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RESEARCH ARTICLE

Exogenous stimulation of *Tanacetum vulgare* roots with pipecolic acid leads to tissue-specific responses in terpenoid composition

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Keywords

Chemical diversity; metabolic atlas; plant-insect interactions; systemic acquired resistance; tansy; terpenoids; volatile organic compounds.

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ABSTRACT

- *Tanacetum vulgare* L., tansy, is a perennial plant with highly variable terpenoid composition, with mono- and sesquiterpenoids being the most abundant. The high diversity of terpenoids plays an important role in mediating ecological interactions. However, the distribution of terpenoids in different tissues and inducibility of terpenoids in these tissues via biotic stress are poorly understood.
- We investigated changes in terpenoid profiles and concentrations in different organs following treatment of roots with pipecolic acid (Pip), a non-proteinogenic amino acid that triggers defence responses leading to induce systemic resistance (SAR) in plants.
- Tansy leaves and midribs contained mainly monoterpenoids, while coarse and fine roots contained mainly sesquiterpenoids. Rhizomes contained terpenoid profiles of both midribs and roots but also unique compounds. Treatment with Pip led to an increase in concentrations of mono- and sesquiterpenoids in all tissues except rhizomes. However, significantly more sesquiterpenoids was formed in root tissues in response to Pip treatment, compared to shoots.
- The metabolic atlas for terpenoids presented here shows that there is exceptionally strong differentiation of terpenoid patterns and terpenoid content in different tissues of tansy. This, together with differential inducibility by Pip, suggests that the chemical diversity of terpenoids may play an important role in tansy ecological interactions and defence against biotic stressors that feed on below- and aboveground organs.

INTRODUCTION

The production of specialised metabolites in plants is regulated by transcriptional mechanisms, resulting in tissue-specific synthesis of compounds – such as alkaloids and terpenoids – which mediate interactions with the biotic and abiotic environment (Erb & Reymond 2019; Wetzel & Whitehead 2020). Previous studies on metabolic variations in response to abiotic and biotic factors have mostly examined metabolic responses in shoot tissues, but for non-destructive sampling of root material, comparatively little is known about how abiotic or biotic factors alter metabolic profiles of belowground tissues (van Dam 2009; Rasmann *et al.* 2012). Considering that shoots and roots are exposed to different herbivore and pathogen communities, they face unique selection pressures. The production and distribution of secondary metabolites, such as flavonoids, alkaloids, and terpenoids, may vary between organs like shoots and roots (Kleine & Müller 2013; Zhang *et al.* 2021).

Among these secondary metabolites, terpenoids represent a significant and highly diverse subgroup of specialised metabolites in plants. Terpenoids play an important role in many biological processes, especially in interactions with their environment and with other organisms (Pichersky & Raguso 2018). This variety of terpenoids is regulated by terpene synthases, which are the most significant enzymes involved in synthesis of hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20) (Bohlmann *et al.* 1998; Tholl 2006). Of these subgroups, mono- and sesquiterpenes are dominant and can be localised in different tissues. For instance, *Tanacetum vulgare* shoot tissues primarily contain monoterpenes (Kleine & Müller 2013; Clancy *et al.* 2016), whereas sesquiterpenes are dominant in roots (Kleine & Müller 2013). Several terpenoids, particularly monoterpenoids, are involved in direct defence against pathogens and herbivores, influencing interactions between plants and their environment (Penuelas *et al.* 2014). Due to their volatility, they also serve as indirect defence ('a cry for help') by attracting predators of herbivores (Dicke & Baldwin 2010). For instance, switchgrass (*Panicum virgatum* L.) emits a combination of mono- and sesquiterpenes when attacked by the generalist herbivore *Spodoptera frugiperda* (J.E. Smith) (fall armyworm) and as a systematic response to root treatment with defence hormones, such as methyl jasmonate (Muchlinski *et al.* 2019).

Plants are constantly under attack from insects, herbivores, viruses, fungi, and bacteria, which can affect plant health and productivity (Poelman & Kessler 2016; Choudhary & Senthil-Kumar 2022; Irulappan *et al.* 2022). When a plant is challenged by biotic attackers, a number of biochemical reactions occur in plant tissues that can lead to systemic acquired resistance (SAR) (Vlot *et al.* 2021) or induced systemic resistance. While the latter is mediated by the jasmonic acid

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(JA)/ethylene pathway, induction of SAR is dependent on salicylic acid (SA) (Vlot *et al.* 2009). For instance, aphid feeding stimulates SA production (Voelckel *et al.* 2004), whereas leaf-chewing herbivores induce the JA/ethylene pathway (Walling 2000). JA-induced defence mechanisms have been shown to impair aphid performance, whereas SA-induced defence mechanisms are less effective (Agrawal 1998; Ali & Agrawal 2014). Pipecolic acid (Pip) is a positive regulator of SA production and leads to an increase in plant defences (Chen *et al.* 2018; Hartmann *et al.* 2018; Vlot *et al.* 2021). For example, when *Arabidopsis thaliana* (L.) Heynh. is exposed to microbial infection, Pip, a non-protein amino acid, is synthesised from L-lysine (Návarová *et al.* 2013) and then undergoes N-hydroxylation to produce N-hydroxypipecolic acid (NHP) (Hartmann *et al.* 2018). Both Pip and NHP are mediators of SAR and accumulate in locally infected and distal tissues during infection (Hartmann *et al.* 2018). Furthermore, Pip/NHP triggers production of volatile organic compounds (VOCs) as part of the SAR mechanism, contributing to intra- and inter-plant defence propagation through emission of VOCs that act as defence signals (Vlot *et al.* 2021; Brambilla *et al.* 2023). When SAR is induced by bacterial infection in *Arabidopsis*, plants emit VOCs containing elevated levels of monoterpenoids, such as α-pinene, β-pinene, and camphene, that trigger plant-to-plant communication (Wenig *et al.* 2019) within and between species (Frank *et al.* 2021). In barley (*Hordeum vulgare* L.), Pip drip irrigation similarly induces defences against subsequent powdery mildew infection (Lenk *et al.* 2019). Given that Pip application to roots provides a robust mimic of biotic induction in many model systems, and that the Pip pathway is common across the plant kingdom, it provides an excellent opportunity to use Pip to study terpenoid inducibility in plant organs.

Tanacetum vulgare L. (tansy, Asteraceae) is a fragrant herb native to Eurasia. Its terpenoid content varies considerably across individuals (Kleine & Müller 2011; Clancy *et al.* 2016; Rahimova *et al.* 2024). Tansy leaves have glandular trichomes that store terpenoids. These terpenoids, such as monoterpenes (e.g., α-thujone, α-thujene, γ-terpinene) and sesquiterpenes (e.g., β-caryophyllene, α-copaene, β-cubebene) can be synthesised and emitted immediately upon herbivory attack (Clancy *et al.* 2016, 2020). Tansy terpenoids exhibit high intraspecific variability among plants, allowing classification of plants into distinct chemotypes based on their dominant or mixed compounds, in the absence of a discernible dominant compound (Dussarrat *et al.* 2023; Rahimova *et al.* 2024). The diverse group of terpenoids in tansy is known to influence plant–insect interactions (Ojeda-Prieto *et al.* 2024). Terpenoid content correlates with plant-associated aphid communities and can also determine aphid feeding preference on leaves (Clancy *et al.* 2016; Jakobs & Müller 2018; Neuhaus-Harr *et al.* 2024). It is still unclear whether terpenoids in tansy are induced by elicitors and whether there are metabolic differences between leaves, rhizomes, and roots. Thus, in this study, we mimicked the SA-dependent response of tansy to aphid attack leading to SAR using Pip as a stimulant and investigated effects of exogenously applied Pip on tansy defence responses. We asked:

1 How does tansy terpenoid content differ between coarse roots, fine roots, rhizome, leaflets, and midribs? We aimed to compile a metabolome atlas on the occurrence of terpenoids and their chemical diversity in tansy. Based on existing literature (Kleine & Müller 2013), which reports disparate terpene distributions between shoots and roots, we hypothesise that terpenoids may exhibit structural differences between tissues, but that additional segregation may exist among above- or belowground tissues.
2 Does Pip equally induce terpenoids as a defence response in tansy shoot and root tissues? We hypothesise that application of Pip will result in increased induction of terpenoids, particularly in root tissues, but also systemically in shoot tissues. We predict that the tissue response to treatment would be stronger for sesquiterpenoids belowground, and stronger for monoterpenoids aboveground.

MATERIAL AND METHODS

Tansy cultivation and pipecolic acid treatment

Tansy plants used in this study originated from 120 plants propagated from seeds collected from 12 mother plants on plots of "The Jena Experiment" (https://the-jena-experiment.de), Jena, Germany in 2020. Five unique genotypes were collected for further propagation. Plants were clonally propagated by rhizome cuttings in a greenhouse (21 °C, 14 h:10 h light: dark) in February 2022. After root formation, plantlets were transplanted into pots (10 × 10 × 11 cm) containing commercial soil (Floradur Bodensubstrat, Floragard Vertriebs-GmbH, Oldenburg, Germany) and fertilised with Hakaphos Red (8% N, 12% P_2O_5, 24% K_2O, 4% MgO, 31% SO_3, 0.01% B, 0.02% Cu, 0.05% Fe, 0.05% Mn, 0.001% Mo, 0.02% Zn; Compo Expert, Münster, Germany). Nine weeks after propagation, 20 of the resulting 40 plants were randomly assigned to a Pipecolic acid (Pip) treatment and 20 to a control group, irrespective of the genetic identity. A total of 40 ml Pip solution (10 μM Pip in H_2O; Sigma Aldrich, Taufkirchen, Germany) was applied to each pot by watering the root system. The control group received the same treatment but without addition of Pip. After 3 days, on 7 and 8 April 2022, all plants were harvested. At harvest, the plants were divided into: (i) leaflets, (ii) leaf midrib, (iii) rhizomes, (iv) coarse roots, and (v) fine roots. To avoid mechanical damage, samples were processed within 1 min (each tissue was cut and packaged separately) and frozen in liquid nitrogen to stabilise molecular processess.

Hexane extraction of terpenoids and GC-MS analyses

Chemical analyses were performed as reported in Clancy *et al.* (2016), with minor modifications. Frozen samples were ground to fine powder and immediately stored at −80 °C until further processing. A total of 600 μl hexane +860 pmoL·μl^{-1} internal standard (monoterpene δ-2-carene) was added to 300 mg frozen samples, and the mixture vortexed and refrigerated at 4 °C for 24 h. Then 150 μl of extract was removed and stored at 4 °C. A further 150 μl of n-hexane were added, vortexed and stored for 24 h. After which 150 μl of hexane extract was again collected and combined with the initial liquid extract. The samples were analysed with gas chromatography–mass spectrometry (GC-MS) by injecting 1 μl into a glass microvial in an empty glass cartridge on an autosampler. Samples were desorbed from 35 to 240 °C at 120 °C·min^{-1} (held for 2 min) in the thermal desorption unit (TDU, Gerstel,

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Mülheim an der Ruhr, Germany) connected to a GC-MS (GC, 7890A, MS: 5975C inert XL MSD with a triple axis detector; Agilent Technologies Palo Alto, CA, USA) using an arylene siloxane capillary column (60 m × 250 µm × 0.25 µm DB-5MS + 10 m DG; Agilent Technologies). Samples were analysed in splitless mode at a constant flow rate of carrier gas (He: 1 ml·min^{-1}). The temperature program was 40 °C (held for 0 min) to 150 °C, with a ramp rate of 10 °C·min^{-1}, then 80 °C·min^{-1} to 175 °C, then 5 °C·min^{-1} to 190 °C, then 80 °C·min^{-1} to 250 °C, then 100 °C·min^{-1} to 300 °C and held for 6 min. The chromatograms from the GC-MS analysis were evaluated, integrated and quantified using Enhanced ChemStation software (MSD ChemStation E.02.01.1177, 1989–2010; Agilent Technologies). The peaks were first checked for chromatogram quality based on peak purity. The detected peaks were then identified by comparing mass spectra of each chromatogram with the Mass Spectral Library (National Institute of Standards and Technology: NIST 20). The compounds were verified by measuring and comparing the Kovats retention indices based on retention times of a saturated alkane mixture added to the samples (C9-C25; Sigma-Aldrich) as reported by Guo *et al.* (2019, 2020). Peak areas were normalised according to the fresh weight of each sample. Six dilutions of external standards – sabinene, α-pinene, linalool, methylsalicylate, β-caryophyllene, α-humulene, geraniol, and bornyl acetate – were used for quantification of the compounds.

Statistical analyses

The data were logarithmically transformed for statistical analysis to fulfil assumptions of normal distribution. Before log transformation, data were tested for normal distribution using the Shapiro–Wilk test. To visualise terpenoid content of all analysed tansy tissues, we first normalised data by log transformation and Pareto scaling then plotted the heatmap in MetaboAnalyst 6.0 (Pang *et al.* 2021). The '*Euclidean*' distance measure and '*Ward*' clustering method were used to plot compounds on the heatmap as default parameters. Partial least squares discrminant analysis (PLS-DA) of multivariate statistics was used as described in Bertić *et al.* (2021, 2023). The model was separately fit to monoterpenoids and sesquiterpenoids (Figs 1b and 2b). The model helped in finding tissue-specific discriminant mono- and sesquiterpenoid compounds and separation between tissues. The PLS-DA model was calculated by maximizing covariance between X and Y variables: X variables (32 compounds of monoterpenoid group and 41 compounds of sesquiterpenoid group) and Y variables (leaflets, midrip, rhizome, coarse root, fine roots). The fit of the model was cross-validated by R2 and Q2 values, which indicated how well variation in a variable is explained and how well a variable can be predicted. Note that well-modelled variables have high levels (as reference: 0.5 or above) of R2 and Q2. The result of the PLS-DA model fitness is given in the legend of each plot. We additionally made a stacked bar plot showing terpenoid distribution across all samples to visualise differences in mean relative abundance between te different tissues (Fig. 3).

Total concentration of mono- and sesquiterpenoids between the five tissues were tested using post-hoc tests ($P < 0.05$). A *t*-test was used for total concentration of mono- and sesquiterpenoids between Pip treated and untreated tissues. Induction of individual compounds between untreated and Pip treated groups were tested using one-way ANOVA ($P < 0.05$). Control plants were pooled and hierarchical clustering used to separately cluster them into monoterpenoid and sesquiterpenoid chemotypes, using the "factoextra" package (Kassambara & Mundt 2020), "hclust" function with the "ward.D2" method of correlation distance in R (Rahimova *et al.* 2024). We also plotted a tanglegram (Figure S2a) to visualise potential links in chemotypic clustering between aboveground (AG) and belowground (BG) sesquiterpenoid chemotype classes.

Statistical tests were carried out using R (version 4.3.2; R Core Team 2023) and MetaboAnalyst 6.0 (Pang *et al.* 2021). All graphs were produced using the R package ggplot2 (Wickham 2016) and resulting images were edited using te "Inkscape" (version 1.1.1) image processing software for enhanced resolution.

RESULTS

Monoterpenoid compositions of different tissues in tansy

First, the terpenoid composition of untreated tansy plants was investigated. The heatmap shows the monoterpenoid profile of leaf, petiole, rhizome, coarse root, and fine root tissues (Fig. 1a). Shoots, leaflets, midribs and rhizomes had very similar monoterpenoid profiles. Compounds, such as γ-terpinene, β-thujone, trans-chrysanthenyl acetate, camphor, were identified in leaflet, midrib and rhizome tissues. However, more monoterpenoid compounds were identified in leaflet and midrib tissues than in rhizomes. In contrast, roots contained comparatively low amounts of monoterpenoids. β-Terpinyl acetate, limonene, and camphene were found in coarse roots, whereas camphene was only identified in fine roots (Fig. 1a). PLS-DA analysis of monoterpenoid profiles of the different tissues showed clear separation between shoot and root tissues. However, monoterpenoids of coarse and fine roots, leaflets, midribs and rhizomes could not be distinguished within each group. (Fig. 1b). Concentrations of monoterpenoids were significantly (Tukey's HSD, $P < 0.05$) higher in leaves than in midribs and rhizomes (Fig. 1c), while shoots and roots contained only trace amounts of monoterpenoids.

Hierarchical cluster analysis showed that the tansy control group could be separated into four classes of monoterpenoid chemotypes in leaflets and leaf midribs (Figure S1a). Class 1 had two main compounds, 31% sabinene hydrate and 27% camphor; class 2 was 70% trans-chrysanthenyl acetate; class 3 was 75% β-thujone; class 4 contained a mixture of monoterpenoids (Figure S1b). This marked variation between monoterpenoid classes is consistent with our previous study (Rahimova *et al.* 2024), showing that intraspecific variation in terpenoids can be used to determine tansy chemotypes.

Sesquiterpenoid compositions of different tissues in tansy

Sesquiterpenoid profiles of shoot and root systems were clearly different (Fig. 2a). In the shoot system, leaflets and midribs had very similar sesquiterpenoid profiles. β-Copaene, β-caryophyllene, β-ylangene, epicubebol, and oxygenated sesquiterpenoids such as spirojatamol, β-selinenol and sabinol isovalerate were identified in almost all leaflet and midrib tissues. In contrast, root systems had different sesquiterpenoid compounds,

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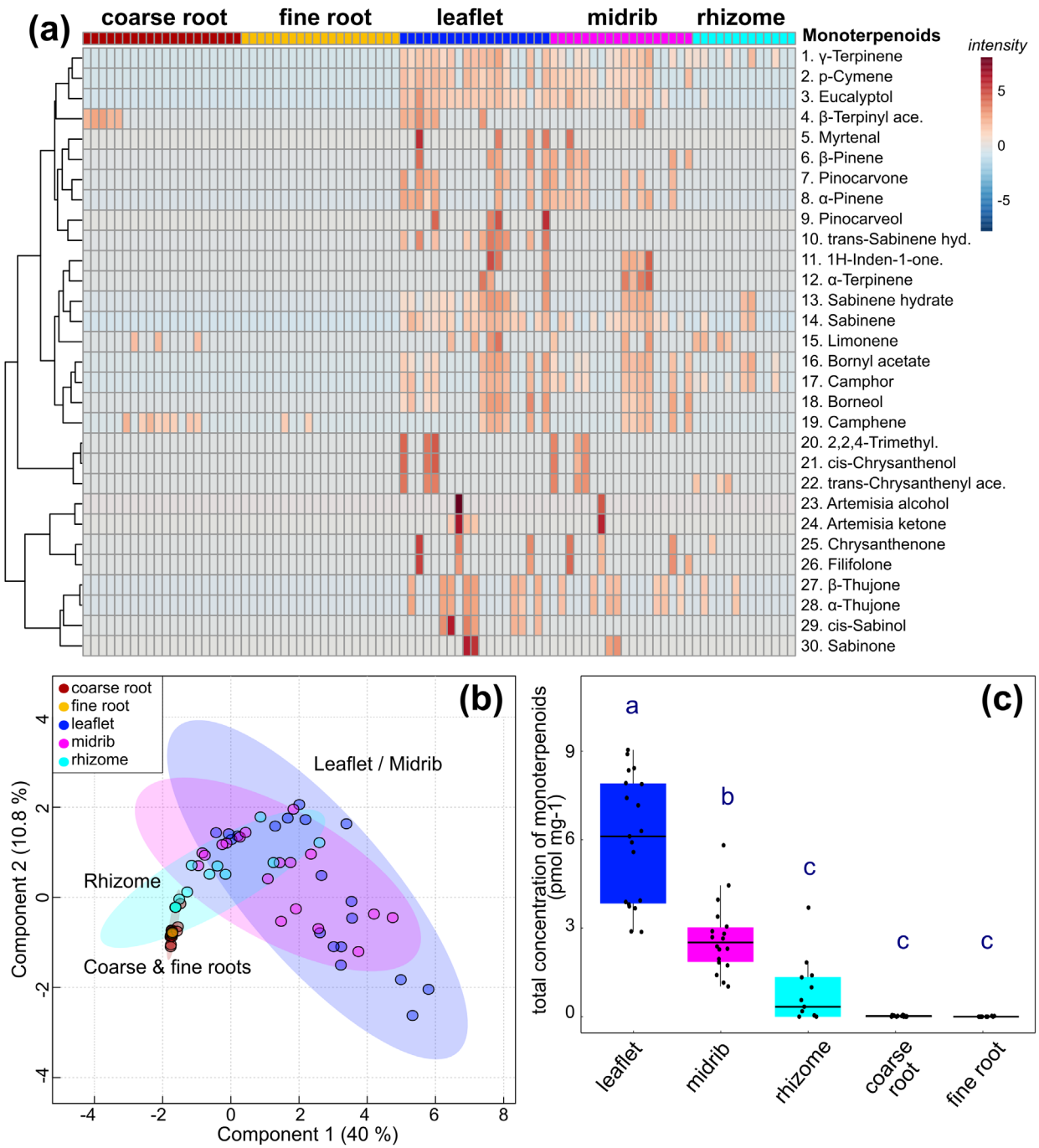

Fig. 1. Tissue atlas of monoterpenoids in all tissues of untreated plants. (a) Heatmap shows monoterpenoid compounds across all plants analysed. The distribution of monoterpenoids is displayed among coarse root, fine root, leaflets, midrib and rhizome tissues as indicated in the legend. The intensity column (right) shows concentration s of the compounds. For instance, dark red cells on map indicate the highest abundance and blue cells show no compound present. Compound names are given for each line on the right axis of the heatmap. The dendogram on the left shows the association between compounds. (b) PLS-DA plot showing grouping between tissues. Leaflets, midrib and rhizome tissues show overlapping points while rhizome tissue also shows separating features in the elipse. Root and shoot tissues show a clear separation. The plot also shows how modelled observations lie in the X-space. Observations close together are more similar than those relatively far apart. Components 1 and 2 explain the largest and second largest variation in the X-space. The two components together describe the data with a total of 51.6% of explained variance. PLS model fitness: R^2X(cum) = 0.821, R^2Y(cum) = 0.556, Q^2(cum) = 0.374. (c) Total concentration of monoterpenoids is significantly (one-factorial ANOVA, $P < 0.05$) elevated in leaflets, and midribs, in comparison to rhizomes, coarse and fine root tissues. Abundance of monoterpenoids is very low in coarse and fine roots. Tissues labelled with the same letter do not differ significantly at $P < 0.05$ (post-hoc test).

such as β-sesquiphellandrene, β-farnesene, α-isocomene and β-isocomene in coarse and fine root tissues, albeit at different concentrations. Terpenoid pattern and content of rhizomes was comparable to that of the shoot and root sesquiterpenoids, as well as compounds unique to rhizomes, i.e. nerol acetate and γ-elemene (Fig. 2a). Shoot-specific compounds, such as

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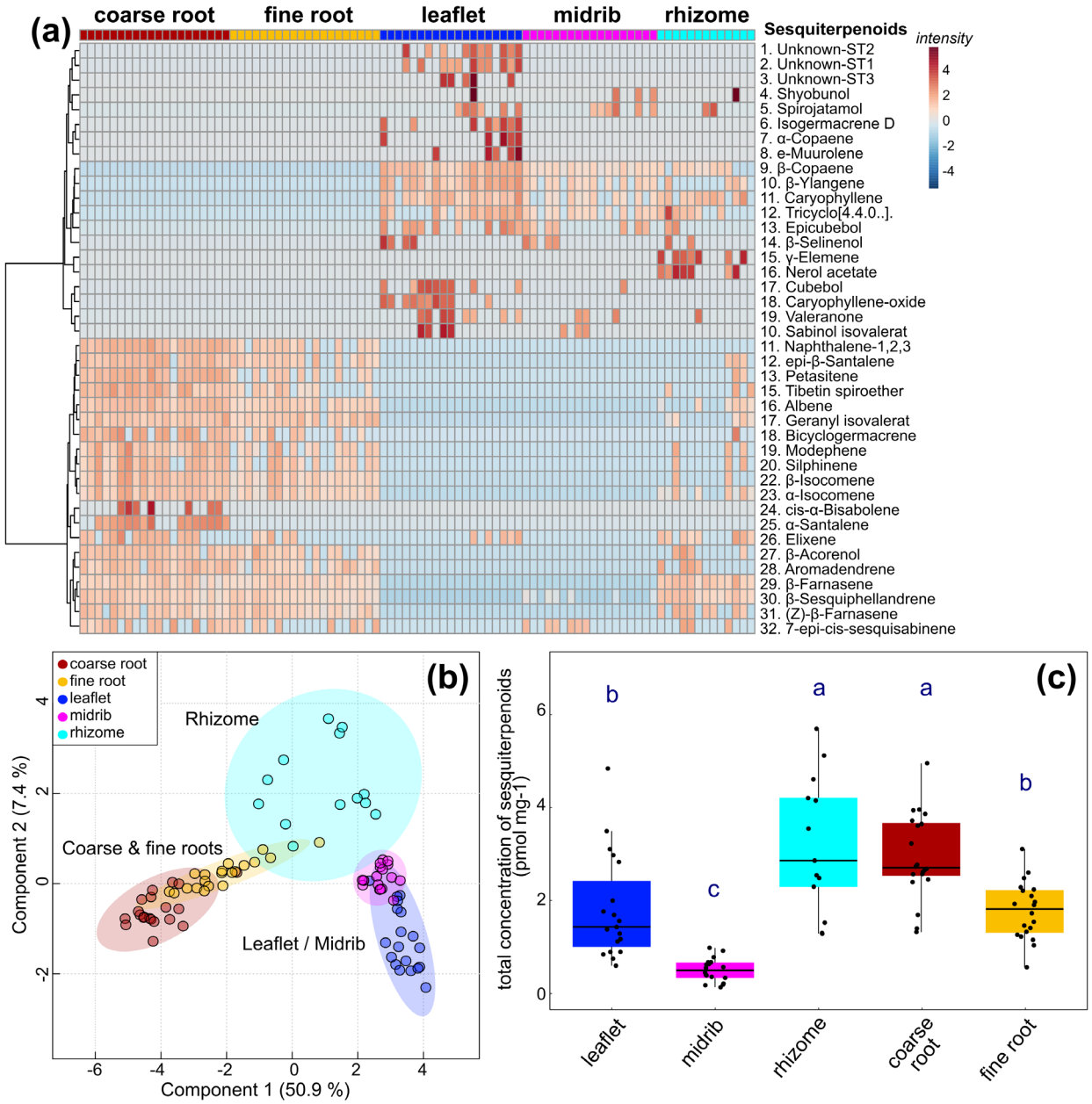

Fig. 2. Tissue atlas of sesquiterpenoids identified in all tissues of untreated plants. (a) Heatmap displays distinct sesquiterpenoid profiles among all plants and tissues analysed. Each coloured cell in the heatmap corresponds to a compound abundance, e.g. compounds in the dark red cell on the map have highest abundance in a sample. (b) PLS-DA shows a clear separation between tissues according to their sesquiterpenoid content. Root, shoot and rhizome tissues show te clear grouping with each other. Coarse and fine roots, leaflets and midribs show th clear clustering, and also overlapping data points. The two components show data with 60.3% explained variance. PLS model fitness: R^2X(cum) = 0.720, R^2Y(cum) = 0.740, Q^2(cum) = 0.709. (c) Total concentration of sesquiterpenoids tested (one-factor ANOVA, $P < 0.05$) between leaflets, midribs, rhizomes, coarse and fine root tissues. Tissues labelled with the same letter do not differ significantly at $P < 0.05$ (post-hoc test).

β-copaene, β-caryophyllene and β-ylangene, and root-specific compounds, such as β-sesquiphellandrene, β-farnesene and α-isocomene were present in the rhizome profile. PLS-DA showed clear separation of sesquiterpenoid content (Fig. 2b). The total concentration of sesquiterpenoids was significantly lower in midribs compared to rhizomes, leaflets and coarse and fine roots (Tukey's HSD, $P < 0.05$; Fig. 2c).

The leaflet and midrib samples were categorised into four sesquiterpenoid chemotype classes (Figure S2b). β-Copaene was dominant in all classes. Classes 2 and 3 contained unique compounds: 27% sabinolisovalerate and 13% valeranone in class 2, and 12% β-selinenol in class 3, as well as β-copaene (Figure S2b). Sesquiterpenoids present in coarse and fine roots also grouped into four chemotype classes (Figure S2c): β-sesquiphellandrene was most prevalent in each class, followed by β-farnesene, geranylisovalerate and β-isocomene. However, abundance of these compounds varied between classes (Figure S2c). A tanglegram plot revealed no direct link

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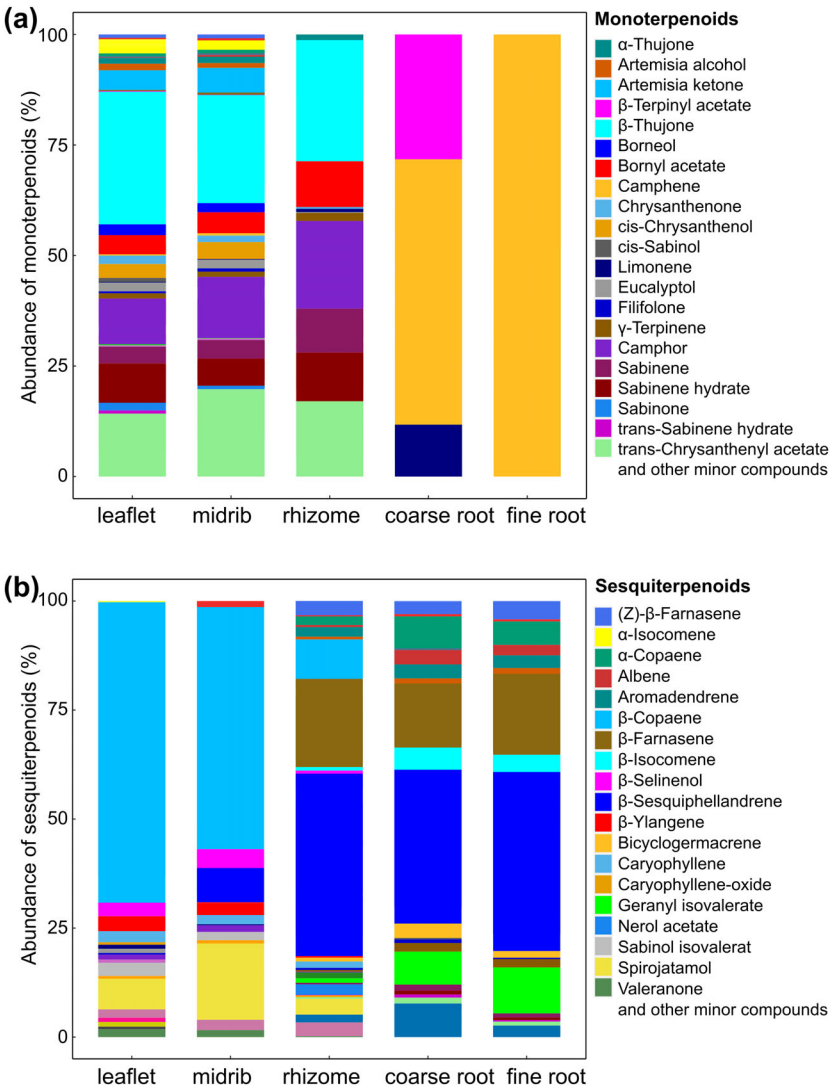

Fig. 3. Stacked bar plot showing content of monoterpenoids and sesquiterpenoids. (a) Cyan, light green, and purple indicate most dominant monoterpenoids in leaflet, midrib and rhizome tissues; orange, magenta and navy blue in root tissues. Fine root has only a single monoterpenoid compound. (b) Blue columns show the most dominant sesquiterpenoid in leaf and midrib and dark blue in coarse and fine root tissues. Minor mono- and sesquiterpenoid compounds are low in abundance and full names of those compounds are listed in Table S1.

between AG and BG chemotype classes, likely because sesquiterpenoids are synthesised by different terpene synthases in shoot and root tissues.

Metabolic atlas of terpenoids

The monoterpenoid content (Fig. 3a) shows that β-thujone, trans-chrysanthenyl acetate and camphor are dominant in leaflet, midrib and rhizome tissues. It is important to note that if, for example, β-thujone is dominant compound in leaflets, it is also dominant in midribs and rhizome of the same individuals, although concentration varies between these tissues. As camphene was the only monoterpenoid identified in fine roots, its abundance is represented as maximum. In comparison to limonene and β-terpinyl acetate, camphene was the dominant monoterpenoid in coarse roots. The sesquiterpenoid β-copaene was very abundant in leaflets and midribs but less in rhizomes. In roots and rhizomes, β-sesquiphellandrene was the most abundant sesquiterpenoid, followed by β-farnesene (Fig. 3b).

Exogenously applied pipecolic acid increased the induction of terpenoids

Following application of Pip there was an increase in total concentrations of mono- and sesquiterpenoids within 3 days of SAR stimulation in all tissues except rhizomes. However, the total concentration of monoterpenoids was significantly elevated by Pip application only in leaflets ($F_{x,y}$, $P = 0.006$), not in midribs, rhizomes, coarse or fine roots (Fig. 4a). In these tissues there was only a tendency towards higher monoterpenoid content. In contrast to monoterpenoids, sesquiterpenoids were significantly induced in Pip-treated plants in leaflets, midribs, coarse roots and fine roots (t test, $P < 0.001$), but not in rhizomes (Fig. 4b). Upon evaluation of each terpenoid, no

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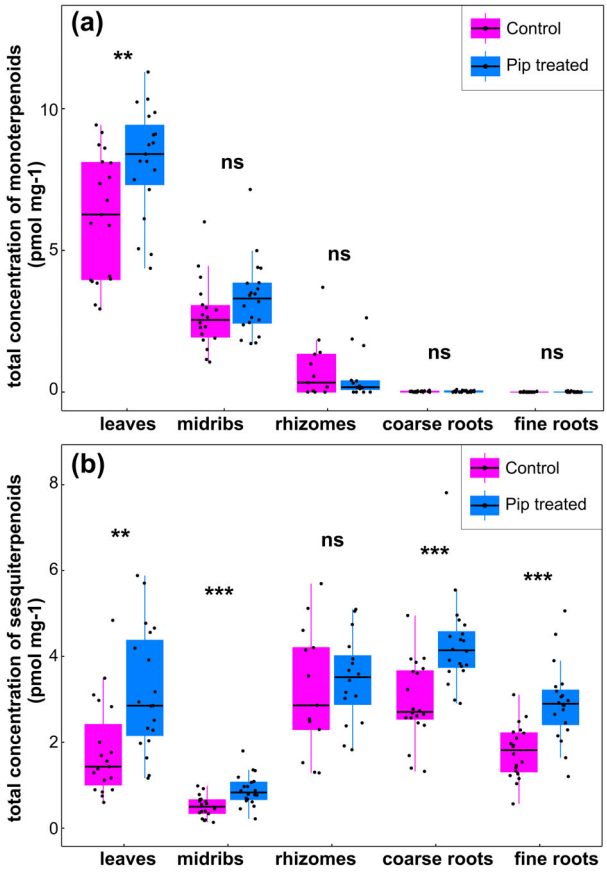

Fig. 4. (a) Total concentration of monoterpenoids is significantly higher in Pip-treated plants compared to control group for leaflets (*t* test, $P = 0.006$), but not for midrib, rhizome, coarse and fine roots. (b) Total concentration of sesquiterpenoids is significantly higher in Pip-treated plants than in control for all tissues except rhizomes (ns). ** $p < 0.01$, *** $p < 0.001$.

discernible differences were observed between terpenoids in the control and the Pip-treated group. Of the identified monoterpenoids, only π-cymene was significantly induced in leaves following Pip application (one-way ANOVA, $P = 0.05$; Fig. 5a). Notably, there were no novel sesquiterpenoids in plants treated with Pip. However, it is possible that levels of new compounds potentially synthesised following Pip treatment were very low or, instead of being directly emitted were stored, and consequently were below the limit of detection.

In contrast to monoterpenoids, numerous sesquiterpenoid concentrations were higher in the shoot and root tissues of Pip-treated plants than in controls. β-Copaene was the most abundant sesquiterpenoid in shoot tissues, with concentrations almost twice those in leaflets (one-way ANOVA, $P = 0.004$; Fig. 5a) and midribs (one-way ANOVA, $P < 0.001$; Fig. 5b) of the Pip-treated plants compared to those of the control group 3 days after the start of treatment. Furthermore, some low-abundance sesquiterpenoids, including β-ylangene, β-caryophyllene, and others, were significantly increased in shoot tissues of Pip-treated plants after 3 days (Fig. 5a, b, Table S1). Moreover, the sesquiterpenoid compounds mentioned above were absent in root tissues and therefore, could not be quantified statistically. Treatment with Pip led to significant increases in sesquiterpenoid content of root tissues compared to shoot tissues and rhizomes, in which concentrations only tended to increase. In addition to the dominant root sesquiterpenoids β-sesquiphellandrene and β-farnesene, 15 other sesquiterpenoid compounds were induced by Pip in coarse and fine roots of treated plants (Fig. 5c, d, Table S1). Statistical test results for each compound between Pip-treated and untreated plants are shown in Table S1.

DISCUSSION

This study investigated the effects on tansy of exogenous Pip application, which mimics effects of aphid infestation (Agrawal 1998; Kloth *et al.* 2016). A further aim was to create a metabolic atlas of terpenoid distribution and chemical diversity by examining terpenoid profiles in leaflets, leaf midribs, rhizome, coarse root and fine roots. The distribution of terpenoids between tissues and their concentrations varied considerably, confirming our initial hypothesis that there are tissue-specific differences in terpenoid composition of tansy. Contrary to our expectations, application of Pip induced only low levels of monoterpenoids in aboveground tissues, particularly leaflets, within 3 days of treatment initiation. In contrast, sesquiterpenoids were strongly induced, particularly in belowground organs. This finding supports our second hypothesis that sesquiterpenoids are induced as plant defence. Our data offer valuable insights into inducibility of terpenoid mixtures in tansy and provide evidence for the involvement of different terpenoid groups in different ecological interactions in above- and belowground tissues.

Our analysis confirms high intraspecific chemical diversity of terpenoids in the aboveground tissues of tansy. Furthermore, it shows that individuals can be categorised into chemotypic classes, in accordance with studies by Clancy *et al.* (2016), Rahimova *et al.* (2024) and Neuhaus-Harr *et al.* (2024). However, tansy roots also exhibit a high degree of intraspecific diversity in terpenoid profiles between tissues. While the aboveground tissues are generally high in monoterpenoids, levels in belowground tissues tend to be low (Kleine & Müller 2013; Muchlinski *et al.* 2019). This is not uncommon and is consistent with studies demonstrating that monoterpenoids are produced in roots in a highly cell type-specific manner (Chen *et al.* 2011). Despite their low abundance, monoterpenoids play an important role in root development and interaction of roots with the rhizosphere (Lin *et al.* 2007). In contrast to monoterpenoids, the distribution of sequiterpenoids across the tissues is markedly different. Our previous study (Rahimova *et al.* 2024) showed that sesquiterpenoid chemotypes defined for leaves (and now also in roots) show little variation between chemotypes, in contrast to the monoterpenoid chemotypes. Instead, there was a single dominant sesquiterpenoid, which varied slightly in abundance across all individuals. This indicates that monoterpenoid and sequiterpenoid chemotypes are formed independently in tansy, as recently corroborated for tansy chemotypes across Germany (Rahimova *et al.* 2024).

The metabolic atlas also indicates distinct patterns of sequiterpenoids in above- and belowground tissues. In particular, sesquiterpenoids are only detectable in roots or green tissues, corroborating the findings of Kleine & Müller (2013) of clear separation of mono- and sesquiterpenoids in tansy leaves and roots. The sequiterpenoids identified in their study were also detected in our study. Furthermore, additional sequiterpenoids, such as silphinene, modephene, β-isocomene and

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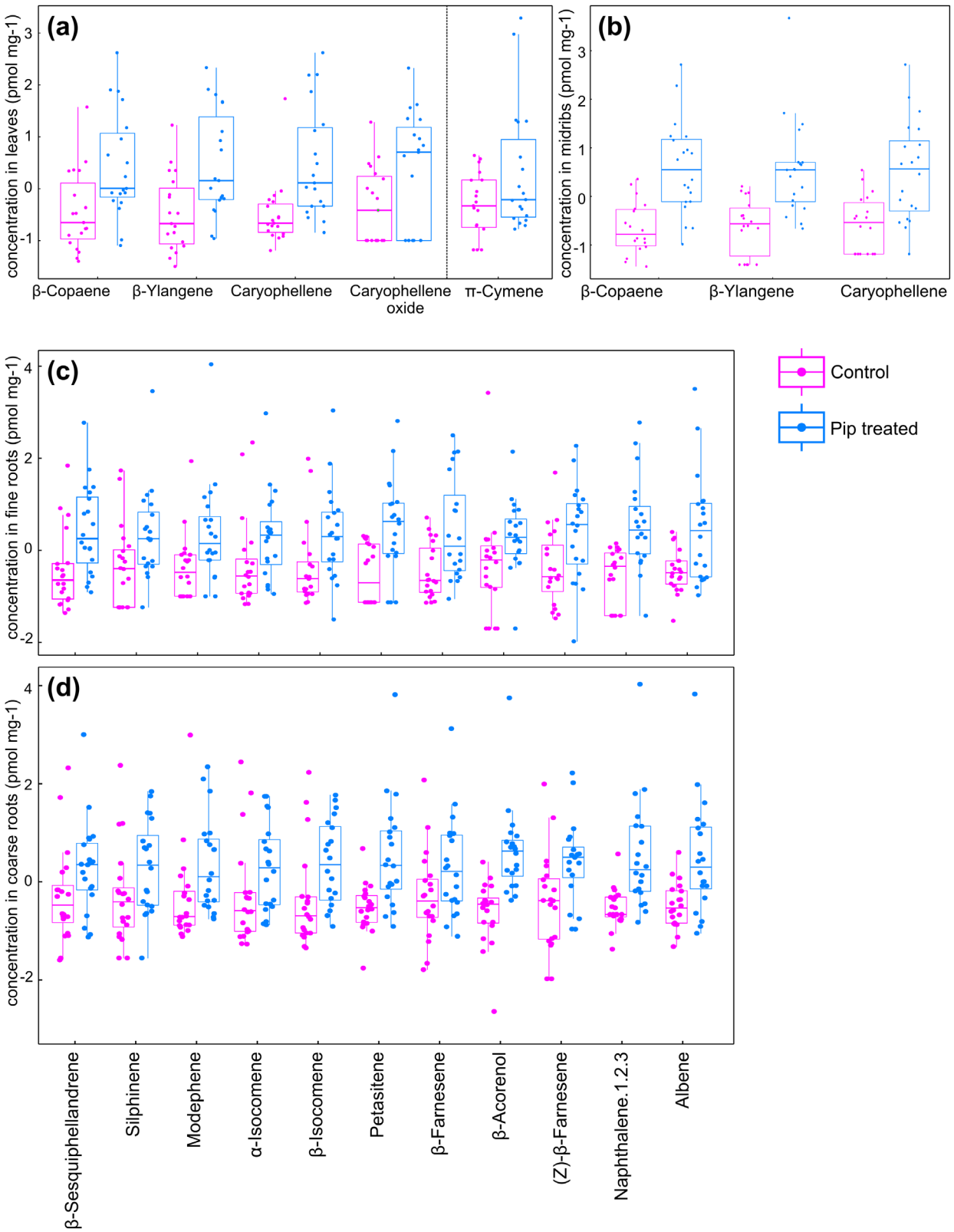

Fig. 5. Concentration of terpenoids significantly increased in Pip-treated plants in comparison to control plants. (a) Four sesquiterpenoid compounds, β-copaene, β-ylangene, caryophyllene, β-caryophyllene oxide (one-way ANOVA, $P < 0.05$) and one monoterpenoid, π-cymene ($P = 0.05$) in leaflets. (b) Three sesquiterpenoid compounds, β-copaene, β-ylangene, β-caryophyllene in midribs. (c) 12 sesquiterpenoid compounds in fine roots. (d) 15 sesquiterpenoid compounds in coarse roots are significantly induced in Pip-treated plants relative to control plants. Y-axis (concentration) is log-scale.

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many others, were detected in the present study, likely because of the larger number of plants examined here. Such distinct profiles of secondary metabolites have been observed in other plant species. For instance, in *Brassica oleracea* L., the quantity of glucosinolate metabolites differs between the shoot and roots, with their concentration being higher in roots than in shoots (Kabouw *et al.* 2010). Such variations can also be demonstrated for phenolic compounds. In *Oenothera biennis* L. shoot tissues contain chlorogenic acid and flavonoids that are not found in roots, while roots contain ellagic acids that are not found in shoots (Parker *et al.* 2012). The metabolite content of the meadow herbs *Centaurea jacea* L, *Knautia arvensis*, (L.) Coult., *Leucanthemum vulgare* Lam. and *Plantago lanceolata* L. exhibited significant differences between shoots and roots, where chemical diversity of secondary metabolites was higher in shoots than in roots (Ristok *et al.* 2019).

The differences in terpenoid profiles of tansy roots and shoots are likely related to the different abiotic and biotic pressures on specialised metabolites in aboveground and belowground tissues and physicochemical properties, such as volatility and solubility of the measured compounds (van Dam 2009; Kleine & Müller 2013; Mofikoya *et al.* 2019). Vapour pressure is a measure of rate of evaporation of a liquid at a given temperature. A vapour pressure below 0.005 kPa at 25 °C indicates semi-volatile compounds (Copolovici & Niinemets 2015), which differ from highly volatile VOCs (boiling point <100 °C) and VOCs with boiling point of 100–240 °C (Lucattini *et al.* 2018). Highly volatile monoterpenoids are typically stored in glands and glandular trichomes in aboveground tissues and evaporate rapidly. In contrast, semi-volatile compounds, such as sesquiterpenoids, have higher boiling points and low Henry's law constants (Mofikoya *et al.* 2019), making them less volatile and less soluble in water. Thus, sesquiterpenoids are not sufficiently volatile to be emitted from undisturbed tissues at high emission rates and are more likely concentrated in the rhizosphere, whereas highly hydrophilic substances, such as monoterpenoids, leach out more rapidly and dissipate into the soil (Qualls 2005). This explains why monoterpenoids are abundant in aboveground tissues, where they can be used to communicate with other organisms, including herbivores and their natural enemies or pollinators (Sasidharan *et al.* 2023; Ziaja & Müller 2023; Neuhaus-Harr *et al.* 2024). Although sesquiterpenoids are not as volatile, they are also induced by herbivory, depending on intensity of the damage.

Application of Pip led to increases in chemical subgroup and tissue-specific terpenoid levels. By mimicking biotic stress through introduction of aphids, we demonstrated that terpenoids, particularly sesquiterpenoids, significantly increased in all plant tissues, with the largest increase in root tissue in response to Pip. The induction of terpenoid biosynthesis and its emission have been reported in many other plants. In *Arabidopsis* monoterpenes (α/β-pinene and camphene) are increasingly emitted after SAR induction (Riedlmeier *et al.* 2017). In tomato (*Solanum lycopericum* L.), plant defence against infection with the Phytoplasma Potato Purple Top is more effective after application of the phytohormone SA (Wu *et al.* 2012). Exogenously applied Pip induced SAR in cucumber (*Cucumis sativus* L.) and enhanced activity of defence-associated enzymes against subsequent pathogen infection (Pazarlar *et al.* 2021).

Although the specific mechanisms of SAR induction in tansy have not yet been investigated and further studies are needed, especially regarding genetic regulation by specific terpene synthases (TPS) and other backbone modifying enzymes, the increasing concentration of terpenoids in leaflets, midribs and coarse and fine roots reflects an inducible defence response. However, this interpretation is complicated by the compartmentalisation of terpenoids in tansy. Tansy leaves, including midribs (the main target of aphid attack), are characterised by a high density of glandular cells, which serve as storage sites for monoterpenoids (Guerreiro *et al.* 2016; Bergman *et al.* 2023). Jakobs *et al.* (2018) showed plant organ-specific responses to aphid infestation, e.g., the composition of sugars and organic acids in tansy was more affected by aphids in phloem exudates of stems than in leaves. This shows that metabolic differences in different tansy organs play an important role in the tansy–insect interaction.

The presence of constitutively present monoterpenoid reservoirs, which can be used as stable chemical markers for differentiation of chemotypes (here and Clancy *et al.* 2016, Rahimova *et al.* 2024) makes it challenging to demonstrate induction of stress-induced monoterpenes. The available data indicate a weak tendency for an increase in monoterpenoid content following application of Pip, which suggests that formation of new monoterpenes was relatively low compared to the existing pool.

Terpene synthases (TPS) are, together with backbone modifying enzymes downstream, responsible for the great diversity of mono- and sesquiterpenoids. Terpene synthases catalyse the conversion of terpenoid compounds from isoprenoid precursors (Degenhardt *et al.* 2009; Chen *et al.* 2011). The TPS gene family has been identified in various plant species, including tansy leaves, and is characterised by mono- and sesquiterpenoid synthases (Clancy *et al.* 2020). It is likely that different TPS genes, which regulate organ-specific biosynthesis of terpenoids, are responsible for the observed differences in tissue-specific terpenoid patterns as well as for inducibility by Pip. For instance, in maize, different TPS enzymes are expressed exclusively in shoot or root tissues (Köllner *et al.* 2009). This work further demonstrates that these TPS enzymes produce sesquiterpenoids that are consistently induced by herbivores. As evidenced by β-caryophyllene, these sesquiterpenoids play a role in defence against the western corn rootworm by attracting insect-killing nematodes (Degenhardt *et al.* 2009). Most recently, Lackus *et al.* (2021) detected a TPS gene (*PtTPS5*) induced in poplar roots during pathogen infection, which also involves sequiterpenoids in the pathogen defence.

The patterns of terpenoids in the different tissues of tansy and their different inducibility by simulated SAR encourage further investigation of these patterns using functional genomics. Expression analysis of TPS with GUS (β-glucuronidase) reporter gene of *Artemisia annua* L., a species closely related to tansy, has provided information on possible distribution of sesquiterpenoids in plant tissues (Wang *et al.* 2013). The promoter activity of β-caryophyllene synthase at different stages of flower development was highest expression at full flowering. T-shaped trichomes, leaf primordia and leaf ribs of older leaves also show GUS staining, whereas there was no promoter activity in glandular cells. In roots, promoter activity was found in vascular tissue. TPS, such as α-farnesene synthase, are expressed in roots, flowers and leaves, but also in glandular cells. Other TPS show different expression patterns and are absent in roots. This shows that the metabolic atlas of terpenoid distribution in tansy presented here can probably be functionally explained by analysis of

the very heterogeneous tissue-specific activity of the TPS. Since it is not clear where terpenoids are localised in root and rhizome tissues, as no storage structures in roots have yet been reported, single cell metabolomics analyses might reveal specialised cell types in tansy. For example, Li *et al.* (2024) showed that a cell type-specific transcription factor regulates expression of two idioblast-specific biosynthetic genes in the monoterpenoid indole alkaloid (MIA) pathway of *Catharanthus roseus* L. (Madagascar periwinkle), providing insights into cell type-specific metabolic regulation. Single cell metabolomics of phloem tissues may be of particular importance, as phloem tissues are preferred by aboveground feeding insects (Jakobs *et al.* 2018).

With the tansy genome available in draft form (personal communication from Andrea Bräutigam, University of Bielefeld, Germany), the necessary prerequisites for a further study have been created to understand the role of tissue-specific chemodiversity and its inducibility in the interaction of tansy with surrounding communities. With such a study it will also be possible to functionally link terpenoid patterns to their role(s) in interactions with aphid, ant and arthropod metacommunities in general (Neuhaus-Harr *et al.* 2024; Ojeda-Prieto *et al.* 2024; Rahimova *et al.* 2024).

CONCLUSIONS

The present study demonstrates that terpenoid profiles exhibit significant variations in compound content and concentration between plant tissues, including leaflets, leaf midribs, rhizomes, coarse roots, and fine roots of tansy. These findings suggest that tansy shoot and root tissues are subject to distinct challenges imposed by contrasting abiotic and biotic factors, including belowground root herbivores, microorganisms, and aboveground insect populations. Furthermore, our findings indicate that pipecolic acid application, mimicking SAR induction, increases terpenoid levels in tansy tissues, suggesting enhanced defence responses that are likely regulated by specific enzymes. Our results highlight that the terpenoid diversity of tansy extends beyond intraspecific differences between individuals, but also occurs within individuals. Our study provides a foundation for further investigations into how the environment selects for chemical fingerprints in different plant tissues and, subsequently, how this mediates plant-associated communities.

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AUTHOR CONTRIBUTIONS

JPS conceived and designed the study. JPS, RH, and WWW reviewed, edited, and supervised the study. BW conducted the calibration and GC-MS analyses. HR performed the extraction, analysed the data, and wrote the manuscript, with substantial input from JPS, RH, and WWW.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. (a) Hierarchical cluster analysis shows that leaf and midrib tissues of control plants clustered into monoterpenoid chemotype classes based on their monoterpenoid profile. Four main classes identified, and each cluster is shown in a different colour; class 1 purple, class 2 orange, class 3 blue and class 4 magenta. Data are normalised to log scale. (b) The abundance of each representative compound of each class is presented in a stacked bar plot.

Figure S2. (a) Hierarchical cluster analysis shows that leaf and midrib samples clustered into aboveground chemotype classes (AG chemotypes) based on their sesquiterpenoid profile, shown in the left side tree with four different classes highlighted in different colours: From bottom to top: class 1 cyan, class 2 yellow, class 3 blue, class 4 magenta. Coarse and fine root samples clustered into belowground chemotypes (BG chemotypes), shown on the right side tree in four colours: class 1 purple, class 2 orange, class 3 forest green, class 4 light blue. The tanglegram shows relationships between AG and BG chemotype classes, e.g. individuals found in BG chemotypes are not connected with the same individuals in AG chemotypes. The abundance of sesquiterpenoids in AG (b) and BG (c) chemotypes is shown for each class in a stacked bar plot.

Table S1. Statistics (*F*-value) and *P*-value for individual terpenoid compounds tested between Pip-treated and untreated plants using one-way ANOVA. Bold letters indicate significant *P*-values ($P < 0.050$). Italic and green numbers indicate mean concentration ± SD of each compound. Dashes indicate absence of the compound in the respective compartment. NA indicates that these compounds were not detected in sufficient plant material to be tested for reliable statistical analysis.

REFERENCES

Agrawal A.A. (1998) Induced responses to herbivory and increased plant performance. *Science*, **279**, 1201–1202. https://doi.org/10.1126/science.279.5354.1201

Ali J.G., Agrawal A.A. (2014) Asymmetry of plant-mediated interactions between specialist aphids and caterpillars on two milkweeds. *Functional Ecology*, **28**, 1404–1412. https://doi.org/10.1111/1365-2435.12271

Bergman M.E., Franks A.E., Phillips M.A. (2023) Biosynthesis, natural distribution, and biological activities of acyclic monoterpenes and their derivatives. *Phytochemistry Reviews*, **22**, 361–384. https://doi.org/10.1007/s11101-022-09849-6

Bertić M., Orgel F., Gschwendtner S., Schloter M., Moritz F., Schmitt-Kopplin P., Zimmer I., Fladung M., Schnitzler J., Schroeder H., Ghirardo A. (2023) European oak metabolites shape digestion and fitness of the herbivore *Tortrix viridana*. *Functional Ecology*, **37**, 1476–1491. https://doi.org/10.1111/1365-2435.14299

Bertić M., Schroeder H., Kersten B., Fladung M., Orgel F., Buegger F., Schnitzler J.-P., Ghirardo A. (2021) European oak chemical diversity – From ecotypes to herbivore resistance. *New Phytologist*, **232**, 818–834. https://doi.org/10.1111/nph.17608

Bohlmann J., Meyer-Gauen G., Croteau R. (1998) Plant terpenoid synthases: molecular biology and phylogenetic analysis. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 4126–4133. https://doi.org/10.1073/pnas.95.8.4126

Brambilla A., Lenk M., Ghirardo A., Eccleston L., Knappe C., Weber B., Lange B., Imani J., Schäffner A.R., Schnitzler J.-P., Vlot A.C. (2023) Pipecolic acid synthesis is required for systemic acquired resistance and plant-to-plant-induced immunity in barley. *Journal of Experimental Botany*, **74**, 3033–3046. https://doi.org/10.1093/jxb/erad095

Chen F., Tholl D., Bohlmann J., Pichersky E. (2011) The family of terpene synthases in plants: a mid-size

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family of genes for specialized metabolism that is highly diversified throughout the kingdom. *The Plant Journal*, **66**, 212–229. https://doi.org/10.1111/j.1365-313X.2011.04520.x

Chen Y.-C., Holmes E.C., Rajniak J., Kim J.-G., Tang S., Fischer C.R., Mudgett M.B., Sattely E.S. (2018) N-hydroxy-pipecolic acid is a mobile metabolite that induces systemic disease resistance in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, E4920–E4929. https://doi.org/10.1073/pnas.1805291115

Choudhary A., Senthil-Kumar M. (2022) Drought attenuates plant defence against bacterial pathogens by suppressing the expression of CBP60g/SARD1 during combined stress. *Plant, Cell & Environment*, **45**, 1127–1145. https://doi.org/10.1111/pce.14275

Clancy M.V., Haberer G., Jud W., Niederbacher B., Niederbacher S., Senft M., Zytynska S.E., Weisser W.W., Schnitzler J.-P. (2020) Under fire – Simultaneous volatilome and transcriptome analysis unravels fine-scale responses of tansy chemotypes to dual herbivore attack. *BMC Plant Biology*, **20**, 551. https://doi.org/10.1186/s12870-020-02745-1

Clancy M.V., Zytynska S.E., Senft M., Weisser W.W., Schnitzler J.-P. (2016) Chemotypic variation in terpenes emitted from storage pools influences early aphid colonisation on tansy. *Scientific Reports*, **6**, 38087. https://doi.org/10.1038/srep38087

Copolovici L., Niinemets U. (2015) Temperature dependencies of Henry's law constants for different plant sesquiterpenes. *Chemosphere*, **138**, 751–757. https://doi.org/10.1016/j.chemosphere.2015.07.075

van Dam N.M. (2009) Belowground herbivory and plant defenses. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 373–391. https://doi.org/10.1146/annurev.ecolsys.110308.120314

Degenhardt J., Köllner T.G., Gershenzon J. (2009) Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry*, **70**, 1621–1637. https://doi.org/10.1016/j.phytochem.2009.07.030

Dicke M., Baldwin I.T. (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science*, **15**, 167–175. https://doi.org/10.1016/j.tplants.2009.12.002

Dussarrat T., Schweiger R., Ziaja D., Nguyen T.T.N., Krause L., Jakobs R., Eilers E.J., Müller C. (2023) Influences of chemotype and parental genotype on metabolic fingerprints of tansy plants uncovered by predictive metabolomics. *Scientific Reports*, **13**, 11645. https://doi.org/10.1038/s41598-023-38790-7

Erb M., Reymond P. (2019) Molecular interactions between plants and insect herbivores. *Annual Review of Plant Biology*, **70**, 527–557. https://doi.org/10.1146/annurev-arplant-050718-095910

Frank L., Wenig M., Ghirardo A., Vlot A.C., Schnitzler P., Rosenkranz M. (2021) Isoprene and β-caryophyllene confer plant resistance via different plant internal signalling pathways. *Plant, Cell & Environment*, **44**, 1151–1164. https://doi.org/10.1111/pce.14010

Guerreiro K.K., Bobek V., Santos V.L.P., Franco C.R.C., Paula J.P., Farago P.V., Budel J.M. (2016) Análise farmacobotânica de folha e caule de *Tanacetum vulgare* (*L.*). *Revista Brasileira de Plantas Medicinais*, **18**, 89–95. https://doi.org/10.1590/1983-084X/15_046

Guo Y., Ghirardo A., Weber B., Schnitzler J.-P., Benz J.P., Rosenkranz M. (2019) *Trichoderma* species differ in their volatile profiles and in antagonism toward ectomycorrhiza *Laccaria bicolor*. *Frontiers in Microbiology*, **10**, 891. https://doi.org/10.3389/fmicb.2019.00891

Guo Y., Jud W., Ghirardo A., Antritter F., Benz J.P., Schnitzler J.-P., Rosenkranz M. (2020) Sniffing fungi – Phenotyping of volatile chemical diversity in *Trichoderma* species. *New Phytologist*, **227**, 244–259. https://doi.org/10.1111/nph.16530

Hartmann M., Zeier T., Bernsdorff F., Reichel-Deland V., Kim D., Hohmann M., Scholten N., Schuck S., Bräutigam A., Hölzel T., Ganter C., Zeier J. (2018) Flavin monooxygenase-generated N-hydroxypipecolic acid is a critical element of plant systemic immunity. *Cell*, **173**, 456–469.e16. https://doi.org/10.1016/j.cell.2018.02.049

Irulappan V., Kandpal M., Saini K., Rai A., Ranjan A., Sinharoy S., Senthil-Kumar M. (2022) Drought stress exacerbates fungal colonization and endodermal invasion and dampens defense responses to increase dry root rot in Chickpea. *Molecular Plant-Microbe Interactions*, **35**, 583–591. https://doi.org/10.1094/MPMI-07-21-0195-FI

Jakobs R., Müller C. (2018) Effects of intraspecific and intra-individual differences in plant quality on preference and performance of monophagous aphid species. *Oecologia*, **186**, 173–184. https://doi.org/10.1007/s00442-017-3998-x

Jakobs R., Schweiger R., Müller C. (2018) Aphid infestation leads to plant part-specific changes in phloem sap chemistry, which may indicate niche construction. *New Phytologist*, **221**, 503–514. https://doi.org/10.1111/nph.15335

Kabouw P., Biere A., van der Putten W.H., van Dam N.M. (2010) Intra-specific differences in root and shoot glucosinolate profiles among white cabbage (*Brassica oleracea* var. *capitata*) cultivars. *Journal of Agricultural and Food Chemistry*, **58**, 411–417. https://doi.org/10.1021/jf902835k

Kassambara A., Mundt F. (2020) factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7 https://doi.org/10.32614/CRAN.package.factoextra

Kleine S., Müller C. (2011) Intraspecific plant chemical diversity and its relation to herbivory. *Oecologia*, **166**, 175–186. https://doi.org/10.1007/s00442-010-1827-6

Kleine S., Müller C. (2013) Differences in shoot and root terpenoid profiles and plant responses to fertilisation in *Tanacetum vulgare*. *Phytochemistry*, **96**, 123–131. https://doi.org/10.1016/j.phytochem.2013.09.018

Kloth K.J., Wiegers G.L., Busscher-Lange J., van Haarst J.C., Kruijer W., Bouwmeester H.J., Dicke M., Jongsma M.A. (2016) AtWRKY22 promotes susceptibility to aphids and modulates salicylic acid and jasmonic acid signalling. *Journal of Experimental Botany*, **67**, 3383–3396. https://doi.org/10.1093/jxb/erw159

Köllner T.G., Gershenzon J., Degenhardt J. (2009) Molecular and biochemical evolution of maize terpene synthase 10, an enzyme of indirect defense. *Phytochemistry*, **70**, 1139–1145. https://doi.org/10.1016/j.phytochem.2009.06.011

Lackus N.D., Morawetz J., Xu H., Gershenzon J., Dickschat J.S., Köllner T.G. (2021) The sesquiterpene synthase PtTPS5 produces (1S,5S,7R,10R)-guaia-4(15)-en-11-ol and (1S,7R,10R)-guaia-4-en-11-ol in Oomycete-infected poplar roots. *Molecules*, **26**, 555. https://doi.org/10.3390/molecules26030555

Lenk M., Wenig M., Bauer K., Hug F., Knappe C., Lange B., Timsy Häußler F., Mengel F., Dey S., Schäffner A., Vlot A.C. (2019) Pipecolic acid is induced in barley upon infection and triggers immune responses associated with elevated nitric oxide accumulation. *Molecular Plant-Microbe Interactions*, **32**, 1303–1313. https://doi.org/10.1094/MPMI-01-19-0013-R

Li C., Colinas M., Wood J.C., Vaillancourt B., Hamilton J.P., Jones S.L., Caputi L., O'Connor S.E., Buell C.R. (2024) Cell-type aware regulatory landscapes governing monoterpene indole alkaloid biosynthesis in the medicinal plant *Catharanthus roseus*, bioRxiv 2024.04.23.590703. https://doi.org/10.1101/2024.04.23.590703.

Lin C., Owen S.M., Peñuelas J. (2007) Volatile organic compounds in the roots and rhizosphere of *Pinus* spp. *Soil Biology and Biochemistry*, **39**, 951–960. https://doi.org/10.1016/j.soilbio.2006.11.007

Lucattini L., Poma G., Covaci A., de Boer J., Lamoree M.H., Leonards P.E.G. (2018) A review of semi-volatile organic compounds (SVOCs) in the indoor environment: occurrence in consumer products, indoor air and dust. *Chemosphere*, **201**, 466–482. https://doi.org/10.1016/j.chemosphere.2018.02.161

Mofikoya A.O., Bui T.N.T., Kivimäenpää M., Holopainen J.K., Himanen S.J., Blande J.D. (2019) Foliar behaviour of biogenic semi-volatiles: potential applications in sustainable pest management. *Arthropod-Plant Interactions*, **13**, 193–212. https://doi.org/10.1007/s11829-019-09676-1

Muchlinski A., Chen X., Lovell J.T., Köllner T.G., Pelot K.A., Zerbe P., Ruggiero M., Callaway L., Laliberte S., Chen F., Tholl D. (2019) Biosynthesis and emission of stress-induced volatile terpenes in roots and leaves of switchgrass (*Panicum virgatum* L.). *Frontiers in Plant Science*, **10**, 1144. https://doi.org/10.3389/fpls.2019.01144

Návarová H., Bernsdorff F., Döring A.-C., Zeier J. (2013) Pipecolic acid, an endogenous mediator of defense amplification and priming, is a critical regulator of inducible plant immunity. *The Plant Cell*, **24**, 5123–5141. https://doi.org/10.1105/tpc.112.103564

Neuhaus-Harr A., Ojeda-Prieto L., Eilers E., Müller C., Weisser W.W., Heinen R. (2024) Chemodiversity affects preference for *Tanacetum vulgare* chemotypes in two aphid species. *Oikos*, **2024**, e10437. https://doi.org/10.1111/oik.10437

Ojeda-Prieto L., Medina-van Berkum P., Unsicker S.B., Heinen R., Weisser W.W. (2024) Intraspecific chemical variation of *Tanacetum vulgare* affects plant growth and reproductive traits in field plant communities. *Plant Biology*. Early view. https://doi.org/10.1111/plb.13646

Pang Z., Chong J., Zhou G., de Lima Morais D.A., Chang L., Barrette M., Gauthier C., Jacques P.-É., Li S., Xia J. (2021) MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Research*, **49**(W1), W388–W396. https://doi.org/10.1093/nar/gkab382

Parker J.D., Salminen J.-P., Agrawal A.A. (2012) Evolutionary potential of root chemical defense: genetic correlations with shoot chemistry and plant growth. *Journal of Chemical Ecology*, **38**, 992–995. https://doi.org/10.1007/s10886-012-0163-1

Pazarlar S., Sanver U., Cetinkaya N. (2021) Exogenous pipecolic acid modulates plant defence responses against *Podosphaera xanthii* and *Pseudomonas syringae* pv. *Lachrymans* in cucumber (*Cucumis sativus* L.). *Plant Biology*, **23**, 473–484. https://doi.org/10.1111/plb.13243

14388677, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/plb.13703 by Helmholtz Zentrum Muenchen Deutsches Forschungszentrum, Wiley Online Library on [01/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Penuelas J., Asensio D., Tholl D., Wenke K., Rosenkranz M., Piechulla B., Schnitzler J.-P. (2014) Biogenic volatile emissions from the soil. *Plant, Cell & Environment*, **37**, 1866–1891. https://doi.org/10.1111/pce.12340

Pichersky E., Raguso R.A. (2018) Why do plants produce so many terpenoid compounds? *New Phytologist*, **220**, 692–702. https://doi.org/10.1111/nph.14178

Poelman E.H., Kessler A. (2016) Keystone herbivores and the evolution of plant defenses. *Trends in Plant Science*, **21**, 477–485. https://doi.org/10.1016/j.tplants.2016.01.007

Qualls R.G. (2005) Biodegradability of fractions of dissolved organic carbon leached from decomposing leaf litter. *Environmental Science & Technology*, **39**, 1616–1622. https://doi.org/10.1021/es049090o

Rahimova H., Neuhaus-Harr A., Clancy M.V., Guo Y., Junker R.R., Ojeda-Prieto L., Petrén H., Senft M., Zytynska S.E., Weisser W.W., Heinen R., Schnitzler J.-P. (2024) Geographic distribution of terpenoid chemotypes in *Tanacetum vulgare* mediates tansy aphid occurrence but not abundance. *Oikos*, **e10320**. https://doi.org/10.1111/oik.10320

Rasmann S., Hiltpold I., Ali J. (2012) The role of root-produced volatile secondary metabolites in mediating soil interactions. In: Montanaro G., Cichio B. (Eds), *Advances in selected plant physiology aspects*. InTech Open Access Publisher, Rijeka, Croatia, pp 269–290. https://doi.org/10.5772/34304

Riedlmeier M., Ghirardo A., Wenig M., Knappe C., Koch K., Georgii E., Dey S., Parker J.E., Schnitzler J.-P., Vlot A.C. (2017) Monoterpenes support systemic acquired resistance within and between plants. *The Plant Cell*, **29**, 1440–1459. https://doi.org/10.1105/tpc.16.00898

Ristok C., Poeschl Y., Dudenhöffer J., Ebeling A., Eisenhauer N., Vergara F., Wagg C., van Dam N.M., Weinhold A. (2019) Plant species richness elicits changes in the metabolome of grassland species via soil biotic legacy. *Journal of Ecology*, **107**, 2240–2254. https://doi.org/10.1111/1365-2745.13185

Sasidharan R., Junker R.R., Eilers E.J., Müller C. (2023) Floral volatiles evoke partially similar responses in both florivores and pollinators and are correlated with non-volatile reward chemicals. *Annals of Botany*, **132**, 1–14. https://doi.org/10.1093/aob/mcad064

Tholl D. (2006) Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Current Opinion in Plant Biology*, **9**, 297–304. https://doi.org/10.1016/j.pbi.2006.03.014

Vlot A.C., Dempsey D.A., Klessig D.F. (2009) Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology*, **47**, 177–206. https://doi.org/10.1146/annurev.phyto.050908.135202

Vlot A.C., Sales J.H., Lenk M., Bauer K., Brambilla A., Sommer A., Chen Y., Wenig M., Nayem S. (2021) Systemic propagation of immunity in plants. *New Phytologist*, **229**, 1234–1250. https://doi.org/10.1111/nph.16953

Voelckel C., Weisser W.W., Baldwin I.T. (2004) An analysis of plant-aphid interactions by different microarray hybridization strategies. *Molecular Ecology*, **13**, 3187–3195.

Walling L.L. (2000) The myriad plant responses to herbivores. *Journal of Plant Growth Regulation*, **19**, 195–216. https://doi.org/10.1007/s003440000026

Wang H., Han J., Kanagarajan S., Lundgren A., Brodelius P.E. (2013) Studies on the expression of sesquiterpene synthases using promoter-β-glucuronidase fusions in transgenic *Artemisia annua* L. *PLoS One*, **8**, e80643. https://doi.org/10.1371/journal.pone.0080643

Wenig M., Ghirardo A., Sales J.H., Pabst E.S., Breitenbach H.H., Antritter F., Weber B., Lange B., Lenk M., Cameron R., Schnitzler J.-P., Vlot A.C. (2019) Systemic acquired resistance networks amplify airborne defense cues. *Nature Communications*, **10**, 3813. https://doi.org/10.1038/s41467-019-11798-2

Wetzel W.C., Whitehead S.R. (2020) The many dimensions of phytochemical diversity: linking theory to practice. *Ecology Letters*, **23**, 16–32. https://doi.org/10.1111/ele.13422

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

Wu W., Ding Y., Wei W., Davis R.E., Lee I.-M., Hammond R.W., Zhao Y. (2012) Salicylic acid-mediated elicitation of tomato defence against infection by potato purple top phytoplasma. *Annals of Applied Biology*, **161**, 36–45. https://doi.org/10.1111/j.1744-7348.2012.00550.x

Zhang Y., Li P., She G., Xu Y., Peng A., Wan X., Zhao J. (2021) Molecular basis of the distinct metabolic features in shoot tips and roots of tea plants (*Camellia sinensis*): characterization of MYB regulator for root theanine synthesis. *Journal of Agricultural and Food Chemistry*, **69**, 3415–3429. https://doi.org/10.1021/acs.jafc.0c07572

Ziaja D., Müller C. (2023) Intraspecific chemodiversity provides plant individual- and neighbourhood-mediated associational resistance towards aphids. *Frontiers in Plant Science*, **14**, 1145918. https://doi.org/10.3389/fpls.2023.1145918

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