



Plant metabolic responses to soil herbicide residues differ under herbivory in two woodland strawberry genotypes

Benjamin Fuchs^{a,*}, Marjo Helander^b, Kari Saikkonen^a, Petre I. Dobrev^c, Radomira Vankova^c, James D. Blande^d, Juha-Pekka Salminen^e, Niko Luntamo^e, Anne Muola^{a,f}

^a Biodiversity Unit, University of Turku, 20014 Turku, Finland

^b Department of Biology, University of Turku, 20014 Turku, Finland

^c Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojova 263, 16502 Prague, Czech Republic

^d Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

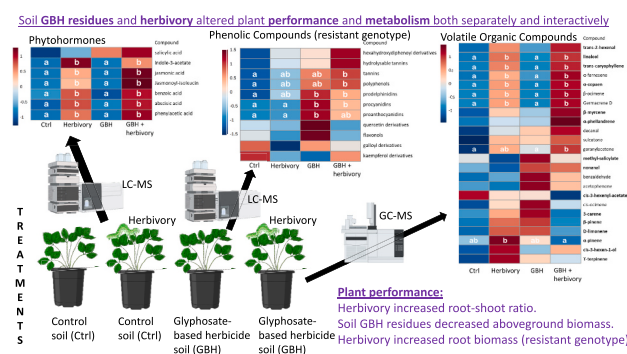
^e Natural Chemistry Research Group, Department of Chemistry, FI-20014, University of Turku, Finland

^f Division of Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research, Ås, Norway

HIGHLIGHTS

- GBH residue pollution of agricultural soils impacts crop resistance
- Soil GBH residues decreased above-ground biomass of woodland strawberries
- Herbivory increased root-shoot ratio and induced volatile emission
- Genotype-specific increase in salicylic acid concentrations by soil GBH residues
- Genotype-specific increase in phenolic compound concentrations by soil GBH residues

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Sergey Shabala

Keywords:

Chemical plant defence
 Herbicide residues
 Plant-insect interactions
 Agricultural ecosystems
 Soil-mediated plant responses
 Plant resistance

ABSTRACT

The use of glyphosate-based herbicides (GBHs) to control weeds has increased exponentially in recent decades, and their residues and degradation products have been found in soils across the globe. GBH residues in soil have been shown to affect plant physiology and specialised metabolite biosynthesis, which, in turn, may impact plant resistance to biotic stressors.

In a greenhouse study, we investigated the interactive effects between soil GBH residues and herbivory on the performance, phytohormone concentrations, phenolic compound concentrations and volatile organic compound (VOC) emissions of two woodland strawberry (*Fragaria vesca*) genotypes, which were classified as herbivore resistant and herbivore susceptible. Plants were subjected to herbivory by strawberry leaf beetle (*Galerucella tenella*) larvae, and to GBH residues by growing in soil collected from a field site with GBH treatments twice a year over the past eight years.

Soil GBH residues reduced the belowground biomass of the susceptible genotype and the aboveground biomass of both woodland strawberry genotypes. Herbivory increased the belowground biomass of the resistant

* Corresponding author.

E-mail address: Benjamin.fuchs@utu.fi (B. Fuchs).

<https://doi.org/10.1016/j.scitotenv.2024.174198>

Received 20 April 2024; Received in revised form 18 June 2024; Accepted 20 June 2024

Available online 22 June 2024

0048-9697/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

genotype and the root–shoot ratio of both genotypes. At the metabolite level, herbivory induced the emission of several VOCs. Jasmonic acid, abscisic acid and auxin concentrations were induced by herbivory, in contrast to salicylic acid, which was only induced by herbivory in combination with soil GBH residues in the resistant genotype. The concentrations of phenolic compounds were higher in the resistant genotype compared to the susceptible genotype and were induced by soil GBH residues in the resistant genotype.

Our results indicate that soil GBH residues can differentially affect plant performance, phytohormone concentrations and phenolic compound concentrations under herbivore attack, in a genotype-dependent manner. Soil GBH altered plant responses to herbivory, which may impact plant resistance traits and species interactions. With ongoing agrochemical pollution, we need to consider plant cultivars with better resistance to polluted soils while maintaining plant resilience under challenging environmental conditions.

1. Introduction

Globally, glyphosate-based herbicides (GBHs) are the most commonly used pesticides because of their affordability and efficiency in killing weeds (Benbrook, 2016; Myers et al., 2016). The effect of glyphosate is based on the inactivation of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway, which is present only in plants and various microbes (Duke and Powles, 2008; Helander et al., 2012; Williams et al., 2000). In addition, glyphosate is assumed to be quickly degraded by soil microorganisms, which limits its bioavailability and phytotoxicity (Borggaard and Gimsing, 2008). For these reasons, GBHs are considered to have minimal ecological risks. However, as a result of the heavy use of GBHs, glyphosate residues and degradation products accumulate in diverse agricultural habitats across the globe (Maggi et al., 2020). Furthermore, several studies have confirmed that the rate of glyphosate degradation depends on environmental conditions and may be especially slow in cold climates (Helander et al., 2019, 2012). Thus, it is of utmost importance to understand the potential effects of GBH residues in soil on the biology and ecology of organisms.

Sublethal glyphosate doses, including low concentrations of GBHs and their residues in soil, can substantially alter plant physiology, for example, by altering photosynthesis (Gomes et al., 2017, 2014; Soares et al., 2020), causing oxidative stress (Spormann et al., 2019), affecting tannin biosynthesis (Ossipov et al., 2003), changing plant volatile composition (D'Alessandro et al., 2006) or affecting phytohormone biosynthesis (Fuchs et al., 2022a). Many of these effects can be directly linked to the mode of action of glyphosate. By inactivating the EPSPS enzyme, glyphosate interrupts the supply of chorismate, which is the essential precursor for the biosynthesis of three aromatic amino acids: tryptophan, phenylalanine and tyrosine (Maeda and Dudareva, 2012). Chorismate and amino acids are key components of numerous primary and secondary compounds, including the phytohormones indole-3-acetic acid (IAA) and salicylic acid (SA), both of which are involved in plant stress responses to numerous abiotic and biotic stressors (Ding and Ding, 2020; Kazan and Manners, 2009; Rekhter et al., 2019). In addition, many other organic compounds, such as flavonoids, are derived from the shikimate pathway (Mouradov and Spangenberg, 2014). Flavonoids are essential compounds involved in plant interactions with various organisms, from beneficial microorganisms and insect pollinators to herbivores and pathogens (Deng and Lu, 2017). Depending on their biosynthesis pathway, the concentrations of phenolic compounds involved in plant defence can be induced or reduced by sublethal doses of glyphosate (Fuchs et al., 2022b; Ossipov et al., 2003). Thus, because of their effects on phytohormones and plant metabolism, GBH residues may indirectly alter plant interactions with other organisms, such as herbivores (Fuchs et al., 2021).

Plant defence against herbivores is multifaceted and consists of direct and indirect strategies, both of which rely largely on specialised plant metabolites (Kessler and Baldwin, 2001; Strauss et al., 2002); direct defences include low nutritional quality and deterrence or toxicity of the plant to the herbivore (Gols, 2018), while the emission of volatile organic compounds (VOCs) that attract the herbivore's natural enemies is considered an indirect defence (Heil, 2008; Karban, 2011). In a

signalling cascade initiated by herbivore feeding on plant tissues, phytohormone biosynthesis is induced, and it mediates both direct and indirect defence responses. The biosynthesis of defence metabolites, such as alkaloids or polyphenols, which mediate direct defence responses, is induced (Berens et al., 2017). If GBH residues alter or disrupt the biosynthesis of plant signalling and defence metabolites, they may affect plant-herbivore interactions and disrupt signalling to beneficial insects (Fuchs et al., 2021; War et al., 2012). However, relatively little is known about how GBH residues in soil might affect plant defence responses to herbivores. Furthermore, as plant genetic variability shapes plant defence traits (Ballhorn et al., 2009), plant responses to GBH residues might vary not only between different plant species (Fuchs et al., 2022a) but also within species.

To address these knowledge gaps, we examined whether exposure to GBH residues via soil affects plant growth, phytohormone biosynthesis, VOC emission and phenolic compounds in herbivore-free and herbivore-treated plants from two different woodland strawberry genotypes. Furthermore, we studied whether potential alterations in plant metabolism affect herbivore feeding (i.e., the amount of damage to the plant). In a controlled greenhouse experiment, we selected two genotypes of woodland strawberry (*Fragaria vesca*) with different levels of resistance to strawberry leaf beetles (*Galerucella tenella*) and contrasting metabolite profiles (Muola et al., 2017; Weber et al., 2020b, 2020a). In our experiment, plants were exposed to GBH residues via soil and folivory by *G. tenella* larvae. Earlier studies have indicated that exposure to high amounts of GBH residues via organic fertiliser has a negative impact on strawberry growth, while low GBH residues in soil do not affect strawberry growth (Mathew et al., 2023; Muola et al., 2021). However, even low GBH residues in soil have been shown to affect strawberry phytohormones and interactions with herbivores (Fuchs et al., 2022a; Muola et al., 2021). We hypothesise that GBH residues in soil (1) do not affect plant growth because of low doses, (2) affect phytohormones derived from the shikimate pathway, (3) affect the emission of VOCs either because of their metabolism deriving from the shikimate pathway or as a consequence of altered hormone signalling, (4) alter the constitutive polyphenol concentrations in plants because of their biosynthetic pathway and (5) alter the polyphenol concentrations induced by herbivory.

2. Materials and methods

2.1. Study species

Woodland strawberry (*Fragaria vesca* L., Rosaceae) is a perennial herb that reproduces sexually and asexually via runners. *Galerucella tenella* L. is an oligophagous herbivore that feeds on the leaves and flowers of several Rosaceae plants (Stenberg and Axelsson, 2008). Woodland strawberry plants show genetic variations in resistance (antibiosis and antixenosis) to *G. tenella* (Muola et al., 2017; Weber et al., 2020a, 2020b). Although the exact resistance mechanism is not known, several primary and specialised metabolites have been identified as being associated with plant resistance (Weber et al., 2020a).

Adult *G. tenella* were collected from a large population of *Filipendula ulmaria* (N 66,90395°, E 23,5768°) in early May 2021. The plants were

placed in meshed cages with garden strawberry 'Bounty' and kept in a greenhouse at Ruissalo Botanical Garden, University of Turku, at +20 °C under ambient light. The beetles were allowed to mate and oviposit on garden strawberry plants. The plants used for oviposition were replaced every second to third day, kept in mesh cages and monitored daily for egg hatching. Newly hatched larvae were used for herbivory treatment.

2.2. Experimental setup

In a controlled greenhouse experiment, we tested how soil GBH residues and herbivory by *Galerucella tenella* affect woodland strawberry growth, the biosynthesis of hormones and metabolites and the emission of volatiles. For the experiment, we selected two woodland strawberry genotypes: one of which has been classified as resistant to *G. tenella* (genotype 34F) and the other as susceptible (genotype 1A; Weber et al., 2020b).

Plant material was produced by cloning the two selected genotypes in a greenhouse at Ruissalo Botanical Garden (University of Turku) at +20 °C and 16:8 L:D. Ten runners per genotype were assigned to each of the four treatments (i.e., 20 plants per treatment): 1) the **control**, where plants were grown in field-collected soil without GBH residues; 2) **herbivory**, where plants were grown in field-collected soil without GBH residues and subjected to herbivory by *G. tenella* larvae; 3) **GBH**, where plants were grown in field-collected soil with GBH residues; and 4) **GBH + herbivory**, where plants were grown in field-collected soil with GBH residues and subjected to herbivory by *G. tenella* larvae. The runners were directly planted in 0.2 l pots filled with field-collected soil with or without GBH residues depending on the treatment and kept in a greenhouse at +20 °C and 16:8 L:D.

Soil was collected from a long-term field experiment with GBH applications in spring and autumn at the Ruissalo Botanical Garden in southwestern Finland (60°26'N, 22°10'E). The experimental field (25 m × 50 m) was established in 2013 by dividing the area into alternating control and GBH treatment plots (10 plots each, measuring 23 m × 1.5 m), with 1.5 m buffer strips between the plots. Twice a year, the plots were tilled to a depth of 5 cm with a hand-held rotary tiller. Following this, the control plots were treated with tap water (5 L/plot), and the GBH plots were treated with Roundup Gold (glyphosate concentration: 450 g l⁻¹, CAS: 3864-194-0, application rate: 6.41 ha⁻¹ in 5 l of tap water per plot) using a hand-operated pressure tank with a plastic hood over the sprinkler tip to prevent GBH from spreading outside the treatment plots. The buffer strips between the plots were mowed several times during each field season to minimise weed invasion. A more detailed description of the establishment of the experimental field and its management, including GBH treatments, can be found in Helander et al. (2019). For this experiment, soil was collected in early May 2021, 11 days after GBH application. In 2020, the soil was characterized as medium clay soil with high amounts of organic matter (>120 g kg⁻¹) and pH 5.9. The nutrient values of the soil were (based on an analysis made in 2016): phosphorus 4.2 mg l⁻¹, potassium 250 mg l⁻¹, calcium 1900 mg l⁻¹, magnesium 570 mg l⁻¹, sulfur 10.6 mg l⁻¹, zinc 2.74 mg l⁻¹, copper 7.5 mg l⁻¹, manganese 15 mg l⁻¹, nitrate 9.1 mg l⁻¹, and ammonium 2.8 mg l⁻¹.

The woodland strawberry runners were allowed to grow for approximately three weeks in greenhouse conditions, during which plants established a root system and developed two new leaf triplets, reaching an average size of 5 ± 1 leaves (data not shown) at the start of the experiment. We started the experiment by placing two newly hatched *G. tenella* larvae (<24 h old) on each plant in the herbivory treatments. The larvae were monitored every second day to record mortality. Overall, mortality was very low. Of the six larvae that died, five were feeding on plants that grew in soil with GBH residues, and one was feeding on plants that grew in soil without GBH residues. The dead larvae were replaced with fresh larvae. Four days after the first two larvae were placed on the experimental plants, one additional newly hatched larva was added to each plant in the herbivory treatment to

ensure sufficient feeding damage for the induction of defence-related metabolite signalling.

2.3. Sampling

After one week of herbivore feeding, that is, one week after the day the first larvae were placed on the plant, we collected the first plant volatiles, then samples for plant hormone and phenolic compound analyses, and measured the above- and belowground biomass.

Before volatile collection, we removed *G. tenella* larvae from plants in the herbivory treatment and estimated the proportion of leaf area consumed by the larvae. This was done by visually estimating the proportion of consumed area for every leaf of each plant in the herbivory treatments. Later, the damage to each leaf was averaged as a proportion of the leaf area consumed per plant. To determine the effect of soil GBH residues and herbivory by *G. tenella* on the emission of VOCs, we collected VOCs from all 80 plants via dynamic headspace sampling. Volatile collection was conducted in batches of four plants at one time. Each batch of four plants consisted of one randomly selected plant per treatment. VOC samples were analysed by gas chromatography–mass spectrometry (GC–MS), and ChemStation software was used for peak identification and quantification. Most of the detected compounds (22 out of 24) belonged to one of three compound classes: green leaf volatiles, monoterpenes or sesquiterpenes.

After the collection of plant volatiles, we sampled approximately 100 mg of undamaged leaf tissue from the tip of the leaf. The samples were immediately flash frozen in liquid nitrogen and stored at –80 °C until analysis. Plant hormones were analysed as described previously Dobrev and Vankova (2012) and Fuchs et al. (2022a). The analyses covered the plant hormones abscisic acid (ABA), indole acetic acid (IAA), phenyl acetic acid (PAA), salicylic acid (SA) and jasmonic acid (JA). Furthermore, we quantified the potential SA precursor benzoic acid (BzA) and the jasmonic acid conjugate jasmonoyl isoleucine (JA-Ile).

Following the collection of plant volatiles and phytohormone samples, three leaflets per plant of similar size and age were removed, weighed and frozen at –20 °C until lyophilisation. All leaf samples were freeze-dried for 72 h and subsequently finely ground with a metal matrix in a ball mill (Retsch GmbH) for 120 s at 21 beats per second. Leaf powder (20 mg) was weighed into a 2 ml reaction tube (Eppendorf GmbH) before the extraction procedure. The sample was extracted and analysed according to Engström et al. (2014, 2015) and Fuchs et al. (2022b), using an Acquity UPLC system (Waters Corp., Milford, MA, USA) interfaced with a Xevo TQ triple-quadrupole mass spectrometer with electrospray ionisation (ESI) (Waters Corp., Milford, MA, USA). The UPLC system consisted of an autosampler, a binary solvent manager, a 100 mm × 2.1 mm i.d., 1.7 µm Acquity UPLC BEH Phenyl column (Waters Corp., Wexford, Ireland), and a diode array detector.

We quantified hydrolysable tannins as the sum of galloyl and hexahydroxydiphenoyl (HHDP) derivatives, proanthocyanidins as the sum of procyanidins and prodelphinidins and the total tannin content as the sum of hydrolysable tannins and proanthocyanidins (Engström et al., 2014). We further quantified flavonol derivatives by summing kaempferol, quercetin and myricetin derivatives (Engström et al., 2015). Finally, we summed the total polyphenol content of the tannins and flavonols.

More detailed descriptions of the plant metabolite analyses can be found in the Supplementary material.

2.4. Biomass sampling

To study the effect of soil GBH residues and folivory by *Galerucella tenella* on woodland strawberry performance, we sampled aboveground and belowground biomass. Aboveground biomass was sampled by clipping the remaining aboveground plant tissues at the base of the stem. For belowground biomass, the excess soil was first carefully separated

Table 1

The results of general linear models testing the effect of treatment (i.e. control, herbivory by *Galerucella tenella* larvae, GBH residue exposure via soil, and combined herbivory and GBH residue exposure) and genotype (one resistant and one susceptible to *G. tenella*) and their interaction on woodland strawberry (*Fragaria vesca*) above- and belowground biomass, and root: shoot ratio. The results of generalised linear model testing the effect of treatment (control and GBH residue exposure via soil), genotype (one resistant and one susceptible to *G. tenella*) and their interaction on proportion of *G. tenella* feeding damage on woodland strawberry.

Dependent variable	Explanatory variable	df	F	P
Aboveground biomass	Treatment	3, 71	3.01	0.0357
	Genotype	1, 71	21.31	<0.0001
	Treatment x genotype	3, 71	1	0.398
Belowground biomass	Treatment	3, 72	2.24	0.0907
	Genotype	1, 72	29.5	<0.0001
	Treatment x genotype	3, 72	2.91	0.0404
Root:shoot ratio	Treatment	3, 71	6.28	0.0008
	Genotype	1, 71	0.36	0.5495
	Treatment x genotype	3, 71	2.56	0.0616
Proportion of damage	Treatment	1, 32	4.75	0.0368
	Genotype	1, 32	0	0.9721
	Treatment x genotype	1, 32	1.63	0.2113

from the roots, after which the rest of the soil was washed away with tap water. The washed roots were dried with tissue paper. Above- and belowground samples were weighed separately (fresh weight; same scale as above), and the weights of the hormone and metabolite samples were added to the aboveground biomass.

2.5. Statistics

Plant biomass and herbivory: The effects of soil GBH residues and herbivory by *Galerucella tenella* on the above- and belowground biomass and root: shoot ratio of woodland strawberry plants were analysed using separate general linear models (PROC GLIMMIX, SAS 9.4). In each model, treatment (control, herbivory, GBH and combined GBH + herbivory), plant genotype (1A, i.e., susceptible to *G. tenella*, and 34F, i.e., resistant to *G. tenella*) and their interaction were used as fixed factors. The effect of soil GBH residues on the proportion of damaged leaf area was analysed using a generalised linear model with a beta distribution and logit link function (PROC GLIMMIX, SAS 9.4). In the model, treatment (soil with and without GBH residues), plant genotype and their interaction were used as fixed factors. The normality and equality of variances of the residuals were assessed by visual examination and Levene's test, respectively.

Volatiles: To examine the effect of soil GBH residues and herbivory on the composition of leaf VOC emissions, we performed nonmetric multidimensional scaling (NMDS) analyses of all 27 compounds (vegan::metaMDS). We used a Bray–Curtis dissimilarity matrix (vegan::vegdist) with Wisconsin double standardisation 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 analysis of variance (PERMANOVA; 9999 permutations; vegan::adonis). Significant PERMANOVA was followed by Tukey's post hoc test to test for differences in the factorial analysis.

Phytohormones: A generalised linear model was used to examine the effects of soil GBH residues, herbivory, genotype and their interactions on phytohormone concentrations in woodland strawberry plants. Tukey's post hoc test was used to test the effect of treatment (GBH, herbivory, or GBH + herbivory) on the SA concentration for each genotype individually.

Phenolics: To examine the effect of soil GBH residues and herbivory on the composition of leaf phenolic compound concentrations, we performed NMDS analyses of all compounds (vegan::metaMDS). We used a Bray–Curtis dissimilarity matrix (vegan::vegdist) with Wisconsin double standardisation and two dimensions (stress = 0.167) for ordination. We

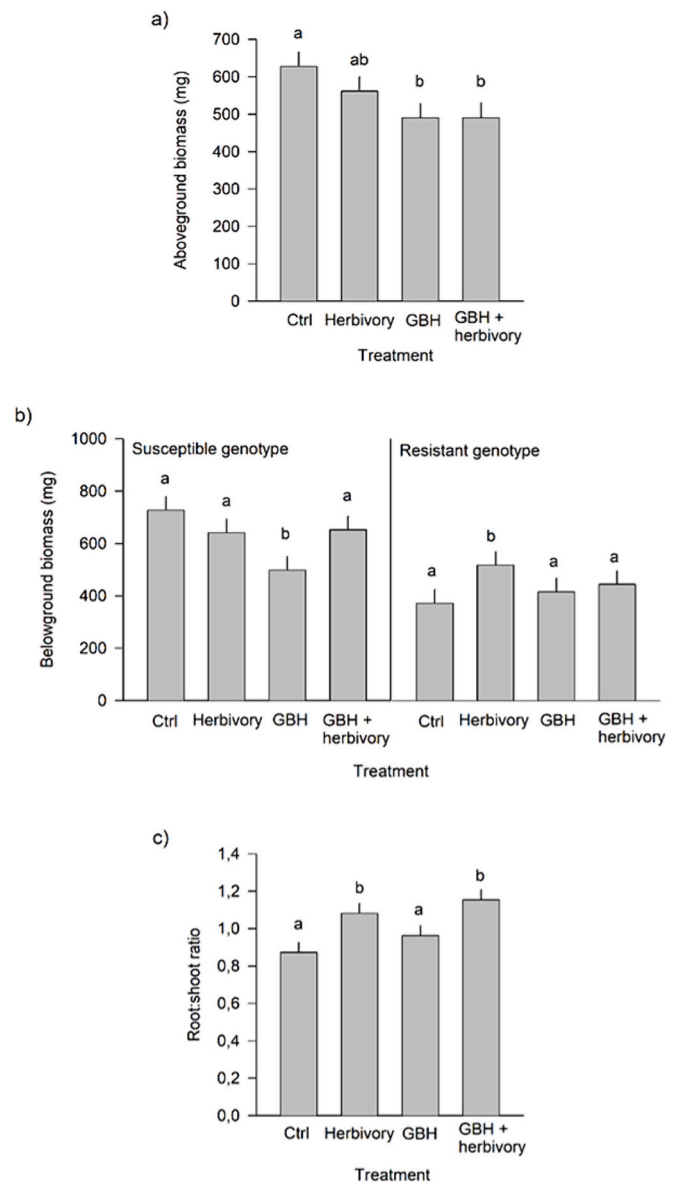


Fig. 1. The effect of treatments (Ctrl - control plants growing in soil without GBH residues; Herbivory -, plants growing in soil without GBH residues and subjected to *Galerucella tenella*; GBH - plants growing in soil with GBH residues; GBH + Herbivory - combined GBH and herbivory treatment) on woodland strawberry (*Fragaria vesca*) a) aboveground biomass (dry weight, mg) b) belowground biomass of susceptible and resistant genotype (note that figure presents significant treatment by genotype interaction; dry weight, mg) and c) root: shoot ratio. Lsmeans \pm SE. Statistical significance between different treatment groups was determined using Tukey's test and is denoted with different letters ($p < 0.05$).

tested whether soil GBH residues, herbivory, genotype and their interactions affected phenolic compound composition via permutational analysis of variance (PERMANOVA; 9999 permutations; vegan::adonis). Because the genotype was significant, we performed the same PERMANOVAs for each genotype separately to disentangle the effects of soil GBH residues and herbivory for each genotype. Significant PERMANOVA was followed by the Tukey post hoc test to test for differences among the four treatments.

Table 2

The results of Permanova analyses testing the effect of treatment (control, i.e. soil without GBH residues; GBH, i.e. soil with GBH residues; herbivory i.e. soil without GBH residues and feeding by *Galerucella tenella*; and combined GBH and herbivory), genotype (susceptible and resistant to *G. tenella*) and their interaction on composition of volatile organic compounds (VOCs) and phenolic compounds of woodland strawberry (*Fragaria vesca*).

Dependent variable	Explanatory variable	Df	F	p
VOC composition	Treatment	3, 76	10.43	0.001
	Genotype	1, 76	1.57	0.195
	Treatment x genotype	3, 76	1.50	0.154
Phenolics composition	Treatment	3, 76	0.08	0.966
	Genotype	1, 76	3.95	0.037
	Treatment x genotype	3, 76	3.89	0.011

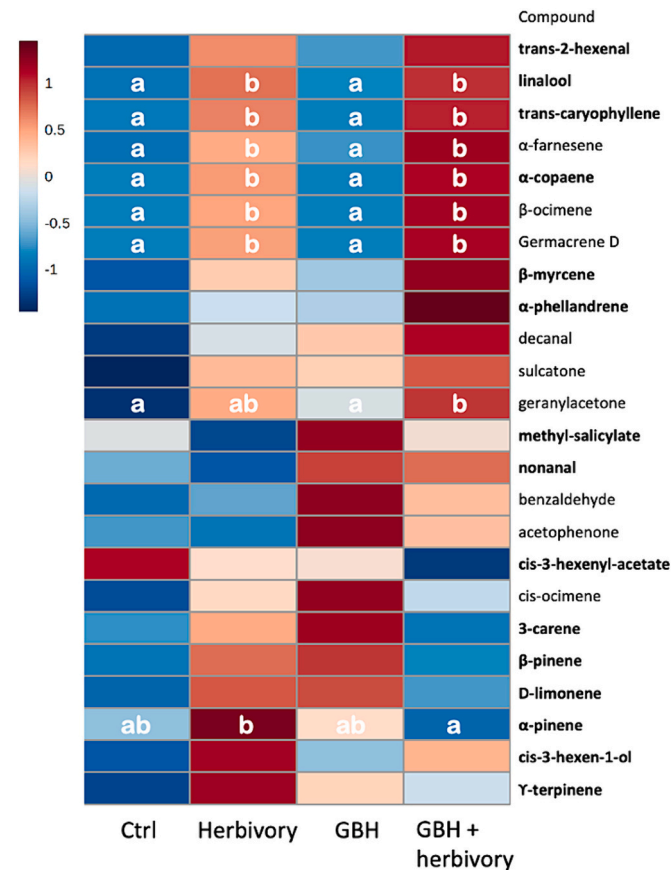


Fig. 2. Heatmap based on Tukey post-hoc test results with single-step adjusted P values showing VOC emissions for both woodland strawberry (*Fragaria vesca*) genotypes in different treatments (Ctrl - control plants growing in soil without GBH residues; Herbivory -, plants growing in soil without GBH residues and subjected to *Galerucella tenella*; GBH - plants growing in soil with GBH residues; GBH + Herbivory - combined GBH and herbivory treatment). VOCs were quantified with external standard curves by GC-MS. Compounds in bold were injected as analytical standards. Corresponding values see Table S1.

3. Results

3.1. Plant growth

GBH residues in the soil decreased the aboveground biomass of woodland strawberry plants, regardless of herbivory (Table 1, Fig. 1a). Herbivore damage did not affect the plant aboveground biomass (Table 1, Fig. 1a). Plants of the genotype classified as susceptible to *G. tenella* (1A) had on average, 39 % higher biomass than plants of the genotype classified as resistant (34F; Table 1). The effect of soil GBH

Table 3

The results of general linear models testing the effect of treatment (i.e. control, herbivory by *Galerucella tenella* larvae, GBH residue exposure via soil, and combined herbivory and GBH residue exposure) and genotype (one resistant and one susceptible to *G. tenella*) and their interaction on woodland strawberry (*Fragaria vesca*) phytohormones (ABA = Abscisic acid, JA = Jasmonic acid, JA_Ile = Jasmonoyl Isoleucine, IAA = Indole-3-acetic acid, SA = Salicylic acid, BzA = Benzoic acid, PAA = Phenylacetic acid).

Dependent variable	Explanatory variable	Df	F	p
ABA	Treatment	3, 72	9.01	<0.001
	Genotype	1, 72	0.10	0.750
	Treatment x genotype	3, 72	0.81	0.490
JA	Treatment	3, 72	12.94	<0.01
	Genotype	1, 72	0.84	0.362
	Treatment x genotype	3, 72	0.32	0.814
JA_Ile	Treatment	3, 72	14.32	<0.001
	Genotype	1, 72	0.11	0.745
	Treatment x genotype	3, 72	0.50	0.682
IAA	Treatment	3, 72	8.60	<0.001
	Genotype	1, 72	<0.01	0.993
	Treatment x genotype	3, 72	0.52	0.669
SA	Treatment	3, 72	2.09	0.110
	Genotype	1, 72	1.44	0.235
	Treatment x genotype	3, 72	4.20	0.044
BzA	Treatment	3, 72	7.27	<0.001
	Genotype	1, 72	2.37	0.078
	Treatment x genotype	3, 72	1.10	0.353
PAA	Treatment	3, 72	10.26	<0.001
	Genotype	1, 72	0.17	0.685
	Treatment x genotype	3, 72	0.80	0.496

residues on woodland strawberry belowground biomass differed between the studied genotypes, with herbivory inducing root biomass in the resistant genotype and soil GBH residues reducing root biomass in the susceptible genotype (Table 1, Fig. 1b). Herbivory increased the root: shoot ratio, both with and without exposure to soil GBH residues (Table 1, Fig. 1c). The proportion of leaf damage was higher in plants exposed to soil GBH residues (14.9 ± 1.2 % of leaf area damaged; $lsmeans \pm SEs$) than in control plants (11.3 ± 1.3 % of leaf area damaged; $lsmeans \pm SEs$; Table 1). The proportion of damage did not differ between the two genotypes (Table 1).

3.2. Plant metabolites

3.2.1. Volatile organic compounds

PERMANOVA revealed a treatment effect on the emitted volatile composition (Table 2), and NMDS showed a clear separation between the herbivore and nonherbivore treatments unaffected by soil GBH residues (Fig. S1). Genotype did not affect the emission of VOCs alone or in combination with treatment (Table 2). We identified 30 compounds, 24 of which were present in at least 10 % of the samples and consequently were used for statistical analyses (Table S1). Among the 30 compounds, 13 were monoterpenes; eight were sesquiterpenes; three were green leaf volatiles; and benzaldehyde, sulcatone, acetophenone, nonanal, decanal and methyl-salicylate were present (Fig. 2). Because genotype did not affect VOC emission, it was removed as a factor from the final generalised linear model. Tukey post hoc tests revealed that six terpenoids were induced by herbivory, irrespective of whether the plant was subjected to soil GBH residues (Fig. 2). Geranylacetone emissions were induced by herbivory, but the effect was only significant when plants were grown in soil with GBH residues. α -Pinene emission was induced by herbivory in plants growing in the control soil but not in plants growing in soil with GBH residues (Fig. 2).

3.2.2. Phytohormones

Herbivory increased the concentrations of ABA, JA, jasmonoyl isoleucine, indole-3-acetic acid, benzoic acid, and phenylacetic acid, irrespective of the soil GBH residue, for both the susceptible and resistant genotypes (Table 3, Fig. 3a). Interestingly, soil GBH residues

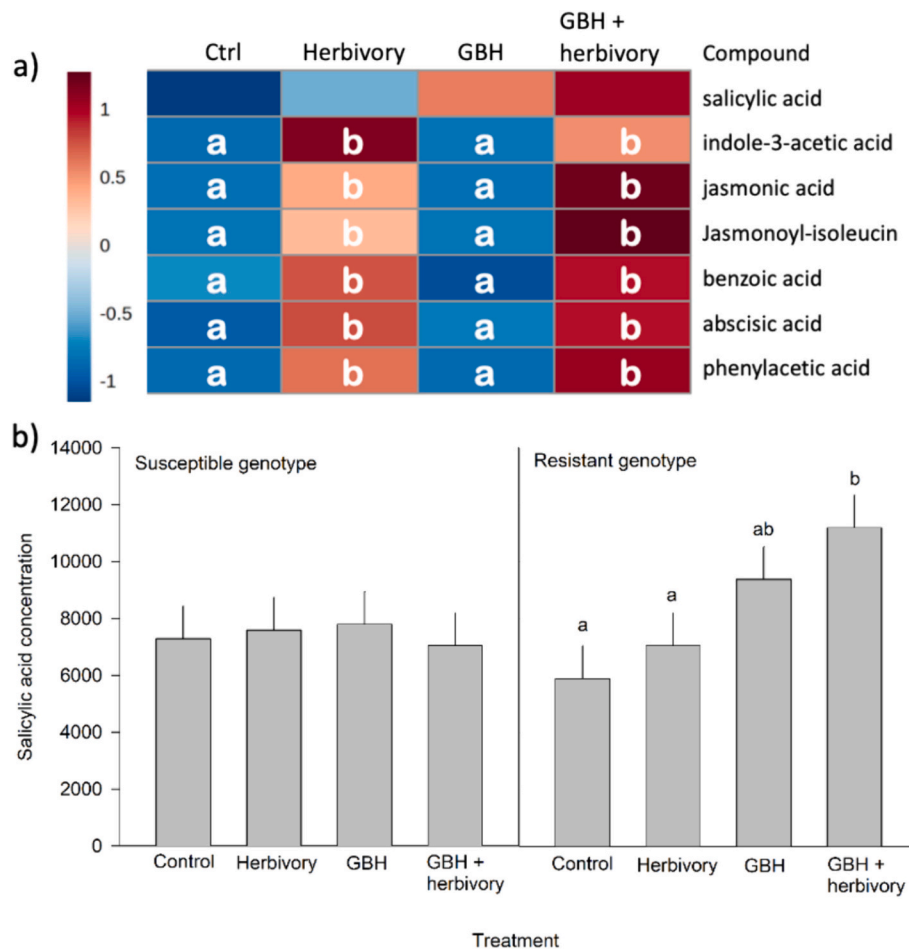


Fig. 3. a) Heatmap based on Tukey post-hoc test results with single-step adjusted P values showing phytohormone concentrations of both woodland strawberry (*Fragaria vesca*) genotypes in different treatments (Ctrl - control plants growing in soil without GBH residues; Herbivory -, plants growing in soil without GBH residues and subjected to *Galerucella tenella*; GBH - plants growing in soil with GBH residues; GBH + Herbivory - combined GBH and herbivory. treatment). Phytohormone concentrations were quantified with reference to internal standard compounds by LC-MS. Corresponding values see Table S2; b) the effect of treatment (Control - plants growing in soil without GBH residues; Herbivory - plants growing in soil without GBH residues and subjected to *Galerucella tenella*; GBH - plants growing in soil with GBH residues; GBH + herbivory - combined GBH and herbivory treatment) on salicylic acid concentration of susceptible and resistant woodland strawberry (*Fragaria vesca*) genotype (significant treatment by genotype interaction). Ls means \pm SE. Statistical significance between different treatment groups was determined using Tukey's test and is denoted with different letters ($p < 0.05$).

Table 4

The difference in phenolic compound concentrations between the susceptible and resistant woodland strawberry (*Fragaria vesca*) genotype based on student's t -test with Bonferroni correction.

Compound group	Genotype		t	p
	Susceptible (1A)	Resistant (34F)		
Galloyl deriv.	1.19 \pm 0.04	1.57 \pm 0.06	5.24	<0.01
HHDP deriv.	48.13 \pm 1.11	55.01 \pm 1.15	4.29	<0.01
Hydrolysable tannins	49.32 \pm 1.14	56.57 \pm 1.19	4.39	<0.01
Procyanidins	6.28 \pm 0.32	6.87 \pm 0.57	0.92	0.36
Prodelfinidins	0.10 \pm 0.01	0.16 \pm 0.02	3.28	<0.01
Proanthocyanidins	6.38 \pm 0.33	7.04 \pm 0.58	0.99	0.33
Tannins	55.70 \pm 1.21	63.62 \pm 1.45	4.19	<0.01
Kaempferol deriv.	1.97 \pm 0.06	2.24 \pm 0.07	3	<0.01
Quercetin deriv.	1.9 \pm 0.06	2.03 \pm 0.10	1.14	0.23
Flavonol deriv.	3.88 \pm 0.09	4.28 \pm 0.10	2.93	<0.01
Polyphenols	59.58 \pm 1.26	67.89 \pm 1.50	4.26	<0.01

increased SA concentrations in the resistant genotype (34F), but not in the susceptible genotype (1A) (Fig. 3b).

3.2.3. Phenolics

PERMANOVA revealed a community difference in phenolic

compounds caused by genotype and an interaction of treatment and genotype (Table 2), while the difference between genotypes was explained by the concentrations of most of the analysed phenolic compounds, which were significantly higher in the resistant genotype than in the susceptible genotype, except for procyanidins and quercetin derivatives (Table 4). Follow-up PERMANOVA of each genotype separately showed that treatment (i.e., soil GBH residues, herbivory or their combination) did not affect the composition of phenolic compounds in the susceptible genotype ($Df = 3,36$; $F = 1.64$; $p = 0.29$). However, for the resistant genotype, the effect of treatment was significant ($Df = 3,35$; $F = 3.13$; $p = 0.05$); the concentrations of tannins and polyphenols were affected by soil GBH residues in combination with herbivory, and the concentrations of proanthocyanidins, procyanidins and prodelfinidins were affected by soil GBH residues irrespective of herbivory (Fig. 4).

4. Discussion

The results of the present study show that soil GBH residues and herbivory affect woodland strawberry performance and metabolic defence responses, both separately and interactively, and that these effects largely depend on the plant genotype. Contrary to hypothesis 1, we found that the aboveground biomass of plants growing in soil with GBH

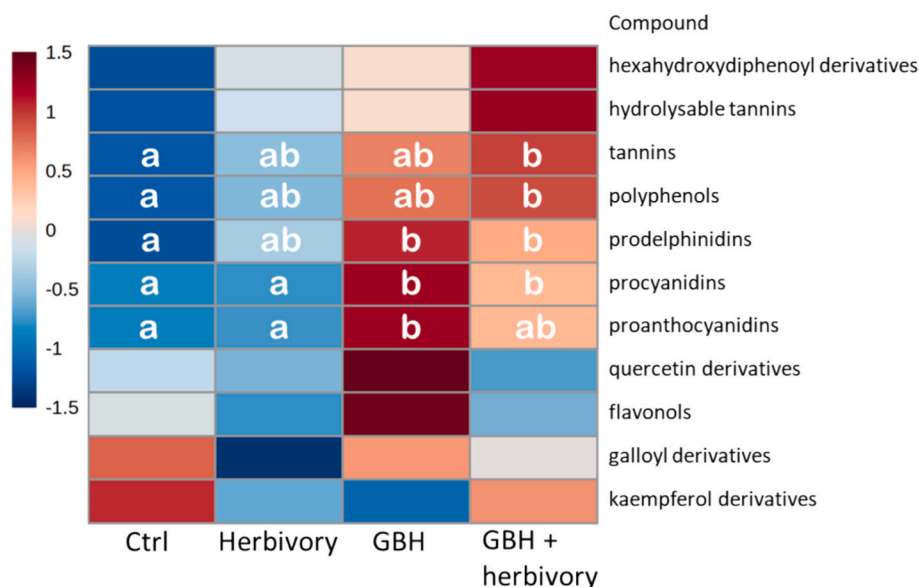


Fig. 4. Heatmap based on Tukey post-hoc test results with single-step adjusted P values showing concentrations of phenolic compounds of resistant woodland strawberry (*Fragaria vesca*) genotype in different treatments (Ctrl - control plants growing in soil without GBH residues; Herbivory -, plants growing in soil without GBH residues and subjected to *Galerucella tenella*; GBH - plants growing in soil with GBH residues; GBH + Herbivory - combined GBH and herbivory treatment). Corresponding values see Table S3.

residues was lower than that of plants growing in soil without GBH residues. In earlier studies, low levels of GBH residues in soil were not shown to affect garden strawberry growth (Fuchs et al., 2022a; Mathew et al., 2023), but higher concentrations of GBH residues had a negative effect (Muola et al., 2021). Moreover, the effect of soil GBH residues on the belowground biomass of woodland strawberry plants was affected by plant genotype and herbivore damage. Soil GBH residues decreased the belowground biomass of the susceptible genotype in the absence of herbivory, which indicates that exposure to soil GBH residues had a direct and herbicidal effect on root growth, as has been shown for meadow fescue (Keronen et al., 2023; Laihonon et al., 2022; Muola et al., 2021). Our findings indicate that herbivory might have overridden the negative effect of GBH residues on belowground biomass. Herbivory can cause the short-term reallocation of resources away from the site of plant damage to storage organs, such as roots (Gómez et al., 2010; Schultz et al., 2013). This might be especially important for young perennial plants that need resources for growth and reproduction (Muola and Stenberg, 2018; Stowe et al., 2000; Tiffin, 2000). Accordingly, herbivory increased the belowground biomass of the resistant genotype and the root: shoot ratio of both genotypes. A higher root: shoot ratio indicates that the plant can better acquire soil nutrients, which are likely to be beneficial for regrowth following herbivore damage (Tiffin, 2000). Our results may be explained either by the direct interaction of soil GBH residues with plant roots or by GBH-residue elicited changes in the soil microbiome (Badri et al., 2013). Both mechanisms can affect plant metabolism, and eventually plant performance. In a study on potato, we demonstrated that GBH-residues in soil induced changes in phytohormone concentrations which showed a weak correlation with changes in the plant endophytic microbiome (Mathew et al., 2024). However, studies on the link between GBH residue elicited changes in the soil microbiome and cascading effects on plant biochemistry are scarce (Ruuskanen et al., 2023).

Volatile emissions were induced by herbivory and not affected by soil GBH residues. Herbivore-induced volatile emissions are known for several plant species and can serve as a signal cue for predatory insects as a form of indirect plant defence (Heil, 2008; Kessler and Heil, 2011). VOCs can also quickly transmit internal signals within plants or among neighbouring plants (Frost et al., 2007). We suspected that GBH residues in soil may interfere with herbivore-induced or constitutive plant

volatile emissions because of either direct interference of the metabolic pathway or indirectly via phytohormone regulation of the metabolic pathway (D'Alessandro et al., 2006; Fuchs et al., 2021, 2022a; Schmelz et al., 2003). The volatiles emitted by woodland strawberry plants were mainly green leaf volatiles as well as terpenoids. Compared with phenylpropanoid-based volatiles, the biosynthesis of these volatile compounds does not rely on aromatic amino acids, which excludes most of the direct effects of GBH residues on their biosynthesis (D'Alessandro et al., 2006; Dudareva et al., 2013; Fuchs et al., 2021). Additionally, we did not observe any indirect effects potentially elicited by differences in phytohormone concentrations as a result of glyphosate residues in the soil (Fuchs et al., 2022a).

Similar to volatiles, herbivory increased the concentrations of several phytohormones irrespective of exposure to soil GBH residues or plant genotypes. However, the increase in the SA concentration of the resistant genotype was induced by soil GBH residues in combination with herbivory. Neither soil GBH residues nor herbivory affected the SA concentration of the susceptible genotype. Based on our results, soil GBH residues enforced the herbivore-induced biosynthesis of SA in the resistant genotype, which correlated with higher concentrations of tannins and proanthocyanidins. In addition to JA and ABA (Erb et al., 2012), SA also plays an important role in plant defence against insect herbivory and induces the biosynthesis of defence compounds (Bauters et al., 2021; War et al., 2012). SA pathways are commonly induced by phloem-feeding herbivores and biotrophic pathogens and were previously shown to increase proanthocyanidin levels (Ullah et al., 2019). Leaf beetle larval feeding has previously been shown to increase SA concentrations in young elm trees, which is correlated with increased flavonoid biosynthesis (Schott et al., 2022).

The resistant plant genotype showed higher concentrations of the majority of the analysed phenolic compounds overall, which likely explains its generally greater resistance (Dixon et al., 2002; Weber et al., 2020b). Phenolic compounds, particularly hydrolysable tannins, are known to reduce a plant's nutritional quality to herbivores and to decrease protein digestion by herbivores (Barbehenn and Constabel, 2011). Interestingly, neither GBH residues nor herbivory affected the concentrations of phenolic compounds in the susceptible genotype (1A), indicating that the biosynthesis of phenolic compounds in the susceptible genotype is constitutively regulated (Morris et al., 2006; Weber

et al., 2020b). However, for the resistant genotype, proanthocyanidin concentrations were induced by soil GBH residues and overall tannin and polyphenol concentrations were induced by soil GBH residues in combination with herbivory. The signalling cascades responsible for this induction need further investigation. In our study, the observed increase in polyphenol concentrations caused by soil GBH residues did not lead to an increase in plant defence (i.e., a decrease in plant damage). Interestingly, the larvae of *G. tenella* were found to cause more damage to plants subjected to GBH residues, indicating compensatory feeding (Lavoie and Oberhauser, 2004).

Our results demonstrate (1) that plant genotype plays a major role in the susceptibility of plants to soil GBH residues, (2) that GBH residues in soil shape the plant response to herbivory at the phytohormone and phenolic compound levels and (3) that soil GBH residues interfere with polyphenol-based plant defence. Considering the ongoing reduction in plant resilience and increasing pest problems in agriculture together with a global increase in agrochemical pollution, future research needs to identify crop varieties and genotypes that can cope with herbicide residues in soil and pest pressure while maintaining yield quantity and quality. Future studies need to transfer our results to the field to provide results from applied scenarios.

Funding statement

We acknowledge the Research Council of Finland (Grant No. 311077 to MH, Grant No. 355917 to BF), the Novo Nordisk Foundation (to BF) and the Maj and Tor Nessling Foundation (Grant no. 201800048 to AM) for funding.

CRediT authorship contribution statement

Benjamin Fuchs: Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marjo Helander:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Kari Saikkonen:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Petre I. Dobrev:** Investigation, Formal analysis. **Radomira Vankova:** Writing – review & editing, Investigation, Formal analysis. **James D. Blande:** Writing – review & editing, Investigation. **Juha-Pekka Salminen:** Writing – review & editing, Formal analysis. **Niko Luntamo:** Formal analysis. **Anne Muola:** Writing – original draft, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are available from the authors upon reasonable request.

Acknowledgments

We thank Johan Stenberg for providing us runners of the woodland strawberry genotypes. We thank Kaisa Honkanen and Lauri Heikkonen for their help during the greenhouse experiment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.174198>.

References

- Badri, D.V., Zolla, G., Bakker, M.G., Manter, D.K., Vivanco, J.M., 2013. Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. *New Phytol.* 198, 264–273. <https://doi.org/10.1111/nph.12124>.
- Ballhorn, D.J., Kautz, S., Heil, M., Hegeman, A.D., 2009. Analyzing plant defenses in nature. *Plant Signal. Behav.* 4, 743–745.
- Barbehenn, R.V., Constabel, C.P., 2011. Tannins in plant–herbivore interactions. *Phytochem. Plant–Insect Interact.* 72, 1551–1565. <https://doi.org/10.1016/j.phytochem.2011.01.040>.
- Bauters, L., Stojilković, B., Gheysen, G., 2021. Pathogens pulling the strings: effectors manipulating salicylic acid and phenylpropanoid biosynthesis in plants. *Mol. Plant Pathol.* 22, 1436–1448. <https://doi.org/10.1111/mpp.13123>.
- Benbrook, C.M., 2016. Trends in glyphosate herbicide use in the United States and globally. *Environ. Sci. Eur.* 28, 3. <https://doi.org/10.1186/s12302-016-0070-0>.
- Berens, M.L., Berry, H.M., Mine, A., Argueso, C.T., Tsuda, K., 2017. Evolution of hormone signaling networks in plant defence. *Annu. Rev. Phytopathol.* 55, 401–425. <https://doi.org/10.1146/annurev-phyto-080516-035544>.
- Borggaard, O.K., Gimsing, A.L., 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Manag. Sci.* 64, 441–456. <https://doi.org/10.1002/ps.1512>.
- 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. *J. Chem. Ecol.* 32, 2733–2748. <https://doi.org/10.1007/s10886-006-9196-7>.
- Deng, Y., Lu, S., 2017. Biosynthesis and regulation of phenylpropanoids in plants. *Crit. Rev. Plant Sci.* 36, 257–290. <https://doi.org/10.1080/07352689.2017.1402852>.
- Ding, P., Ding, Y., 2020. Stories of salicylic acid: a plant defense hormone. *Trends Plant Sci.* 25, 549–565. <https://doi.org/10.1016/j.tplants.2020.01.004>.
- Dixon, R.A., Achnine, L., Kota, P., Liu, C.-J., Reddy, M.S.S., Wang, L., 2002. The phenylpropanoid pathway and plant defence—a genomics perspective. *Mol. Plant Pathol.* 3, 371–390. <https://doi.org/10.1046/j.1364-3703.2002.00131.x>.
- Dobrev, P.I., Vankova, R., 2012. Quantification of abscisic acid, cytokinin, and auxin content in salt-stressed plant tissues. In: Shabala, S., Cui, T.A. (Eds.), *Plant Salt Tolerance: Methods and Protocols*, Methods in Molecular Biology. Humana Press, Totowa, NJ, pp. 251–261. https://doi.org/10.1007/978-1-61779-986-0_17.
- Dudareva, N., Klempien, A., Muhlemann, J.K., Kaplan, I., 2013. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytol.* 198, 16–32. <https://doi.org/10.1111/nph.12145>.
- Duke, S.O., Powles, S.B., 2008. Glyphosate: a once-in-a-century herbicide. *Pest Manag. Sci.* 64, 319–325. <https://doi.org/10.1002/ps.1518>.
- Engström, M.T., Pälljärvi, M., Frygasas, C., Grabber, J.H., Mueller-Harvey, I., Salminen, J.-P., 2014. Rapid qualitative and quantitative analyses of proanthocyanidin oligomers and polymers by UPLC-MS/MS. *J. Agric. Food Chem.* 62, 3390–3399. <https://doi.org/10.1021/jf500745y>.
- Engström, M.T., Pälljärvi, M., Salminen, J.-P., 2015. Rapid fingerprint analysis of plant extracts for ellagitannins, gallic acid, and quinic acid derivatives and quercetin, kaempferol- and myricetin-based flavonol glycosides by UPLC-QqQ-MS/MS. *J. Agric. Food Chem.* 63, 4068–4079. <https://doi.org/10.1021/acs.jafc.5b00595>.
- Erb, M., Meldau, S., Howe, G.A., 2012. Role of phytohormones in insect-specific plant reactions. *Trends Plant Sci.* 17, 250–259. <https://doi.org/10.1016/j.tplants.2012.01.003>.
- Frost, C.J., Appel, H.M., Carlson, J.E., De Moraes, C.M., Mescher, M.C., Schultz, J.C., 2007. Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. *Ecol. Lett.* 10, 490–498. <https://doi.org/10.1111/j.1461-0248.2007.01043.x>.
- Fuchs, B., Saikkonen, K., Helander, M., 2021. Glyphosate-modulated biosynthesis driving plant defense and species interactions. *Trends Plant Sci.* 26, 312–323. <https://doi.org/10.1016/j.tplants.2020.11.004>.
- Fuchs, B., Laihonon, M., Muola, A., Saikkonen, K., Dobrev, P.I., Vankova, R., Helander, M., 2022a. A glyphosate-based herbicide in soil differentially affects hormonal homeostasis and performance of non-target crop plants. *Front. Plant Sci.* 12.
- Fuchs, B., Saikkonen, K., Helander, M., Tian, Y., Yang, B., Engström, M.T., Salminen, J.-P., Muola, A., 2022b. 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.
- Gols, R., 2018. Direct and indirect chemical defences against insects in a multitrophic frame-work. *Plant Cell Environ.* 37, 1741–1752. <https://doi.org/10.1111/pce.12318>.
- Gomes, M.P., Smedbol, E., Chalifour, A., Hénault-Ethier, L., Labrecque, M., Lepage, L., Lucotte, M., Juneau, P., 2014. Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: an overview. *J. Exp. Bot.* 65, 4691–4703. <https://doi.org/10.1093/jxb/eru269>.
- Gomes, M.P., Le Manach, S.G., Hénault-Ethier, L., Labrecque, M., Lucotte, M., Juneau, P., 2017. Glyphosate-dependent inhibition of photosynthesis in willow. *Front. Plant Sci.* 8, 207. <https://doi.org/10.3389/fpls.2017.00207>.
- Gómez, S., Ferrieri, R.A., Schueller, M., Orians, C.M., 2010. Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato. *New Phytol.* 188, 835–844. <https://doi.org/10.1111/j.1469-8137.2010.03414.x>.
- Heil, M., 2008. Indirect defence via tritrophic interactions. *New Phytol.* 178, 41–61. <https://doi.org/10.1111/j.1469-8137.2007.02330.x>.
- Helander, M., Saloniemi, I., Saikkonen, K., 2012. Glyphosate in northern ecosystems. *Trends Plant Sci.* 17, 569–574. <https://doi.org/10.1016/j.tplants.2012.05.008>.

- Helander, M., Pauna, A., Saikkonen, K., Saloniemi, I., 2019. Glyphosate residues in soil affect crop plant germination and growth. *Sci. Rep.* 9, 1–9. <https://doi.org/10.1038/s41598-019-56195-3>.
- Karban, R., 2011. The ecology and evolution of induced resistance against herbivores. *Funct. Ecol.* 25, 339–347.
- Kazan, K., Manners, J.M., 2009. Linking development to defense: auxin in plant–pathogen interactions. *Trends Plant Sci.* 14, 373–382. <https://doi.org/10.1016/j.tplants.2009.04.005>.
- 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. *J. Sustain. Agric. Environ.* 2, 16–25. <https://doi.org/10.1002/sae.212035>.
- Kessler, A., Baldwin, I.T., 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291, 2141–2144. <https://doi.org/10.1126/science.291.5511.2141>.
- Kessler, A., Heil, M., 2011. The multiple faces of indirect defences and their agents of natural selection. *Funct. Ecol.* 25, 348–357. <https://doi.org/10.1111/j.1365-2435.2010.01818.x>.
- Laihonen, M., Rainio, K., Birge, T., Saikkonen, K., Helander, M., Fuchs, B., 2022. Root biomass and cumulative yield increase with mowing height in *Festuca pratensis* irrespective of *Epichloë* symbiosis. *Sci. Rep.* 12, 21556. <https://doi.org/10.1038/s41598-022-25972-y>.
- Lavoie, B., Oberhauser, K.S., 2004. Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. *Environ. Entomol.* 33, 1062–1069. <https://doi.org/10.1603/0046-225X-33.4.1062>.
- Maeda, H., Dudareva, N., 2012. The shikimate pathway and aromatic amino acid biosynthesis in plants. *Annu. Rev. Plant Biol.* 63, 73–105. <https://doi.org/10.1146/annurev-arplant-042811-105439>.
- Maggi, F., la Cecilia, D., Tang, F.H.M., McBratney, A., 2020. The global environmental hazard of glyphosate use. *Sci. Total Environ.* 717, 137167. <https://doi.org/10.1016/j.scitotenv.2020.137167>.
- Mathew, S.A., Fuchs, B., Nissinen, R., Helander, M., Puigbò, P., Saikkonen, K., Muola, A., 2023. Glyphosate-based herbicide use affects individual microbial taxa in strawberry endosphere but not the microbial community composition. *J. Appl. Microbiol.* [lxad006](https://doi.org/10.1093/jambio/lxad006) <https://doi.org/10.1093/jambio/1xad006>.
- Mathew, S.A., Jeevanavar, A., Helander, M., Tamminen, M., Puigbò, P., Rainio, M., Saikkonen, K., Fuchs, B., 2024. Glyphosate residues in soil and phosphate fertilizer affect foliar endophytic microbial community composition and phytohormone levels in potato. *Phytochemistry* <https://doi.org/10.1094/PBIOMES-11-23-0118-R>.
- Morris, W.F., Traw, M.B., Bergelson, J., 2006. On testing for a tradeoff between constitutive and induced resistance. *Oikos* 112, 102–110. <https://doi.org/10.1111/j.0030-1299.2006.14253.x>.
- Mouradov, A., Spangenberg, G., 2014. Flavonoids: a metabolic network mediating plants adaptation to their real estate. *Front. Plant Sci.* 5, 620. <https://doi.org/10.3389/fpls.2014.00620>.
- Muola, A., Stenberg, J.A., 2018. Folivory has long-term effects on sexual but not on asexual reproduction in woodland strawberry. *Ecol. Evol.* 8, 12250–12259. <https://doi.org/10.1002/ece3.4687>.
- Muola, A., Weber, D., Malm, L.E., Egan, P.A., Glinwood, R., Parachnowitsch, A.L., Stenberg, J.A., 2017. Direct and pollinator-mediated effects of herbivory on strawberry and the potential for improved resistance. *Front. Plant Sci.* 8 <https://doi.org/10.3389/fpls.2017.00823>.
- Muola, A., Fuchs, B., Laihonen, M., Rainio, K., Heikkonen, L., Ruuskanen, S., Saikkonen, K., Helander, M., 2021. Risk in the circular food economy: glyphosate-based herbicide residues in manure fertilizers decrease crop yield. *Sci. Total Environ.* 750, 141422. <https://doi.org/10.1016/j.scitotenv.2020.141422>.
- Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G., Hansen, M., Landrigan, P.J., Lanphear, B.P., Mesnage, R., Vandenberg, L.N., vom Saal, F.S., Welshons, W.V., Benbrook, C.M., 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environ. Health* 15, 19. <https://doi.org/10.1186/s12940-016-0117-0>.
- Ossipov, V., Salminen, J.-P., Ossipova, S., Haukioja, E., Pihlaja, K., 2003. Gallic acid and hydrolysable tannins are formed in birch leaves from an intermediate compound of the shikimate pathway. *Biochem. Syst. Ecol.* 31, 3–16. [https://doi.org/10.1016/S0305-1978\(02\)00081-9](https://doi.org/10.1016/S0305-1978(02)00081-9).
- Rekhter, D., Lüdke, D., Ding, Y., Feussner, K., Zienkiewicz, K., Lipka, V., Wiermer, M., Zhang, Y., Feussner, I., 2019. Isochorismate-derived biosynthesis of the plant stress hormone salicylic acid. *Science* 365, 498–502. <https://doi.org/10.1126/science.aaw1720>.
- Ruuskanen, S., Fuchs, B., Nissinen, R., Puigbò, P., Rainio, M., Saikkonen, K., Helander, M., 2023. Ecosystem consequences of herbicides: the role of microbiome. *Trends Ecol. Evol.* 38, 35–43.
- Schmelz, E.A., Alborn, H.T., Banchio, E., Tumlinson, J.H., 2003. Quantitative relationships between induced jasmonic acid levels and volatile emission in *Zea mays* during *Spodoptera exigua* herbivory. *Planta* 216, 665–673. <https://doi.org/10.1007/s00425-002-0898-y>.
- Schott, J., Fuchs, B., Böttcher, C., Hilker, M., 2022. Responses to larval herbivory in the phenylpropanoid pathway of *Ulmus minor* are boosted by prior insect egg deposition. *Planta* 255, 16. <https://doi.org/10.1007/s00425-021-03803-0>.
- Schultz, J., Appel, H., Ferrieri, A., Arnold, T., 2013. Flexible resource allocation during plant defense responses. *Front. Plant Sci.* 4.
- Soares, C., Pereira, R., Martins, M., Tamagnini, P., Seródio, J., Moutinho-Pereira, J., Cunha, A., Fidalgo, F., 2020. Glyphosate-dependent effects on photosynthesis of *Solanum lycopersicum* L.—an ecophysiological, ultrastructural and molecular approach. *J. Hazard. Mater.* 398, 122871. <https://doi.org/10.1016/j.jhazmat.2020.122871>.
- Spormann, S., Soares, C., Fidalgo, F., 2019. Salicylic acid alleviates glyphosate-induced oxidative stress in *Hordeum vulgare* L. *J. Environ. Manag.* 241, 226–234. <https://doi.org/10.1016/j.jenvman.2019.04.035>.
- Stenberg, J.A., Axelsson, E.P., 2008. Host race formation in the meadowsweet and strawberry feeding leaf beetle *Galerucella tenella*. *Basic Appl. Ecol.* 9, 560–567. <https://doi.org/10.1016/j.baae.2007.10.007>.
- Stowe, K.A., Marquis, R.J., Hochwender, C.G., Simms, E.L., 2000. The evolutionary ecology of tolerance to consumer damage. *Annu. Rev. Ecol. Syst.* 31, 565–595. <https://doi.org/10.1146/annurev.ecolsys.31.1.565>.
- Strauss, S.Y., Rudgers, J.A., Lau, J.A., Irwin, R.E., 2002. Direct and ecological costs of resistance to herbivory. *Trends Ecol. Evol.* 17, 278–285. [https://doi.org/10.1016/S0169-5347\(02\)02483-7](https://doi.org/10.1016/S0169-5347(02)02483-7).
- Tiffin, P., 2000. Mechanisms of tolerance to herbivore damage: what do we know? *Evol. Ecol.* 14, 523–536. <https://doi.org/10.1023/A:1010881317261>.
- Ullah, C., Tsai, C.-J., Unsicker, S.B., Xue, L., Reichelt, M., Gershenzon, J., Hammerbacher, A., 2019. Salicylic acid activates poplar defense against the biotrophic rust fungus *Melampsora larici-populina* via increased biosynthesis of catechin and proanthocyanidins. *New Phytol.* 221, 960–975. <https://doi.org/10.1111/nph.15396>.
- War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S., Sharma, H.C., 2012. Mechanisms of plant defense against insect herbivores. *Plant Signal. Behav.* 7, 1306–1320. <https://doi.org/10.4161/psb.21663>.
- Weber, D., Egan, P.A., Muola, A., Ericson, L.E., Stenberg, J.A., 2020a. Plant resistance does not compromise parasitoid-based biocontrol of a strawberry pest. *Sci. Rep.* 10, 5899. <https://doi.org/10.1038/s41598-020-62698-1>.
- Weber, D., Egan, P.A., Muola, A., Stenberg, J.A., 2020b. Genetic variation in herbivore resistance within a strawberry crop wild relative (*Fragaria vesca* L.). *Arthropod Plant Interact.* 14, 31–40. <https://doi.org/10.1007/s11829-019-09724-w>.
- Williams, G.M., Kroes, R., Munro, I.C., 2000. Safety evaluation and risk assessment of the herbicide roundup and its active ingredient, glyphosate, for humans. *Regul. Toxicol. Pharmacol.* 31, 117–165. <https://doi.org/10.1006/rtp.1999.1371>.