plant biology

Plant Biology ISSN 1435-8603

RESEARCH ARTICLE

Glyphosate residues in soil alter herbivore-induced plant volatiles and affect predatory insect behaviour

B. Fuchs[1,2], J. D. Blande[3] & V. Weijola[2]

1 Department of Agroecology, Aarhus University, Slagelse, Denmark
2 Biodiversity Unit, University of Turku, Turku, Finland
3 Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland

Keywords
crop health; ecosystem services; plant defence; plant volatile emission; sustainable agriculture; trophic interactions.

Correspondence
B. Fuchs, Department of Agroecology, Aarhus University, 4200 Slagelse, Denmark
E-mail: bf@agro.au.dk

Editor
V. Pastor

Received: 10 June 2025;
Accepted: 9 September 2025

doi:10.1111/plb.70117

ABSTRACT

- Plants under herbivore attack emit distinct blends of herbivore-induced plant volatiles (HIPVs) which serve as signalling cues for predatory insects. This concept of indirect plant defence has tremendous potential in sustainable insect pest control. It represents a cornerstone of alternatives to synthetic pesticides in integrated pest management (IPM) strategies. The composition of HIPVs determines the effectiveness of predatory insect attraction and is vulnerable to disturbance by multiple biotic and abiotic factors above- and belowground. Residues of the most widely used herbicide (glyphosate) are persistent pollutants in agricultural soils, where they increasingly affect plant physiology, with cascading effects on species interactions.
- Here, we tested whether herbicide legacy in soil affects plant performance, aphid herbivory, and aphid-induced volatile organic compound (VOC) emissions in oat plants, and tested whether the preference of predatory ladybirds towards aphid-infested plants is affected by herbicide legacy in the soil.
- Soil herbicide legacy reduced chlorophyll activity and plant height, but did not affect plant biomass nor aphid populations. Five compounds in the emitted VOC profile were significantly affected by soil history of herbicide use, which, in turn, affected ladybird orientation behaviour. In a choice assay, ladybirds preferred the odour of plants growing in herbicide-free soil.
- These results reveal a subtle layer of effects of herbicide legacy in soil on emission of HIPVs, with cascading effects on predatory insect behaviour. Our results demonstrate that essential ecosystem services in the aboveground plant space, such as natural pest control, may be reduced by soil pollution with anthropogenic pesticides such as glyphosate, causing mismatches in plant–insect communication.

INTRODUCTION

By the beginning of this millennium 15.5% of ice-free land was devoted to growing crops, and 47.6% to agriculture as a whole, making agroecosystems the dominant terrestrial biome on earth (Ellis *et al.* 2010). In Europe, farmland constitutes the most extensive habitat for wildlife and harbours more than 50% of bird species (Emmerson *et al.* 2016). Furthermore, the intensification of agriculture, which started after the second world war, has had significant impact on biodiversity in agroecosystems (Emmerson *et al.* 2016; Raven & Wagner 2021). In fact, agriculture is now the single biggest driver of biodiversity loss globally, with long-term studies showing drastic declines in insect taxonomic diversity and biomass over the past few decades (Kehoe *et al.* 2017; Ziesche *et al.* 2024). This loss of biodiversity can have serious ramifications for ecosystem services provided by insects and other arthropods. Predators and parasitoids are important natural control agents of insect pest outbreaks in agroecosystems but, similar to many other taxa, their abundance and diversity have declined over the past decades (Bianchi *et al.* 2006; Rusch *et al.* 2016; Ali *et al.* 2018). Meanwhile the abundance of pest insects such as aphids have increased (Lundgren & Fausti 2015; Ziesche *et al.* 2024). Ecosystem services are essential components of functional agricultural landscapes (Bennett *et al.* 2021). Two vital ecosystem services executed by insects are pollination and pest control, both of which are highly reliant on olfactory cues (Kielty *et al.* 1996; Dickens 1999; Francis *et al.* 2004; Wright & Schiestl 2009; Mäkinen *et al.* 2024).

The vast majority of plants are sessile organisms, unable to relocate under unfavourable conditions. This has prompted adaptations to the environment, including high physiological and phytochemical plasticity, which ensures persistence in conditions at the edge of their physiological range (Nawaz *et al.* 2023). Examples include the induction of specialized metabolites for protection against abiotic stressors such as UV-light and ozone, and against biotic stressors such as pathogen infection and herbivore infestation (Hartmann 2007). Herbivore control, however, is often provided by natural enemies, which either prey on or parasitize herbivorous insects to the benefit of the plant (Boldorini *et al.* 2024). To locate their prey, predatory insects orientate using olfactory cues released by plants under herbivore attack (Pare & Tumlinson 1999; War *et al.* 2011). These cues, known as herbivore-induced plant volatiles (HIPVs), are comprised of volatile organic compounds (VOCs) and released in a highly specific manner, determined by plant species, insect species, feeding type, salivary proteins, microbial composition of the feeding organelle, and diverse other biotic and abiotic factors (Alborn *et al.* 2007; Takemoto & Takabayashi 2015; Turlings & Erb 2018).

The emitted HIPV blend is a complex and specific mixtures of compounds from multiple compound classes, including various indole derivatives (terpenoids) with high chemical diversity, aromatic compounds, and green leaf volatiles (GLVs), which are enzymatic degradation products of cell wall lipids (Dudareva *et al.* 2013; Liu *et al.* 2019). In line with their diversity, their biosynthesis is regulated by at least three pathways (Maffei 2010). Green leaf volatiles are a product of the lipoxygenase pathway, where the hydroperoxide lyase enzyme produces hexanol derivatives from C18 cell wall lipids (Bate & Rothstein 1998; Blée 1998). Terpenoids are synthesized from isoprene units mainly within the mevalonate pathway (Lichtenthaler *et al.* 1997). Sesquiterpenes have been found to be derived from the methylerythritol phosphate pathway or a metabolic crosstalk between the mevalonate pathway and the methylerythritol phosphate pathway (Maffei 2010). Aromatic compounds, such as methyl salicylate (MeSA), indole and phenolic acid derivatives, are products of the shikimate pathway (Bennett & Wallsgrove 1994).

In addition to herbivore feeding, recent studies show that biotic and abiotic plant growth conditions, such as the microbial rhizosphere setting and agrochemical use, respectively, can impact the amount and composition of emitted VOCs (Mauch-Mani *et al.* 2017; Meier & Hunter 2019; Fuchs *et al.* 2024). In this study, we focus on the effects of persistent agrochemical pollution of agricultural soils on HIPVs – a topic which has only recently started to gain attention (Cesco *et al.* 2021; Fuchs *et al.* 2021). Many agrochemicals are intended to interact with plant biochemical regulation, either to boost crop performance and resistance, or to kill undesired plants in crop production areas (Lamberth *et al.* 2013). Herbicides are excessively used in GMO cropping systems, but also in non-GMO crop production (Van Bruggen *et al.* 2018; Ospina *et al.* 2024). The regular use of herbicides has been established as a fast and affordable method of weed control (Bonny 2016). In particular, the use of glyphosate-based herbicides has exponentially grown in the past decades, which has resulted in global pollution of agricultural soils by glyphosate residues (Benbrook 2016; Maggi *et al.* 2020). The effects of these residues on VOC emissions, insect behaviour and multitrophic interactions have been little studied, and their impact on plant physiology and ecosystem services is virtually unknown.

Glyphosate residues are now found in the majority of agroecosystems globally (Maggi *et al.* 2020). These compounds kill plants by blocking an enzyme in the shikimate pathway which prevents the biosynthesis of essential aromatic amino acids (Benbrook 2016). In a long-term field trial and in greenhouse studies, we have shown that glyphosate residues in soil affect phytohormone concentration in strawberry, potato, and oat (Fuchs *et al.* 2022), herbivore-induced phenolic compound concentrations in strawberry leaves and fruits (Fuchs *et al.* 2022; Fuchs, Helander, *et al.* 2024), and alkaloid concentrations in potato leaves (Rainio *et al.* 2020). Moreover, in a functional lab study it has been shown that plant VOCs in particular are affected by glyphosate application, which may affect beneficial insect behaviour, such as predatory insects, essential for sustainable pest control (D'Alessandro *et al.* 2006). However, it is not known whether the existence of glyphosate residues in soil affect HIPV emissions and the olfactory behavioural responses of predatory insects.

Aphids are a common pest on cereal species, transfering destructive diseases, such as barley yellow dwarf virus (Nancarrow *et al.* 2021). They are efficiently controlled by multiple predatory insects, where ladybird beetles and their larvae are among the most effective representatives (Fuchs *et al.* 2017; Krey *et al.* 2021). Many aphid predators, including ladybirds, locate their prey via HIPVs (Züst & Agrawal 2016; Aljbory & Chen 2018). In a greenhouse study, we investigated the soil-mediated impact of long-term use – 8 consecutive years – of glyphosate-based herbicide on: (1) plant and aphid performance, (2) plant emission of VOCs infested by aphids, and (3) the behaviour of aphid predators in response to HIPVs released by aphid-infested oat plants.

MATERIAL AND METHODS

Soil herbicide history

Soil was collected from a long-term common garden study site established in 2014 to functionally study the impact of glyphosate residues in soil on non-target organisms. The study site is located in southwest Finland, with 24 plots where glyphosate-based herbicide (GBH) (Roundup Gold) has been used annually according to manufacturer's recommendations on 12 plots, whereas the same number of plots were sprayed with the corresponding amount of water and served as control plots (for details see Helander *et al.* 2019). From each of the 24 plots, soil was collected in spring 2022, 2 weeks after treatment of the area with the recommended dose of glyphosate-based herbicides (6.4 L ha^{-1}, corresponding to 3 kg glyphosate active ingredient per hectare; Helander *et al.* 2019). Approx. 100 L of soil was randomly collected from all 12 treated plots and 100 L from control plots. The soil was immediately transferred to 24 pots per soil treatment in the greenhouse.

Plant setup

On 15 May, soil was transferred to the greenhouse and distributed into 48 square pots, with an edge length of 15 cm. Greenhouse conditions were ambient light and temperature, with minimum temperature set to 16°C and active ventilation from 26°C. Each pot received nine oat (*Avenae sativa*) seedlings arranged in three rows with three seedlings per row. Plants were grown in a greenhouse for 6 weeks before being enclosed in aphid proof cages. At the end of the experiment, in July, after the insect behaviour bioassay (see below), we measured the foliar chlorophyll content with a hand-held optical chlorophyll meter (Soil Plant Analysis Development SPAD-502 Plus; Konica Minolta, Japan) (Keronen *et al.* 2023). Measurements with the SPAD-502 produce relative values that are proportional to the amount of chlorophyll in the leaf. As we were only interested in relative differences between the treatments, we did not convert the measurements into absolute values. We abbreviate the presented measurements as the SPAD value. Following collection of SPAD values, plant height was measured, and aboveground biomass collected and dried for 6 days in a dehumidified room at 28°C.

Herbivory bioassay

The experiment started with 48 pots, 12 per treatment for the four treatments (Control, Control + aphid, GBH, GBH + aphid). For the aphid and VOC studies, we neglected the aphid-free pots, reducing pot number to 24. Additionally, we reduced the number of experimental pots based on availability of insect cages to eight per treatment. 50 adult bird-cherry oat aphids (*Rhopalosiphum padi*) were added to each of the 16 pots (8 pots filled with GBH soil and 8 pots with control soil). Aphids were transferred individually with a fine paintbrush and added to the experimental plants. Each pot was placed individually in a perforated cage (Bug Dorm 4E3074) to prevent aphid movement between the pots. All aphids had been reared on young oat plants grown in commercially available plant substrate (Kekkilä Garden Viherkasvimulta). Aphid numbers were recorded once per week through 3 consecutive weeks. Aphids were counted for 15 min per plant. In cases when 15 min did not suffice to count all aphids per pot, the percentage of the remaining plant surface was estimated and aphid numbers extrapolated for the entire plant surface per pot (Fuchs *et al.* 2024). Due to the high number of aphids, this method was applied to all the pots. To ensure reliable results from this method, we counted all aphids on one of the pots after applying the estimation method, and deviation was found to be <3%.

Volatile organic compound measurements

After the last aphid count on 20 July, VOCs were collected in a shaded greenhouse chamber at 18–20°C for 1 h per pot, following published protocols (Giron-Calva *et al.* 2017). Three plants per pot for a total of 16 pots (8 per treatment control + aphid and GBH + aphid) were enclosed in an airtight polyethylene terephthalate oven bag (25 × 38 cm; Look® Uunipussi Eskimo Oy). One opening in the bag was used for the introduction of charcoal filtered air (220 mL min^{-1}) and on the opposite side of the bag we inserted a stainless-steel tube filled with 200 mg Tenax TA 60/80 adsorbent (Markes International), which trapped the VOCs and was connected to a vacuum pump (KNF) with a flow rate of 200 mL min^{-1} for 1 h. VOCs were collected from four pots simultaneously, allowing for eight samples per day on two consecutive mornings between 08:00 h and 10:00 h. We calibrated airflows with a flowmeter every morning, before the trapping started, using a bubble film calibrator (mini-Buck Calibrator, Buck). VOCs were collected by dynamic headspace sampling. VOC samples were analysed by gas chromatography–mass spectrometry (GC–MS), and ChemStation software was used for further compound identification and quantification. The majority of the detected compounds belonged to one of three compound classes: green leaf volatiles, monoterpenes, or sesquiterpenes.

GC–MS analysis

To prevent loss of VOCs from the sampling tubes, samples were stored at 4°C until analysis by GC–MS. The compounds trapped in the tubes were desorbed with a thermal desorption unit (TD-100; Markes International) at 250°C for 10 min, and cryofocused at −10°C in split-less mode onto an HP-5 capillary column (60 m, 250 μm × 0.25 μm; Agilent) (Saunier *et al.* 2023). We identified compounds by comparing their mass spectra with those of pure standards and compounds in the NIST library (v. 20). The standard mix consisted of 21 terpenoid and 9 green leaf volatiles. Integrated peak areas were converted into amounts (ng) using external pure standards (Sigma Aldrich, Germany). For compounds without pure standards, we used data from chemically similar compounds for quantification (i.e., α-pinene for monoterpenes). We calculated VOC emissions in ng g^{-1} min^{-1} (g of dry leaf mass). The majority of the detected compounds belonged to one of three compound classes: green leaf volatiles, monoterpenes, or sesquiterpenes. Total VOC emission was calculated as the sum of all integrated peak areas per sample. Emissions were normalized to plant dry weight (ng g^{-1} min^{-1}).

Predatory insect behaviour assay

Subsequent to VOC collection, we removed the Tenax adsorbent filter and the suction tube from the bagged plants and connected the opening in the oven bags to the upper ends of a Y-shaped glass tube with a diameter of ca. 4 cm (Y-tube olfactometer). The airflow of clean air into the plant bag was increased to 400 mL min^{-1} to increase the transport of plant VOC-enriched air into each arm of the Y-tube olfactometer. Airflow was analysed with the mini-Buck Calibrator and adjusted to minimize any difference in airflow between the two arms. Adult individuals of the two-spotted ladybirds (*Adalia bipunctata*) were purchased from Katz Biotech AG and maintained on an *ad libitum* aphid diet until the experiment. To ensure no additional factors were affecting the behavioural decision of ladybirds before the experiments, 10 ladybirds were tested for their preferences in a controlled air test, where empty bags were connected to the air flow and the Y-tube olfactometer (Meza *et al.* 2020; Roberts *et al.* 2023).

For the behavioural assay towards plant odour, ladybirds were excluded from food for 48 h before the Y-tube test (Mäkinen *et al.* 2024). Tests were conducted in a shaded greenhouse chamber at 25°C with an artificial light source directly over the test area to minimize light bias. Insect behaviour was recorded for a maximum of 5 min per ladybird and a behavioural decision was recorded when the insect spent 10 s or longer in one of the arms of the olfactometer. In total we tested 10 adult ladybirds for each treatment combination (Control vs. air, GBH vs. air, Control vs. GBH). Plants were replaced after every second ladybird was tested, and the position of the odour sources was alternated to prevent directional bias in the Y-tube setup.

Statistics

All statistical analyses were conducted in R v. 4.4.2. Plant performance parameters (height, biomass, SPAD) were analysed with ANOVA. Normality of residuals was assessed using the Shapiro–Wilk test. The results indicated no significant deviation from normality, confirming that the assumption of normality was met. In the case where ANOVA showed significance differences, we performed a Tukey post-hoc test comparing the individual treatments. Aphid

Table 1. The results of ANOVA for differences between treatments (Control, Control + aphid, GBH, GBH + aphid) for biomass (g plant^{-1} dry weight), SPAD value (representing plant photosynthetic activity) and plant height (in cm) are shown.

variable	sum of squares	df	*F*-value	*P*-value
Biomass	6.275	3	1.337	0.3477
SPAD	34.3239	3	2.9844	**0.0432**
Height	396.5208	3	6.8459	<**0.001**

P-values in bold indicate significant differences. *N* = 8.

populations were tested in a generalized linear model (GLM). Aphid numbers were tested against soil treatment, including week as a random factor to account for multiple testing of the same plants. Plant VOCs were first analysed by performing nonmetric multidimensional scaling (NMDS) analyses of all 16 compounds (vegan::metaMDS). We used a Bray–Curtis dissimilarity matrix (vegan::vegdist) with Wisconsin double standardization and two dimensions (stress = 0.167) for ordination. We tested whether soil GBH residues, herbivory, plant genotype, or their interactions affected VOC emissions via permutational ANOVA (PERMANOVA; 9999 permutations; vegan::adonis). Emission rate of individual VOCs was compared with a *t*-test corrected for multiple testing (Benjamini-Hochberg correction). Total VOC emission was compared between the two treatments using a two-sample *t*-test. Assumptions of normality and homogeneity of variances were tested prior to analysis. Behavioural decision of ladybirds between treatments were recorded as binomial data and analysed with a Chi-square test.

RESULTS

Plant and aphid performance

Plant biomass did not differ between treatments (Table 1). Plants had increased height in the control treatment without aphids compared to all other plants (Table 1, Fig. 1). Chlorophyll activity measured as SPAD value differed between control plants and plants growing in soil containing GBH residues, but only in the aphid-free treatment (Table 1, Fig. 1).

Following the addition of 50 adult aphids, aphid numbers increased rapidly to several thousand individuals per pot within 3 weeks, but their numbers did not differ significantly between the treatments (Fig. 2).

VOC analysis

In all 16 samples, 16 VOCs were consistently found (Table 2). PERMANOVA revealed a significant difference in the emitted VOCs between the treatments (Pseudo-$F = 3.88$, $R^2 = 0.28$, $P = 0.024$). NMDS analysis revealed a marginal significant difference in the emitted volatile composition ($F = 4.25$; $P = 0.08$; Fig. 3). Total VOC emission rate did not significantly differ between the treatments ($t(14) = 0.74$, $P = 0.49$). The *t*-tests revealed that three green leaf volatile compounds, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate, were emitted in higher concentrations by plants growing in soil with a GBH history, while concentrations of the sesquiterpene (*E*)-β-ocimene and the benzoate ester methyl-salicylate were emitted in larger quantities by control plants (Table 2).

Insect behaviour assay

Comparing the behaviour of adult ladybirds subjected to an airflow of cleaned air against cleaned air showed no difference in preference of the beetles, which demonstrates the unbiased behavioural responses of ladybirds in the Y-tube olfactometer (Fig. 4). When testing aphid-infested plants of any of the treatments against clean air, ladybirds significantly oriented towards the plant odour (Fig. 4). However, when testing plants of different treatments against each other, a significantly larger orientation towards the plant odour emitted by aphid-infested oat plants growing in control soil was observed when compared to those growing in GBH soil (Fig. 4).

DISCUSSION

Our study shows that the state of the soil impacts plant biochemistry and aboveground plant–insect interactions. Specifically, the use of glyphosate-based herbicides altered the emission of herbivore-induced plant volatiles (HIPVs) in aphid-infested oat plants, with a cascading effect on the olfactory preference of aphid predatory ladybirds (*Adalia bipunctata*). Among the tested plant performance parameters, height and chlorophyll activity were highest in aphid-free plants growing in control soil. Aphid numbers were not affected by GBH treatment.

Our results demonstrate that soil pollution with GBH residues does not affect crop biomass, but chlorophyll value (SPAD) and plant height were reduced. Furthermore, SPAD value was only reduced by soil GBH treatments without aphid herbivory, indicating that aphid herbivory may have a stimulatory effect on photosynthesis (Nietupski *et al.* 2022). However, there are many indirect effects that are seldomly studied in relation to agrochemical pollution that can affect ecological dynamics and ecosystem services, such as pest control and pollination (Fuchs *et al.* 2021). Our results indicate that these mechanisms may cause an erosion of plant–insect communication. We show that residues of the herbicide glyphosate affected the emission of HIPVs in oat plants, decreasing their attractiveness to ladybirds. This is consistent with recently published theoretical work highlighting the potential of pesticide residues to disrupt biochemical pathways vital for plant–insect communication (Fuchs *et al.* 2021; Ramos *et al.* 2023). Efficacy in natural pest control of pest insects relies on timing and abundance of natural enemies, as well as functioning communication between plants and predators and parasitoids (de Vos & Jander 2010; Fuchs *et al.* 2017). Many of these animal groups locate their prey by perception of an HIPV signature specific to the species and abundance of the pest (Pérez-Hedo *et al.* 2021).

At the biochemical level, glyphosate blocks an enzyme in the shikimate pathway and thereby inhibits the biosynthesis of essential aromatic amino acids (Duke & Powles 2008). These amino acids are the building blocks for a tremendous number of plant metabolites, including many specialized secondary metabolites that play a role in plant–insect interactions (Sharon *et al.* 1992; Marchiosi *et al.* 2009; Tzin & Galili 2010; Vogt 2010; Fuchs *et al.* 2021). Consequently,

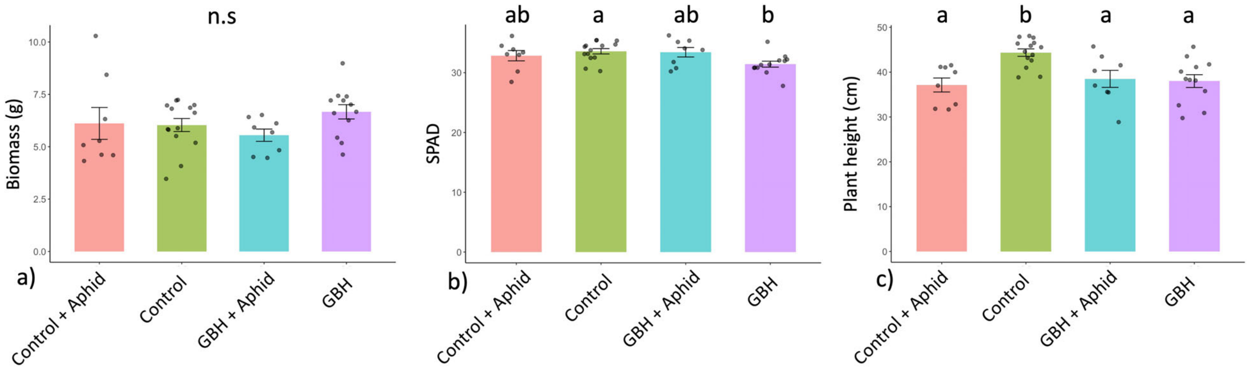

Fig. 1. Mean (mean ± SE) biomass (g dry weight), chlorophyll activity (SPAD value), and plant height (cm) between treatments (Control + Aphid, Control, GBH + Aphid, GBH) with Tukey post-hoc test. Different letters indicate significant differences between treatments (*N* = 8).

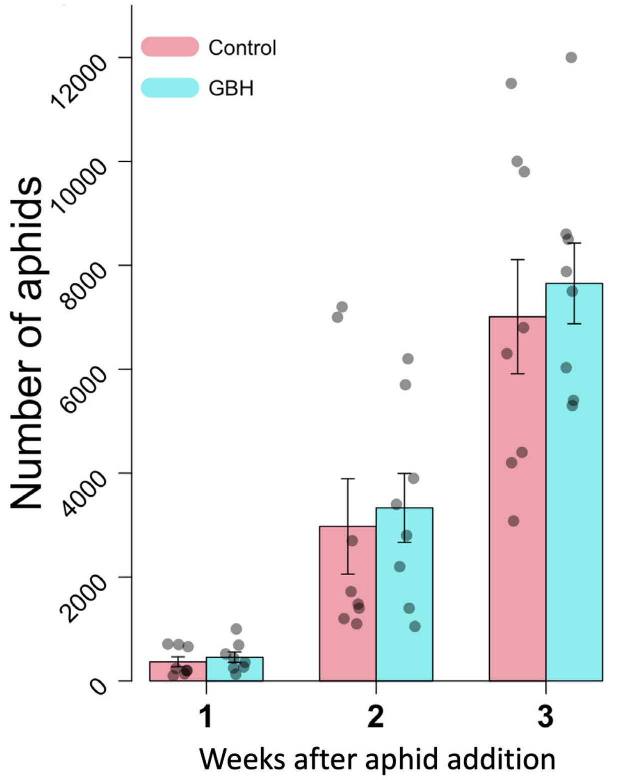

Fig. 2. Mean aphid populations (mean ± SE) per pot for 3 weeks following inoculation with 50 aphids per pot. Population size was compared with a generalized linear model (*N* = 8).

Table 2. Emission rate of individual VOCs was compared with *t*-test corrected for multiple testing (Benjamini-Hochberg correction) between oat plants growing in soil with GBH residues and control soil.

compound	GBH + Aphids	Control + Aphids	*P*-value
(*E*)-2-HEXENAL	**3.06 ± 0.62***	**0.97 ± 0.11**	**0.015**
(*Z*)-3-hexen-1-ol	**23.23 ± 3.30***	**12.48 ± 2.20**	**0.021**
Hexanol	3.64 ± 0.96	3.49 ± 1.06	0.915
α-pinene	1.70 ± 0.25	1.57 ± 0.19	0.692
Benzaldehyde	25.17 ± 2.21	29.46 ± 3.01	0.275
(*Z*)-3-hexenyl acetate	**65.82 ± 7.66***	**42.16 ± 6.67**	**0.038**
Limonene	3.13 ± 0.38	2.13 ± 0.37	0.081
Octanal	3.93 ± 0.84	4.27 ± 0.44	0.732
(*E*)-β-ocimene	**119.40 ± 12.71**	**192.07 ± 15.66***	**0.004**
Acetophenone	79.74 ± 7.62	65.98 ± 8.82	0.261
Linalool	10.13 ± 1.28	8.94 ± 1.96	0.625
Nonanal	37.50 ± 4.19	32.13 ± 2.99	0.320
Methyl-salicylate	**2.63 ± 0.37**	**11.46 ± 2.53***	**0.013**
Decanal	117.32 ± 20.42	110.17 ± 10.28	0.762
Borneol	4.85 ± 0.96	3.13 ± 0.28	0.128
β-farnesene	11.00 ± 0.80	8.96 ± 1.42	0.238

Compound emission rate is presented in ng g^{-1} min^{-1}. Compounds with a significantly different emission rate are highlighted in bold. *N* = 8.
**P* ≤ 0.05.

sublethal doses of glyphosate and its residues have been linked to changes in phytohormone concentrations and phenolic compounds (Fuchs, Laihonen, *et al.* 2022; Fuchs, Helander, *et al.* 2024). We expected that glyphosate would affect the emission of VOCs derived from the shikimate pathway because of their biosynthetic link to the mode of action of glyphosate (Fuchs *et al.* 2021). In line with this, our results show a reduction in methyl salicylate, which is a derivative of the phytohormone salicylic acid and a biosynthetic product of the shikimate pathway (Huang *et al.* 2020). Increased GLV emission related to glyphosate residues in soil may be an indirect consequence of altered cell wall lipid composition, which determines the ratios of emitted GLVs (D'Alessandro *et al.* 2006; Zobiole *et al.* 2010).

Green leaf volatiles (GLVs) are ubiquitously found in volatile blends in the plant kingdom related to their biosynthetic origin as breakdown products of cell wall lipids (Ameye *et al.* 2018). Their concentrations in volatile blends indicates the degree of plant damage from cell wall damage, which often correlates with the degree of herbivory and may serve as a foraging cue for generalist predatory insects and higher-order predators (Ameye *et al.* 2018; Matsui & Engelberth 2022). Our results demonstrate an induction of GLVs in plants growing in soil with a glyphosate history, which did not cause higher attraction of aphid-predatory ladybirds. GLV emission is often found to be higher in plants attacked by chewing and biting insects (Matsui & Engelberth 2022), which are generally not the preferred prey of ladybirds (Sloggett 2022). Increased GLV concentrations may consequently not be an olfactory cue eliciting preference in the behaviour

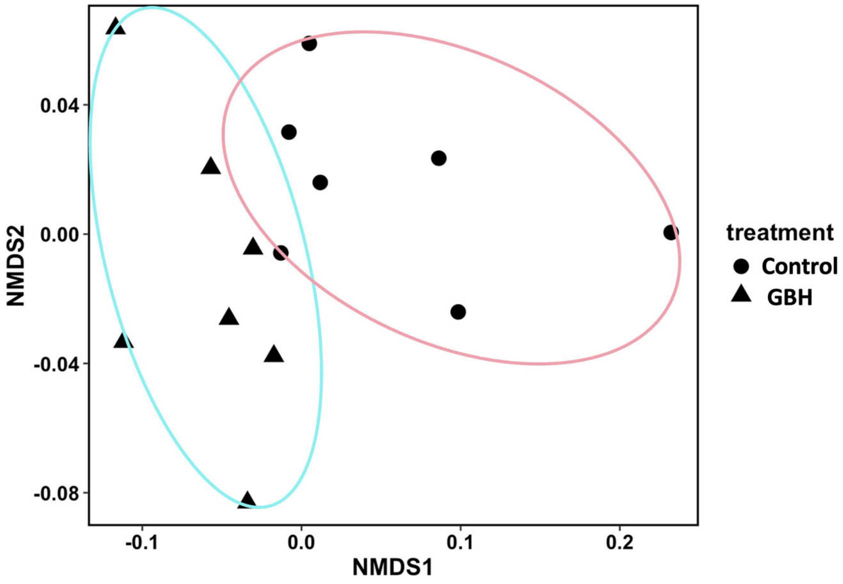

Fig. 3. Non-metric multidimensional scaling ordination based on 16 leaf VOC compound emissions rates (ng g^{-1} min^{-1}), collected from 9 oat (*Avena sativa*) plant per pot. Shapes are clustered by treatment (Control, GBH). Circle colours correspond to treatment affiliation in Figs. 1 and 2 (N = 8).

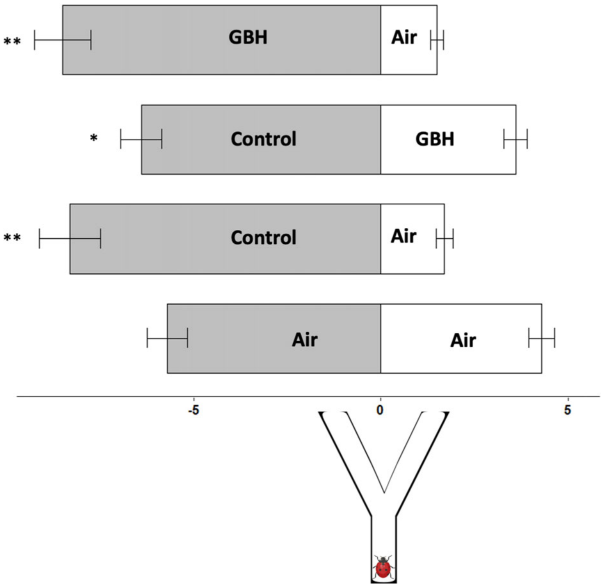

Fig. 4. Olfactory orientation of ladybirds in Y-tube olfactometer. Ten insects were tested per combination (Control vs. air, GBH vs. air, Control vs. GBH). Asterisks indicate significance: $^*P \leq 0.05$, $^{**}P \leq 0.01$.

of ladybirds. Instead, higher concentrations of (*E*)-β-ocimene and methyl salicylate (MeSA) emitted by plants in the control treatment appeared to be attractive to the ladybirds in our study. Salicylic acid is generally involved in plant responses to piercing-sucking herbivores such as aphids, and an induction in the emission of MeSA has been shown to increase predatory insect abundance in a field trial (Lee *et al.* 2022). This may also be the main metabolite driving ladybird attraction in our study. Additionally, (*E*)-β-Ocimene is a common plant volatile, which has effects on the behaviour of a variety of insects and may contribute to the higher attraction of predatory ladybirds to control plants (Shimoda *et al.* 2012; Farré-Armengol *et al.* 2017). In functional behavioural studies, (*E*)-β-Ocimene attracted the predatory mite *Phytoseiulus persimilis* and the parasitoid *Aphytis melinus* (Dicke *et al.* 1990; Mohammed *et al.* 2020). However, further functional studies are required to determine the specific role of the individual compounds in the volatile blend.

Our results provide the first empirical evidence that a soil legacy of glyphosate use alters the composition of HIPVs in oat plants as induced by aphid infestation. This resulted in a behavioural preference of ladybirds towards control plants during a choice assay attributed to changes in five VOCs. Compromising the ability of plants to attract natural enemies due to herbicide residues could lead to more damage from pests and, consequently, the need for increased use of insecticides. This is alarming as it would put further pressure on the already threatened biodiversity in agroecosystems. This study was made under controlled conditions in a greenhouse, focusing on the interactions between one species of plant (oat), a herbivorous insect (bird cherry-oat aphid) and an insect predator (two-spotted ladybird). Further studies are required to investigate how widespread this phenomenon is among different crop plant species and predators of their pests. Of particular importance would be studies on insect communities, which could shed light on the impact of glyphosate residues on pest pressure, and abundance and diversity of predators and parasitoids in realistic field conditions.

AUTHOR CONTRIBUTIONS

BF performed the greenhouse study, performed the collection of volatile organic compounds and conducted the insect behavioral assays. JDB performed the analysis of volatile organic compounds via gas chromatography coupled to mass spectrometry. BF analyzed the data. BF and VW wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

ACKNOWLEDGMENTS

We thank Salla Malik, Emilia Mäkkinen, and Eva-Maria Vaajamo for help during the greenhouse experiment. We thank the staff at University of Turku Botanical Gardens for their support in technical questions. The study was funded by the Novo Nordisk Foundation (grant no. 0079712 to B.F.) and the Research Council of Finland (grant no. 355917 to B.F.).

REFERENCES

Alborn H.T., Hansen T.V., Jones T.H., Bennett D.C., Tumlinson J.H., Schmelz E.A., Teal P.E.A. (2007) Disulfooxy fatty acids from the American bird grasshopper Schistocerca americana, elicitors of plant volatiles. *Proceedings of the National Academy of Sciences*, **104**, 12976–12981.

Ali A., Desneux N., Lu Y., Wu K. (2018) Key aphid natural enemies showing positive effects on wheat yield through biocontrol services in northern China. *Agriculture, Ecosystems & Environment*, **266**, 1–9.

Aljbory Z., Chen M.-S. (2018) Indirect plant defense against insect herbivores: A review. *Insect Science*, **25**, 2–23.

Ameye M., Allmann S., Verwaeren J., Smagghe G., Haesaert G., Schuurink R.C., Audenaert K. (2018) Green leaf volatile production by plants: A meta-analysis. *New Phytologist*, **220**, 666–683.

Bate N.J., Rothstein S.J. (1998) C-volatiles derived from the lipoxygenase pathway induce a subset of defense-related genes. *The Plant Journal*, **16**, 561–569.

Benbrook C.M. (2016) Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*, **28**, 3.

Bennett E.M., Baird J., Baulch H., Chaplin-Kramer R., Fraser E., Loring P., Morrison P., Parrott L., Sherren K., Winkler K.J., Cimon-Morin J., Fortin M.J., Kurylyk B.L., Lundholm J., Poulin M., Rieb J.T., Gonzalez A., Hickey G.M., Humphries M., Bahadur KC K., Lapen D. (2021) Chapter one - ecosystem services and the resilience of agricultural landscapes. In: Bohan D.A., Vanbergen A.J. (Eds), *The future of agricultural landscapes, part II*. Academic Press, Advances in Ecological Research, pp 1–43.

Bennett R.N., Wallsgrove R.M. (1994) Secondary metabolites in plant defence mechanisms. *New Phytologist*, **127**, 617–633.

Bianchi F.J.J.A., Booij C.J.H., Tscharntke T. (2006) Sustainable pest regulation in agricultural landscapes: A review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 1715–1727.

Blée E. (1998) Phytooxylipins and plant defense reactions. *Progress in Lipid Research*, **37**, 33–72.

Boldorini G.X., Mccary M.A., Romero G.Q., Mills K.L., Sanders N.J., Reich P.B., Michalko R., Gonçalves-Souza T. (2024) Predators control pests and increase yield across crop types and climates: A meta-analysis. *Proceedings of the Royal Society B: Biological Sciences*, **291**, 20232522.

Bonny S. (2016) Genetically modified herbicide-tolerant crops, weeds, and herbicides: Overview and impact. *Environmental Management*, **57**, 31–48.

Cesco S., Lucini L., Miras-Moreno B., Borruso L., Mimmo T., Pii Y., Puglisi E., Spini G., Taskin E., Tiziani R., Zangrillo M.S., Trevisan M. (2021) The hidden effects of agrochemicals on plant metabolism and root-associated microorganisms. *Plant Science*, **311**, 111012.

D'Alessandro M., Held M., Triponez Y., Turlings T.C.J. (2006) The role of indole and other shikimic acid derived maize volatiles in the attraction of two parasitic wasps. *Journal of Chemical Ecology*, **32**, 2733–2748.

de Vos M., Jander G. (2010) Volatile communication in plant–aphid interactions. *Current Opinion in Plant Biology*, **13**, 366–371.

Dicke M., Van Beek T.A., Posthumus M.A., Ben Dom N., Van Bokhoven H., De Groot A. (1990) Isolation and identification of volatile kairomone that affects acarine predatorprey interactions involvement of host plant in its production. *Journal of Chemical Ecology*, **16**, 381–396.

Dickens J.C. (1999) Predator–prey interactions: Olfactory adaptations of generalist and specialist predators. *Agricultural and Forest Entomology*, **1**, 47–54.

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.

Duke S.O., Powles S.B. (2008) Glyphosate: A once-in-a-century herbicide. *Pest Management Science*, **64**, 319–325.

Ellis E.C., Klein Goldewijk K., Siebert S., Lightman D., Ramankutty N. (2010) Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography*, **19**, 589–606.

Emmerson M., Morales M.B., Oñate J.J., Batáry P., Berendse F., Liira J., Aavik T., Guerrero I., Bommarco R., Eggers S., Pärt T., Tscharntke T., Weisser W., Clement L., Bengtsson J. (2016) Chapter two - how agricultural intensification affects biodiversity and ecosystem services. In: Dumbrell A.J., Kordas R.L., Woodward G. (Eds), *Large-scale ecology: Model systems to global perspectives*. Academic Press, Advances in Ecological Research, pp 43–97.

Farré-Armengol G., Filella I., Llusià J., Peñuelas J. (2017) β-Ocimene, a key floral and foliar volatile involved in multiple interactions between plants and other organisms. *Molecules*, **22**, 1148.

Francis F., Lognay G., Haubruge E. (2004) Olfactory responses to aphid and host Plant volatile releases: (E)-β-Farnesene an effective Kairomone for the predator Adalia bipunctata. *Journal of Chemical Ecology*, **30**, 741–755.

Fuchs B., Breuer T., Findling S., Krischke M., Mueller M.J., Holzschuh A., Krauss J. (2017) Enhanced aphid abundance in spring desynchronizes predator-prey and plant–microorganism interactions. *Oecologia*, **183**, 469–478.

Fuchs B., Damerau A., Yang B., Muola A. (2024) Reduced seed viability in exchange for transgenerational plant protection in an endophyte-symbiotic grass: Does the defensive mutualism concept pass the fitness test? *Annals of Botany*, **134**, 993–1002.

Fuchs B., Helander M., Saikkonen K., Dobrev P.I., Vankova R., Blande J.D., Salminen J.P., Luntamo N., Muola A. (2024) Plant metabolic responses to soil herbicide residues differ under herbivory in two woodland strawberry genotypes. *Science of the Total Environment*, **946**, 174198.

Fuchs B., Laihonen M., Muola A., Saikkonen K., Dobrev P.I., Vankova R., Helander M. (2022) A glyphosate-based herbicide in soil differentially affects hormonal homeostasis and performance of non-target crop plants. *Frontiers in Plant Science*, **12**, 787958.

Fuchs B., Saikkonen K., Helander M. (2021) Glyphosate-modulated biosynthesis driving Plant defense and species interactions. *Trends in Plant Science*, **26**, 312–323.

Fuchs B., Saikkonen K., Helander M., Tian Y., Yang B., Engström M.T., Salminen J.P., Muola A. (2022) Legacy of agrochemicals in the circular food economy: Glyphosate-based herbicides introduced via manure fertilizer affect the yield and biochemistry of perennial crop plants during the following year. *Chemosphere*, **308**, 136366.

Giron-Calva P.S., Li T., Blande J.D. (2017) Volatile-mediated interactions between cabbage plants in the field and the impact of ozone pollution. *Journal of Chemical Ecology*, **43**, 339–350.

Hartmann T. (2007) From waste products to ecochemicals: Fifty years research of plant secondary metabolism. *Phytochemistry*, **68**, 2831–2846.

Helander M., Pauna A., Saikkonen K., Saloniemi I. (2019) Glyphosate residues in soil affect crop plant germination and growth. *Scientific Reports*, **9**, 19653.

Huang W., Wang Y., Li X., Zhang Y. (2020) Biosynthesis and regulation of salicylic acid and N-Hydroxypipecolic acid in Plant immunity. *Molecular Plant*, **13**, 31–41.

Kehoe L., Romero-Muñoz A., Polaina E., Estes L., Kreft H., Kuemmerle T. (2017) Biodiversity at risk under future cropland expansion and intensification. *Nature Ecology & Evolution*, **1**, 1129–1135.

Keronen S., Helander M., Saikkonen K., Fuchs B. (2023) Management practice and soil properties affect plant productivity and root biomass in endophyte-symbiotic and endophyte-free meadow fescue grasses. *Journal of Sustainable Agriculture and Environment*, **2**, 16–25.

Kielty J.P., Allen-Williams L.J., Underwood N., Eastwood E.A. (1996) Behavioral responses of three species of ground beetle (Coleoptera: Carabidae) to olfactory cues associated with prey and habitat. *Journal of Insect Behavior*, **9**, 237–250.

Krey K.L., Smith O.M., Chapman E.G., Crossley M.S., Crowder D.W., Fu Z., Harwood J.D., Jensen A.S., Lynch C.A., Snyder G.B., Snyder W.E. (2021) Prey and predator biodiversity mediate aphid consumption by generalists. *Biological Control*, **160**, 104650.

Lamberth C., Jeanmart S., Luksch T., Plant A. (2013) Current challenges and trends in the discovery of agrochemicals. *Science*, **341**, 742–746.

Lee J.C., Flores S.M., Velasco Graham K., Skillman V.P. (2022) Methyl salicylate can benefit ornamental Pest control, and does not Alter per capita predator consumption at close-range. *Frontiers in Ecology and Evolution*, **9**, 788187.

Lichtenthaler H.K., Rohmer M., Schwender J. (1997) Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. *Physiologia Plantarum*, **101**, 643–652.

Liu C.-M., Matsuyama S., Kainoh Y. (2019) Synergistic effects of volatiles from host-infested plants on host-searching behavior in the parasitoid wasp Lytopylus rufipes (hymenoptera: Braconidae). *Journal of Chemical Ecology*, **45**, 684–692.

Lundgren J.G., Fausti S.W. (2015) Trading biodiversity for pest problems. *Science Advances*, **1**, e1500558.

Maffei M.E. (2010) Sites of synthesis, biochemistry and functional role of plant volatiles. *South African Journal of Botany*, **76**, 612–631.

Maggi F., la Cecilia D., Tang F.H.M., McBratney A. (2020) The global environmental hazard of glyphosate use. *The Science of the Total Environment*, **717**, 137167.

Mäkinen J.K., Saussure S., Ruhanen H., Räty E., Blande J.D. (2024) Effects of diversified volatile profiles on olfactory orientation of flea beetles Phyllotreta spp. and the diamondback moth Plutella xylostella. *Plant Biology (Stuttgart, Germany)*, **27**, 883–890.

Marchiosi R., Lucio Ferrarese M.d.L., Bonini E.A., Fernandes N.G., Ferro A.P., Ferrarese-Filho O. (2009) Glyphosate-induced metabolic changes in susceptible and glyphosate-resistant soybean (*Glycine max* L.) roots. *Pesticide Biochemistry and Physiology*, **93**, 28–33.

Matsui K., Engelberth J. (2022) Green leaf volatiles-the forefront of Plant responses against biotic attack. *Plant & Cell Physiology*, **63**, 1378–1390.

Mauch-Mani B., Baccelli I., Luna E., Flors V. (2017) Defense priming: An adaptive part of induced resistance. *Annual Review of Plant Biology*, **68**, 485–512.

Meier A.R., Hunter M.D. (2019) Mycorrhizae Alter constitutive and herbivore-induced volatile emissions by milkweeds. *Journal of Chemical Ecology*, **45**, 610–625.

Meza F.C., Roberts J.M., Sobhy I.S., Okumu F.O., Tripet F., Bruce T.J.A. (2020) Behavioural and electrophysiological responses of female Anopheles gambiae mosquitoes to volatiles from a mango bait. *Journal of Chemical Ecology*, **46**, 387–396.

Mohammed K., Agarwal M., Li B., Newman J., Liu T., Ren Y. (2020) Evaluation of d-limonene and β-Ocimene as attractants of Aphytis melinus (hymenoptera: Aphelinidae), a parasitoid of Aonidiella aurantii (Hemiptera: Diaspididae) on citrus spp. *Insects*, **11**, 44.

Nancarrow N., Aftab M., Hollaway G., Rodoni B., Trębicki P. (2021) Yield losses caused by barley yellow dwarf virus-PAV infection in wheat and barley: A three-year field study in south-eastern Australia. *Microorganisms*, **9**, 645.

Nawaz M., Sun J., Shabbir S., Khattak W.A., Ren G., Nie X., Bo Y., Javed Q., du D., Sonne C. (2023) A review of plants strategies to resist biotic and abiotic environmental stressors. *Science of the Total Environment*, **900**, 165832.

Nietupski M., Ludwiczak E., Olszewski J., Gabryś B., Kordan B. (2022) Effect of aphid foraging on the intensity of photosynthesis and transpiration of selected crop plants in its early stages of growing. *Agronomy*, **12**, 2370.

Ospina M., Schütze A., Morales-Agudelo P., Vidal M., Wong L.-Y., Calafat A.M. (2024) Temporal trends of exposure to the herbicide glyphosate in the United States (2013–2018): Data from the National Health and nutrition examination survey. *Chemosphere*, **364**, 142966.

Pare P.W., Tumlinson J.H. (1999) Plant volatiles as a defense against insect herbivores. *Plant Physiology*, **121**, 325–332.

Pérez-Hedo M., Alonso-Valiente M., Vacas S., Gallego C., Pons C., Arbona V., Rambla J.L., Navarro-Llopis V., Granell A., Urbaneja A. (2021) Plant exposure to herbivore-induced plant volatiles: A sustainable approach through eliciting plant defenses. *Journal of Pest Science*, **94**, 1221–1235.

Rainio M.J., Margus A., Virtanen V., Lindström L., Salminen J.P., Saikkonen K., Helander M. (2020) Glyphosate-based herbicide has soil-mediated effects on potato glycoalkaloids and oxidative status of a potato pest. *Chemosphere*, **258**, 127254.

Ramos S.E., Bakhtiari M., Castañeda-Zárate M., Iriart V., Ashman T.-L. (2023) Herbicides and their potential to disrupt plant–insect chemical communication. *Journal of Systematics and Evolution*, **61**, 550–560.

Raven P.H., Wagner D.L. (2021) Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, **118**, e2002548117.

Roberts J.M., Clunie B.J., Leather S.R., Harris W.E., Pope T.W. (2023) Scents and sensibility: Best practice in insect olfactometer bioassays. *Entomologia Experimentalis et Applicata*, **171**, 808–820.

Rusch A., Chaplin-Kramer R., Gardiner M.M., Hawro V., Holland J., Landis D., Thies C., Tscharntke T., Weisser W.W., Winqvist C., Woltz M., Bommarco R. (2016) Agricultural landscape simplification reduces natural pest control: A quantitative synthesis. *Agriculture, Ecosystems & Environment*, **221**, 198–204.

Saunier A., Grof-Tisza P., Blande J.D. (2023) Effect of ozone exposure on the foraging behaviour of *Bombus terrestris*. *Environmental Pollution*, **316**, 120573.

Sharon A., Amsellem Z., Gressel J. (1992) Glyphosate suppression of an elicited defense response 1: Increased susceptibility of Cassia obtusifolia to a Mycoherbicide. *Plant Physiology*, **98**, 654–659.

Shimoda T., Nishihara M., Ozawa R., Takabayashi J., Arimura G. (2012) The effect of genetically enriched (E)-β-ocimene and the role of floral scent in the attraction of the predatory mite Phytoseiulus persimilis to spider mite-induced volatile blends of torenia. *New Phytologist*, **193**, 1009–1021.

Sloggett J.J. (2022) Diet and chemical defence in ladybird beetles (Coleoptera: Coccinellidae). *European Journal of Entomology*, **119**, 362–367.

Takemoto H., Takabayashi J. (2015) Parasitic wasps Aphidius ervi are more attracted to a blend of host-induced Plant volatiles than to the independent compounds. *Journal of Chemical Ecology*, **41**, 801–807.

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.

Tzin V., Galili G. (2010) New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. *Molecular Plant*, **3**, 956–972.

Van Bruggen A.H.C., He M.M., Shin K., Mai V., Jeong K.C., Finckh M.R., Morris J.G., Jr. (2018) Environmental and health effects of the herbicide glyphosate. *Science of the Total Environment*, **616–617**, 255–268.

Vogt T. (2010) Phenylpropanoid biosynthesis. *Molecular Plant*, **3**, 2–20.

War A.R., Sharma H.C., Paulraj M.G., War M.Y., Ignacimuthu S. (2011) Herbivore induced plant volatiles. *Plant Signaling & Behavior*, **6**, 1973–1978.

Wright G.A., Schiestl F.P. (2009) The evolution of floral scent: The influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology*, **23**, 841–851.

Ziesche T.M., Ordon F., Schliephake E., Will T. (2024) Long-term data in agricultural landscapes indicate that insect decline promotes pests well adapted to environmental changes. *Journal of Pest Science*, **97**, 1281–1297.

Zobiole L.H.S., Oliveira R.S., Visentainer J.V., Kremer R.J., Bellaloui N., Yamada T. (2010) Glyphosate affects seed composition in glyphosate-resistant soybean. *Journal of Agricultural and Food Chemistry*, **58**, 4517–4522.

Züst T., Agrawal A.A. (2016) Mechanisms and evolution of plant resistance to aphids. *Nature Plants*, **2**, 1–9.