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Communication between undamaged plants can elicit changes in volatile emissions from neighbouring plants, thereby altering their susceptibility to aphids

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Abstract

Plant volatiles play an important role in intra- and interspecific plant communication, inducing direct and indirect defenses against insect pests. However, it remains unknown whether volatile interactions between undamaged cultivars alter host plant volatile emissions and their perception by insect pests. Here, we tested the effects of exposure of a spring barley, *Hordeum vulgare* L., cultivar, Salome, to volatiles from other cultivars: Fairytale and Anakin. We found that exposing Salome to Fairytale induced a significantly higher emission of *trans*-β-ocimene and two unidentified compounds compared when exposed to Anakin. Aphids were repelled at a higher concentration of *trans*-β-ocimene. Salome exposure to Fairytale had significant repulsive effects on aphid olfactory preference, yet not when Salome was exposed to Anakin. We demonstrate that volatile interactions between specific undamaged plants can induce changes in volatile emission by receiver plants enhancing certain compounds, which can disrupt aphid olfactory preferences. Our results highlight the significant roles of volatiles in plant–plant interactions, affecting plant–insect interactions in suppressing insect pests. This has important implications for crop protection and sustainable agriculture.

KEYWORDS

aphid, aphid host plant orientation, barley, insect–plant interaction, plant–plant communication, plant–plant interaction, volatile organic compounds, volatile profiles

1 | INTRODUCTION

Plants share complex habitats, where interactions with neighbouring plants and other organisms are inevitable. They also use diverse signals and cues to interact with their neighbouring plants including: responses to the quality of light (Keuskamp et al., 2010), acoustic signals (Appel & Cocroft, 2014), root-exudates (Biedrzycki et al., 2010), root emitted volatile organic compounds (VOCs) (Delory et al., 2016), mechanical touch (Markovic et al., 2014) and airborne VOCs (Ninkovic et al., 2013). A diverse range of different VOCs are produced and released by plants, providing information about the plant's current physiological state (Dudareva et al., 2013). Such plant-emitted VOCs serve important roles in the detection of neighbours, as well as inter- and intraspecific plant interaction mediators, as they carry important information about neighbouring plants (Ninkovic et al., 2016, 2020). The VOCs perceived from neighbouring plants,

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therefore, can be essential for host plants to prepare for resource competition, self-protection against herbivores and microbes, or natural enemy attractions (Turlings & Erb, 2018).

Damaged as well as undamaged plants have been shown to release distinct volatile compounds that can affect tritrophic interactions in the ecosystem (Erb et al., 2015; Ninkovic et al., 2013). Plant VOCs play vital roles as cues and signals in plant–plant interactions (Ninkovic et al., 2020) and can stimulate or prime defense responses in neighbouring plants (Brilli et al., 2019; Heil & Karban, 2010). When plants are attacked by herbivores, herbivore-induced plant volatiles (HIPVs) are released (Clavijo McCormick et al., 2012), which function as direct repellents for herbivores or as attractants for natural enemies (Aartsma et al., 2017; Turlings & Erb, 2018). Furthermore, plants use volatile cues from undamaged plants to gather information on their neighbours and adapt to competition with proper morphological and physiological responses (Kegge & Pierik, 2010). This phenomenon, so-called allelobiosis, could possibly account for insect suppression in mixed plantings (Pettersson et al., 2003). Volatiles from onion plants can induce changes in potato volatile emissions making them less attractive to aphids in the laboratory and field experiments (Ninkovic et al., 2013). Previous studies further suggest a high specificity of such volatile-based plant–plant interactions depending on the species or even cultivar identity of interacting plants (Karban et al., 2006; Kheam et al., 2023; Ninkovic et al., 2013). For example, volatiles emitted from one specific barley cultivar significantly affect biomass allocation in another cultivar, leading to increased root biomass production, while other cultivars do not (Ninkovic, 2003). A growing body of literature provides substantial evidence that volatiles from undamaged plants can shift a variety of properties in neighbouring plants; however, it is still not well documented whether volatile interactions between undamaged plants from different cultivars affect plant volatile emissions and subsequent interactions in insect herbivores.

The evolutionary history of insects and plants is highly interconnected in nature. Insects use plant volatiles as cues to assess not only whether they are selecting the right host plant species but also to determine the nutritional quality of the host and the presence/absence of other insects (Bruce & Pickett, 2011). For instance, the damaged *Rumex confertus* released high levels of VOCs, that potentially repelled the weevil *Hypera rumicis* L. at high concentrations of 25 and 125 ng mn^{-1} (Piesik et al., 2015). Another weevil (*Sitophilus granaries*) was also repelled by high concentrations (100 and 1000 ng mn^{-1}) of most cereal volatiles (Piesik & Wenda-Piesik, 2015). The filbert aphid, *Myzocallis coryli* Goetze, showed low acceptance of leaves of certain cultivars of hazel (*Corylus* L.) with a high content of phenolic acids (Gantner et al., 2019). Phloem-feeding insects can use olfactory cues to identify suitable host plants, despite the evaluation of food quality, determined by the abundance of primary and secondary metabolites in the plant sap (Gallinger & Gross, 2020; Gallinger et al., 2020). Therefore, insects have evolved sensitive olfactory systems, located mainly on the antenna and maxillary palpi that consist of different types of olfactory receptor neurons has evolved in insects (Singh et al., 2019), to precisely recognize chemical cues in the surrounding environments (Field et al., 2000). The different olfactory receptor neurons with unique molecular structures in sensillae are responsible for insect volatile detections (Bruce & Pickett, 2011). In particular, aphids can utilize the sensory receptors to detect colour, shape, texture and odour released by plants (Bruce et al., 2005) and they can use specific volatile blends or single compounds to locate their host plants (Pettersson et al., 2017). These studies indicate that certain concentrations of specific plant volatiles could affect different groups of insect host-searching behaviours.

Aphids with their rapid growth and potential to spread, are major pests that pose substantial challenges to food security, and the development of sustainable agricultural practices (Pettersson et al., 2017). Chemical insecticides are still extensively used in cropping systems and have resulted in evolutionary responses in aphids. Aphids have led to the development of individual resistance (Foster et al., 2017) and their defense against natural enemies through the acquisition of heritable symbionts (Oliver et al., 2003). Therefore, the rapid development of alternative strategies to control aphid populations is needed for sustainable crop protection. One of those potential biological alternatives against aphids could be based on the effects of plant–plant communication via volatile interactions in genotypically diverse cultivar mixtures. Previous studies reported that plant–plant communication in certain cultivar mixtures can increase the resilience of the crop to insect pests (Dahlin et al., 2018; Grettenberger & Tooker, 2016; Kheam et al., 2023; Shoffner & Tooker, 2013). Field studies showed that significant decreases in aphid populations were observed in the mixtures of Salome and Fairytale barley cultivars as compared to their pure stands. There was no such pattern in the Salome and Anakin mixtures compared to pure stands. Laboratory studies showed that the volatile interactions reduced plant acceptance by aphids in Salome exposed to Fairytale (SeF), but not in Salome exposed to Anakin (SeA) (Dahlin et al., 2018). Additionally, aphid feeding behaviour was disrupted and performance was reduced in SeF, but not in SeA (Kheam et al., 2023). The results of these studies suggest that plant–plant volatile interactions between specific cultivars may contribute to changes in host plants that make them less suitable for aphids. However, the mechanisms of volatile interactions in specific cultivar mixtures against aphid host plant searching behaviours has yet to be established. A better understanding of the effects of volatile interactions in cultivar mixtures on aphid's host choices may, in the future, contribute to the development of sustainable management strategies.

Here we investigate the potential effects of volatile interactions between different undamaged barley cultivars on (i) the plant volatile emissions and (ii) consequences for aphid's olfactory attraction to host plants. We hypothesize that VOCs from emitting cultivars can induce changes of volatile emissions in receiving cultivars depending on the genotypic identity of the emitter and that such changes in olfactory cues can affect aphid host choice.

2 | METHODS

2.1 | Plants and insects

We used three different spring barley cultivars (*Hordeum vulgare* L.) in this study including: cultivars Salome (Nordaat Saatzucht GmbH), Fairytale and Anakin (Sejet Plant Breeding), since some of these cultivars in combination can decrease aphid plant acceptance in the laboratory and lower population size in the field (Dahlin et al., 2018; Kheam et al., 2023). All three cultivars have different pedigrees: Auriga × (Publican × Beatrix) for Salome, Colston × (Receipt × Power) for Fairytale, and (Tumbler × Response) for Anakin. Scandinavian Seed AB supplied seeds of the three cultivars. Before sowing, seeds were germinated between two filter papers in Petri dishes for 24 h at room temperature. Twelve seeds were sown per pot (9 × 9 × 7 cm), filled with P-soil (Hasselfors), and kept in the growing chamber for 9 days at 18–22°C, 50%–60% relative humidity, and L16:D8 h photoperiod.

The bird cherry-oat aphids (*Rhopalosiphum padi* L.), one of the most important pests in cereals were used as a model study insect. The wingless aphids used in the experiments were the fourth instar aphids and the adult aphids. The aphids were reared on oat (*Avena sativa* L.) cultivar Belinda in a separate growing chamber under the same growing conditions as the barley plants.

2.2 | Plant volatile exposure

Twin Perspex cages (Ninkovic, 2003) were used to explore the effects of volatile interactions between different barley cultivars on the receiving plant's volatile profiles and aphid's preference and repellency. In brief (Figure 1a), 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^{-1}. 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 the exposing system at the one-leaf stage (7 days old). The plant exposure time was 5 days. Salome, the receiving cultivar, was exposed to Fairytale volatiles (SeF), Anakin volatiles (SeA), or clean air (Se0). Se0 was used as the absolute control (Dahlin et al., 2018; Kheam et al., 2023).

2.3 | VOCs collections

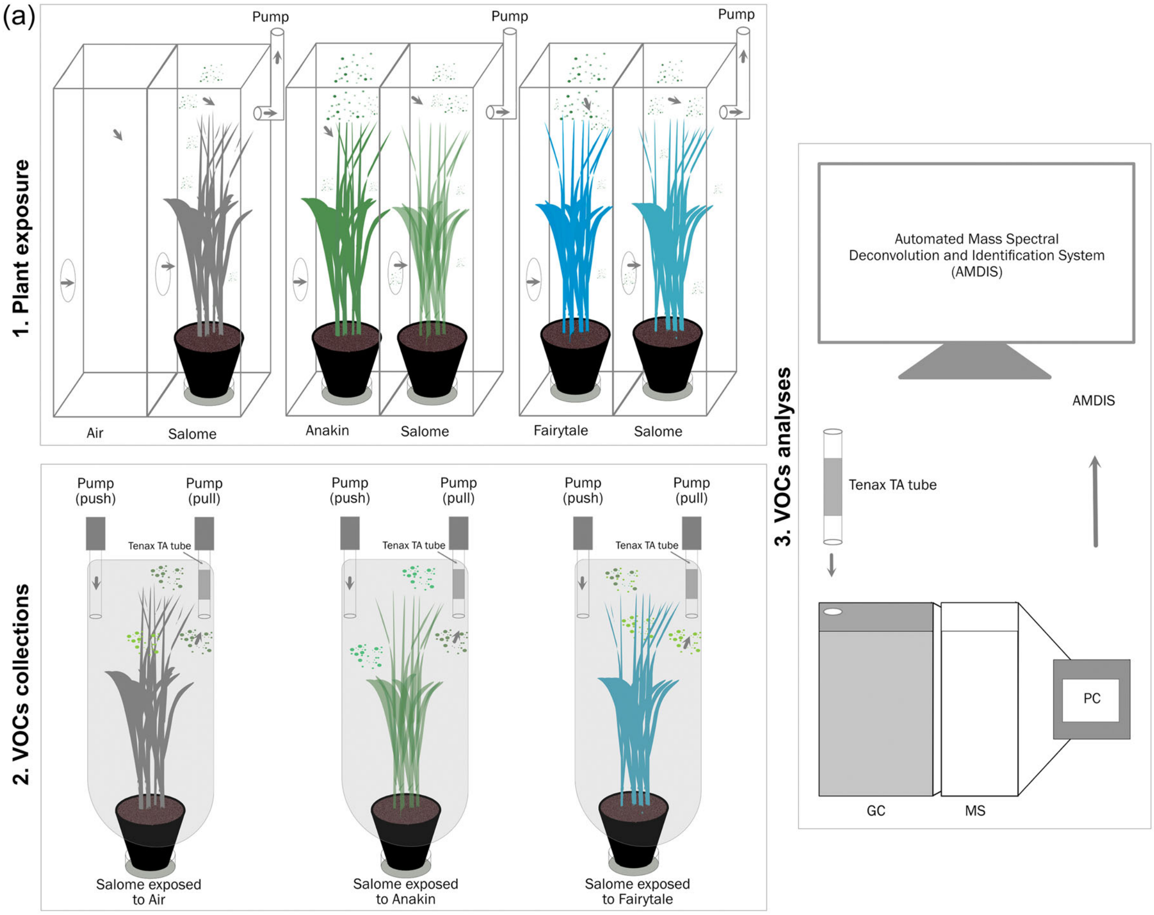
The impact of exposure on the VOC release of receiving Salome plants was investigated by gas chromatography–mass spectrometry (GC-MS). Headspace samples were collected from 12 plants per pot from unexposed Salome plants and Salome that were previously exposed to VOCs from Anakin or Fairytale, after 5 days of exposure (Figure 1a). Four replicates of each treatment were sampled in parallel per day and repeated three times resulting in 9–11 replicates per exposing treatment (unexposed Salome: $n = 9$, SeA: $n = 10$, SeF: $n = 11$). Pots with 12 barley plants were enclosed in polyethylene terephthalate oven plastic bags (35 × 43 cm; Melita) and these bags were baked in the oven for 2 h at 140°C before the volatile collection. The volatiles were collected with a push–pull system for 24 h. Charcoal-filtered air was pushed in to the oven bags with a flow of 600 mL min^{-1} while pulling the air out of the bags over an adsorbent trap with 400 mL min^{-1}. The VOCs were trapped on Tenax TA sample tubes (60/80 mesh size; GLScience) containing 80 mg adsorbent. 1-nonene was used as an internal standard for quality control purposes, by injecting 20 ng on the top of the collection tube mesh right before headspace collections. Volatiles were released from the adsorbent tubes by thermal desorption with an Optics 3 Injector (GLScience) at 250°C. The thermal desorbed compounds were separated using an Agilent 7890 N GC system equipped with an HP-1MS capillary column (30 × 0.25 mm id × 0.25 µm film thickness, 100% dimethylpolysiloxane) coupled to an Agilent 5975C mass spectrometer (Agilent Technologies Inc.). Injection was employed using helium as carrier gas (Helium 6.0) with a flow of 1.3 mL min^{-1}. The GC temperature programme was as follows: Initial oven temperature of 30°C was held for 2 min, increased at a rate of 5 K min^{-1} to 150°C, followed by a rate of 10 K min^{-1} to the final temperature of 250°C and held for 15 min. The GC inlet line temperature was 250°C, and the ion source temperature was 180°C. The quadrupole mass detector was operated in the electron impact mode at 70 eV, MS gain was set to 10. All data were obtained by collecting the full-scan mass spectra within the range of 40–500 *m*/*z*.

2.4 | Volatile analyses

The volatile compounds from the chromatograms were identified and quantified with the 'Automated Mass Spectral Deconvolution and Identification System' (V. 2.71; National Institute of Standards and Technology) according to (Gross et al., 2019). The settings for deconvolution were set to medium sensitivity and resolution, and a high shape requirement with a component width of 32 and one adjacent peak subtraction. Identification criteria were applied as follows: match factor ≥ 80% with relative retention index deviation ≤ 5% + 0.01 from the reference value. The match factor penalty was set to very strong with a maximum penalty of 25. A signal-to-noise ratio filter of <300 was applied. Only compounds where identities were confirmed by the analysis of authentic standards were reported as identified.

2.5 | Olfactory bioassay with barley plants

A two-way airflow olfactometer was used to measure the olfactory responses of aphids. The olfactometer consisted of two stimulus zones, in two arms directly opposite to each other connected by a



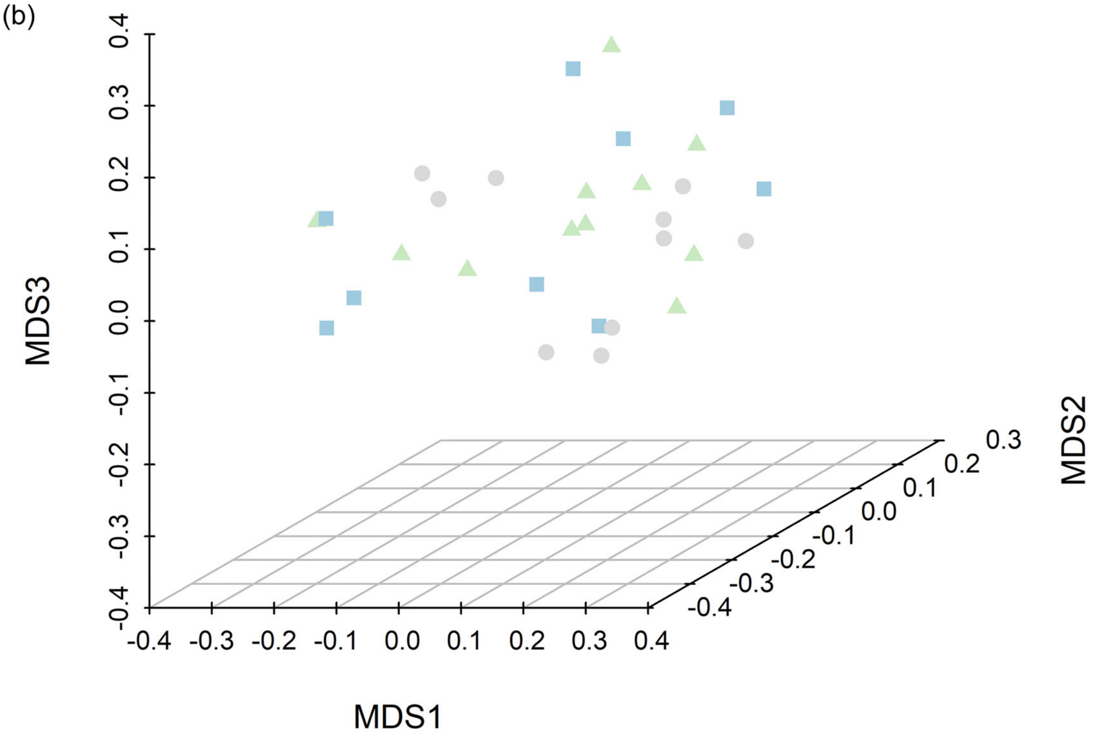


FIGURE 1 (See caption on next page).

neutral central zone separating them. The airflow of 180 mL min^{-1} was set in the olfactometer to ensure discrete air currents in the side zones (Dahlin et al., 2015; Ninkovic et al., 2013; Tous-Fandos et al., 2023).

We conducted three olfactory experiments to compare aphid preferences and repellency for different plant odours. We first compared the aphid's cultivar preferences by offering the volatiles from (a) Fairytale versus Salome, and (b) Anakin versus Salome by offering odours from both cultivars simultaneously against each other (Figure 4a). Second the aphid's preferences for odours from previous exposed plants: (a) SeF versus a Se0, and (b) SeA versus Se0 - (Figure 4c). Finally, we investigated the potential repellency of odours from previously exposed plants: (a) SeF, and (b) SeA when offered against air from an empty cage (0) (Figure 4e). The arms of the olfactometer were connected to the odours sources from the two-chamber cages containing plants. The sucking pump and air flowmeters were used to control the airflow and provide a consistent flow of 180 mL min^{-1} in all olfactometers. We used different sets of plants in each experiment.

The wingless aphids were randomly collected from the stock cultures using a fine paintbrush and placed in Petri dishes with moist filter paper to prevent dehydration. The aphids were then left in the experiment room for about 30 min to acclimatize before the experiments. A single wingless aphid was introduced into the central zone of the olfactometer through a hole on the top and after an adaptation period of 10 min the position of the aphid in the olfactometer was recorded at 3 min intervals over a 30 min period. One aphid was tested once. A clean olfactometer was used for each aphid. The olfactometers were cleaned with 10% Teepol L (TEEPOL) and rinsed with 70% ethanol solution and distilled water and left to air dry. To avoid the influence of lighting conditions outside the laboratory on aphid behaviours, the olfactory experiments were conducted in a dark room under artificial light (Osram FQ80W/840 HO Constant Lumilux Cool White (4000 K)) at 60 μmol m^{-2} s^{-1} above the olfactometer. These experiments were carried out between 9:00 and 17:00 during the day. The average room temperature was about 20°C with the 40%–50% of relative humidity.

The total number of visits of a single aphid per a single arm after 10 recordings was regarded as one replicate. If an aphid did not move for longer than 10 min, these individuals were discarded and not included in the analyses. The aphid visits into middle (central) zone were not taken for the analyses. Data were expressed as the total of individual aphid visits per olfactometer arm during the observation period. Each comparison was replicated with 20 to 24 individuals.

2.6 | Olfactory bioassay with *trans*-β-ocimene compound

To confirm the olfactory responses of aphids to the more abundance volatile in SeF, the dose olfactory responses of *trans*-β-ocimene experiments were conducted by using serial dilutions based on the quantified amount in the headspace. *Trans*-β-ocimene (≥99% purity; Toronto Research Chemical Inc.) was diluted to five different concentrations (0.01, 0.1, 1, 10 and 100 ng µL^{-1}) in methanol. Due to the dynamic characteristics of plant volatile emission over diurnal and ontogenetic time (Schuman et al., 2016), these five concentrations were tested in the olfactometer experiments to determine the effects of *trans*-β-ocimene and its relevant concentrations on aphid preference. Aphid olfactory responses to the different concentrations of *trans*-β-ocimene were tested against methanol as a control (Figure 5a). Each diluted concentration of *trans*-β-ocimene and methanol was dosed at a volume of 10 µg on small pieces of filter paper, allowed to evaporate for 1–2 min and placed into glass tubes (2.5 mm diameter) connected to holes in the sides of the olfactometer arms. The observation and experiment protocol is the same as the testing aphid responses to barley plant's odour. Each pair comparison was replicated with 19–22 individuals.

2.7 | Statistical analyses

The Olfactory response and volatile profile data were analyzed by using R statistical software (R Core Team, 2022). Data visualization was realized using functions from 'ggplot2' package (Wickham, 2016).

2.7.1 | Aphid olfactory responses

Wilcoxon matched-pairs test was used to analyze the pair-choice test for aphid olfactory responses.

FIGURE 1 The overall composition of volatile emissions from Salome is not different after exposing to other emitter cultivars or air. (a) Volatile collection process. 1. Plant exposure: Before the volatile collection, one pot with 12 barley plants (9-day) was exposed to volatiles from other barley cultivars or to air for 5 days. The exposures included Salome exposed to air (Se0), Salome exposed to Anakin (SeA) and Salome exposed to Fairytale (SeF). 2. Volatile organic compound (VOC) collection: Push–pull volatile collections of plant headspace were taken from unexposed Salome plants and Salome plants that were previously exposed to VOCs from Anakin or Fairytale. 3. VOC analyses: Thermal desorption–gas chromatography (GC)–mass spectrometry (MS) was used for chemical analysis of plant VOCs followed by identification and quantification with 'Automated Mass Spectral Deconvolution and Identification System'. (b) Nonmetric multidimensional scaling (NMDS) plot visualizing Bray–Curtis dissimilarities calculated from proportional VOC compositions emitted from barley plants. Each point represents the volatile profile emitted from 12 cv. Salome after 5 days exposure to air (unexposed = grey circles, $n = 9$), to VOCs from cv. Anakin (green triangles, $n = 10$) or VOCs from cv. Fairytale (blue squares, $n = 11$); 3-day stress: 0.119.

2.7.2 | VOCs of profile compositions

To calculate and visualize differences in plant VOC compositions we used the 'vegan' package (Oksanen et al., 2022). Nonmetric multidimensional scaling plots were used to visualize Bray–Curtis dissimilarities of the VOC composition from Salome plants after exposure to air or volatiles from Anakin or Fairytale calculated using the *metaMDS* function. Wisconsin double standardization was used for scaling. Permutational multivariate analysis of variance (PERMANOVA) was used to test for discrimination between differently exposed plant volatile profiles. Bray–Curtis distance matrix was calculated with *vegdist* function and was used for permutation testing using *adonis2* function with 10 000 permutations. The sampling day was included as strata to preserve the effect of the sampling day.

2.7.3 | Fold change of single compounds

The peak area per gram dry weight was used to calculate the fold change in the emission of single volatile compounds. Therefore, the mean peak area per gram dry biomass from unexposed plants was calculated per day, accounting for daily variation. The logarithmic ratio of the peak area per dry weight from each exposed plant sample to the mean of unexposed plants from the corresponding day was calculated as

$$\text{Fold-change} = \log\left(\frac{\text{exposure}_{\text{day}[i]}\left(\frac{\text{peak area}}{\text{DW}}\right) + 1}{\text{mean}\left(\text{control}_{\text{day}[i]}\left(\frac{\text{peak area}}{\text{DW}}\right) + 1\right)}\right). \qquad (1)$$

To visualize the change of the emission of single compounds from Salome induced by exposure to different cultivars a heatmap was generated based on the mean fold-change. Generalized linear models with Gamma (log link) distributions were fitted to compare the fold-change between Anakin and Fairytale exposed Salome plants. The identity of the emitter cultivar and the sampling day were fitted as fixed effects. The model fit was visually inspected as recommended by Zuur et al. (2010). Differences in fold-change of single compounds from Salome plants exposed to Anakin or Fairytale were calculated using estimated marginal means and 95% confidence intervals with the *emmeans* function from the 'emmeans' package (Length, 2022). Additionally, the peak are per g dry weight of *trans*-β-ocimene was analysed in the same way.

3 | RESULTS

3.1 | Volatile emissions of host plants after exposure

To identify changes in the volatile emission of Salome plants after exposure to VOCs from Fairytale, Anakin or air, the headspace of previously exposed Salome plants was collected and analyzed via GC. In total, 62 components were analyzed from the Salome headspace samples. The exposure to air or VOCs from the other barley cultivars did not lead to a differentiation of the overall composition of VOCs released from Salome (Figure 1b, PERMANOVA, $F = 0.51$, $p = 0.58$, $n = 30$).

As changes in the release of single compounds, or rather, shifts in specific blends of plant VOCs can be important for host recognition of insects, induced fold changes of single volatile compounds were analyzed and visualized as heatmap (Figure 2). Only three compounds, *trans*-β-ocimene and two unidentified compounds (RI 855 and RI 1093) changed significantly different after exposure to Fairytale or Anakin VOCs (Figure 2). *Trans*-β-ocimene, RI 855 and RI 1093 were statistically increased in SeF compared to SeA (Figure 3b,c, general linear model [GLM], Estimate = 6.27, SE = 2.59, $p = 0.02$; Estimate = 0.51, SE = 0.19, $p = 0.01$; Estimate = 0.02, SE = 0.01, $p = 0.04$), respectively. Comparing the peak area per biomass (g dry weight) of *trans*-β-ocimene released from unexposed and exposed Salome plants, SeF was significantly higher in *trans*-β-ocimene compared to Salome exposed to air (Figure 3a, GLM, Estimate = 4.47, SE = 2.22, $p = 0.01$). No differences in the peak area of *trans*-β-ocimene were detected between SeA and Se0 (Figure 3a, GLM, Estimate = 2.29, SE = 1.16, $p = 0.25$) and between SeF and SeA (Figure 3a, GLM, Estimate = 0.51, SE = 0.24, $p = 0.36$).

3.2 | Olfactory preference of *R. padi* for barley plants

A series of olfactory experiments were conducted to assess the preferences of *R. padi* for odours from individual unexposed cultivars and exposed Salome plants. We first tested whether *R. padi* has a general preference for cv. Salome or the cultivars used as emitters (Anakin and Fairytale), odours of unexposed plants were offered simultaneously in olfactometer trials. *R. padi* individuals did not show an olfactory preference for Anakin over Salome (Figure 4b, Wilcoxon, $V = 63$, $p = 0.53$, $n = 20$), nor Fairytale over Salome (Figure 4b, Wilcoxon, $V = 50.5$, $p = 0.37$, $n = 20$).

To confirm with the volatile emission changes in plant exposure results, the impact of exposure to volatiles from other cultivars (plant–plant volatile interaction) on the olfactory attraction of *R. padi* for Salome plants was then investigated. Aphids were significantly less attracted to the odours of Salome plants that were previously exposed to Fairytale VOCs than unexposed Salome plants (Figure 4d, Wilcoxon, $V = 127.5$, $p = 0.01$, $n = 24$). Aphids did not show a preference for SeA VOCs over unexposed Salome plants when offered simultaneously (Figure 4d, Wilcoxon, $V = 40.5$, $p = 0.09$, $n = 24$).

We additionally evaluated the avoidance responses for *R. padi* to the odours of previously exposed Salome plants. Aphids significantly preferred air over odours from Salome exposed previously to Fairytale VOCs (Figure 4f, Wilcoxon, $V = 131$, $p = 0.04$, $n = 20$). In contrast, odour of Salome exposed previously to Anakin VOCs were more significantly attractive for *R. padi* individuals than air (Figure 4f, Wilcoxon, $V = 39.5$, $p = 0.04$, $n = 22$).

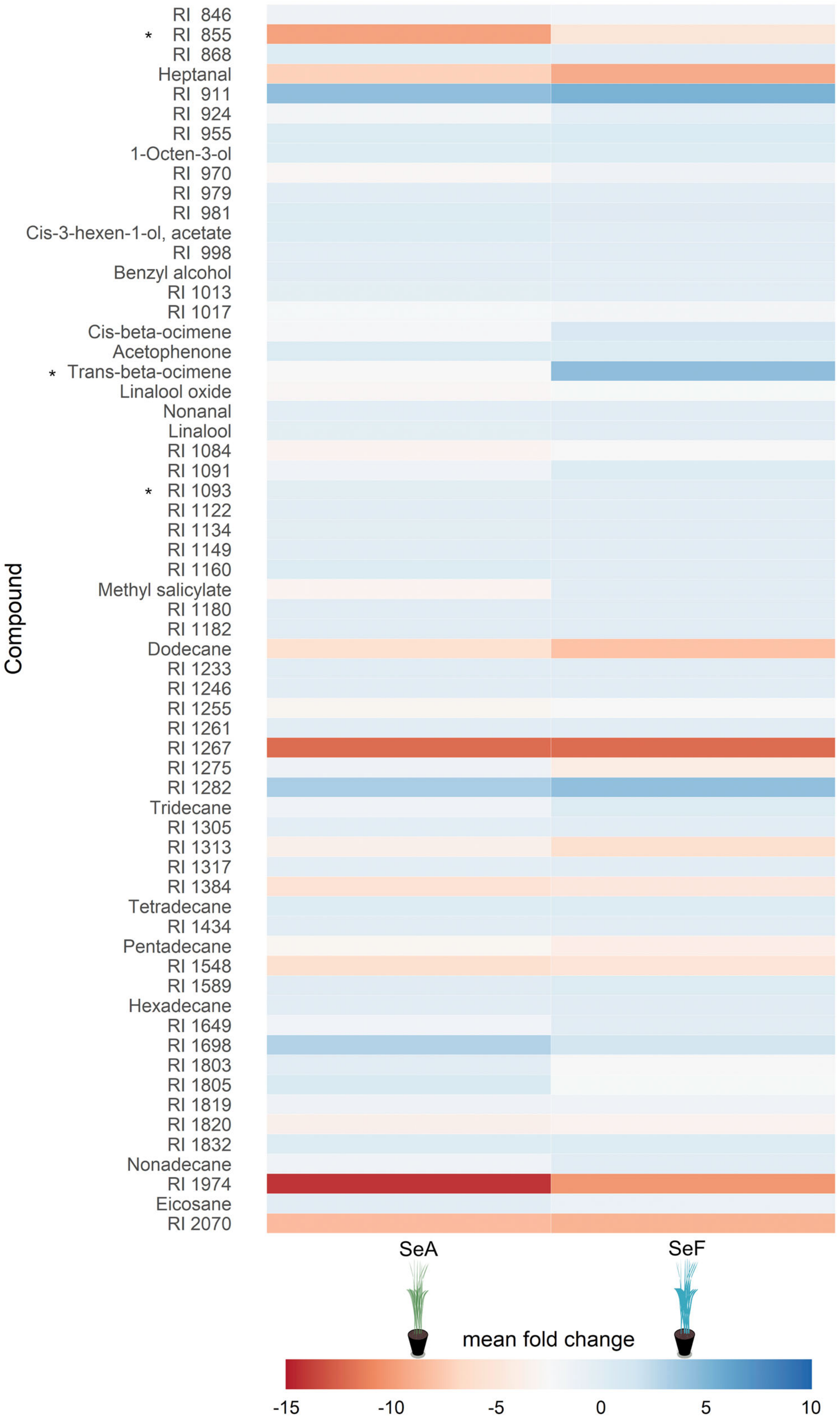


FIGURE 2 (See caption on next page).

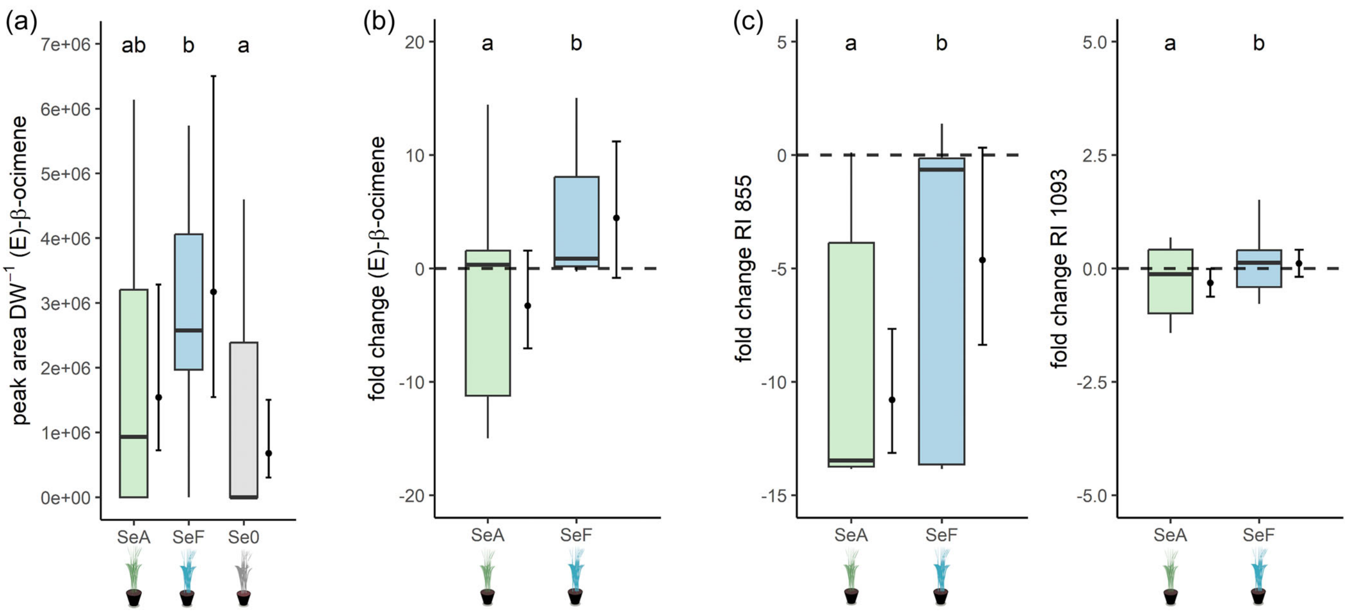


FIGURE 3 Exposure to Fairytale volatile organic compounds (VOCs) increases *trans*-β-ocimene, RI 855 and RI 1093 released from Salome. (a) Amount (peak area) of *trans*-β-ocimene per biomass (g dry weight) released from Salome after exposure to Anakin (SeA), Fairytale (SeF), or air (Se0). (b) Fold change in *trans*-β-ocimene, (c) RI 855 and (d) RI 1093 release from Salome in response to Fairytale or Anakin VOC exposure. Boxes represent the interquartile range (IQR) and whiskers extend to 1.5 × IQR. Bars represent the 95% confidence intervals with the estimated marginal means (EMMs) as dots, obtained from generalized linear models fitted with Gamma (log link) distributions. Letters indicate statistical differences between EMMs of groups at the 0.05 significance level. [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | Olfactory preference of *R. padi* for pure *trans*-β-ocimene compound

To confirm whether *trans*-β-ocimene has an impact on aphid behaviour, we performed a series of olfactory experiments with five different concentrations of *trans*-β-ocimene (0.01, 0.1, 1, 10 and 100 ng μL^{-1}) against Methanol (solvent control). No difference was detected at lower concentration of *trans*-β-ocimene (0.01, 0.1 and 1 ng μL^{-1}) compared to methanol (Figure 5b, Wilcoxon, $V = 78.5$, $p = 0.32$, $n = 22$; $V = 45$, $p = 0.23$, $n = 22$; $V = 52.5$, $p = 0.15$, $n = 20$), respectively. At the two highest concentrations (10 and 100 ng μL^{-1}), *R. padi* significantly preferred the control arm and avoided the *trans*-β-ocimene (Figure 5b, Wilcoxon, $V = 49.5$, $p = 0.03$, $n = 22$; $V = 31.5$, $p = 0.01$, $n = 19$), respectively.

4 | DISCUSSION

Our findings demonstrate that volatile interactions between specific undamaged cultivars can enhance the emission of certain volatile compounds in receiving plants, resulting in the disruption of aphid olfactory responses. The phenomenon is context specific, depending on the genotypic identity of the emitting plants. This study, therefore, highlights the significant roles of VOCs in plant–plant communications, affecting plant–insect interactions in cultivar mixture cropping systems.

4.1 | Airborne interactions between undamaged plants alter volatile emission of receiving plants

Plants have the capacity to detect and respond to the surrounding environments by chemical cues, including emitted volatiles from neighbouring plants (Brosset & Blande, 2022; Ninkovic et al., 2016). Constitutively emitted VOCs contain unique information about genotype identity (Dahlin et al., 2018), which receiving plants can detect and use to identify neighbours, then respond through growth adaptation (Ninkovic, 2003). It has been previously shown that the three individual cultivars examined in this study (Salome, Anakin, and Fairytale) had distinct constitutively produced volatile emissions (Dahlin et al., 2018). Our fold change analyses highlighted that the

FIGURE 2 Exposure to emitter volatiles induces changes in the volatile organic compound (VOC) emission of receiver. Heatmap visualization of the mean values of fold change of single compounds emitted from Salome exposed to Anakin (SeA) ($n = 10$) and Salome exposed to Fairytale (SeF) ($n = 11$). The fold change value represents the logarithmic ratio of the peak area per dry weight from each exposed plant sample to the mean of unexposed plants. The scale ranges from dark red, indicating a strong decrease of VOC emission to a highly increased (dark blue) emission of the compound in response to exposure to VOCs from Anakin or Fairytale relative to the emission from unexposed Salome plants. Asterisks(*) indicate significant changes from GLM analyses ($p < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

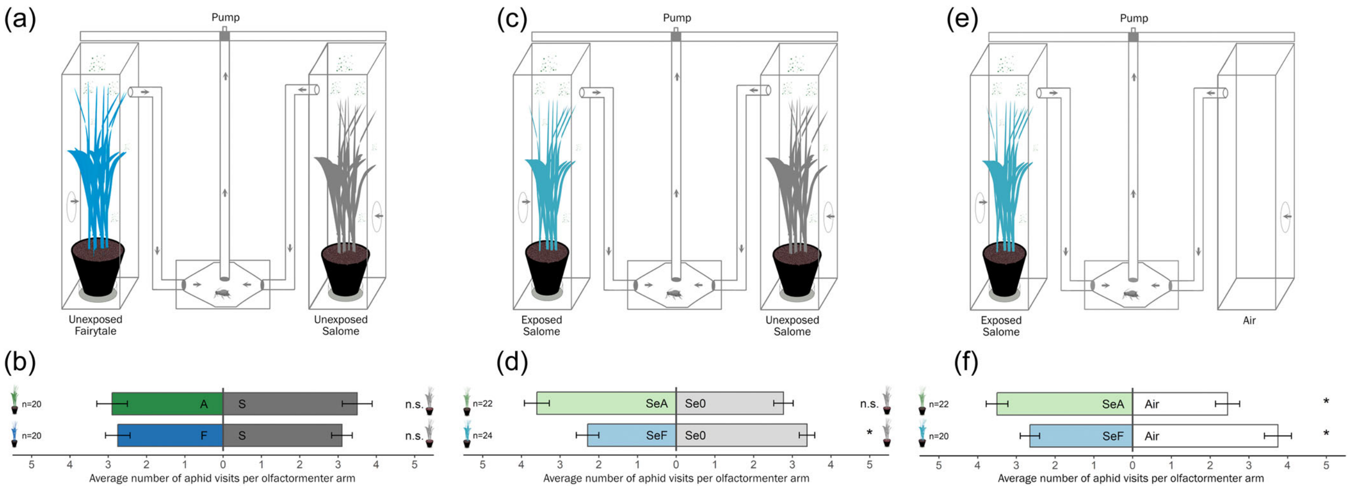


FIGURE 4 Aphid shows repellent effects from odours in Salome exposed to Fairytale (SeF) against air and less attraction in Salome exposed Fairytale (SeF) against Salome exposed to air (Se0). Before the olfactory experiment, one pot with 12 barley plants (9-day) was exposed to or not exposed to other barley cultivars as emitter for 5 days. The exposed/unexposed pot with 12 barley cultivar (14-day) was placed in each cage connected to an olfactometer and a suction pump that was used to facilitate airflow from the plants through the olfactometer. A wingless adult aphid was placed in the middle of the olfactometer. (a) Aphid preference test between individual cultivars: unexposed Fairytale (emitter) against unexposed Salome (receiver). (b) Aphid preference test on odours between individual cultivars: unexposed Anakin (A) against unexposed Salome (S) ($n = 20$), and unexposed Fairytale (F) against unexposed Salome (S) ($n = 20$). (c) Aphid preference test between exposed Salome against unexposed Salome as control. (d) Aphid preference test on odours between receiving cultivars: SeA against Se0 ($n = 22$), and SeF against Se0 ($n = 24$). (e) Aphid avoidance test between exposed Salome cultivar against Air. (f) Aphid avoidance test on odours between receiving cultivars against air: SeA against Air ($n = 22$), and SeF against Air ($n = 20$). Error bars indicate the standard error of the mean. Asterisks indicate significant differences according to the Wilcoxon signed-rank test ($p < 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

emission of *trans*-β-ocimene and two unidentified compounds were significantly increased in Salome exposed Fairytale, but not when Salome was exposed to Anakin. It, therefore, seems that Salome can distinguish between different neighbouring cultivars by perceiving volatiles and activating specific biological pathways, altering its own volatile emissions in response. To our knowledge, this study presents the first evidence that volatiles released by specific undamaged cultivars can prompt neighbouring cultivars to alter their physiological state by emitting greater quantities of specific volatile compounds. This highlights the significant involvement of VOCs in interactions between undamaged plants.

Plants discriminate between the emitted volatiles of different neighbours (Kigathi et al., 2019; Ninkovic et al., 2016, 2020). In studies of interactions between different plant species, Ninkovic et al. (2013) revealed that volatile compounds from undamaged onions can enhance the release of certain volatile compounds from receiving potatoes. Ninkovic (2003) also showed that exposure to specific cultivars could alter the pattern of biomass allocation for receiving plants, away from leaves and in favour of roots. As a result, these receiving plants become more competitive for limited nutrition and water resources. Given the evidence from these previous studies and our own, it is clear that plants are capable of detecting, responding to, and distinguishing between neighbouring plants, depending on the identity of the emitters. The identification of a competitive neighbour can therefore lead to specific morphological and physiological adaptations to better tolerate resource competition.

While the mechanisms of VOC perception in plants are not well understood, the effects are context-dependent (Hemachandran et al., 2017). The observed rise in specific volatile emissions in receiving plants may be attributed to active mechanisms of volatile-mediated plant–plant interactions, which encourage specific physiological pathways, such as induced or primed defenses. In this case, the observed increased emission of certain volatiles in our study could possibly be a mechanism to induce or prime defenses in receiving plants. Another possible mechanism, which cannot be excluded at this stage, is a passive one: the adhesion of volatiles to the surface of recipient plants, which could be re-emitted later (Li & Blande, 2015). Therefore, further research is needed to uncover the underlying mechanisms of the observed effects between specific undamaged plants.

4.2 | Aphid olfactory responses to specific airborne-induced plant volatiles

Plant volatiles are olfactory cues that can be detected and utilized by insects in host plant location and selection (Bruce et al., 2005). In line with the emitter-specific impact on the VOC emission of receiving Salome plants, *R. padi* showed emitter-dependent preferences for differently-exposed Salome plants. While aphids were less attracted to Salome after exposure to Fairytale, compared to Se0, no preference was detected when offering Salome previously exposed

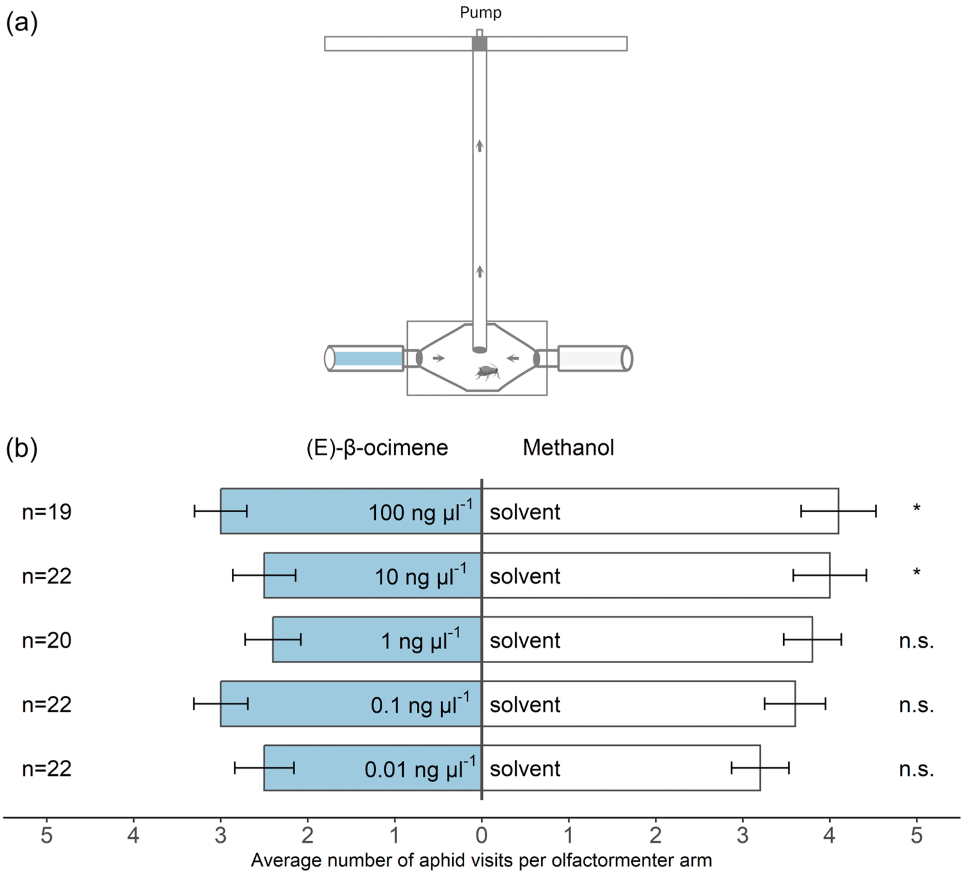


FIGURE 5 *Trans*-β-ocimene repels *Rhopalosiphum padi*; aphid shows repellent effects from *trans*-β-ocimene (≥ 10 ng μL^{-1}) against solvent (methanol). (a) Aphid avoidance setup. Two-arm olfactometer was used: The light blue colour represents the arm of *trans*-β-ocimene and the grey colour represents the arm of solvent as control (methanol). Five series of *trans*-β-ocimene dilutions (0.01, 0.1, 1, 10 and 100 ng μL^{-1}) against solvent were tested in olfactometer. Each 10 μg of each dilution concentration was placed on a small piece of filter paper in each glass tube connected to an olfactometer and a suction pump that was used to facilitate airflow from the glass tube through the olfactometer. A wingless adult aphid was placed in the middle of the olfactometer. (b) Aphid avoidance tests were conducted on *trans*-β-ocimene at five different concentrations against solvents: 0.01 ng μL^{-1} against solvent (n = 22), 0.1 ng μL^{-1} against solvent (n = 22), 1 ng μL^{-1} against solvent (n = 20), 10 ng μL^{-1} against solvent (n = 22) and 100 ng μL^{-1} against solvent (n = 19), respectively. Error bars indicate the standard error of the mean. The n.s. represent no significant differences and asterisks(*) represent significant differences according to the Wilcoxon signed-rank test (p < 0.05). [Color figure can be viewed at wileyonlinelibrary.com]

to Anakin or to air. This highlights that the changes in volatile emissions induced by certain emitters can impact the behavioural responses of herbivorous insects. Accordingly, Tous-Fandos et al. (2023) showed that the alternation in the odour profile of specific wheat cultivar mixtures affects the host preference of aphids.

The reduced attraction and increased avoidance of aphids to the host plant odour could be due to the effects of specific chemical compounds (Webster et al., 2010). *trans*-β-ocimene, emitted at higher levels in SeF, induced a significant avoidance response in aphids, indicating the potential role of *trans*-β-ocimene in aphid host preference. The olfactory responses of aphids to the other two unidentified compounds remain unknown. Further chemical identification is needed to discern the roles of these compounds as cues for aphid detection and the location of their host plants. Nonetheless, our findings support the hypothesis that aphids can respond to particular compounds at specific concentrations, influencing their search for a host plant (Webster, 2012; Webster et al., 2010).

Trans-β-ocimene is known to be a HIPV that effectively repels insect pests (Thompson et al., 2022). Externally applying β-ocimene can trigger induced defense in recipient plants, resulting in subsequent adverse effects on the olfactory responses, settling, feeding behaviours and overall performance of aphids (Cascone et al., 2015; Kang et al., 2018). Interestingly, exposure to the volatiles of a specific undamaged cultivar can enhance *trans*-β-ocimene production in undamaged plants as well. As *trans*-β-ocimene has repulsive effects on aphids, it may serve as a relevant compound for sustainable pest control in crop production.

The specific ratios of each VOC could contribute to aphid behavioural responses (Dardouri et al., 2019), and certain compound blends could be more repellent than single VOCs (Bruce & Pickett, 2011; Dardouri et al., 2019; Deletre et al., 2016). As not all volatile compounds used by *R. padi* for host plant detection are currently identified, we compared the composition of all detected plant volatiles, which showed no significant difference in response to the exposure. However, the shifts in emissions of specific combinations of VOCs influential to *R. padi* behavioural activity can be relevant for the observed olfactory preferences.

4.3 | Ecological aspects of cultivar mixtures in pest control

This study provides fresh knowledge of the potential specificity of volatile-mediated plant–plant interactions, affecting plant–insect interactions and highlighting the significant roles of VOCs in pest control. Our results support the hypothesis that plants can use emitted volatiles from specific neighbouring plants to prepare for growth adaptation, future threat or stress (Ninkovic et al., 2020). Aphids, like many other herbivorous insects, use plant volatiles as olfactory cues to make crucial decisions regarding host plant selection, feeding and overall development (Pettersson et al., 2017). The volatile components of plants carry information about their quality for the aphids. The observed repellent effects on aphids in certain cultivar mixtures suggest that aphids struggle to find suitable host plants in these instances. This could reduce the establishment of new aphid colonies due to prolongation of searching for host plants and increased exposure to natural enemies in the agroecosystem. Volatile interactions between undamaged Salome plants exposed to Fairytale can induce responses in receiving plants which disrupt aphid feeding and reduce their performance (Kheam et al., 2023). In the field, the reduction of aphid populations observed in specific cultivar mixtures, such as Salome–Fairytale mixtures (Dahlin et al., 2018), is consistent with the mechanistic explanation proposed from the results of our laboratory experiments. All of these findings indicate that volatile interactions between plants constitute a potential underlying mechanism of insect pest suppression in cultivar mixtures and, on a broader scale, suggest their potential in pest management to enhance the sustainability of cropping systems.

5 | CONCLUSION

Plants detect VOCs emitted by their specific, undamaged neighbours and respond by altering their own volatile emissions, thus enhancing their defenses. Our results suggest that volatile interactions in specific cultivar mixtures have a major implication for plant–insect interactions and bear potential as an effective approach for the development of integrated pest management in crop protection. The observed increasing *trans*-β-ocimene in plant–plant communication and its repellent effects on insect pests suggest this compound is a suitable candidate for sustainable agricultural pest control. Currently, control measures for insect pests by cultivar mixtures are still limited, but exploring volatile-mediated plant–plant communications to understand interactions among plants, insect pests, natural enemies, and diseases will help us determine the novel points of control that will open the door for wider adoption of cultivar mixtures in sustainable agricultural practices.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

Aartsma, Y., Bianchi, F.J.J.A., van der Werf, W., Poelman, E.H. & Dicke, M. (2017) Herbivore-induced plant volatiles and tritrophic interactions across spatial scales. *New Phytologist*, 216, 1054–1063. Available from: https://doi.org/10.1111/nph.14475

Appel, H.M. & Cocroft, R.B. (2014) Plants respond to leaf vibrations caused by insect herbivore chewing. *Oecologia*, 175, 1257–1266. Available from: https://doi.org/10.1007/s00442-014-2995-6

Biedrzycki, M.L., Jilany, T.A., Dudley, S.A. & Bais, H.P. (2010) Root exudates mediate kin recognition in plants. *Communicative & Integrative Biology*, 3, 28–35. Available from: https://doi.org/10.4161/cib.3.1.10118

Brilli, F., Loreto, F. & Baccelli, I. (2019) Exploiting plant volatile organic compounds (VOCS) in agriculture to improve sustainable defense strategies and productivity of crops. *Frontiers in Plant Science*, 10, 264. Available from: https://doi.org/10.3389/fpls.2019.00264

Brosset, A. & Blande, J.D. (2022) Volatile-mediated plant-plant interactions: volatile organic compounds as modulators of receiver plant defence, growth, and reproduction. *Journal of Experimental Botany*, 73, 511–528. Available from: https://doi.org/10.1093/jxb/erab487

Bruce, T.J.A. & Pickett, J.A. (2011) Perception of plant volatile blends by herbivorous insects—finding the right mix. *Phytochemistry*, 72, 1605–1611. Available from: https://doi.org/10.1016/j.phytochem.2011.04.011

Bruce, T.J.A., Wadhams, L.J. & Woodcock, C.M. (2005) Insect host location: a volatile situation. *Trends in Plant Science*, 10, 269–274. Available from: https://doi.org/10.1016/j.tplants.2005.04.003

Cascone, P., Iodice, L., Maffei, M.E., Bossi, S., Arimura, G. & Guerrieri, E. (2015) Tobacco overexpressing β-ocimene induces direct and indirect responses against aphids in receiver tomato plants. *Journal of Plant Physiology*, 173, 28–32. Available from: https://doi.org/10.1016/j.jplph.2014.08.011

Clavijo McCormick, A., Unsicker, S.B. & Gershenzon, J. (2012) The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. *Trends in Plant Science*, 17, 303–310. Available from: https://doi.org/10.1016/j.tplants.2012.03.012

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

Dahlin, I., Vucetic, A. & Ninkovic, V. (2015) Changed host plant volatile emissions induced by chemical interaction between unattacked plants reduce aphid plant acceptance with intermorph variation. *Journal of Pest Science*, 88, 249–257. Available from: https://doi.org/10.1007/s10340-014-0625-z

Dardouri, T., Gomez, L., Schoeny, A., Costagliola, G. & Gautier, H. (2019) Behavioural response of Green peach aphid *Myzus persicae* (Sulzer) to volatiles from different rosemary (*Rosmarinus officinalis* L.) clones. *Agricultural and Forest Entomology*, 21, 336–345. Available from: https://doi.org/10.1111/afe.12336

Deletre, E., Schatz, B., Bourguet, D., Chandre, F., Williams, L., Ratnadass, A. et al. (2016) Prospects for repellent in pest control: current developments and future challenges. *Chemoecology*, 26, 127–142. Available from: https://doi.org/10.1007/s00049-016-0214-0

Delory, B.M., Delaplace, P., Fauconnier, M.L. & du Jardin, P. (2016) Root-emitted volatile organic compounds: can they mediate belowground plant-plant interactions? *Plant and Soil*, 402, 1–26. Available from: https://doi.org/10.1007/s11104-016-2823-3

Dudareva, N., Klempien, A., Muhlemann, J.K. & Kaplan, I. (2013) Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist*, 198, 16–32. Available from: https://doi.org/10.1111/nph.12145

Erb, M., Veyrat, N., Robert, C.A.M., Xu, H., Frey, M. & Ton, J. et al. (2015) Indole is an essential herbivore-induced volatile priming signal in maize. *Nature Communications*, 6, 6273. Available from: https://doi.org/10.1038/ncomms7273

Field, L.M., Pickett, J.A. & Wadhams, L.J. (2000) Molecular studies in insect olfaction. *Insect Molecular Biology*, 9, 545–551. Available from: https://doi.org/10.1046/j.1365-2583.2000.00221.x

Foster, S.P., Devine, G. & Devonshire, A.L. (2017) Inseciticide resistance. In: Emden, H.F.V. & Harrington, R., (Eds.) *Aphids as crop pests*. Wallingford: CAB International. pp. 426–447.

Gallinger, J. & Gross, J. (2020) Phloem metabolites of *Prunus Sp.* rather than infection with candidatus phytoplasma prunorum influence feeding behavior of *Cacopsylla pruni* nymphs. *Journal of Chemical Ecology*, 46, 756–770. Available from: https://doi.org/10.1007/s10886-020-01148-8

Gallinger, J., Jarausch, B., Jarausch, W. & Gross, J. (2020) Host plant preferences and detection of host plant volatiles of the migrating psyllid species *Cacopsylla pruni*, the vector of European stone fruit yellows. *Journal of Pest Science*, 93, 461–475. Available from: https://doi.org/10.1007/s10340-019-01135-3

Gantner, M., Najda, A. & Piesik, D. (2019) Effect of phenolic acid content on acceptance of hazel cultivars by filbert aphid. *Plant Protection Science*, 55, 116–122. Available from: https://doi.org/10.17221/150/2017-PPS

Grettenberger, I.M. & Tooker, J.F. (2016) Inter-varietal interactions among plants in genotypically diverse mixtures tend to decrease herbivore performance. *Oecologia*, 182, 189–202. Available from: https://doi.org/10.1007/s00442-016-3651-0

Gross, J., Gallinger, J. & Rid, M. (2019) Collection, identification, and statistical analysis of volatile organic compound patterns emitted by phytoplasma infected plants. In: Musetti, R. & Pagliari, L. (Eds.) *Phytoplasmas methods and protocols*. Berlin: Springer Nature.

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

Hemachandran, H., Doss, C.G.P. & Siva, R. (2017) Plant communication: an unresolved mystery. *Current Science*, 112, 1990–1991. Available from: https://doi.org/10.1007/978-3-642-12162-3.Future

Kang, Z.W., Liu, F.H., Zhang, Z.F., Tian, H.G. & Liu, T.X. (2018) Volatile β-ocimene can regulate developmental performance of peach aphid *Myzus persicae* through activation of defense responses in Chinese cabbage *Brassica pekinensis*. *Frontiers in Plant Science*, 9, 708. Available from:. https://doi.org/10.3389/fpls.2018.00708

Karban, R., Shiojiri, K., Huntzinger, M. & McCall, A.C. (2006) Damage-induced resistance in sagebrush: volatiles are key to intra- and interplant communication. *Ecology*, 87, 922–930. Available from: https://doi.org/10.1890/0012-9658(2006)87[922:DRISVA]2.0.CO;2

Kegge, W. & Pierik, R. (2010) Biogenic volatile organic compounds and plant competition. *Trends in Plant Science*, 15, 126–132. Available from: https://doi.org/10.1016/j.tplants.2009.11.007

Keuskamp, D.H., Sasidharan, R. & Pierik, R. (2010) Physiological regulation and functional significance of shade avoidance responses to neighbors. *Plant Signaling & Behavior*, 5, 655–662. Available from: https://doi.org/10.4161/psb.5.6.11401

Kheam, S., Markovic, D., Rubene, D., Ith, S. & Ninkovic, V. (2023) Volatile interactions between specific undamaged barley cultivars affect aphid feeding behavior and performance. *Journal of Pest Science*, 96, 1049–1059. Available from: https://doi.org/10.1007/s10340-023-01592-x

Kigathi, R.N., Weisser, W.W., Reichelt, M., Gershenzon, J. & Unsicker, S.B. (2019) Plant volatile emission depends on the species composition of the neighboring plant community. *BMC Plant Biology*, 19, 58. Available from: https://doi.org/10.1186/s12870-018-1541-9

Length, R., (2022). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.8.3 [WWW Document]. https://cran.r-project.org/package=emmeans [Accessed 09th May 2023].

Li, T. & Blande, J.D. (2015) Associational susceptibility in broccoli: mediated by plant volatiles, impeded by ozone. *Global Change Biology*, 21, 1993–2004. Available from: https://doi.org/10.1111/gcb.12835

Markovic, D., Glinwood, R., Olsson, U. & Ninkovic, V. (2014) Plant response to touch affects the behaviour of aphids and ladybirds. *Arthropod-Plant Interactions*, 8, 171–181. Available from: https://doi.org/10.1007/s11829-014-9303-6

Ninkovic, V. (2003) Volatile communication between barley plants affects biomass allocation. *Journal of Experimental Botany*, 54, 1931–1939. Available from: https://doi.org/10.1093/jxb/erg192

Ninkovic, V., Dahlin, I., Vucetic, A., Petrovic-Obradovic, O., Glinwood, R. & Webster, B. (2013) Volatile exchange between undamaged plants—a new mechanism affecting insect orientation in intercropping. *PLoS ONE*, 8, e69431. Available from: https://doi.org/10.1371/journal.pone.0069431

Ninkovic, V., Markovic, D. & Dahlin, I. (2016) Decoding neighbour volatiles in preparation for future competition and implications for tritrophic interactions. *Perspectives in Plant Ecology, Evolution and Systematics*, 23, 11–17. Available from: 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 & Environment*, 44, 1030–1043. Available from: https://doi.org/10.1111/pce.13910

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Weedon, J. (2022) Package "vegan". https://github.com/vegandevs/vegan [Accessed 09th May 2023].

Oliver, K.M., Russell, J.A., Moran, N.A. & Hunter, M.S. (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 1803–1807. Available from: https://doi.org/10.1073/pnas.0335320100

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. pp. 1135–1144.

Pettersson, J., Tjallingii, W.F. & Hardie, J. (2017) Host-plant selection and feeding. In: Emden, H.F.V. & Harrington, R. (Eds.) *Aphids as crop pests*. Wallingford: CAB International, pp. 173–189.

Piesik, D. & Wenda-Piesik, A. (2015) *Sitophilus granarius* responses to blends of five groups of cereal kernels and one group of plant volatiles. *Journal of Stored Products Research*, 63, 63–66. Available from: https://doi.org/10.1016/j.jspr.2015.05.007

Piesik, D., Wenda-Piesik, A., Krasińska, A., Wrzesińska, D. & Delaney, K.J. (2015) Volatile organic compounds released by *Rumex confertus* following *Hypera rumicis* herbivory and weevil responses to volatiles. *Journal of Applied Entomology*, 140, 308–316. Available from: https://doi.org/10.1111/jen.12238

R Core Team. (2022) *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Schuman, M.C., Allmann, S., & Baldwin, I.T. (2016) Correction: Plant defense phenotypes determine the consequences of volatile emission for individuals and neighbors. *ELife*, 5, e16606. https://doi.org/10.7554/elife.16606

Shoffner, A.V. & Tooker, J.F. (2013) The potential of genotypically diverse cultivar mixtures to moderate aphid populations in wheat (*Triticum aestivum* L.). *Arthropod-Plant Interactions*, 7, 33–43. Available from: https://doi.org/10.1007/s11829-012-9226-z

Singh, S.S., Mittal, A.M., Chepurwar, S. & Gupta, N. (2019) Olfactory systems in insects: similarities and differences between species. In: Picimbon, J.-F. (Ed.) *Olfactory concepts of insect control—alternative to insecticides*. New York: Springer International Publishing, pp. 19–48. https://doi.org/10.1007/978-3-030-05165-5

Thompson, M.N., Grunseich, J.M., Marmolejo, L.O., Aguirre, N.M., Bradicich, P.A. & Behmer, S.T. et al. (2022) Undercover operation: belowground insect herbivory modifies systemic plant defense and repels aboveground foraging insect herbivores. *Frontiers in Ecology and Evolution*, 10, 1033730. https://doi.org/10.3389/fevo.2022.1033730

Tous-Fandos, A., Gallinger, J., Enting, A., Chamorro-Lorenzo, L., Sans Serra, F.X. & Ninkovic, V. (2023) Alterations in the odor profile of plants in cultivar mixtures affect aphid host-location behavior. *Frontiers in Plant Science*, 14, 1186425. Available from: https://doi.org/10.3389/fpls.2023.1186425

Turlings, T.C.J. & Erb, M. (2018) Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. *Annual Review of Entomology*, 63, 433–452. Available from: https://doi.org/10.1146/annurev-ento-020117-043507

Webster, B. (2012) The role of olfaction in aphid host location. *Physiological Entomology*, 37, 10–18. Available from: https://doi.org/10.1111/j.1365-3032.2011.00791.x

Webster, B., Bruce, T., Pickett, J. & Hardie, J. (2010) Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. *Animal Behaviour*, 79, 451–457. Available from: https://doi.org/10.1016/j.anbehav.2009.11.028

Wickham, H. (2016) *ggplot2: elegant graphics for data analysis*. New York: Springer-Verlag.

Zuur, A.F., Ieno, E.N. & Elphick, C.S. (2010) A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1, 3–14. Available from: https://doi.org/10.1111/j.2041-210x.2009.00001.x

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