



Aphid Colony Size in Tansy is Affected by Plant Chemical Composition but not by Belowground Herbivory

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Abstract

Plants are hosts for above- and belowground insect communities that can influence each other via above-belowground plant-physiological dynamics. To mediate interactions, plants produce secondary metabolites, including terpenoids, and mixtures can differ intraspecifically. While intraspecific variation in plant chemistry gained increased interest, the extent to which intraspecific differences in plant chemistry mediate above-belowground interactions of herbivores remains unclear. We used a full factorial design with six distinct terpenoid chemotypes, differing in their chemical diversity of tansy (*Tanacetum vulgare*). We exposed these to the aboveground herbivore *Macrosiphoniella tanacetaria* (Hemiptera: Aphididae), the belowground herbivore *Agriotes* sp. (Coleoptera: Elateridae), no herbivore or both herbivores, to determine if chemotypes or the chemical diversity of plant compounds affected aphid performance and if the interactions between herbivores were mediated by the chemical profile. We found that aphid colony size differed between chemotypes, with the strongest colony increase over time in a mixed chemotype, and the weakest in a β -thujone chemotype. Root herbivory had no effect on aphid colony size, regardless of the chemotype. Aphid colony size was positively correlated with terpenoid evenness, but not with terpenoid Shannon diversity, terpenoid richness, or relative terpenoid concentration. Tansy chemotypes differed in their morphological responses (final plant height and final plant dry weight) and average leaf chlorophyll content to aboveground herbivory, whereas belowground herbivory exerted minimal impacts. Overall, our results show that intraspecific variation in terpenoid profiles directly modify ecological interactions on a plant, with plant chemistry mediating aphid performance and chemotypes differing in their morphological responses to herbivory.

Keywords Herbivory · Intraspecific chemodiversity · Terpenoids · Plant–insect interactions · *Tanacetum vulgare*

Introduction

Plants play a central role in multitrophic interactions, serving as hosts for complex insect communities across trophic levels. Specialised plant metabolites are important for regulating interactions between plants and their living environment (Agrawal & Weber 2015). Within a single plant species, individuals can exhibit differences in specialised

metabolite profiles (Weng et al. 2021), and this intraspecific variation can lead to significant differences in the outcome of interactions within plant species (Bączek et al. 2019; Christensen et al. 2019; Kleine & Müller 2011; Rahimova, Neuhaus-Harr, et al., 2024; Schoonhoven et al. 2005). How different aspects of plant chemical profiles, particularly their metabolic diversity, relate to ecological plant interactions is currently receiving a lot of interest (Jakobs & Müller 2018; Kessler & Kalske 2018; Petrén, Anaia, et al., 2023a; Petrén, Köllner, et al. 2023b; Richards et al. 2015; Wetzal & Whitehead 2020; Whitehead et al. 2021; Ziaja & Müller 2023). For instance, in a recent study, the terpenoid diversity and distinct composition of terpenoid mixtures in tansy plants (*Tanacetum vulgare*) affected host preference of specialised tansy aphids in choice assays (Neuhaus-Harr et al., 2024). *Macrosiphoniella tanacetaria* aphids preferred the chemotypes dominated by α -thujone/ β -thujone and

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β -trans-chrysanthenyl acetate, while avoiding the chemotype with a mixed terpenoid profile (Neuhaus-Harr et al., 2024).

Chemotypes refer to groups of plants of the same species that can be differentiated based on the (typically heritable) composition of specialized compounds within a specific, ecologically relevant class (Müller et al. 2020). For example, individuals of Common Tansy (*Tanacetum vulgare*) can be categorized into chemotypes based on their terpenoid composition, and are often named by dominant monoterpenoids. In a recent study, this was done for monoterpenoids such as α -thujone, camphor, and trans-chrysanthenyl acetate, and sesquiterpenoids, such as bicyclosesquiphellandrene, longiverbenone, and calarene (Rahimova, Neuhaus-Harr, et al., 2024). These chemotypes may be characterized by the dominance of one or several key compounds or by diverse blends without a single predominant compound, resulting in mixed chemotypes (Dussarrat et al. 2023). Single compounds and mixtures are known to affect aphid colonies. Senft et al. (2019) have found that a chemotype characterized by L-camphor, β -terpineol and eucalyptol boost colony size in *Metopeurum fuscoviride* aphids. In another experiment, *M. tanacetaria* and *Uroleucon tanacetii* aphids both preferred chemotypes dominated by β -thujone, over trans-carvyl acetate (Jakobs & Müller, 2018).

The chemical diversity (i.e., diversity of chemical compounds) of a plant individual can be described in a number of ways, including by the distinct difference of chemical profiles, but can further be described by its three main diversity components: richness, evenness, and disparity (Petrén, Köllner, et al. 2023b). Chemical richness, a straightforward measure of phytochemical diversity, refers to the number of compounds in a tissue. It is hypothesised that chemically richer plants benefit when having e.g. multiple herbivore species as attackers, compared with plants that produce fewer compounds (Junker 2016). Chemical evenness describes the number of compounds and takes into account their relative abundance. Evidence also exists for chemical evenness to affect interactions between plants and insects. For example, specialised tansy aphids tend to avoid tansy plants with higher terpenoid evenness levels (Neuhaus-Harr et al., 2024). Chemical disparity considers the qualitative differences of tissues in terms of chemical compounds that are present, but to date very few studies have taken the ecological role of chemical disparity into account (Petrén, Köllner, et al. 2023b). Though numerous studies provide valuable insights into different aspects of plant chemistry and its role in ecology (Dyer 2018; Junker 2018), we still lack a comprehensive understanding of how different components of plant chemical diversity shape plant–insect interactions and which aspects are most relevant as mediators of plant–herbivore interactions (Petrén, Köllner, et al. 2023b).

While the effects of secondary metabolites on plant–herbivore interactions are documented, less is known about how intraspecific differences in chemical profiles affect the interactions between multiple simultaneous attackers on the same plant, especially if these herbivores feed on different plant parts. It is plausible that plant chemotype composition may determine the outcome of above-belowground herbivore interactions on the same plant. Aboveground and belowground herbivores can induce local and/or systemic defences in plants, leading to altered plant metabolism, changes in plant morphology, or resource allocation towards defence (Lehndal & Ågren 2015; Maron & Crone 2006; Zhou et al. 2015). This, in turn, can affect herbivores feeding on other plant parts. For example, root-feeding herbivores such as the endo-parasitic nematode species *Pratylenchus penetrans* or the larvae of the cabbage root fly (*Delia radicum*) significantly alter the nutritional quality of plant shoots in *Brassica nigra*, through changes in glucosinolate levels, which in turn negatively affect the growth and reproduction rate of caterpillars of the small cabbage white, *Pieris rapae* (Van Dam et al. 2005). According to a meta-analysis, the outcome of above-belowground herbivore interactions depends on multiple factors such as herbivore feeding guild (Johnson et al. 2012). For instance, belowground chewing larvae of beetle species had a positive effect on aboveground Homoptera, such as aphids, but a negative effect on aboveground Hymenoptera (Johnson et al. 2012). Furthermore, Yang et al. (2024) recently suggested that species-specific plant responses to herbivores are more important than herbivore identity or herbivore specialization in determining the plant response to sequential attacks. How these interactions are affected by intraspecific differences in plant chemistry, are not yet fully understood.

Insect herbivores, above- and below-ground typically have multiple negative effects on plants. In their review, Nability et al. (2009) point out that herbivory reduces photosynthetic rates due to tissue loss and disruption of photosynthesis around the missing tissue. Herbivory also reduces plant size, growth, and seed production (Hodkinson & Hughes 1982; Myers & Sarfraz 2017). It remains unclear, how chemical diversity of plants might mitigate these effects, as plants that differ in their chemical composition could also differ in their resistance and resilience to above- or belowground herbivory.

This study uses *Tanacetum vulgare* L. (Asteraceae), a perennial plant known for its variable aromatic terpenoid composition. Tansy has a wide geographical distribution and hosts a diverse community of herbivores, including aphids with varying host specificity (Keskitalo et al. 2001; Kleine & Müller 2011; Schmitz 1998). Tansy plants are characterised by their richness in mono- and sesquiterpenoids and can be classified

into chemotypes based on their terpenoid composition (Keskitalo et al. 2001; Kleine & Müller 2011). It is hypothesised that specialised aphids have adapted to the potentially harmful metabolites in tansy and may even use plant volatiles to locate their hosts (Jakobs & Müller 2019; Schoonhoven et al. 2005). Aphid preference, colonisation, growth rate, survival, and genotype structure have been partially attributed to the chemotypes of tansy (Benedek et al. 2015; Clancy et al. 2018; Neuhaus-Harr et al., 2024; Senft et al. 2017, 2019; Zytynska et al. 2019). For example, it has been found that when given the choice between different chemotypes, the tansy aphid *Macrosiphoniella tanacetaria* preferred the two chemotypes dominated by trans-chrysanthenyl acetate (Chrys_acet) and α -thujone/ β -thujone (Athu_Bthu) over the others (Neuhaus-Harr et al., 2024).

Using six biologically replicated *T. vulgare* chemotypes that differ in their leaf terpenoid composition, total terpenoid concentration, terpenoid richness, terpenoid evenness and Shannon diversity, we test the effects of the presence of generalist belowground root herbivores (wireworm larvae: a mixture of *Agriotes lineatus* and *Agriotes obscurus*, Coleoptera—Elateridae) on the aboveground herbivore performance of the tansy aphid *M. tanacetaria* (Hemiptera – Aphididae) and whether chemotypes mitigate these relationships. Furthermore, we test whether the effects of herbivory on the plant morphology differ between chemotypes. We address the following hypotheses:

(H1) We expect aphids to perform best on the chemotypes they preferred in choice assays in a previous study, i.e., trans-chrysanthenyl acetate (Chrys_acet) and α -thujone/ β -thujone (Athu_Bthu) chemotypes (Neuhaus-Harr et al., 2024).

(H2) Belowground coleopteran herbivores will positively affect aphid colony size and colony growth (as suggested in a meta-analysis by Johnson et al. 2012), but these relationships will differ in their strength between chemotypes.

(H3) More chemically diverse plants (i.e., higher terpenoid richness, higher terpenoid evenness, and higher terpenoid Shannon diversity index) and plants with higher terpenoid concentration will result in smaller aphid colonies but the interaction with belowground treatment will modify this relationship.

(H4) Above- and belowground herbivores will have a detrimental effect on plant growth and morphology, but the strength of these effects differs across chemotypes. Specifically, plants infested with both herbivores will have the least chlorophyll content in their leaves, grow less tall, and have lower dry weight compared to plants with only one or no herbivore, but we predict that chemically less diverse plants will suffer less from herbivory as they possibly use more resources towards growth and not defence.

Methods and Materials

Plant Material

In 2019, 27 tansy plants were collected in different fields in Jena, Germany, and their terpenoid profiles were analysed to determine chemotypes (described in Neuhaus-Harr et al. 2024). Briefly, leaf material was freeze-dried, homogenised and weighed and by adding one-bromodecane as internal standard, terpenoids were extracted in heptane. Extracts were centrifuged and by using gas chromatography and mass spectrometry, supernatants were analysed with Helium as carrier gas, using an alkane standard mix as a reference. Retention indices, and mass spectra were compared with compounds in Pherobase (El-Sayed 2012), entries of the National Institute of Standards and Technology 2014 and mass spectra reported in Adams (2017). Using unsupervised hierarchical k-means clustering with the 'hclust()' function, the plants were grouped into seven clusters based on their terpenoid profiles ($k = 7$). We selected six clusters, with two plants per cluster (twelve mother plants). Seeds from these mothers were used to generate the daughters from which we selected plants for this experiment. From twelve mothers, we grew ten seedlings each, leading to 120 daughter plants. After chemotyping these 120 plants (for details see Neuhaus-Harr et al. 2024), three plants were selected from the same mother in each of six clusters. Further details regarding the characterization of these established chemotype lines are described in Neuhaus-Harr et al. 2024. Chemotypes varied in their dominant compound(s), total terpenoid concentration, terpenoid richness, terpenoid evenness, and terpenoid Shannon diversity (Fig. 5 in the Supplementary Information, from Neuhaus-Harr et al. 2024). Chemotype terpenoid profiles ranged in compound richness between 21–29 terpenoid compounds, and based on their relative concentration, diversity components were calculated for each daughter plant from their terpenoid profiles, using `diversity()` from the 'vegan' package (Oksanen et al. 2022).

Propagation of Plant Material

In May 2022, 40 shoot cuttings were taken from each of the three daughter lines from the six selected chemotypes, which were maintained in a common garden in Freising, Germany. The stems of fresh plants were cut into parts with 1–2 cm below and 4–5 cm above a leaf node. The leaf size was reduced by clipping the pinnate leaves to decrease evaporation and the risk of mould. The cuttings were then planted into seedling trays filled with standard potting substrate (Stender potting substrate C 700 coarse structure, 1 kg

NPK minerals m-3, pH 5.5–6.0). All cuttings were kept in a greenhouse with bottom watering and additional lighting (16:00:8:00 h L:D) following standard protocols described in Neuhaus-Harr 2024. Three weeks later, 25 rooted cuttings from each daughter were transplanted into individual 11 cm-diameter pots. To maintain a target electrical conductivity of 1.0 dS/m, the plants were fertilised with Universol Blue fertiliser (18% N – 11% P – 18% K; ICL Deutschland). In July, all plants were repotted into 19 cm pots to avoid pot limitation. Clones from the same daughter line were grown in pots randomly distributed over different tables in the greenhouse to avoid initial growth bias due to environmental variation within the greenhouse. After the plants were well established, we placed the pots into a covered vegetation hall with iron mesh (5 cm) walls. From each chemotype, we randomly selected 40 established cuttings (with 13–14 out of the 25 cloned individuals from each of the three daughter lines; see Table 5). Clones were obtained by taking stem cuttings from the respective daughter, which were propagated as mentioned above. More details on the individual terpenoid composition of each chemotype, as well as their terpenoid Shannon diversity, terpenoid evenness, terpenoid richness and terpenoid concentration can be found in the supplement (Fig. 5). While the chemotypes Bthu_High and Bthu_Low were dominated by β -thujone, the chemotype Athu_Bthu was dominated by α - and β -thujone. Chrys_Acet was dominated by trans-chrysanthenyl acetate. Both mixed chemotypes (Mixed_High and Mixed_Low) have multiple compounds that are more even in concentration (Fig. 5a). Consequently, the mixed chemotypes also show a significant higher terpenoid Shannon diversity (Fig. 5b) and terpenoid evenness (Fig. 5c). Chemotypes did not significantly differ in their terpenoid richness (Fig. 5d), but the chemotypes Bthu_Low and Mixed_Low had a lower relative terpenoid concentration (Fig. 5e).

Experimental Design

We established a fully factorial design with either no herbivore, only the aboveground herbivore (aphid *Macrosiphoniella tanacetaria*), only the belowground herbivore (wireworm *Agriotes* sp), or both herbivores. Each plant was a priori assigned to one of four different treatments and arranged in a block design with 10 replicated blocks, totaling 240 plants (2 aboveground treatment levels (aphid/no aphid) $\times$ 2 belowground treatment levels (wireworm/no wireworm) $\times$ 6 chemotypes $\times$ 3 biologically replicated daughters each with 3 or 4 clonal replicates; totaling 10 replicates per chemotype; see Table 5).

For the belowground herbivory treatment, wireworms (a mixture of *Agriotes lineatus* and *Agriotes obscurus*) were obtained in 2022 from Wageningen University, Lelystad,

The Netherlands. Upon arrival, the wireworms were kept in sandy soil at 20 °C with two sliced potatoes as a food source until they were used in the experiment. For the aboveground herbivory treatment, we collected *M. tanacetaria* aphids from Jena, Germany. Aphids were kept in cages in a climate-controlled lab at room temperature with supplemental light (16:00: 8:00 h L:D) provided by two tubes (T5 FQ 80 W/865 HO High Output LUMILUX Daylight G5, OSRAM GmbH, Munich, Germany) and with 2–4 tansy plants obtained from local Freising populations, which were unrelated to the chemotypes used in this study to avoid an influence of preference. A minimum of 100 adult aphids were collected and transferred to Petri dishes with fresh leaves to generate age-specific cohorts. One day later, all adults were removed, and the remaining aphid nymphs were kept in the dishes for three more days in a Fitotron standard growth chamber (21/16 °C, 60% RH, Weiss Technik). Aphid cohorts were supplied with fresh leaves of unrelated chemotypes daily until they were used in the experiment.

Above- and belowground Treatments

Four days before aphid infestation (day 0; Fig. 1), two 1 cm deep holes were made in the soil surface in all plant pots, and those pots assigned to the belowground treatment were infested with two wireworms each. During the experiment, all plants were placed on a plant saucer to prevent wireworms from escaping. After the belowground treatment was started, all plants were watered twice daily with up to 400 ml water per watering event, depending on the plant's water demand and soil humidity.

On the day of the aphid treatment (Fig. 1; day 4), we carefully attached a fine mesh bag (11 cm $\times$ 9.5 cm) with breathable and see-through fabric on the second youngest, fully expanded leaf, without squeezing the leaf or petiole. We did this to every plant, to maintain consistency in leaf and bag placement and reduce potential differences in mesh bag effects. Plants that were allocated to the aphid treatment additionally received two three-day-old aphid nymphs inside their mesh bag. The mesh bags protected the nymphs from predators and kept the colony in place, allowing for controlled observations.

Unfortunately, the experimental plants became infested with another tansy-specific aphid, *Colorado tanacetina*. This is a small, green aphid, feeding between leaflets, which makes them hard to detect early and remove. To make sure that the results of our experiment were not distorted by the presence of another aphid species, we monitored *C. tanacetina* numbers, and included their numbers as covariates where appropriate.

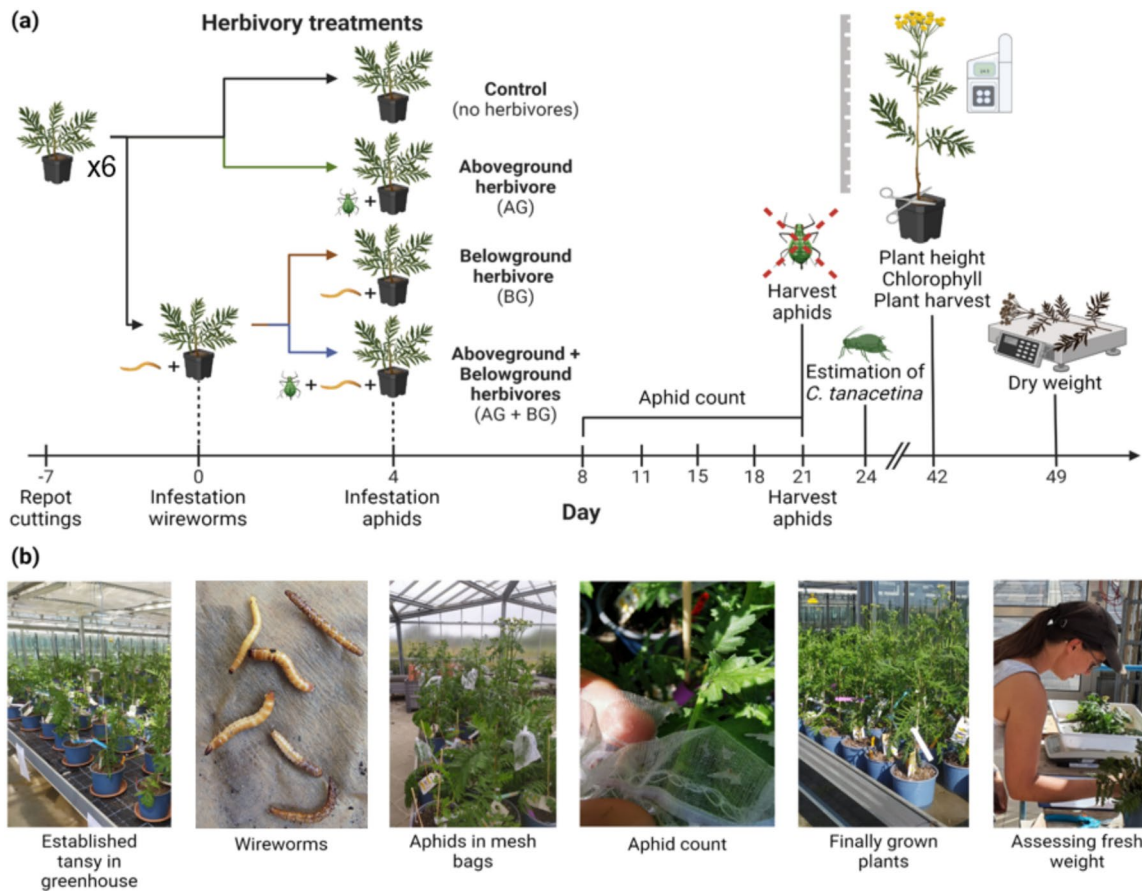


Fig. 1 Experimental design and timeline of the above-belowground herbivore experiment **(a)** The experimental timeline, including the last repotting event, infestation with wireworms and *M. tanacetaria* aphids, aphid counts, assessment of incidental infestation levels by *C. tanacetina* and measurements of plant height, chlorophyll and dry weight. Created with Biorender®. **(b)** Pictures depicting parts of the

experimental procedure; the established tansy plants growing in the vegetation hall; wireworms (image courtesy Wikimedia Commons—© Rasbak 2009); *M. tanacetaria* aphids in mesh bags; aphid counting inside the opened mesh bag; growing plants before harvesting; and the assessment of aboveground fresh weight

Insect Measurements

The experimental timeline started on the day during which plants with belowground treatment were infested with two wireworms (day 0, Fig. 1), which occurred one week after the last repotting event. Aboveground treatment (infestation with two three-day *M. tanacetaria* in a closed mesh bag on the second youngest fully expanded leaf) was performed on day 4. The colony size of *M. tanacetaria* was counted on days 8, 11, 15, 18, and 21. On day 21, *M. tanacetaria* aphids were harvested from each plant. The numbers of *C. tanacetina* aphids were estimated on day 24.

After harvesting the plants (day 49, Fig. 1), we traced back wireworms by going through the soil by hand, and noted that a disproportionate number had pupated or even enclosed as adults – which is not common in such belowground treatments (R. Heinen, pers. obs.), as wireworms typically spend several years in larval stage (Furlan 1998).

This high level of pupation may have been caused by a series of heatwaves that took place during the experiment. For this reason, at the end of the experiment, we counted the number of larvae, pupae, and adult beetles from each pot to ensure that no wireworms were missing and we could account for it statistically. We retrieved 68 wireworms that had pupated or even reached adulthood, while 90 wireworms remained in their larval stadium. Seventy-four individuals went missing and could not be found back, which may indicate that they reached adulthood and escaped the pots, or died. We tested the effect of these pupation events in separate models (referred to below as Model A for belowground treatment and Model B for retrieved number of larvae).

Plant Measurements

Three days after harvesting the leaves with infested aphids, we noted whether plants were infested by the tansy leaf

margin aphid *Coloradoa tanacetina* and estimated infestation numbers to statistically assess the effect of these unplanned infestations on *M. tanacetaria* performance and plant morphology. *C. tanacetina* estimation was done by picking three random leaves per plant and calculating the average number of aphids in steps of 10 for numbers between 0 and 100, in steps of 50 for numbers above 100, up to 300. On day 42 (Fig. 1), once plant growth stagnated, we assessed plant height by measuring the distance from the soil to the highest point of the plant, without straightening the plant. We further measured the average chlorophyll content of three random leaves per plant using a chlorophyll meter (Konica Minolta SPAD-502Plus, Tokyo, Japan) as a proxy of plant health. Aboveground dry weight was assessed after drying the samples for 78 h at 60 °C. Furthermore, we calculated total terpenoid concentration, terpenoid richness, terpenoid evenness, and terpenoid Shannon diversity from the absolute terpenoid profiles for each daughter using the ‘vegan’ package (Oksanen et al. 2022).

Statistical Analysis

All statistical analyses were performed in R version 4.1.2. We used linear mixed models, as detailed below, to test our hypotheses with the ‘lmer()’ command from the ‘lme4’ package (Bates et al. 2014). As aphid counts were strongly left-skewed, we square root-transformed this variable in every model to meet model assumptions. The assumptions of all models were assessed by plotting QQ plots, residual plots, and scale-location plots. We used ‘Anova()’ from the ‘car’ package to calculate p-values (Fox & Weisberg 2019). All models can be found in the supplement Table 6.

To address **H1**, whether chemotypes would drive aphid colony size, we created a model where we included the final aphid count as the response variable and chemotype as a fixed factor. To test whether natural colonisation of the experimental plants by the aphid *C. tanacetina* affected *M. tanacetaria* colony size, we included *C. tanacetina* abundance as a covariate in this model. Block and daughter were included as random effects to account for variation between blocks and clonal replicates.

To simultaneously test **H1** and **H2**, we created two model variants to test the effect of belowground treatment (Model variant A), or the number of retrieved wireworm larvae (Model variant B) on aphid colony size over time. Model variant A included chemotype, belowground treatment, observation day, and their interactions as fixed effects. Block and daughter were included as random effects to account for variation between blocks and clonal replicates. Furthermore, unique plant ID, nested in observation day, was used as a random effect, to account for the fact that aphids were counted more than once on the same plant over

time. In Model variant B we replaced belowground treatment with the number of retrieved wireworm larvae to investigate whether pupation during the experimental procedure affected the treatment effect on aphid colony size.

To address **H3**, whether components of plant chemical diversity (terpenoid richness, terpenoid Shannon diversity, terpenoid evenness) and total terpenoid concentration mediate the effect of wireworms on aphid colony size, we set up two multiple regression models. In Model variant A, we included belowground treatment and all chemical diversity components as fixed effects. Block and daughter were included as a random effect. In Model variant B, we replaced belowground treatment with the number of retrieved wireworm larvae to investigate whether pupation during the experimental procedure affected the treatment effect on aphid colony size. In the next step we used variance inflation factors, ‘vif()’ to diagnose multicollinearity in our models. We excluded the factors with the highest VIF, until all factors reached a VIF < 2. In both models, we therefore excluded terpenoid Shannon diversity.

To address **H4** whether above- or belowground herbivory affected plant traits (i.e., chlorophyll, plant height, plant biomass) we used linear mixed models. In Model variant A, we included *C. tanacetina* as a covariate, treatment (aboveground herbivory, belowground herbivory, above- and belowground herbivory, and control), chemotype, and the interaction of these three variables as fixed factors. Block and daughter were included as random effects. As the retrieved number of larvae were not homogeneously distributed across all treatment and chemotype combinations, this limited our analytical power at this level. Therefore, we tested the effect of retrieved wireworm larvae (Model B variant) to investigate whether pupation during the experimental procedure affected the treatment effect on plant variables. Block and daughter were included as random effects.

Results

Experimental Procedure

We infested 120 plants with aphids, two of which died during the experiment and were excluded from analysis. On 37 out of the remaining 118 plants, no aphids survived until the end of the aphid assay. In three of these plants, predatory mirid bugs were found in the mesh bags that were installed to protect the aphids. Hence, we excluded these three plants from further analyses. After three weeks, the remaining aphid colonies ranged from 0 to 77. We tested whether aphid survival differed between chemotypes. We found that aphid survival (recorded as 0 and 1) did not significantly differ between chemotypes ($\chi^2_5 = 7.26$, $p > 0.05$;

see Table 7). Within chemotypes the survival rates between daughters varied significantly ($\chi^2_1 = 11.30$, $p < 0.001$, see Table 7), but not between plants with or without BG treatment ($\chi^2_1 = 0.08$, $p = 0.784$, see Table 7) or between different numbers of retrieved wireworm larvae ($\chi^2_1 = 0.011$, $p = 0.916$, see Table 7). As aphid colonies that consist of zero individuals throughout the experiment provide no meaningful insights in aphid colony growth on different chemotypes, we analysed aphid colony growth data with and without the non-surviving aphids included. As patterns did not differ between the two approaches, we present the aphid colony growth data in the main text excluding the non-surviving aphids. However, for transparency we also present all analyses including the non-surviving aphids in the supplementary information (Tables 8, 9, Fig. 6, 7).

Over the course of the experiment *C. tanacetina* aphids incidentally infested our experimental plants, with colony sizes ranging from zero (on 25 plants) up to 200 individuals (on 3 plants) per leaf. We tested for each variable, whether its presence had a significant effect. Where appropriate, *C. tanacetina* numbers were included as covariate in the respective models.

Aphid performance across chemotypes (H1) and belowground treatment (H2)

We found no evidence that the degree of incidental infestation by *C. tanacetina* affected responses in *M. tanacetaria* final aphid colony size, neither when belowground treatment was included ($\chi^2_1 = 2.09$, $p > 0.05$; see Table 10 Model A) nor when the number of wireworms were included instead of the belowground treatment ($\chi^2_3 = 1.53$, $p > 0.05$; see Table 10 Model B). Furthermore, the numbers of *C. tanacetina* did not differ across chemotypes ($\chi^2_5 = 1.49$, $p > 0.05$; see Table 11), colony sizes of *M. tanacetaria* ($\chi^2_1 = 2.21$, $p > 0.05$; see Table 11) or the interplay of chemotype and *M. tanacetaria* colony sizes ($\chi^2_1 = 7.66$, $df = 5$, $p > 0.05$; see

Table 11). For this reason, *C. tanacetina* numbers were not included in the further aphid analyses below. A graph with *C. tanacetina* abundance for all chemotypes can be found in the supplementary information (Fig. 8).

Four days after adding two aphids to plants with aphid treatments, we counted the aphid numbers. After eleven days, aphids had matured and first offspring was recorded. On day 24, the colony sizes ranged from one to 77. Colony size of *M. tanacetaria* significantly increased with time the experiment (Model A, day: $\chi^2_1 = 180.35$, $p < 0.001$; Table 1). Aphid colony size also significantly differed between chemotypes over time, indicated by the interaction between day and chemotype (Model A, chemotype * day: $\chi^2_5 = 16.97$, $p = 0.005$; Table 1), and visible as different slopes in Fig. 2a). Aphid colony sizes increased faster on chemotypes with a mixed terpenoid profile, particularly the mixed chemotype with low terpenoid concentration. In line with this, final aphid colony sizes were higher on the mixed low chemotype than on the others (Fig. 2b). The number of winged adult aphids within a colony on the chemotype “Mixed_Low” was significantly higher than on the chemotypes “Bthu_High” and “Bthu_Low” (Fig. 9; $F = 3.19$, $df = 5$, $p = 0.010$).

However, belowground herbivory treatment did not affect aphid colony size (Table 1). When the number of retrieved wireworm larvae was included in the model instead of belowground herbivory treatment, we observed very similar patterns (Model B, day: $\chi^2_1 = 168.97$, $p < 0.001$; chemotype * day: $\chi^2_5 = 13.43$, $p = 0.020$; Table 1). We compared both models using AIC, and found that model A had a lower AIC, suggesting this model provides a better fit. Model B is not significantly better than Model A, suggesting that replacing BG with Wireworm Larvae does not meaningfully improve model fit (AIC_Model A = 1111.2, AIC_Model B = 1144.1, model comparison: $p = 0.973$). Note that plants with zero aphids were excluded. A table with zero aphids included can be found in the supplementary (Table 8).

Table 1 Output from a mixed linear model for *M. tanacetaria* colony size over time, using either belowground herbivory treatment (belowground) (Model A) or the number of retrieved wireworm larvae

Model A	d.f	χ^2 (p-value)	Model B	d.f	χ^2 (p-value)
Belowground	1	0.02 (0.891)	Wireworm larvae	3	0.43 (0.934)
Chemotype	5	4.30 (0.507)	Chemotype	5	4.29 (0.509)
Day	1	180.35 (< 0.001)	Day	1	168.97 (< 0.001)
Belowground * Chemotype	5	1.81 (0.874)	Wireworm larvae * Chemotype	14	4.95 (0.987)
Belowground * Day	1	1.47 (0.225)	Wireworm larvae * Day	3	3.71 (0.295)
Chemotype * Day	5	16.97 (0.005)	Chemotype * Day	5	13.43 (0.020)
Belowground * Chemotype * Day	5	1.28 (0.937)	Wireworm larvae * Chemotype * Day	14	4.64 (0.990)

(Wireworm larvae) (Model B), and day and chemotype, and the interaction terms as fixed effects. In both models, block, daughter, and individual id (nested within day) were used as random effects

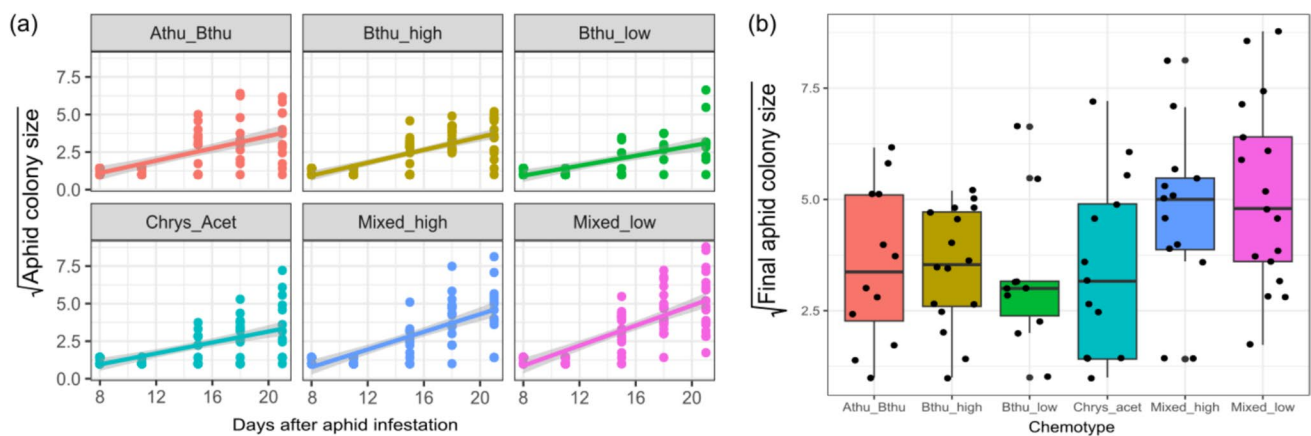


Fig. 2 (a) Square root-transformed *M. tanacetaria* colony size over time in days after aphid infestation, across chemotypes. (b) Final aphid colony size at the time of the experimental harvest for different tansy chemotypes. Boxes represent the variation in data, where the lower hinge corresponds to the first quartile (25 th percentile) and the upper hinge depicts the third quartile (75 th percentile). Whisk-

ers indicate the 5% and 95% percentiles; solid lines within boxes represent the medians. Black dots indicate individual sample values. The six chemotypes are depicted in different colours for convenience. Note that in both graphs plants with zero aphids were excluded. Graphs with zero aphids included can be found in the supplementary (Fig. 6)

Table 2 Output from a mixed linear model for final *M. Tanacetaria* colony size, using belowground herbivory treatment (BG) (Model A) or the number of retrieved wireworm larvae (Model B), and terpenoid richness, terpenoid evenness and total terpenoid concentration calculated based on the terpenoid profile of the 18 daughter plants (three for each of the six chemotypes) as fixed effects and the block and daughter as random effect

Model A	d.f	χ^2 (p-value)	Model B	d.f	χ^2 (p-value)
BG	1	2.22 (0.136)	Wireworm larvae	3	4.77 (0.189)
Evenness	1	4.34 (0.037)	Evenness	1	3.21 (0.073)
Richness	1	0.04 (0.837)	Richness	1	0.09 (0.764)
Concentration	1	1.97 (0.160)	Concentration	1	1.60 (0.206)

Chemical Diversity Components (H3) and Their Effects on Aphid Colony Size

When investigating the relationships between different components of chemical diversity, we found that *M. tanacetaria* colonies were significantly larger on plants that had a higher leaf terpenoid evenness ($\chi^2_1 = 4.34$, $p = 0.037$; Table 2; Fig. 3a). We observed no effects for terpenoid richness, or total terpenoid concentration (Table 2). Terpenoid Shannon diversity was excluded as this is highly correlated to terpenoid richness and evenness. There was no effect of the belowground herbivory treatment on aphid colony size.

When including the number of retrieved wireworm larvae instead of belowground herbivory treatment (Model B), we found that terpenoid evenness was nearly significant ($\chi^2_1 = 3.21$, $p > 0.05$; Table 2). Wireworm larvae did not affect final aphid colony sizes, regardless if we checked for treatments

(Table 2 Model A) or included the number of wireworm larvae retrieved (Table 2, Model B, Fig. 3b). We compared models using AIC and found that there was no significant difference in model fit (AIC_Model A = 332.8, AIC_Model B = 333.7, model comparison: $p = 0.210$). Note that plants with zero aphids were excluded. A table with zero aphids included can be found in the supplementary (Table 9).

Effect of Above- and Belowground Herbivory Treatments on Plant Morphology (H4)

As our experimental plants were incidentally infested with *C. tanacetina* during the experiment, we assessed whether this aphid species had a significant effect on plant performance, before analysing the effects of experimental aboveground herbivore treatment (AG). We observed that the strength of infestation by *C. tanacetina* did not affect aboveground plant dry weight, but significantly negatively affected plant height and marginally affected chlorophyll content of the experimental plants, and hence was included as a covariate in all plant response models below (Table 3; Fig. 10).

We found that the interaction of aboveground treatment and chemotype significantly affected the aboveground plant dry weight ($\chi^2_5 = 11.70$, $p = 0.039$; Table 3). Specifically, plants from the Mixed_high chemotype had a higher aboveground dry weight when they received an aboveground treatment with aphids, compared to control plants, while in all other chemotypes aboveground treatment with aphids had either a negative or no effect on plant aboveground dry weight (Fig. 4a). Plant height was significantly affected by aboveground treatment ($\chi^2_1 = 7.75$, $p = 0.005$; Table 3), and its interaction with chemotype ($\chi^2_5 = 13.59$, $p = 0.018$; Table 3). While control

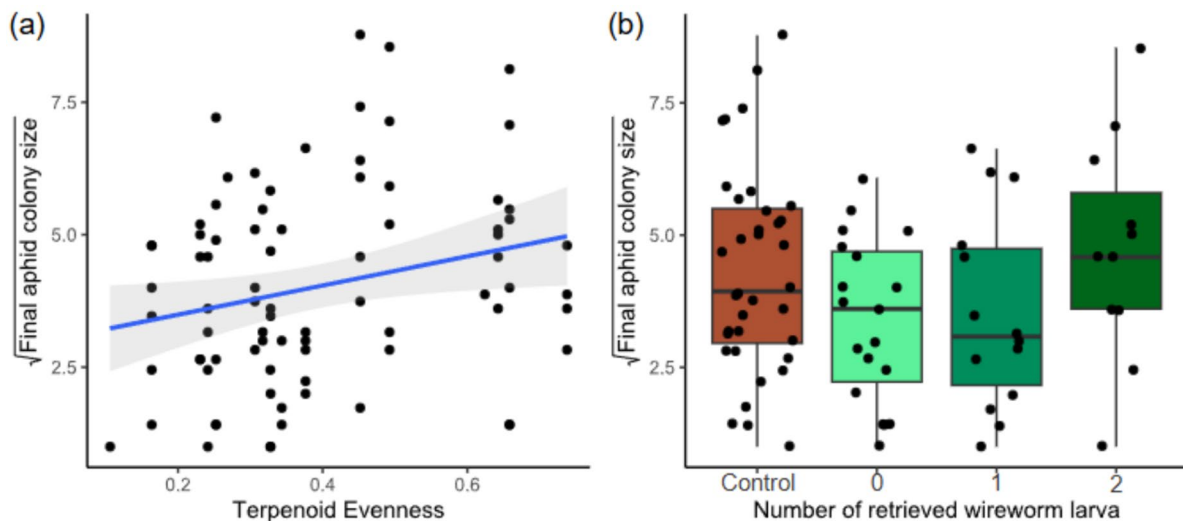


Fig. 3 (a) Square root-transformed *M. tanacetaria* colony size on plants differing in leaf terpenoid evenness. The quadratic trendline depicts average predicted values based on a linear model with quadratic term for evenness, and the shaded area depicts the 95% confidence interval. (b) Box plots visualizing square root-transformed *M. tanacetaria* colony size on plants with no added wireworms, compared to plants on which 0, 1 or 2 wireworm larvae were retrieved

after the harvest. Boxes represent the variation in data, where the lower hinge corresponds to the first quartile (25 th percentile) and the upper hinge depicts the third quartile (75 th percentile). Whiskers indicate the 5% and 95% percentiles; solid lines within boxes represent the medians. Black dots indicate individual sample values. Note that in both graphs plants with zero aphids were excluded. Graphs with zero aphids included can be found in the supplementary (Fig. 7)

Table 3 Output from a mixed linear model for average leaf chlorophyll content (units), plant height (cm), and aboveground dry weight (g) taking the *C. tanacetina* abundance, treatment (aboveground herbivory, belowground herbivory, both herbivory treatments, and control) and chemotype as fixed effects and block and daughter as random effects

		Plant dry weight		Plant height		Chlorophyll content	
	d.f	χ^2 (p-value)	d.f	χ^2 (p-value)	d.f	χ^2 (p-value)	
<i>C. tanacetina</i> abundance	1	0.22 (0.638)	1	13.42 (< 0.001)	1	2.90 (0.089)	
AG	1	0.50 (0.480)	1	7.75 (0.005)	1	0.82 (0.364)	
BG	1	0.33 (0.563)	1	0.33 (0.569)	1	1.54 (0.215)	
Chemotype	5	1.36 (0.929)	5	2.26 (0.812)	5	18.77 (0.002)	
AG * BG	1	2.42 (0.12)	1	1.03 (0.311)	1	6.30 (0.012)	
AG * Chemotype	5	11.70 (0.039)	5	13.59 (0.018)	5	6.99 (0.230)	
BG * Chemotype	5	6.10 (0.296)	5	2.84 (0.725)	5	9.16 (0.103)	
AG * BG * Chemotype	5	2.49 (0.778)	5	3.42 (0.636)	5	6.75 (0.240)	

plants grew taller for most chemotypes than those exposed to aboveground treatment with aphids, plants from the Mixed_low chemotype grew taller in the aboveground treatment, compared to control plants (Fig. 4b). The average leaf chlorophyll content significantly differed across chemotypes ($\chi^2_5 = 18.77$, $p = 0.002$, Table 3) and was also affected by an interaction between above- and below-ground treatments ($\chi^2_1 = 6.30$, $p = 0.012$; Table 3). Specifically, plants exposed to belowground treatment with wireworms seemed to have a higher chlorophyll content than plants that received aboveground treatment with aphids (with and without wireworms), or than control plants (Fig. 4c). Further, the Bthu_low chemotype had a significantly lower average leaf chlorophyll content than all other chemotypes, whereas the Mixed_low chemotype showed the highest average leaf chlorophyll content (Fig. 4d).

Discussion

In this study, we tested the effects of chemotypic variation in leaf terpenoid profiles on the interactions between a belowground coleopteran root herbivore and an aboveground phloem feeding aphid in Common Tansy (*T. vulgare*). We found that aphid colony size development over time significantly differed across chemotypes, as well as the numbers of winged aphids. However, contrary to our expectations, belowground infestation with wireworms did not have any effect on aphid colony size. Therefore, chemotypes did not mediate interactions between belowground herbivores and aboveground herbivores as we hypothesized. In addition to the observed chemotype effects, our multiple regression models also indicated a positive

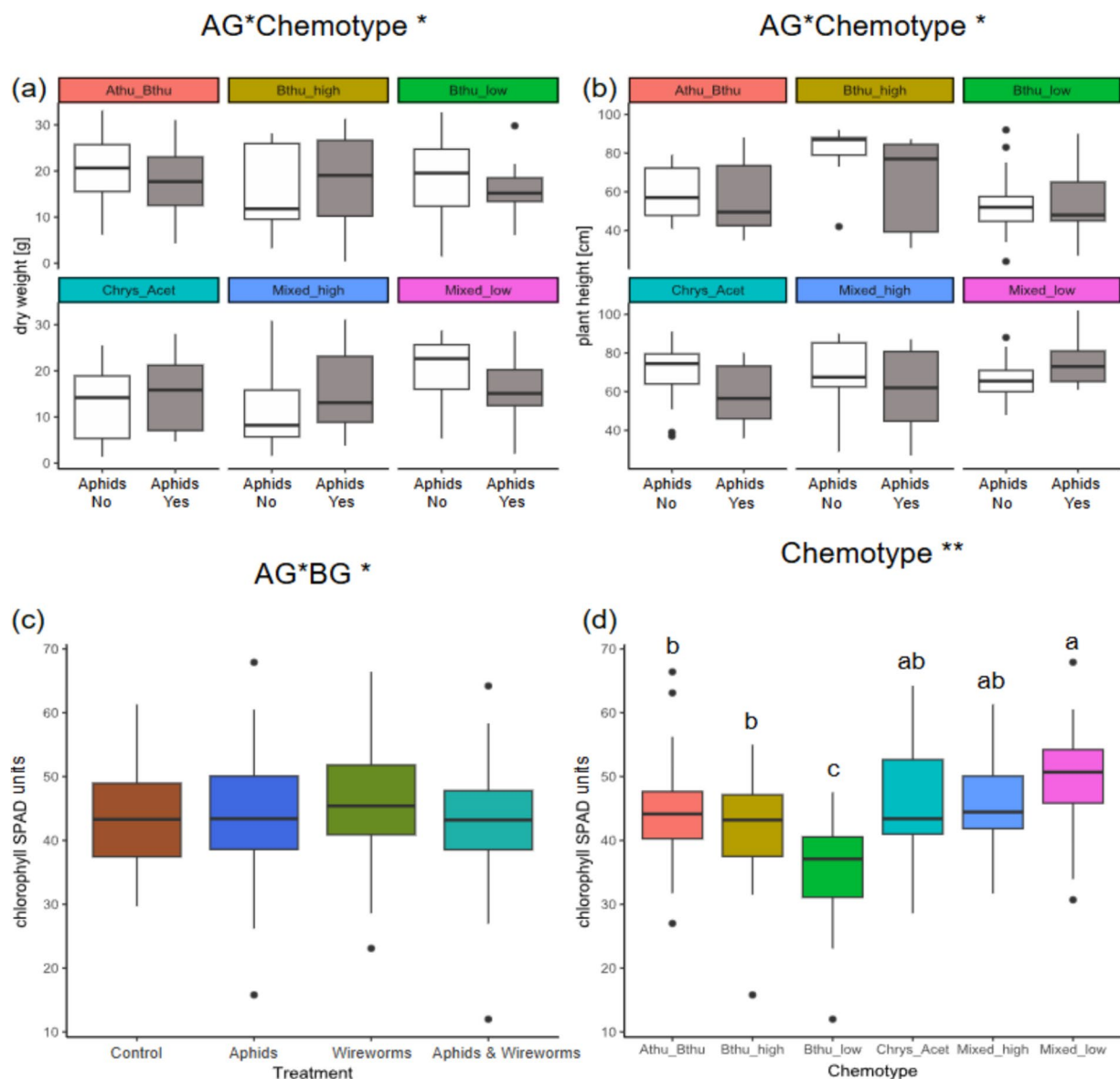


Fig. 4 Effects of plant chemotype, aboveground (aphid) and below-ground (wireworm) treatment on **(a)** plant dry weight, **(b)** plant height, **(c, d)** chlorophyll content. White boxes represent plants without aphids; grey boxes represent plants with aphids. Panel **(c)** represents an interactive effect between above- and belowground treatment on average leaf chlorophyll content (SPAD units), and **(d)** depicts differences in chlorophyll content across chemotypes. Boxes represent

the variation in data, where the lower hinge corresponds to the first quartile (25 th percentile) and the upper hinge depicts the third quartile (75 th percentile). Whiskers indicate the 5% and 95% percentiles; solid lines within boxes represent the medians. Black dots indicate outliers. Letters depict statistical significance based on posthoc Tukey tests

relationship between tansy leaf terpenoid evenness and final aphid colony size, suggesting that aphids perform better when the compounds in the terpenoid mixtures are more evenly distributed in concentration. We found that aphid presence significantly affected plant dry weight and plant height, but that the patterns differed between chemotypes. Root herbivore presence had surprisingly little effect on plant growth of any chemotype. Taken together, our results suggest an important role of plant chemotype as

a determinant of aphid colony dynamics, that corresponds to the distribution of the relative abundance of terpenoid compounds in the mixtures.

In line with our first hypothesis, we found that *M. tanacetaria* colony size development significantly differed between different *T. vulgare* chemotypes. This is supporting by a previous study showing that this aphid species was significantly affected by an interaction between tansy chemotype and plant part (Jakobs & Müller 2018), but in our study, the

effects of chemotypes were substantially more pronounced than in the aforementioned. Aphid colonies grew largest on Mixed_high and Mixed_low chemotypes that represent a high terpenoid evenness and diversity. In a previous study that used the same Tansy chemotype lines as used in the present study we found that, when given a pairwise choice, *M. tanacetaria* adults preferred to feed on leaves from the chemotype Athu_Bthu compared to Bthu_high or Mixed_low, and generally showed a higher attraction towards the chemotypes Athu_Bthu and Chrys_acet (Neuhaus-Harr et al., 2024). This is interesting, because this seems to indicate that what aphids *prefer* to feed on does not seem to match how they *perform* on it. According to the "mother knows best" or "preference-performance" hypothesis, adult insects should prefer plants on which their offspring have maximum performance, which is believed to be true for many aboveground specialist insects (Birke & Aluja 2018; Gripenberg et al. 2010). However, taking into account that the number of winged adult on the Mixed_Low chemotype was significantly higher than on the Bthu_High and Bthu_Low chemotypes, this could indicate a dispersal response to a poor quality resource. Previous research found that winged aphids are produced as a stress response, e.g. if the quality of a host is not good enough to support a viable colony (Wadley 1923; Williams et al. 2000). By generating more dispersal morphs (i.e., alates), aphids may be able to optimize their colony health, by reducing densities, and migrating for more suitable hosts. How colony success is assessed, raises important questions regarding the costs and benefits of attraction to specific chemistry in aphids: Possibly, reaching peak numbers quickly may not be the optimal strategy in aphids, as it deteriorates the host plant, and requires relocating to a new host. Future studies should focus on how (terpenoid) chemical cues relate to other plant qualitative components and inform insects on optimal host plant choice.

The previous prediction that belowground herbivores should positively influence aboveground herbivores (Masters et al. 1993), has since been challenged in many subsequent studies indicating that above- and belowground interactions are highly context dependent (Johnson et al. 2012). In line with this, but contrary to our second hypothesis, we did not find that belowground herbivores had a positive influence on *M. tanacetaria* colony size. There could be several explanations for this. First, although Coleoptera (such as wireworm larvae) as belowground herbivores typically have a positive influence on aboveground Homoptera (e.g., aphids) (Johnson & Murray 2008), this effect is often found when the both herbivores arrive at the same time, which indicates that the systemic plant response to root herbivores is early, and potentially short-lived (Erb et al. 2011; Johnson et al. 2012). As we infested plants with wireworms three days before aphids, plants might have already recovered from the root attack and the increase of leaf nutrients due

to herbivore stress (which benefits the aphids), had already faded out (Johnson et al. 2012). Second, when ending the experiment and retrieving the wireworms, many of them had pupated over the course of the experimental duration, or, in some cases, had even turned into adults. This is possibly due to local heat waves that occurred during the experiment, in August 2022. Although wireworms typically live for many years, the warm conditions may have sped up their larval cycle, as temperature is typically negatively correlated to the length of larval life cycles in insects (Furlan 1996; Meikle & Patt 2011). As wireworms do not feed during pupation, the resulting numbers of herbivory might have been too low to have a significant effect on the aboveground aphids. However, in as of yet unpublished follow-up studies, we added nine instead of two wireworms to Tansy in a temperature-regulated environment, completely avoiding pupation events, but in this follow-up, effects of belowground treatments on aphids were also not significant (A. Neuhaus-Harr, pers. obs.). It could also be that wireworm feeding on tansy roots is not consistent, although we have observed in the aforementioned follow-up that wireworms readily feed on Tansy roots, and particularly on the fine root hairs (J-P Schnitzler, pers. obs.). A final explanation may be that responses to root herbivory in tansy are local, rather than systemic. As we did not find differences among chemotypes, this could indicate that belowground and aboveground plant responses might be compartmentalised. As described in a recent study, Tansy terpenoid profiles differ strongly between above- (shoot tissues) and below-ground compartments (root tissues), following different biosynthetic pathways (Rahimova et al. 2024a, b). It is possible that there is minimal resource allocation or defence pathways overlap. Further studies unravelling how and where wireworm feeding affects plant physiological processes are needed to draw definitive conclusions.

We predicted that more chemically diverse plants (i.e., higher terpenoid richness, higher terpenoid evenness, and higher terpenoid Shannon diversity) and plants with higher terpenoid concentration would be more strongly defended, which we hypothesized would lead to reductions in *M. tanacetaria* colony size. If only certain compounds are detrimental to aphids, it might be more likely for these compounds to occur in plants with a higher terpenoid richness. Furthermore, if a compound is detrimental to aphid colony growth, a higher concentration might be even more detrimental. We predicted that belowground treatment with root herbivores would modify this relationship. Contrary to our predictions, we found that *M. tanacetaria* colonies tended to be larger on plants with higher terpenoid evenness. No significant effects were observed for the other chemodiversity components. There was no effect of belowground herbivory treatment, nor any interaction between belowground treatment and the chemical diversity components on aphid colony size, similar to H2 above.

The role of evenness in ecological contexts is highly dependent on the organisms and functions involved (Petrén, Köllner, et al. 2023b). If specific functions, like suppressing aphid growth, depend on a few key compounds, high evenness could dilute the relative abundance of these critical compounds, resulting in more favourable conditions for aphids. However, one caveat in our study is that terpenoid evenness at any point in the distribution is reflected by a small number of chemotypes, some of which may have substantial overlap in composition. Although several studies show positive relationships between chemical profiles dominated by individual compounds and aphid colony sizes, these results seem to suggest that across a range of different chemotypes, single compound-dominated mixtures (i.e., low evenness) are detrimental to aphid colony development. One important caveat is that evenness may also be partly confounded by disparity, i.e., the effect of the origin of the compound on its ecological effect (Petrén, Anaia, et al., 2023a). For instance, in our study, low-evenness profiles were typically dominated by β -thujone, or by chrysanthenyl acetate, which, although both monoterpenoids derived from geranyl diphosphate, are the result of different downstream pathways, and may have different ecological effects on herbivores (Rahimova, Neuhaus-Harr, et al., 2024). Similarly, mixtures dominated by other compounds occurring in nature may have even different impacts on aphids. Disentangling the effects of evenness from the effect of disparity would require large-scale studies that include a broader selection of chemical profiles, with representative replication across the distribution of chemical evenness and diversity, testing their impacts on aphid colonies under standardised conditions, and this would be an important direction for the future.

We found that the infestation of tansy by *M. tanacetaria* significantly influenced plant height and plant dry weight, although the direction of the effect differed between chemotypes. The leaf chlorophyll content also differed between chemotypes and was lower when a plant experienced above- and belowground herbivory. As the leaf chlorophyll content is commonly seen for a proxy of plant quality (Pavlovic et al. 2014; Takayama & Nishina 2009; Xu et al. 2024), our results imply that plant quality decreased when plants were attacked by above- and belowground herbivores in combination. It is plausible that two herbivores attacking a plant at the same time pose more pressure on the plant than single herbivores, and our results suggest that one herbivore can be dealt with without strong negative consequences for plant health (i.e., chlorophyll status). Our findings also imply that chemotypes might differ in their growth and defence strategies as has been found in multiple other plant species (He et al. 2022; Huot et al. 2014; Züst & Agrawal 2017). Both of the mixed chemotypes, i.e., the chemotypes with the highest richness, diversity and evenness of compounds, grew either

taller or had a higher dry weight when infested with aphids, compared to control plants. Interestingly, these chemotypes also had the largest *M. tanacetaria* colony sizes of all. Producing chemical defence is typically considered to be costly, and it is often associated with a restriction in growth (Havko et al. 2016; Herms & Mattson 1992; Huot et al. 2014; Sestari & Campos 2022), which has been found in many plant species (Campos et al. 2016; Haak et al. 2012; Hayashi et al. 2020; Mihaliak & Lincoln 1989). Here, we observe the opposite. Although it may seem counterintuitive, perhaps Tansy plants with more diverse terpenoid chemotypes may save resources by the production of a diverse mixture of compounds in low relative abundance. This could allow these chemotypes to invest resources into growth and compensation, while other chemotypes possibly invest more into chemically defence through production of a select number of dominant compounds in high concentrations. However, recent research shows that growth vs. defence is not simply a consequence of limited resources but a strategy of plants to maximise their fitness, that is context-dependent and aims to ensure greatest fitness of a plant in its environment (Campos et al. 2016; Guo et al. 2018; Kliebenstein 2016). Our findings also indicate that individuals within species can display very different growth responses when faced with herbivory, and that this response might be connected to secondary metabolites. A cost–benefit analysis of the maintenance of chemical diversity, for instance relative to other well-characterized processes such as tolerance and compensation for herbivory in plants, would greatly help us understand chemical profiles in the context of defence optimization strategies.

To conclude, we found that intraspecific plant chemistry plays an important role in how plants interact with their biotic and abiotic environment. Secondary metabolites not only serve as a defence system, through repelling herbivores or attracting herbivore predators, but also seem to be connected to other life history traits such as plant growth. This study might help us understand the role of chemotypes in the growth-defence trade-off of aboveground herbivory. While belowground herbivory had a small effect on the plant and none on the aboveground herbivore, these effects did not differ between plants with different leaf chemotypes. This may be an indication that plant defence is locally compartmentalised, as the chemotypic profile of roots highly differs from that found in leaves (Rahimova et al. 2024a, b). It might be that minimal above-ground defence signalling takes place in this system for this reason, or that aboveground herbivory would signal belowground defence. Our study sheds light on the role of plant chemotypes on plant responses to above- and belowground herbivory, but we call for further research on root and shoot chemistry and their respective roles in governing above-belowground insect-plant interactions.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10886-025-01609-y>.

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Author Contributions ANH, LOP and RH originally formulated the idea, with input from WWW and JPS. XZ, ANH, and LOP conducted the experiment with help from RH. ANH led the statistical analyses with input from LOP and RH. LOP prepared Fig. 1, ANH prepared all other figures and tables, with input from LOP and RH. ANH wrote the manuscript with input from RH and LOP. XZ, WWW and JPS provided structural feedback and revisions on the first draft. All authors have read the final version and support publication.

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Data Availability Once the manuscript is accepted, all data used will be uploaded to Dryad and will be made openly accessible.

Declarations

Competing interest The authors declare no competing interests.

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