). Under field conditions, several potential mechanisms could contribute to reduced aphid performance (e.g., root interactions, direct competition). However, our findings suggest that volatile interactions between specific undamaged cultivars present one of the underlying mechanisms responsible for disrupting aphid feeding and population development in cultivar mixtures.

Both constitutive and induced resistance to aphids can be located in specific plant tissues, and monitoring aphid stylet activity by electrical penetration graph (EPG) technique has been used for the identification of plant tissues where resistance factors against aphids are expressed (Tjallingii 2006). Constitutive resistance factors located in the peripheral layers of the plant tissues make *R. padi* probed slower on resistant than on susceptible genotypes of *Triticum aestivum* L. (Singh et al. 2020). In the present study, volatile interactions between undamaged barley cultivars triggered a similar probing behavior in *R. padi.* Thus, in SeF aphids spent more time in the pathway phase, and longer to reach the first sustained phloem ingestion phase than on Se0, while there was no difference between SeA and Se0. This suggests that only volatiles from specific cultivar could induce resistance factors in epidermis and mesophyll. Volatiles from damaged plants can induce late responses by regulating the primary and secondary metabolism in the receiving plants (Brosset and Blande 2022). The observed negative effects of aphid feeding behavior from mesophyll to phloem on SeF suggest that volatiles from undamaged Fairytale could trigger a response in the receiving plant (Salome) through enhancing resistance factors against aphids. These changes in the aphid feeding behaviors during the pathway could suggest both inter- and intracellular factors. Plant susceptibility to aphids can depend on the morphological characteristics of plant tissues. It has been shown that large intercellular space appearing with a smaller number of mesophyll cells, thinner leaves and thinner guard cells in vascular bundles could make plants more susceptible to aphids (Singh et al. 2020).

Also, changes in plant physiology may affect signaling pathways, expression of defense-related genes, and phloem sap quality (Dinant et al. 2010; Leybourne et al. 2019). These changes could be induced by volatile interactions, and disrupt aphid feeding behavior from epidermis and mesophyll to phloem, resulting in reduced aphid weight and offspring production. Our EPG data showed that volatiles from Fairytale can induce resistance in Salome and interfere in aphid feeding behavior from mesophyll to phloem, and thus reduce aphid performance.

Along the pathway to the phloem, aphid's stylet punctures cells, which is indicated by potential drops (Tjallingii and Esch 1993). In the pre-phloem phase, the number and total duration of potential drops were significantly higher on SeF compared to Se0 (experiment 1), whereas no differences

were detected in SeA compared to Se0 (experiment 2). However, the importance of the increased number of potential drops in plant resistance against aphids is still unknown (Sun et al. 2018). Volatiles from damaged plants, such as methyl salicylate, induce effects on the leaf surface resistance factor, prolonging the time until the first probe on exposed, compared to unexposed, barley plants (Ninkovic et al. 2021). The recent review paper suggested that volatile cues can induce early responses by changing the receiving plant surface (Brosset and Blande 2022). Conversely, the EPG data in the current study showed no differences in the duration of non-probing between SeF and Se0 or between SeA and Se0, indicating the absence of induced surface resistance by the volatiles from undamaged plants.

Aphids must overcome host plant defenses associated with phloem to succeed in phloem sap ingestion (Tjallingii 2006). The salivation period (E1) is recognized as the initiation of phloem activities and it is usually followed by the phloem sap ingestion period (E2). Our results showed that the frequency and duration of salivation (E1) were significantly higher in SeF compared to Se0, but not significantly higher in SeA compared to Se0. Several proteins are found in watery saliva, some of which play important roles in biochemical activity and could either function as elicitors or suppressors of plant defense (Goodspeed et al. 2012). For instance, the salivation into the sieve elements during feeding suppresses phloem wound responses causing phloem occlusion, which is considered as a physical barrier preventing blockage of the sieve elements (Pettersson et al. 2017). It is suggested that higher levels of glycerol, trehalose, asparagine, and octopamine play important roles as defensive chemical compounds for phloem resistance (Greenslade et al. 2016). The observed higher frequency of aphid salivation in Salome exposed to Fairytale plants may suggest that receiver plant resistance occurs in phloem through activating defensive chemicals.

Phloem resistance factors could be due to the mechanical blocking of the sieve element after puncturing and the changes in the composition of the phloem sap (Van Helden and Tjallingii 1993), e.g., ratios of phloem sap components (amino acids and sugar) (Will and Van Bel 2006; Dinant et al. 2010), or the presence of certain proteins responsible for phloem sealing (Mutti et al. 2008). The changes in host plant morphology and physiology could also induce phloem-based resistance, by reducing phloem sap ingestion (Guo et al. 2012; Greenslade et al. 2016; Simon et al. 2017). The observed shorter duration in phloem ingestion and sustained phloem ingestion suggest that phloem-based resistance could occur in SeF. Still, it is unknown whether volatile interactions between undamaged plants may induce phloem resistance factors, which may create difficulties for aphids to engage in phloem after salivation and to maintain phloem ingestion.

Aphids may ingest xylem in order to balance the osmotic effects related to a huge amount of phloem sap ingestion (Pompon et al. 2010). Our data revealed that aphids spent a significantly shorter duration in phloem ingestion and a longer duration of xylem ingestion in SeF compared to Se0. This result is in line with a recent study suggesting that aphids increase xylem ingestion due to the reduction in phloem sap ingestion (Escudero-Martinez et al. 2021). In addition, aphid starvation is shown to increase xylem ingestion (Ramírez and Niemeyer 2000). According to these findings, we can speculate that the increase in xylem ingestion could be due to the poor quality of phloem sap, which is also indicated by decreased phloem sap ingestion on SeF.

The development time, fecundity, individual size, life span and reproduction of aphids can be related to the quality of the host plant (Berminghnam and Wikinson 2009; Srisakrapikoop et al. 2021). Our data show that there is a significant reduction in aphid relative growth rate and intrinsic rate in SeF, compared to Se0 and SeA (Figs. 1 and 3). The observed reduction in aphid growth corresponds to the disruption of feeding behavior on SeF, showing that there is a linkage between aphid feeding behavior and performance. It is possible that certain volatiles from Fairytale directly affect Salome as host, which effectively delays aphid feeding behavior and growth. The observed significantly lower number of aphid offspring and weight on SeF could be due to the changes in phloem sap quality, which is also indicated by the shorter duration of phloem ingestion and sustained phloem ingestion. This supports our hypothesis that volatiles from a specific emitter could negatively affect the phloem sap quality and shorten phloem ingestion duration, which consequently reduces aphid weight and offspring production.

The volatile cues from *Geranium macrorrhizum* (Ameline et al. 2002) and the volatiles from *Ocimum basilicum*, marigold and *Tagetes patula*, basil (Dardouri et al. 2020), as companion plants, have been shown to disrupt feeding behavior and reduce the performance of *Myzus persicae* on sweet pepper. In these studies, the negative effects of volatile interactions on aphids could be observed in a certain companion plant, which is similar to the reduction in *R. padi* performance in laboratory tests on certain wheat cultivar mixtures (Cascone et al. 2015; Grettenberger and Tooker 2017). Our results also confirmed the findings of the field study by Dahlin et al. (2018) where aphid population size was significantly reduced in the mixture of Salome and Fairytale, but not in a combination of Salome and Anakin, and pure stands, suggesting that the induced resistance responses in receiving plants are emitter and receiver specific/dependent. This provides good evidence that certain volatiles from specific emitters could potentially directly affect aphid feeding and indirectly influence the phloem sap quality of the receiving plant, which contributes to reducing aphid weight and number of offspring.

Our study showed strong indications that volatile interactions between cultivars of the same crop species affect aphid response and performance through induced changes in their host plants, but these effects are specific to neighboring cultivar identity. Future studies should focus on volatiles-induced physiological changes within plants that are responsible for reduced aphid feeding and performance. An improved understanding of the underlying mechanisms of volatile interactions between cultivars in cultivar mixtures will contribute to the development of the integrated pest management, leading to the development of crop management systems at higher levels of integration.

Author contributions

VN and SK designed the study. SK and DM conducted the experiments. SK, DR, and VN analyzed the data. SK, DM, and VN wrote the manuscript. SK, DM, DR, SI, and VN edited the manuscript. All authors read, contributed to revisions, and approved the manuscript.

Acknowledgements We thank Maria Kedmark for her assistance with rearing insects and plants. We are grateful to Merlin Rensing, Jannicke Gallinger, and Ayse Gül Ünlü for constructive discussions. We are thankful to two anonymous reviewers for their comprehensive reviews and insightful comments, which definitely helped us to improve the manuscript.

Funding Open access funding provided by Swedish University of Agricultural Sciences. This work was supported by The Swedish International Development Agency (SIDA) through the "Sweden-Royal University of Phnom Penh Bilateral program" (Contribution No. 11599) and "Stacking of ecosystem services: mechanisms and interactions for optimal crop protection, pollination enhancement, and productivity," with "EcoStack" Grant Agreement Number: 773554.

Availability of data and materials The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Conflict of interest All authors declare that there is no conflict of interest.

Ethical approval This study does not contain any studies with human participants or large animals performed by any of the authors. No approval of research ethics committees was required to accomplish the goals of this study because this study was conducted with insects and crop plants.

References

Ameline A, Dorland J, Werrie PY et al (2022) *Geranium macrorrhizum*, a potential novel companion plant affecting preference and performance of *Myzus persicae* on sweet pepper. J Pest Sci. https://doi.org/10.1007/s10340-022-01522-3

Berminghnam J, Wikinson TL (2009) Embryo nutrition in parthenogenetic viviparous. Physiol Entomol 34:103–109. https://doi.org/10.1111/j.1365-3032.2008.00669.x

Brilli F, Loreto F, Baccelli I (2019) Exploiting plant volatile organic compounds (VOCS) in agriculture to improve sustainable defense strategies and productivity of crops. Front Plant Sci 10:1–8. https://doi.org/10.3389/fpls.2019.00264

Brosset A, Blande JD (2022) Volatile-mediated plant–plant interactions: volatile organic compounds as modulators of receiver plant defence, growth, and reproduction. J Exp Bot 73:511–528. https://doi.org/10.1093/jxb/erab487

Cascone P, Iodice L, Maffei ME et al (2015) Tobacco overexpressing-ocimene induces direct and indirect responses against aphids in receiver tomato plants. J Plant Physiol 173:28–32. https://doi.org/10.1016/j.jplph.2014.08.011

Cook-Patton SC, Mcart SH, Parachnowitsch AL et al (2011) A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. Ecology 92:915–923. https://doi.org/10.1890/10-0999.1

Dahlin I, Rubene D, Glinwood R, Ninkovic V (2018) Pest suppression in cultivar mixtures is influenced by neighbor-specific plant–plant communication. Ecol Appl 28:2187–2196. https://doi.org/10.1002/eap.1807

Dardouri T, Gomez L, Ameline A et al (2020) Non-host volatiles disturb the feeding behavior and reduce the fecundity of the green peach aphid, *Myzus persicae*. Pest Manag Sci. https://doi.org/10.1002/ps.6190

Dicke M, Baldwin IT (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help.' Trends Plant Sci 15:167–175. https://doi.org/10.1016/j.tplants.2009.12.002

Dinant S, Bonnemain JL, Girousse C, Kehr J (2010) Phloem sap intricacy and interplay with aphid feeding. Comptes Rendus Biol 333:504–515. https://doi.org/10.1016/j.crvi.2010.03.008

Escudero-Martinez C, Leybourne DJ, Bos JIB (2021) Plant resistance in different cell layers affects aphid probing and feeding behaviour during non-host and poor-host interactions. Bull Entomol Res 111:31–38. https://doi.org/10.1017/S0007485320000231

Giordanengo P (2014) EPG-Calc: a PHP-based script to calculate electrical penetration graph (EPG) parameters. Arthropod Plant Interact 8:163–169. https://doi.org/10.1007/s11829-014-9298-z

Goodspeed D, Chehab EW, Min-Venditti A et al (2012) Arabidopsis synchronizes jasmonate-mediated defense with insect circadian behavior. Proc Natl Acad Sci U S A 109:4674–4677. https://doi.org/10.1073/pnas.1116368109

Greenslade AFC, Ward JL, Martin JL et al (2016) *Triticum monococcum* lines with distinct metabolic phenotypes and phloem-based partial resistance to the bird cherry-oat aphid *Rhopalosiphum padi*. Ann Appl Biol 168:435–449. https://doi.org/10.1111/aab.12274

Grettenberger IM, Tooker JF (2017) Variety mixtures of wheat influence aphid populations and attract an aphid predator.

Arthropod Plant Interact 11:133–146. https://doi.org/10.1007/s11829-016-9477-1
Grettenberger IM, Tooker JF (2020) Cultivar mixtures of soybeans have inconsistent effects on herbivore and natural-enemy populations. Agric Ecosyst Environ 292:106835. https://doi.org/10.1016/j.agee.2020.106835
Guo S-M, Kamphuis LG, Gao L-L et al (2012) Identification of distinct quantitative trait loci associated with defence against the closely related aphids *Acyrthosiphon pisum* and *A. kondoi* in Medicago truncatula. J Exp Bot 63:3913–3922. https://doi.org/10.1093/jxb/err313
Hambäck PA, Inouye BD, Andersson P, Underwood N (2014) Effects of plant neighborhoods on plant-herbivore interactions: resource dilution and associational effects. Ecology 95:1370–1383. https://doi.org/10.1890/13-0793.1
Heil M, Karban R (2010) Explaining evolution of plant communication by airborne signals. Trends Ecol Evol 25:137–144. https://doi.org/10.1016/j.tree.2009.09.010
Isbell F, Adler PR, Eisenhauer N et al (2017) Benefits of increasing plant diversity in sustainable agroecosystems. J Ecol 105:871–879. https://doi.org/10.1111/1365-2745.12789
Karban R, Yang LH, Edwards KF (2014) Volatile communication between plants that affects herbivory: a meta-analysis. Ecol Lett 17:44–52. https://doi.org/10.1111/ele.12205
Koricheva J, Hayes D (2018) The relative importance of plant intraspecific diversity in structuring arthropod communities: a meta-analysis. Funct Ecol 32:1704–1717. https://doi.org/10.1111/1365-2435.13062
Leybourne DJ, Valentine TA, Robertson JAH et al (2019) Defence gene expression and phloem quality contribute to mesophyll and phloem resistance to aphids in wild barley. J Exp Bot 70:4011–4026. https://doi.org/10.1093/jxb/erz163
Malézieux E, Crozat Y, Dupraz C et al (2009) Mixing plant species in cropping systems: concepts, tools and models. A review. Agron Sustain Dev 29:43–62. https://doi.org/10.4995/agt.2018.7146
Mansion-Vaquié A, Wezel A, Ferrer A (2019) Wheat genotypic diversity and intercropping to control cereal aphids. Agric Ecosyst Environ 285:106604. https://doi.org/10.1016/j.agee.2019.106604
Mutti NS, Louis J, Pappan LK et al (2008) A protein from the salivary glands of the pea aphid, *Acyrthosiphon pisum*, is essential in feeding on a host plant. Proc Natl Acad Sci U S A 105:9965–9969. https://doi.org/10.1073/pnas.0708958105
Ninkovic V (2003) Volatile communication between barley plants affects biomass allocation. J Exp Bot 54:1931–1939. https://doi.org/10.1093/jxb/erg192
Ninkovic V, Al Abassi S, Pettersson J (2001) The influence of aphid-induced plant volatiles on ladybird beetle searching behavior. Biol Control 21:191–195. https://doi.org/10.1006/bcon.2001.0935
Ninkovic V, Glinwood R, Pettersson J (2006) Communication in plants. In: Mancuso S, Volkmann D (eds) Communication in plants. Springer, Berlin. https://doi.org/10.1007/978-3-540-28516-8.pdf
Ninkovic V, Al AS, Ahmed E et al (2011) Effect of within-species plant genotype mixing on habitat preference of a polyphagous insect predator. Oecologia 166:391–400. https://doi.org/10.1007/s00442-010-1839-2
Ninkovic V, Markovic D, Dahlin I (2016) Decoding neighbour volatiles in preparation for future competition and implications for tritrophic interactions. Perspect Plant Ecol Evol Syst 23:11–17. https://doi.org/10.1016/j.ppees.2016.09.005
Ninkovic V, Markovic D, Rensing M (2020) Plant volatiles as cues and signals in plant communication. Plant Cell Environ 44:1030–1043. https://doi.org/10.1111/pce.13910
Ninkovic V, Glinwood R, Ünlü AG, Ganji S (2021) Effects of methyl salicylate on host plant acceptance and feeding by the aphid *Rhopalosiphum padi*. Front Plant Sci 12:1–14. https://doi.org/10.3389/fpls.2021.710268
Pettersson J, Tjallingii W, Hardie J (2017) Host-plant selection and feeding. In: van Emden H, Harrington R (eds) Aphids as crop pests. CABI, Wallingford, pp 173–196
Pettersson J, Ninkovic V, Glinwood R (2003) Plant activation of barley by intercropped conspecifics and weeds: allelobiosis. In: The BCPC International Congress: Crop Science and Technology, vol. 2. Proceedings of an international congress. Glasgow, Scotland, UK, 10–12 pp 1135–1144
Pompon J, Quiring D, Giordanengo P, Pelletier Y (2010) Role of xylem consumption on osmoregulation in *Macrosiphum euphorbiae* (Thomas). J Insect Physiol 56:610–615. https://doi.org/10.1016/j.jinsphys.2009.12.009
Prieto I, Violle C, Barre P et al (2015) Complementary effects of species and genetic diversity on productivity and stability of sown grasslands. Nat Plants. https://doi.org/10.1038/nplants.2015.33
Radford PJ (1967) Growth analysis formulae—their use and abuse. Crop Sci 7:171–175. https://doi.org/10.2135/cropsci1967.0011183x000700030001x
Ramírez CC, Niemeyer HM (2000) The influence of previous experience and starvation on aphid feeding behavior. J Insect Behav 13:699–709. https://doi.org/10.1023/A:1007844027368
Ratnadass A, Fernandes P, Avelino J, Habib R (2012) Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. Agron Sustain Dev 32:273–303. https://doi.org/10.1007/s13593-011-0022-4
R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical computing, Vienna, Austria. http://www.R-project.org/
Schliephake E, Habekuss A, Scholz M, Ordon F (2013) Barley yellow dwarf virus transmission and feeding behaviour of *Rhopalosiphum padi* on *Hordeum bulbosum* clones. Entomol Exp Appl 146:347–356. https://doi.org/10.1111/eea.12033
Shoffner AV, Tooker JF (2013) The potential of genotypically diverse cultivar mixtures to moderate aphid populations in wheat (*Triticum aestivum* L.). Arthropod Plant Interact 7:33–43. https://doi.org/10.1007/s11829-012-9226-z
Simon AL, Wellham PAD, Aradottir GI, Gange AC (2017) Unravelling mycorrhiza-induced wheat susceptibility to the English grain aphid *Sitobion avenae*. Sci Rep 7:1–11. https://doi.org/10.1038/srep46497
Singh B, Simon A, Halsey K et al (2020) Characterisation of bird cherry-oat aphid (*Rhopalosiphum padi* L.) behaviour and aphid host preference in relation to partially resistant and susceptible wheat landraces. Ann Appl Biol 177:184–194. https://doi.org/10.1111/aab.12616
Snyder LD, Gómez MI, Power AG (2020) Crop varietal mixtures as a strategy to support insect pest control, yield, economic, and nutritional services. Front Sustain Food Syst 4:1–14. https://doi.org/10.3389/fsufs.2020.00060
Srisakrapikoop U, Pirie TJ, Fellowes MDE (2021) Aphids show interspecific and intraspecific variation in life history responses to host plant infection by the fungal pathogen *Botrytis cinerea*. Entomol Sci 24:228–234. https://doi.org/10.1111/ens.12476
Sun M, Voorrips RE, Steenhuis-broers G et al (2018) Reduced phloem uptake of *Myzus persicae* on an aphid resistant pepper accession. BMC Plant Biol 18:1–14. https://doi.org/10.1186/s12870-018-1340-3
Tjallingii WF (1990) Continuous recording of stylet penetration activities by aphids. In: Campbell RDE (ed) Aphid-plant genotype interactions. Elsevier, Amsterdam, pp 89–99
Tjallingii WF (2006) Salivary secretions by aphids interacting with proteins of phloem wound responses. J Exp Bot 57:739–745. https://doi.org/10.1093/jxb/erj088
Tjallingii WF, Esch TH (1993) Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. Physiol Entomol 18:317–328. https://doi.org/10.1111/j.1365-3032.1993.tb00604.x

Tooker JF, Frank SD (2012) Genotypically diverse cultivar mixtures for insect pest management and increased crop yields. J Appl Ecol 49:974–985. https://doi.org/10.1111/j.1365-2664.2012.02173.x

Van Helden M, Tjallingii WF (1993) Tissue localisation of lettuce resistance to the aphid *Nasonovia ribisnigri* using electrical penetration graphs. Entomol Exp Appl 68:269–278. https://doi.org/10.1111/j.1570-7458.1993.tb01713.x

War AR, Paulraj MG, War MY, Ignacimuthu S (2011) Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* L.). Plant Signal Behav 6:1787–1792. https://doi.org/10.4161/psb.6.11.17685

Will T, Van Bel AJE (2006) Physical and chemical interactions between aphids and plants. J Exp Bot 57:729–737. https://doi.org/10.1093/jxb/erj089

Wyatt JI, White PF (1977) Simple estimation of intrinsic increase rates for aphids and tetranychid mites. J Appl Ecol 14:757–766

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x