Technische Universität München

TUM School of Life Sciences

Effects of tansy chemodiversity on plant-insect and insect-insect interactions

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Vollständiger Abdruck der von der TUM School of Life Sciences der Technischen Universität München zur Erlangung einer

Doktorin der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitz: Prof. Dr. Sara D. Leonhardt

Prüfende der Dissertation:

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Die Dissertation wurde am 14.03.2025 bei der Technischen Universität München eingereicht und durch die TUM School of Life Sciences am 11.09.2025 angenommen.

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Tansy picture © Robin Heinen

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Summary

Biodiversity is essential for ecosystem functioning, and intraspecific diversity plays a crucial role in shaping species interactions, community structures, and ecosystem processes. In plants, individuality is largely determined by secondary metabolites, which influence communication with biotic and abiotic factors. Tansy (*Tanacetum vulgare*) exhibits distinct chemotypes, characterized by blends of mono- and sesquiterpenoids, which mediate herbivore interactions. These chemical profiles exist as complex mixtures, making it important to assess their composition, abundance, and ecological effects. While belowground herbivores can influence aboveground chemistry, it remains unclear whether chemotypes mediate these interactions differently.

In this thesis, I used *Tanacetum vulgare*, its associated aphids, and wireworms to investigate the role of plant chemotypes in shaping herbivore interactions. By conducting two greenhouse experiments, I examined how tansy chemotypes influence aphid preference, performance, and how plant chemistry mediated multi-herbivore interactions. I also assessed potential morphological differences among chemotypes. Additionally, in a large-scale field study across Germany, I analyzed the distribution of monoterpenoid and sesquiterpenoid chemotypes, their influence on insect communities, and the environmental factors shaping these interactions.

Across Germany, we identified four distinct monoterpenoid and four sesquiterpenoid chemotypes in *Tanacetum vulgare*. While monoterpenoid chemotypes varied in their latitudinal distribution, sesquiterpenoid chemotypes were evenly spread. Aphid and ant presence was influenced primarily by volatile MTs, likely because these compounds serve as host-location cues. However, aphid abundance was linked to soil type. As different soil types vary in their levels of nutrients, this could affect plant health and ultimately translate into insect abundance.

The greenhouse experiments revealed that the effect of chemotypes on aphid preference and performance was species-specific. Furthermore, a higher terpenoid concentration attracted *Macrosiphoniella tanacetaria* aphids, possibly because high concentrations of terpenoids in the ambient air makes the host plant easier to detect. *Uroleucon tanaceti* did not show differences in their preference. Aphid attraction was additionally influenced by the overall blend rather than single terpenoid compounds, suggesting that volatiles in a blend could counteract or add to each other´s function. Nevertheless, aphids preferred chemotypes dominated by a few key terpenoids possible because highly dominant compounds serve as more easily detectable cues for plant recognition. Interestingly, *Metopeurum fuscoviride* aphids did not show a correlation between preference and performance, challenging the "mother knows best" hypothesis.

In my experiment, belowground wireworms had no significant effect on *Metopeurum fuscoviride* colony size. While root herbivores often enhance aphid performance, this typically occurs only when both arrive simultaneously, suggesting a short-lived plant response. Tansy may exhibit localized rather than systemic reactions to root herbivory, and the lack of differences among chemotypes implies compartmentalized defense mechanisms. These findings suggest minimal overlap in resource allocation and signaling between aboveground and belowground plant parts.

Herbivores affected plant morphology, but chemotypes did not mediate the strength or direction of these effects. Plant size and bushiness correlated positively with terpenoid concentration and diversity, challenging the growth-defense trade-off hypothesis.

This thesis highlights the crucial role of intraspecific chemodiversity in shaping plant growth, plant-insect interactions, and the structure of plant-arthropod communities. The findings enhance our understanding of how chemical variation within a species affects their surroundings, demonstrating the importance of intraspecific diversity in ecological studies.

Zusammenfassung

Biodiversität ist essenziell für die Stabilität und Funktionalität von Ökosystemen. Dabei spielt die intraspezifische Diversität eine entscheidende Rolle in der Gestaltung von Pflanzen-Insekten-Interaktionen und der Strukturierung von Ökosystemen. Pflanzen unterscheiden sich stark in der Produktion sekundärer Metaboliten, die eine zentrale Funktion in der Kommunikation mit ihrer Umwelt übernehmen. Daher stehen diese Verbindungen im Fokus vieler ökologischer Studien. Einige Pflanzen, wie der Rainfarn (*Tanacetum vulgare*), lassen sich anhand spezifischer Mischungen aus Mono- und Sesquiterpenoiden in verschiedene Chemotypen unterteilen. Diese chemischen Profile bestehen aus komplexen Gemischen, deren Zusammensetzung, Häufigkeit und ökologische Effekte genauer untersucht werden müssen. Unklar ist bislang, ob bodenbewohnende Pflanzenfresser die chemische Zusammensetzung der oberirdischen Pflanzenteile beeinflussen und damit indirekt auch Auswirkungen auf oberirdische Pflanzenfresser haben.

In der vorliegenden Dissertation wurde die Rolle der Chemotypen von *T. vulgare* in Pflanzen-Insekten-Interaktionen untersucht. Mithilfe zweier Gewächshausexperimente analysierte ich, ob bestimmte Blattlausarten spezifische Chemotypen bevorzugen und auf welchen Chemotypen sich Blattlauskolonien besonders erfolgreich entwickeln. Zudem untersuchte ich, ob unterirdisch lebende Drahtwürmer die überirdischen Blattlauspopulationen beeinflussen und ob Chemotypen diese indirekte Interaktion modulieren. Darüber hinaus wollte ich wissen, ob sich die Chemotypen morphologisch unterscheiden und welche Konsequenzen dies für Blattläuse hat. In einer groß angelegten Feldstudie in Deutschland wurde zudem die geographische Verteilung von Mono- und Sesquiterpenoid-Chemotypen analysiert sowie deren Einfluss auf Insektengemeinschaften und die Umweltfaktoren, die diese Interaktionen prägen.

Deutschlandweit konnten vier unterschiedliche Mono- und vier Sesquiterpenoid-Chemotypen von *T. vulgare* identifiziert werden. Während Monoterpenoid-Chemotypen eine geographische Variabilität entlang des Breitengrads aufwiesen, waren Sesquiterpenoid-Chemotypen gleichmäßig verbreitet. Das Vorkommen von Blattläusen und Ameisen hing primär von den Monoterpenoid-Chemotypen ab, vermutlich weil diese flüchtigen Verbindungen den Insekten als Signal zur Wirtserkennung dienten. Die Größe der Blattlauskolonien auf einer Pflanze korrelierte hingegen mit dem Bodentyp, was darauf hindeutet, dass verschiedene Bodentypen durch ihren variierenden Nährstoffgehalt die Pflanzengesundheit und damit indirekt die Blattlauspopulation beeinflussen.

Die Gewächshausexperimente zeigten, dass der Einfluss der Chemotypen auf Präferenz und Entwicklungsrate von Blattläusen artspezifisch ist. Höhere Terpenoidkonzentrationen schienen die Blattlausart *Macrosiphoniella tanacetaria* anzulocken – möglicherweise, weil eine hohe Terpenoidkonzentration in der Umgebungsluft die Wirtspflanze leichter auffindbar macht. Die Blattlausart *Uroleucon tanaceti* hingegen zeigte keine spezifische Präferenz für einzelne Chemotypen. Die Attraktivität einer Pflanze wurde weniger durch einzelne Terpenoide als durch die gesamte chemische Mischung und deren Konzentration bestimmt, was darauf hindeutet, dass flüchtige Stoffe innerhalb eines Gemisches sich gegenseitig verstärken oder abschwächen können. Gleichzeitig bevorzugten Blattläuse Chemotypen, die von wenigen Terpenoiden dominiert wurden, vermutlich weil diese stark vorherrschenden Verbindungen als deutliche Wirtssignale fungieren. Interessanterweise wuchsen Kolonien von *M. tanacetaria* auf nicht bevorzugten Chemotypen besonders gut, was die „Mother Knows Best“-Hypothese in Frage stellt.

Unterirdische Drahtwürmer hatten in meinen Experimenten keinen signifikanten Einfluss auf die Koloniegröße von *Metopeurum fuscoviride*. Obwohl bekannt ist, dass Wurzelschädlinge in vielen Fällen die Entwicklung von Blattläusen fördern, tritt dieser Effekt meist nur auf, wenn beide Herbivoren gleichzeitig auf die Pflanze treffen, was auf eine kurzfristige pflanzliche Reaktion hindeutet. Weiterhin deuteten meine Ergebnisse darauf hin, dass *T. vulgare* auf Wurzelschädlinge mit lokalisierten statt systemischen Abwehrmechanismen reagiert. Da keine Unterschiede zwischen den Chemotypen festgestellt wurden, legen diese Ergebnisse, dass die Ressourcennutzung und Signalübertragung zwischen ober- und unterirdischen Pflanzenteilen weitgehend unabhängig voneinander ablaufen.

Sowohl Blattläuse als auch Drahtwürmer beeinflussten die Pflanzenmorphologie, doch die Chemotypen modifizierten weder die Stärke noch die Richtung dieser Effekte. Größere und buschigere Pflanzen wiesen höhere Terpenoidkonzentrationen und eine größere Terpenoidvielfalt auf, was die „Growth-Defense“-Hypothese in Frage stellt.

Diese Arbeit hebt die zentrale Bedeutung der intraspezifischen chemischen Diversität für das Pflanzenwachstum, Pflanzen-Insekten-Interaktionen und die Struktur von Pflanzen-Insekten-Gemeinschaften hervor. Die Ergebnisse tragen zu einem besseren Verständnis der ökologischen Relevanz chemischer Variation innerhalb einer Art bei und unterstreichen die Notwendigkeit, intraspezifische Diversität in ökologischen Studien verstärkt zu berücksichtigen.

1. Introduction

Understanding how plant species and their associated arthropod communities interact is an important key to ecological research. Why? Traditionally, many studies have focused on the role of species diversity in ecosystems (Cardinale et al., 2012; Weisser et al., 2017). However, recent research emphasizes the importance of intraspecific diversity, i.e. the phenotypic variation within a single species, in shaping ecological relationships (Petrén, Anaia, et al., 2023; Westerband et al., 2021). A critical topic in intraspecific diversity research is intraspecific chemical diversity, which focuses on the variation in secondary metabolites within a species and how this in turn shapes plant interactions (Müller et al., 2020; Walker et al., 2022). This thesis aims to provide an overview of the chemodiversity concept using tansy (*Tanacetum vulgare*) as example and to deepen our understanding of plant-insect interactions driven by tansy plant chemistry.

Tansy & Aphid drawing © Theresa Rohrberg

1.1 Biodiversity and its Importance

Currently, the earth is experiencing a global biodiversity crisis. Researchers estimate the global biodiversity loss in the last century to be 100-1000 times higher than it would be before industrialization (Ceballos et al., 2015; De Vos et al., 2015). While the loss of biodiversity is lamented on, why should it be ecologically important or even be important for us humans? However, at first, what exactly is biodiversity?

1.1.1 Definition of Biodiversity

What is biodiversity? Biodiversity refers to the variety of living organisms in a given habitat or, globally, on earth (Titley et al., 2017). Biodiversity is defined at different scales within ecosystems: Most commonly, as species diversity (the number of different species), but also as population diversity (the genetic variation between two populations) and genetic diversity (the variation of genes within species). Furthermore, on a higher scale, it can also be defined as ecosystem diversity (the variety of habitats and biotic communities) (Poiani et al., 2000; Titley et al., 2017). As mentioned, biodiversity research often focused on the number of species or ecosystems. However, intraspecific trait variation, i.e., the difference of individuals within one species, has recently been brought into focus (Gugerli et al., 2008). For instance, the difference of chemical profiles within one plant and its effect on associated insects (Boncan et al., 2020; Eilers, 2021; Glassmire et al., 2016; Koricheva & Hayes, 2018; Ojeda-Prieto, Moreno et al., 2024).

1.1.2 Biodiversity's importance

Why is biodiversity important? Biodiversity provides a vast range of benefits to us humans. Even though the number of different species is the most commonly used meaning (Titley et al., 2017), each level is crucial for the resilience and stability of ecosystems (Griffin et al., 2009). To integrate ecological understanding and the economic value of biodiversity, the term “ecosystem services” has been coined and defined as processes of ecosystems and their species that sustain and fulfil human life (Chee, 2004). Ecosystem services can include a range of processes, such as cleaning the air, purifying water, fertilising the soil, or providing goods, such as food-like vegetables, fruits or animals, or materials such as timber (Chee, 2004; Fu et al., 2013). Biodiversity furthermore ensures a stable food supply (Muluneh, 2021). Here, two important reasons are (1) a greater diversity of plants within a region can make it more challenging for pests to locate their specific hosts, leading to smaller pest populations compared to monocultures (Risch et al., 1983); and (2) a variety of insects serve as natural pest controllers, with a higher number of predator species increasing the likelihood of effectively regulating pest populations (Steffan-Dewenter et al., 2024). Importantly, the number of plant species at a site can reduce pests, and the variation within a single plant species can significantly structure insect communities (Fritz & Price, 1988). Plants from the same species can differ in their chemical compounds, which, on the one hand, provide important raw materials and bioactive substances, such as alkaloids, that might be toxic to herbivores (Abidah & Azzreena, 2018). On the other hand, the plant uses these chemical, organic compounds to interact with their living environment. For example, by releasing volatile organic compounds, plants can attract pollinators or natural enemies of herbivores (Dudareva et al., 2006; Pichersky & Gershenzon, 2002).

A range of plants and their chemical compounds have been found to be important for human health. For instance, they provide antioxidants, but also anti-inflammatory or protective substances (Riaz et al., 2023). In order to synthesise some necessary bioactive compounds, like common vitamin C, the human body relies heavily on plants (Butnariu & Bocso, 2022). From a large list of examples, only two shall be mentioned here. For example, the alkaloid quinine, which is isolated from the autumn crocus (*Colchicum autumnale),* is used as antimalarial treatment. Different opiates found in the opium poppy (*Papaver somniferum*) serve as painkiller (Butnariu & Bocso, 2022). This extensive list of secondary plant metabolites is yet another reason, next to the other ecosystem services listed above, why biodiversity is important for humankind.

1.2 Intraspecific Diversity

1.2.1 Definition of Intraspecific Diversity

What is intraspecific diversity, and why does it matter? Intraspecific diversity refers to the difference or variation in traits within a single species, as opposed to interspecific diversity, which focuses on differences between species. Plants and animals can vary in many traits within their species, such as genes, development, behaviour or chemistry (Westerband et al., 2021). For example, in yarrow (*Achillea millefolium*), different populations show significant differences in their developmental traits (e.g., fresh mass, number of shoots per m^2) but also in their chemistry (e.g., concentration and presence/absence of essential oil compounds) (Bączek et al., 2015). The intraspecific as well as the interspecific diversity affect the interaction among individuals or species and therefore the functioning of the ecosystem.

1.2.2 Ecological Importance of Intraspecific Diversity

Intraspecific diversity has the power to affect the performance of the individual or the population, influence species interactions and community structures or impact ecosystem functions (Crutsinger et al., 2008; C. Z. Hahn et al., 2017; Noto & Hughes, 2020; Schöb et al., 2015). For example, a meta-analysis showed that agricultural yield increased by 2.2% when using cultivar mixtures, i.e., plants from the same species but with different traits (Reiss & Drinkwater, 2018). The harsher the conditions in the field– abiotic stress, disease pressure or low nutrient availability – the stronger the effect (Reiss & Drinkwater, 2018). One reason for this finding could be that the diversity of traits increases a population's ability to use resources through increased functional traits related to resource use (Díaz & Cabido, 2001; Reiss & Drinkwater, 2018). A community with higher functional diversity exhibits improved resource partitioning, which can enhance overall productivity and functionality (Cadotte et al., 2011). Specific traits might become disadvantageous in certain instances, but other individuals with different traits will be able to compensate (Cadotte et al., 2012). Thus, only in favourable environments and uniform conditions, less diverse modern cultivars outperform diversified populations. In contrast, the population’s resilience against disturbances relies on diversity (Mohammadi et al., 2014; Yahiaoui et al., 2014).

How important is intraspecific diversity compared to interspecific diversity?

Intraspecific richness – the number of genotypes/phenotypes – shapes ecological dynamics at the same magnitude as interspecific richness (Raffard et al., 2018). Therefore, intraspecific trait variation has received more attention lately. Unfortunately, positive effects of intraspecific variation cannot be generalised (Raffard et al., 2018). Depending on whether one looks at the ecosystem or the community level, investigating primary (e.g., caterpillars) or secondary consumers (e.g., birds), the magnitude of the effect increases or decreases (Raffard et al., 2018). Further, it is not easy to link species and ecosystem functions. Which traits should be considered to link intraspecific differences to function, and, importantly, how can they be adequately measured? Here, recent findings suggest that plant individuality significantly relies on their differences in their chemistry of secondary plant metabolites (Blanchard & Holeski, 2024; Kessler & Kalske, 2018). Thus, chemical substances produced by plants are important players in shaping the function of the ecosystem.

1.3 *Tanacetum vulgare* as Model System for Chemical Diversity

Tanacetum vulgare, commonly known as tansy, is an aromatic plant notable for its diverse terpenoid profiles and mixtures. Tansy chemotypes are characterised by blends of mono- and sesquiterpenoids, often defined by the dominant monoterpene compounds present in the plant. Some of the most common monoterpenes include α-thujone, β-thujone, camphor, and trans-chrysanthenyl acetate (Dussarrat et al., 2023; Muresan, 2015; Neuhaus-Harr et al., 2024; Rahimova, Neuhaus-Harr, et al., 2024). Studies have revealed variations in chemotype occurrence across small and large geographic areas. Tansy chemotypes have been studied extensively in countries such as Canada, Brazil, Finland, Estonia, Poland, Slovakia, Lithuania, Romania, Turkey, Iran, France, and Germany, each with varying dominant compounds (Muresan, 2015; Thompson et al., 2019). Remarkably, fourteen distinct chemotypes were identified in a German study site covering only three square kilometres (Kleine & Müller, 2011), highlighting the exceptional variability of tansy's terpenoid mixtures. Despite this knowledge, the extent to which abiotic factors drive chemotypic plasticity and its effects on associated insect communities remains unclear.

Tansy chemotypes are crucial in shaping biotic interactions through direct and indirect mechanisms (Clancy et al., 2016; Keskitalo et al., 2001; Kleine & Müller, 2011). The interactions mediated by tansy chemistry are complex and vary significantly between chemotypes, underscoring the importance of chemodiversity in ecological dynamics. Tansy's leaf, root, and flower head chemotypes affect a wide range of organisms, including aphids, their predators, mutualists, flower visitors, pollinators, and root herbivores (Clancy et al., 2018; Eilers et al., 2021; Kleine & Müller, 2011; Neuhaus-Harr et al., 2024; Ojeda-Prieto, Moreno et al., 2024; Rahimova, Neuhaus-Harr, et al., 2024; Senft et al., 2019; Ziaja & Müller, 2023). For example, the floral volatile compounds of tansy plants and their associated pollen nutrients influence the behaviour of mutualistic and antagonistic flower visitors (Eilers et al., 2021). Flower beetles such as *Olibrus aeneus* have shown a preference towards certain chemotypes and perform better on these chemotypes (Mahdavi-Arab et al., 2014). Conversely, *Cassida stigmatica* exhibited chemotype preferences but showed no performance differences among chemotypes (Wolf, Gassmann, & Müller, 2012). These findings illustrate the species-dependent effects of tansy chemistry and raise questions about the conditions under which herbivore preferences align with performance. By addressing these questions, I wanted to gain deeper insights into the ecological significance of tansy's chemodiversity, which, in turn, shall help to uncover general principles in plant-insect interactions.

1.4 Plant Chemistry

To communicate with their environment and to adjust to abiotic and biotic factors, plants use a variety of chemical compounds (Pichersky & Raguso, 2018; Wink, 2018). These play a crucial role in mitigating interactions with the (living) environment (Salam et al., 2023) and are an important trait in intraspecific diversity.

1.4.1 Plant Metabolites

Plant chemistry can be divided into three groups of substances: primary metabolites, secondary metabolites and plant hormones (Erb & Kliebenstein, 2020), which are detailed in the following.

Primary plant chemistry refers to metabolites that serve essential functions such as growth, development and reproduction. These metabolites include carbohydrates (such as glucose, starch, cellulose, etc.), proteins, lipids, nucleotides and amino acids (Butnariu & Bocso, 2022). Even though these metabolites mainly serve physiological functions, they can also help facilitate a plant´s response to its living environment (Salam et al., 2023) or can act as a defence (Zaynab et al., 2019). For example, after a leaf-herbivore attack, coyote tobacco (*Nicotiana attenuata*) decreased the concentrations of sugar and starch in the roots. Additionally, plants that were attacked showed a reduction in non-structural carbohydrates, which makes the plant less attractive to herbivores (Machado et al., 2013).

Secondary metabolites are organic compounds produced by plants that are not directly involved in their primary growth, development, or reproduction (Erb & Kliebenstein, 2020). Instead, they play crucial roles in mediating interactions between plants and their environment (Erb & Kliebenstein, 2020; Iason et al., 2012; Theis & Lerdau, 2003). These interactions include defence mechanisms against herbivores and pathogens, attraction of pollinators, and protection against environmental stresses such as UV radiation (Akula & Ravishankar, 2011; Iason et al., 2012; Kliebenstein, 2004). Examples for secondary metabolites are alkaloids, terpenoids, phenolics and others, which, for example, often directly shape plant-insect interactions (see below).

Plant hormones are chemical messengers that regulate various physiological processes within the plant. They control growth, development, and metabolism (Davies, 1987, 1995). The major plant hormones include auxins, cytokinins, gibberellins, abscisic acid, and ethylene (Davies, 1995; Kende & Zeevaart, 1997). In essence, while secondary metabolites are thought to mediate plant-environment interactions, plant hormones regulate organismal processes and metabolism (Erb & Kliebenstein, 2020).

1.4.2 Secondary Metabolites as Mediators of Plant-Environment Interactions

Secondary metabolites mitigate environmental stress.

As plants are the centre stage of many interactions in an ecosystem since they are primary producers, they use many secondary metabolites to interact with their environment. Secondary metabolites can have diverse roles (Salam et al., 2023). For example, terpenoids and other secondary metabolites have been found to mitigate environmental stressors, such as heat stress, drought tolerance, UV protection or even salt stress (Abbas et al., 2017; Boncan et al., 2020; C. Li et al., 2023; Pichersky & Raguso, 2018; Wink, 2018). Volatile terpenoids can mitigate oxidative stress by modulating the oxidative status, interacting with oxidants inside cells or on leaf surfaces, stabilising membranes, and influencing reactive oxygen species signalling (Blande et al., 2014; Sewelam et al., 2016). In simpler terms, terpenoids help plants neutralise harmful molecules, protect cellular membranes, and regulate intercellular communication regarding these threats. Abscisic acid, a terpenoid hormone, regulates the closure of stomata to reduce water loss (C. Li et al., 2023). When plants face tough conditions (such as drought or extreme temperatures), they might upregulate their terpenoid and phytohormone production. These chemicals promote stress-induced leaf senescence and abscission, helping the plant save resources (Boncan et al., 2020). Even though this process is targeted to battle harsh abiotic conditions, such ageing processes can make plants more attractive to pests, such as aphids (Boncan et al., 2020). However, through resources allocation, plants are able to make their leaves less attractive to herbivores (Boncan et al., 2020; Pegadaraju et al., 2005). Once the extreme environmental conditions have passed, plants can reverse the senescence process if not proceeded beyond a certain point (Balazadeh et al., 2014).

Secondary metabolites facilitate biotic interactions.

Some secondary metabolites also likely evolved to mediate plant-organism interactions and can directly affect herbivores through mechanisms such as deterrence, toxicity and host recognition cues (Herms & Mattson, 1992; Kessler & Baldwin, 2001; Wetzel & Whitehead, 2020a; Whitehead et al., 2021). Orchids, for example, produce phytoalexin enzymes with antifungal properties when attacked by pathogenic fungi, but they must regulate these enzymes to maintain mutualism with mycorrhizal fungi (Reinecke, 1994; Teoh, 2016). Other compounds, such as alkaloids, phenolics, and terpenoids, can deter herbivores by disrupting their digestion and metabolism (Hartmann, 2007; War et al., 2012), but herbivores may adapt to these defences, leading to co-evolutionary arms races (Erb & Robert, 2016). Many chemical compounds plants produce are thought to repel herbivores (War et al., 2018). Secondary metabolites can also contribute to the plant’s colour, scent, and flavour (Kessler & Kalske, 2018a). Unsurprisingly, secondary metabolites are ubiquitous in the plant kingdom, and a huge variety has been detected. In 2007, more than 20.000 secondary metabolites were described, while in 2016, researchers identified already over 50.000 different compounds (Teoh, 2016; Waterman, 2007). This stunning number indicates how important secondary metabolites in the plant kingdom must be and that they fulfil a huge variety of roles, helping plants to survive, thrive, defend and propagate (Moore et al., 2014; Teoh, 2016; Waterman, 2007). Considering these numbers, it is not surprising that plants exhibit great inter- and intraspecific diversity in their secondary metabolite profiles. Even within one plant, individual secondary metabolites can vary in their presence or concentration and can be partitioned within different tissues (Moore et al., 2014). Secondary compounds are often focused on in chemodiversity research, as these compounds are mainly the involved in the mediation of plants and their environments (Kessler & Kalske, 2018a; Richards et al., 2015; L. Yang et al., 2018).

1.4.3 Examples of Secondary Compounds and their Functions

Secondary metabolites include several categories, such as alkaloids, flavonoids, tannins, glycosides, resins and terpenoids. Common examples of **alkaloids** are caffeine or nicotine, which have physiological activity but can also deter insects from feeding on leaves (Dalton et al., 2003). Similarly, **glycosides** are often involved in plant defence, as they can be toxic to herbivores (Sivaramakrishnan et al., 2024), while **resins** protect plants from pathogens and herbivores and often emerge after wounding (Vázquez-González et al., 2020). **Terpenoids** represent another large group and often mediate the plant's interactions with its environment. Volatile terpenoids can serve as host-recognition cues but attract herbivore enemies, indirectly contributing to plant defence (Baldwin, 2010; Ghirardo et al., 2012; Heil & Bueno, 2007). These properties make terpenoids an interesting class to study relationships between their diversity and their impact on insect behaviour.

Secondary metabolites can sometimes have dual roles, inadvertently attracting herbivores or other antagonists (Schiestl, 2015; Theis, 2006). For instance, glucosinolates in *Brassicaceae* can deter generalist herbivores, such as the cabbage looper (*Trichoplusia ni*), while attracting parasitoids of specialist herbivores (Hopkins et al., 2009; Mithöfer & Boland, 2012). Both processes, bottom-up (e.g. deterrence of herbivores through toxic metabolites) and top-down (e.g. suppressing herbivore growth through attracting predators and parasitoids), shape ecosystem dynamics and are influenced directly or indirectly by secondary metabolites.

1.4.4 Terpenoids – a functionally relevant group

Terpenoid synthases and modifying enzymes are very diverse and produce numerous monoterpenoids and sesquiterpenoids (Degenhardt et al., 2009; Lange & Srividya, 2019). Terpenoids serve a broad range of ecological functions, including defence against herbivores and pathogens and attraction of pollinators or herbivore enemies. For example, the non-volatile mixtures in tansy leaves (*T. vulgare)* correlated with the abundance of aphids in the field and structured the genetic population of *Metopeurum fuscoviride* aphids (Clancy, Zytynska, Moritz, Witting, Schmitt-Kopplin, et al., 2018; Zytynska et al., 2019a). Furthermore, distinct volatile terpenoid blends lead to a species-specific distribution of herbivores (Kleine & Müller, 2011). Terpenoids can be stored in specialised structures such as glandular trichomes on the leaf surface or induced and emitted when biotic stressors such as herbivory occur (Clancy et al., 2016; Guerreiro et al., 2016).

What remains unknown?

While terpenoids are divided into mono- and sesquiterpenoids, it is unclear how these two groups are related and whether mixtures within a plant relate to each other (Rahimova, Neuhaus-Harr, et al., 2024). As mono- and sesquiterpenoids stem from different biosynthetic pathways, these compounds might be indeed unrelated (e.g., regulated differentially). Furthermore, we do not yet fully understand how the distinct groups of mono- and sesquiterpenoids shape insect colonisation patterns. Are insects affected by either mono- or by sesquiterpenoid profiles only, or do both groups, in combination, shape herbivore communities? Further, insect preference might be more driven by volatile terpenoids, using specific blends as host-finding cues, while performance (e.g., reproduction or growth of larvae) could be more driven by primary compounds or terpenoids in the sap. It remains unknown whether insect preference towards distinct terpenoid blends – so-called chemotypes – is indeed related to higher performance on the specific host plant chosen.

1.5 Chemotypes – distinct Terpenoid Profiles

In natural environments, plant compounds exist as chemical mixtures rather than in isolation. Hence, it is important to describe the variation within these mixtures, including the number of metabolites, their relative abundance, and the specific composition of the blend (Moore et al., 2014; Müller & Junker, 2022), for which the term “chemotype” was coined.

1.5.1 Definition – what is a Chemotype?

Chemotypes refer to groups of conspecific plants that can be distinguished by their secondary metabolite profiles, which often involve heritable differences in the composition or concentration of specialised compounds (Müller et al., 2020; Polatoglu, 2013). Within the same species, subspecies, or variety, chemotypes may exhibit either a blend of secondary metabolites dominated by specific compounds or a mixed profile containing multiple compounds in lower relative concentrations (Dussarrat et al., 2023; Polatoglu, 2013). For example, individuals of common thyme (*Thymus vulgaris*) can be categorised into chemotypes based on the dominant monoterpenoids, such as geraniol, α-terpineol, sabinene hydrate, linalool, carvacrol, or thymol (Thompson et al., 2003). While a single or few dominant compounds define some chemotypes, others lack clear dominance and exhibit a more balanced, mixed composition of terpenoids.

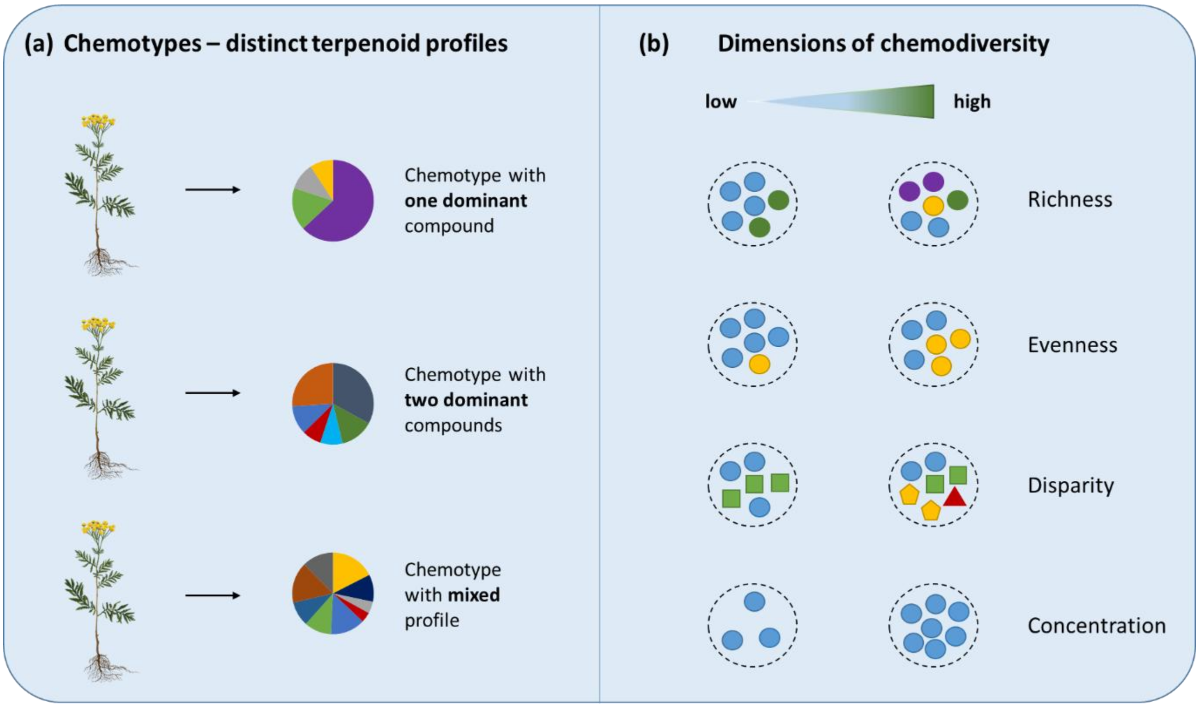

Figure 1: (a) Schematic illustration of different chemotype profiles of *Tanacetum vulgare*). The colours of the pie chart indicate the relative abundance of secondary metabolites, such as terpenoids. Chemodiversity can be described through different dimensions. **(b) Important dimensions of chemodiversity** include richness (the number of different chemicals present), evenness (the relative abundance of chemicals), disparity (the dissimilarity among secondary metabolites, e.g. in their molecular structure) and concentration (the amount of each component present) (Tansy drawing © Theresa Rohrberg)

1.5.2 Important Dimensions of Chemistry

Determination of Richness, Evenness & Disparity

Chemodiversity research encompasses several dimensions, which need to be considered. 1) **Richness** describes the total number of different chemical compounds present. 2) **Evenness** refers to the distribution of these compounds' concentrations. 3) **Disparity** is the degree of difference between the chemical compounds regarding their structure, function, or metabolic pathway (Petrén, Anaia, et al., 2023). Richness, evenness and disparity have been found to significantly influence the arthropod community, and chemotypes differ in these variables (Dyer & Jeffrey, 2021; Fernandez-Conradi et al., 2022; Petrén, Anaia et al., 2023). For example, higher richness is hypothesised to attract a more diverse array of arthropods due to the increased likelihood of producing compounds that serve as recognition cues for different species, potentially enhancing pollination and, therefore, the plant's reproductive success (Dyer, 2018; Jacobsen & Raguso, 2018). A diverse chemical profile may also help deter herbivores, providing more comprehensive protection against herbivory (Wetzel & Whitehead, 2020a). The increased richness can enhance a plant's resistance to herbivores by making it more difficult for insects to adapt to a single defence compound (Kessler & Halitschke, 2009; Poveda et al., 2003).

Levels of intraspecific chemodiversity can be observed at multiple scales, ranging from individual variation within an individual to differences observed across populations (Eilers, 2021; Moore et al., 2014; Müller et al., 2020; Ojeda-Prieto, Moreno et al., 2024). Examining these chemodiversity patterns across populations offers valuable insights into adaptive mechanisms and evolutionary processes (Bakhtiari et al., 2019; Keskitalo et al., 2001; Wolf et al., 2011).

Measurements of Chemical Diversity

Traditional indices like Shannon's and inverse Simpson diversity focus primarily on richness and evenness (Petrén, Anaia, et al., 2023). However, only including specific components of diversity while neglecting others potentially leads to misleading ecological interpretations (Steel et al., 2013; Tuomisto, 2010). For example, studies have shown that only specific measures of chemodiversity in glucosinolates of *Cardamine* species correlate with herbivore performance (Bakhtiari et al., 2021), emphasising the need for indices that capture a broader range of diversity parameters. An integrated approach is critical to understand how the specific diversity parameters impact ecological outcomes (Bakhtiari et al., 2021; Wetzel & Whitehead, 2020a). Here, addressing key limitations of Shannon's and inverse Simpson diversity, the **functional hill diversity** has been proposed (Petrén, Anaia, et al., 2023). This measure integrates compound richness, evenness and disparity into a single framework, which helps describing how functionally different or similar compounds affect ecology.

Concentration of volatiles

Concentration of volatiles reflects the abundance of terpenoids. In this work, the **terpenoid's concentration** is used as one main character for the description of the different chemotypes of tansy. Such volatiles significantly shape herbivore behaviour, life history traits, and survival of herbivores. High concentrations of terpenoids can act as chemical defences, directly affecting herbivores through deterrence or toxicity while influencing their physiological and developmental processes. For instance, in the butterfly painted lady (*Vanessa cardui)*, a higher terpenoid concentration in its host plant was shown to prolong the development time of the herbivore, potentially affecting its fitness and population dynamics (Irving et al., 2023). Thus, it is important to include different angles of chemodiversity in research about ecological effects of chemotypes.

1.5.3 Ecological Effects of Chemotypes

Differences in plant chemical profiles, driven by genetic variation, play a critical role in shaping plant-environment interactions. By producing distinct blends of secondary metabolites, such as terpenoids, plants can significantly influence their performance and interactions with pollinators, herbivores, and pathogens (Kessler & Kalske, 2018a). For example, different chemotypes of *Thymus vulgaris* produce varying mixtures of essential oils, which affect their resistance to pests and attractiveness to pollinators (Etri & Pluhár, 2024; Linhart et al., 2005; Thompson et al., 2003). Similarly, in *Solanum dulcamara*, slugs preferentially consume specific chemotypes with unique glycoalkaloid compositions (Calf et al., 2018).

Chemotypes are crucial for resource competition, defence mechanisms, and mutualistic interactions. In tansy, distinct chemical profiles directly affect aphid populations by influencing aphid abundance, preference, and colonisation dynamics (Clancy et al., 2016; Jakobs & Müller, 2018; Kleine & Müller, 2011; Ojeda-Prieto, Moreno et al., 2024). Moreover, tansy plants can actively alter volatile compound emissions to attract aphid predators, indirectly reducing aphid populations and influencing herbivore communities (Clancy, 2021). The terpenoid composition in tansy leaves further affects the body mass of herbivorous caterpillars and determines chemotypic susceptibility to drought stress (Kleine & Müller, 2014).

Chemotypes also mediate complex ecological interactions by responding differently to herbivore attacks. For instance, aphids feeding on tansy plants trigger an increase in volatile compound emissions, priming the plants for subsequent attacks by caterpillars (Clancy et al., 2020). These intraspecific differences in metabolomic profiles can even shape the genetic population structure of associated herbivores, as seen in aphids feeding on tansy (Zytynska et al., 2019a).

To make things more complex, even surrounding plants can shape herbivore communities. In an experiment, which used homogenous plots (i.e., the same chemotype in each plot) and heterogeneous plots (i.e., different chemotypes), researchers found that aphid abundance was mediated by both the chemotype of its host and the plot type (Ziaja & Müller, 2023a). In other studies, plot-level chemodiversity influenced pollinator visitation and herbivore abundance (Ojeda-Prieto, Moreno et al., 2024; Sasidharan et al., 2023), which emphasises that many more variables play a role than chemotypes alone. Currently, the strength, direction and interplay of all variables in a living environment are not well understood. These and other examples underscore the importance of understanding the chemical diversity within plant species, which influence ecological relationships and adaptive strategies.

1.5.4 Growth-Defence Trade-offs: Are Plant Chemistry and Plant Morphology Linked?

Linkages between plant morphological traits and plant chemistry

Plant morphology and chemical traits can shape plant-herbivore interactions and influence herbivore abundance and community composition individually and collectively (Ruiz-Guerra et al., 2020). Morphological traits such as growth rate, structural defences, and plant architecture not only directly influence herbivore communities. They can also interact with plant chemical profiles, enhancing or modulating the effects of chemodiversity. Often, chemotypes exhibit specific growth traits. For instance, in *Salix sachalinensis*, trichome density and leaf mass per area differ between chemotypes, demonstrating the close link between morphological and chemical defences (Hayashi et al., 2005). Similarly, in tansy, plants with higher camphor storage exhibit taller shoots, while those with other chemotypes, like davadone-D or artemisia ketone, develop taller corymbs and delayed flowering (Keskitalo et al., 2001). Such interactions raise the question of whether certain chemotypes allocate more resources toward growth or chemical defence, reflecting potential trade-offs between these traits.

To grow or to defend?

Trade-offs between plant growth and defence are important in ecology, as plants must allocate limited resources between these competing priorities. Heavily investing in defence can limit growth and reproductive success, while prioritising growth often increases susceptibility to herbivores (Coley et al., 1985; Herms & Mattson, 1992; Huot et al., 2014). For instance, reproductive biomass and terpenoid concentrations in tansy correlated negatively, supporting the idea of a trade-off between these functions (Wolf et al., 2011). This trade-off is further evident in wheat plants, where fast-maturing individuals host higher numbers of aphids than slower-maturing ones, underscoring the impact of growth-related traits on herbivore abundance (Batyrshina et al., 2020). Nevertheless, investing in certain morphological traits can also help a plant to optimise its defence (see below).

Morphological traits can act as a defence.

Morphological traits also directly influence herbivore interactions by shaping the physical environment of insects. Structural defences, such as more rigid leaves, can physically deter herbivores, while plant architecture can influence herbivore community composition (Hanley et al., 2007). In the Coyote brush (*Baccharis pilularis)*, for example, erect plants attract more moth galls, while prostrate plants have a higher density of gall-forming midges (Rudgers & Whitney, 2006). Morphology also interacts with chemical traits to determine insect preference; for instance, in tansy, tall shoots or more flower heads can enhance herbivore attraction depending on the chemotype (Ojeda-Prieto, Medina-van Berkum et al., 2024). Additionally, the surrounding vegetation modulates plant volatile emissions, further influencing herbivore interactions (Kigathi et al., 2019; Ziaja & Müller, 2023a).

Trade-off or positive relationships of growth and defence?

This complex interplay between morphological and chemical traits and their combined effects on herbivore presence and performance is yet understudied. Depending on the ecological context, both traits can have a positive or antagonistic relationship. For example, chemical defences may act in concert with structural defences to increase resistance, as seen in *Salix sachalinensis (syn. Salix udensis)*, or operate independently to target specific herbivores (Hayashi et al., 2005). Understanding these interactions is key to unravelling how plants balance resource allocation to minimise herbivore infestations with the least resource expenditure. By considering both morphological and chemical traits, researchers can better understand how plants optimise their defences while maintaining growth and reproductive success.

What happens if plants receive unlimited nutrients?

The growth-defence hypothesis is built on the assumption that nutrients and other resources are limited, and plants, therefore, have to decide towards which function they allocate resources (He et al., 2022). However, in some cases, nutrients might help strengthen the physical defence mechanisms of plants. In a recent review, researchers pointed out that while an increase in nitrogen leads to increased plant growth but at the price of reducing lignin, making the plant more susceptible to herbivores, the opposite was true when plants received additional potassium. Potassium strengthened cell walls and lignification, leading to better-defended plants (Z.-X. Li et al., 2024). Allocating more nutrients towards growth *vs.* chemical defence and *vice versa* might not always result in a trade-off but is more part of an optimal defence strategy of the plant. Interestingly, in another study on nutrient availability, plant biomass increased with fertiliser application, but so did aphid infestation (Mahdavi-Arab et al., 2014). Once fertilisation reached a certain level, the harmful effects of aphids outweighed the benefits of the fertiliser (Mahdavi-Arab et al., 2014). This underscores the complexity of finding the optimal defence strategy. Despite it has been recognized that chemotypes differ in growth and defence strategies, it remains yet unclear, how plants react of increased amounts of nutrients. Especially it is unknown, if the chemotypes become more or less similar in morphology or their chemical defenses.

1.6. Linking the Above and Below: Effects of Secondary Chemistry on Arthropod-Arthropod Interactions

Plants consist of aboveground (AG) and belowground (BG) compartments. BG herbivores, such as nematodes, have been found to influence secondary metabolites in AG plant parts, indirectly affecting herbivores feeding there (X. Li et al., 2020). While the soil type influenced the insect communities (Rahimova, Neuhaus-Harr, et al., 2024) it is yet unclear, whether chemotypes differ in their mediation of the AG *vs*. BG herbivores.

Plant-mediated AG-BG interactions

Insect herbivores, regardless of AG and BG, typically have multiple adverse effects on plants. For instance, herbivory reduces photosynthetic rates due to tissue loss and also disruption of photosynthesis around the missing tissue, affecting plant size, growth, and seed production (Hodkinson & Hughes, 1982; Myers & Sarfraz, 2017; Nabity et al., 2009). In addition, both AG and BG herbivores induce local and systemic defences in plants, which change the biomass, nutritional value, or secondary metabolites of the plants. These changes, in turn, affect herbivores feeding on other plant parts (Bezemer & van Dam, 2005; Geem et al., 2013; Kostenko et al., 2012; Van Ruijven et al., 2005). For example, root-feeding herbivores, like the nematode *Pratylenchus penetrans* or the cabbage root fly *Delia radicum*, alter the nutritional quality of plant shoots in *Brassica nigra* (Soler et al., 2007), which then affects the growth and reproduction of the aboveground caterpillar *Pieris rapae* (Soler et al., 2007).

Stress response hypothesis vs. *defence induction hypothesis*

Several hypotheses have been proposed to explain the complex plant-mediated AG-BG interactions. The **stress response hypothesis** suggests that root biomass removal by root-feeding organisms causes a drought stress-like response, which increases the nutritional quality of the plant for AG herbivores, benefiting them in turn (Masters et al., 1993). This aligns with findings that BG coleopterans positively affect AG aphids, although this effect only occurs if both herbivores arrive simultaneously (Johnson et al., 2012). Similarly, other studies found that root herbivory through chafer larvae (from Scarabaeoidea) can positively correlate with the pupal weight of leaf miners (Gange & Brown, 1989; Masters & Brown, 1992). Conversely, the **defence induction hypothesis** posits that herbivores in opposite compartments negatively influence each other through toxic secondary plant compounds (Bezemer et al., 2003; Bezemer & van Dam, 2005). For instance, AG herbivores may negatively affect BG herbivores by priming the plants to reduce carbohydrate availability in the roots. Concerning this it was found that leaf-mining AG decreases the performance of chafer larvae feeding BG (Gange & Brown, 1989; Masters & Brown, 1992). In a nutshell, if one herbivore attacks the plant, the plant may increase the production of toxic or deterrent compounds that negatively influence the other herbivores.

No clear effect direction of AG-BG herbivore effects

The direction and strength of AG-BG herbivory effects can vary, and a meta-analysis done by Johnson and colleagues (2012) found no overall direction of effects. The directionality and strength of the effects depended on several variables such as the sequence of arrival, how herbivore performance was measured, the plant life history, laboratory *vs*. field studies and herbivore type (Johnson et al., 2012). Furthermore, effect directionality can vary between levels when multiple trophic levels are included. For instance, aphid offspring production was low on plants with nematode inoculation, while aphid parasitoids performed bester in microcosms with nematodes (Bezemer & van Dam, 2005).

As detailed above, plant-mediated relationships between above- and belowground organisms are well documented. However, a critical aspect is the strikingly high variation in these interactions, an issue that has received relatively little attention.

1.7 Understanding the Drivers of Chemical Diversity in the Landscape

1.7.1 Abiotic Factors

Understanding the relationship between secondary plant chemistry and abiotic factors is essential for comprehending how plants interact with their environment. Exploring these effects can reveal whether intraspecific differences lead to varying ecosystem dynamics and whether the environment itself could act as a selective force.

Location-specific abiotic factors strongly influence plant chemical profiles. For example, studies on *Thymus vulgaris* in southern France found that the composition of secondary compounds depended on soil composition and winter temperatures (Thompson et al., 2007, 2019). In the US, the chemical profiles of *Pinus banksiana* were correlated with abiotic factors like precipitation (Taft et al., 2015). Similarly, the secondary metabolites of the tallow tree (*Triadica sebifera*) vary significantly based on the experimental site and soil type (Xiao et al., 2024). Root flavonoids were most abundant in plants grown in Guilin, while tannins were highest at the Wuhan site, but both sites had higher soil nitrogen and organic matter than Kaifeng (Xiao et al., 2024). Root flavonoids, in turn, can promote beneficial microbial associations (Xiao et al., 2024), demonstrating the close interlink between abiotic and biotic factors.

Several studies have focused on the chemical profile of tansy and its interactions with abiotic factors. A key area of exploration is the spatial variation in chemical composition among individuals within a population or across different geographic locations (Hahn & Maron, 2016). Spatial variation often leads to differing abiotic conditions. Consequently, some populations may face more significant abiotic stressors than others (Wolf et al., 2011). Different tansy chemotypes exhibit distinct responses to abiotic factors. For instance, the ‘Thujone’ and ‘Carvyl-acetate’ chemotypes responded differently to drought in terms of their root-to-shoot ratio, with the ‘Carvyl-acetate’ chemotype being more sensitive to droughts (Kleine & Müller, 2014). Furthermore, warmer temperatures have been linked to higher concentrations of essential oils and phenolic compounds, enhancing antioxidant activity (Nurzyńska-Wierdak et al., 2022). Despite these findings, how specialised plant metabolism and plant-environment interactions contribute to geographic variation remains poorly understood (Rahimova, Neuhaus-Harr, et al., 2024).

The interaction between tansy chemistry and abiotic factors is complex and multifaceted, involving a dynamic interplay between the plant’s chemical defences and its environment. Understanding these interactions is critical for predicting how the environment might shape tansy and associated communities and how they could respond to environmental changes. Nonetheless, plants are influenced by abiotic variables and biotic interactions, e.g., through varying levels of herbivory and competition pressures across landscapes (Wolf et al., 2011). It may not always be clear if herbivore levels vary due to the difference in host chemistry or if the plant chemistry varies because of varying herbivory pressure. Kleine and Müller (2011) point out that the chemotypical pattern of tansy influences the distribution of herbivores but that, in turn, the exceptionally high chemical diversity of tansy found in their study could also result from varying selection pressures through generalist and specialist herbivores.

1.7.2 Biotic Factors

Plants are embedded in complex communities and food webs, and secondary metabolites are their way of mitigating and communicating. The complex interplay of several factors, such as plant chemistry, morphology and ecological context, can strongly shape these interactions. These interactions are not one-sided. Herbivores and other organisms influence and potentially alter plant chemical variation. Observational experiments often run into the classic "chicken-or-egg" problem: Does plant chemistry determine herbivore communities, or do herbivores shape plant chemistry? Biotic differences between regions, such as soil community composition, aboveground herbivores, and pathogens, may further drive radiation in plant chemotypes (Heimes et al., 2016; Mmbando, 2023; Zhang et al., 2016). The complexity deepens when we consider additional factors. For example, precipitation, plant size, mutualist presence, and seasonal timing influenced aphid communities on tansy (Senft et al., 2017a). Their community dynamics, in turn, may alter plant chemical composition.

While experimental studies on individual plants have provided insights into the effects of chemodiversity in tansy, research at the population level remains less common. Studies focusing on a plot-/population-level investigated how the combined chemical diversity of plant individuals of the same species within a plot/population influences ecological interactions and ecosystem functioning (Crutsinger et al., 2006; Koricheva & Hayes, 2018; Raffard et al., 2019). For example, two studies, manipulating the chemodiversity richness in tansy plots by planting individuals with the same or different chemotypes within a plot, have found that aphid abundance depends on both the chemotype and plot type (Ojeda-Prieto, Moreno et al., 2024; Ziaja & Müller, 2023). Hence, it is important to understand if and how the chemodiversity of natural populations differs across a bigger range and how that shapes the associated insect community.

1.8 Aims of Thesis and Research Objectives

My thesis aimed to explore the distribution of tansy chemotypes, to understand how plant chemistry shapes herbivore relationships, and to get a comprehensive understanding of how chemistry is related to plant morphology. Considering both plant and population levels is essential, because plant-level studies help us understand the direct effects of chemodiversity on individual plants. In contrast, population-level studies provide a broader perspective on how these interactions scale up to influence community dynamics and ecosystem functioning. To ensure a comprehensive understanding of the ecological roles of plant chemodiversity, I focused on natural communities of tansy across Germany and subsequently conducted controlled experiments on the plant level. To answer my questions, I conducted five experiments, of which two are published in a peer-reviewed journal, one is currently under review, and two have been analysed but not written up yet.

1.8.1 What are we still missing: the Open Questions

The first Chapter – Large-scale study

My first study was co-led with another PhD candidate. The data was already collected some years back and helped answer some of my questions about tansy and tansy-associated insects. I wanted to explore how chemotypes were distributed over a larger scale, such as in Germany, whether the differences in chemistry were driven by local environmental characteristics and how this related to aphid colonisation. Further, I was interested in whether the monoterpenoid (MT) or sesquiterpenoid (ST) profiles in plants affected associated aphids, as MT and ST can serve different functions in a plant. I expected MT and ST chemotypes to be distributed differently over Germany. If chemotypes were distributed differently over Germany, what would that mean for the herbivore community that relies on tansy as a host? To answer this question, I aimed to explore which variables shape associated aphids and ants and whether plant chemistry, plant morphology or abiotic factors, such as temperature and precipitation, shape the tansy-herbivore relationship. Further, I wanted to know whether plant morphology is linked to plant chemistry. Do certain chemotypes grow bigger than others? Do some individuals invest more resources in their chemistry to defend themselves or more into their growth?

The second Chapter – a study on preference

As I found that aphid presence/absence differed across the MT chemotypes we found across Germany, I wanted to explore whether host preference would also be driven by plant chemistry in my second Chapter. Further, as I focused on one aphid species in the large-scale study, I was interested in whether I would find chemotype preferences in two other dominant aphid species. To answer this, I conducted an experiment in the greenhouse and hypothesised that aphid preference would differ between chemotypes but that different aphid species would prefer different chemotypes. I aimed to understand whether terpenoid richness, evenness, Shannon diversity or concentration would also mediate aphid preference. I hypothesised that a high terpenoid concentration would be used by aphids as a host-finding cue and, therefore, attract aphids. Further, I wanted to understand if growth-defence trade-offs limit plants. Some chemotypes might upregulate their defences, while others might compensate for an herbivore attack with additional growth. To understand this, I wanted to link chemodiversity and chemical descriptors to plant growth and morphology, such as plant height or the number of stems. Would plants show a growth-defence trade-off, meaning that plants with high terpenoid diversity would exhibit smaller growth and fewer stems? Alternatively, do we find growth-defence syndromes with certain chemotypes showing distinct morphological features?

The third Chapter – a study on performance and aboveground-belowground insect interactions

Now that we found differences in colonisation patterns in the field, and knew that this was possibly due to attraction, I wanted to know more about the bottom-up effects. Would aphid colonies grow differently on different chemotypes, and would preference and performance match? Further, I wanted to explore if and how plant chemistry could shape herbivore-herbivore relationships. For my third manuscript, I worked with plants in greenhouses to look into these effects in a more standardised way. I aimed to explore whether aphid colonies grow differently on the chemotypes used in the second study and to get a more comprehensive picture of what drives these effects. Is it the number of terpenoids a plant produces, the concentration, or the evenness of terpenoid compounds that mediate aphid colony growth? As specialised metabolites are known to repel antagonists, I hypothesised that chemotypes with many terpenoids would suppress aphid performance. However, in nature, plants rarely experience only one but usually multiple herbivores and/or pest species, which they must defend themselves against. Hence, I wanted to understand if different chemotypes vary in mediating multiple attackers. It is known that, for example, a belowground coleopteran herbivore has positive effects on aphid colony sizes. However, I wanted to take it one step further and understand whether the chemotypes mediate the strength and the direction of these effects. Plants with certain chemotypic profiles may invest more into repelling the belowground herbivore and would upregulate their defences more than others, resulting in a detrimental effect on aboveground aphids. If this were true and chemotypes varied in their defence signalling, I would expect detrimental herbivore effects on plant health and growth to differ between chemotypes. To understand these effects comprehensively, I conducted another experiment with similar questions and treatments in the greenhouse of the facilities of the Helmholtz Centre in Munich. This experiment has not yet been written up, but I will discuss the significant findings in the discussion.

1.8.2 Research Objectives of this thesis

To answer my questions and enhance the current knowledge in the broad field of chemodiversity research, I developed two main research objectives:

1. Evaluate the ecological consequences on associated herbivores
2. Examine the effects of chemotypes on aphid presence, preference and performance.
    a. Understand which chemical trait is most important in driving aphid presence, preference and performance.
3. Explore how tansy chemistry affects the relation of above- and belowground herbivores.
4. Investigate the links of morphology and chemistry
    a. Assess whether and how morphological and chemical traits are linked in individuals.
5. Understand how resource availability shapes growth-defence trade-offs and growth-defence syndromes.

2. Material and Methods

2.1. Model organisms

2.1.1 *Tanacetum vulgare*

T. vulgare (L.) Bernh., commonly known as tansy, belongs to the *Asteraceae* family. This perennial herbaceous plant thrives in various habitats, including roadsides, pastures, riverbanks and agricultural fields (Devrnja et al., 2012; Senft et al., 2019a). Tansy leaves are divided and possess pinnate with numerous leaflets (Figure 2a). Typically, this plant grows 50-150 cm in height and grows multiple, sometimes branched stems (Figure 2b, c). Tansy propagates sexually by producing bright yellow flower heads, typically arranged in corymbs (Figure 2d), and asexually through rhizome growth (Weisser & Härri, 2005). Tansy is well adapted to full sun and well-drained soils but can grow in partially shaded areas and less fertile soils (Tutin, 1964). This has enabled tansy to spread widely across different continents. Originally from Europe and Asia, tansy can now be found in many temperature regions, including North America (Wolf, Gassmann, Clasen, et al., 2012) and can even become invasive in some areas.

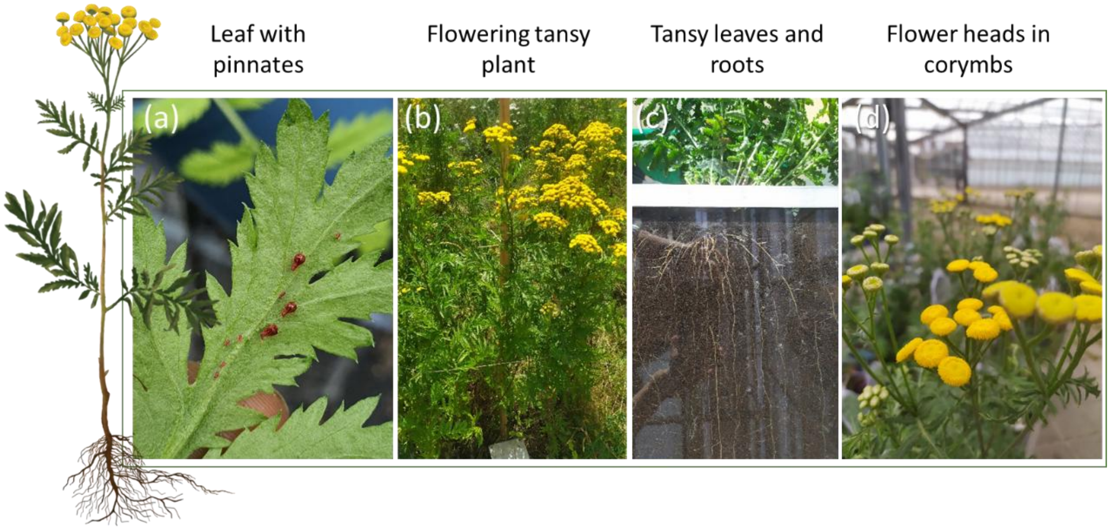

Figure 2: Morphology of tansy (*T. vulgare*) (**a**) leaf with pinnates, (**b**) flowering tansy plant, (**c**) tansy leaves and roots, (**d**) flower head in corymbs (pictures © Robin Heinen, Annika Neuhaus-Harr, Tansy drawing © Theresa Rohrberg).

Tansy is rich in its leaf and headspace terpenoid diversity (Ojeda-Prieto, Medina-van Berkum et al., 2024). Terpenoids can be volatile, and their emission from glandular trichomes and stomata can be continuous or herbivore/stress-induced, or they can be stored, which can affect herbivore behaviour and plant defence (Boncan et al., 2020; Cheng et al., 2007; Clancy et al., 2016; Lange & Turner, 2013; Pichersky & Raguso, 2018). A diverse chemical profile is thought to more effectively deter herbivores, offering broader protection and making it harder for insects to adapt to one specific compound (Kessler & Halitschke, 2009; Poveda et al., 2003; Wetzel & Whitehead, 2020a). The differences in terpenoid profiles among tansy can also influence food webs, as different chemotypes of *T. vulgare* attract distinct herbivore and predator communities (Bálint et al., 2016; Benedek et al., 2015; Senft et al., 2019a; Zytynska et al., 2019a).

In former times, tansy was used in companion planting to repel pests, and it was found that some tansy chemotypes deter the Colorado potato beetle, which helps decrease pests on potatoes (Schearer, 1984). Nevertheless, tansy harbours a diverse insect and arthropod community, some specialised and feed exclusively on tansy (Keskitalo et al., 2001; Kleine & Müller, 2011; Schmitz, 1998). In nature, plants often have to deal with various herbivore species, some of which may feed simultaneously. When aboveground (AG) and belowground (BG) herbivory co-occur, a dynamic interaction can be created between AG insects and BG insects via the host plant. Changes in plant chemistry and physiology often mediate these interactions, as AG insect feeding can influence BG herbivore behaviour and vice versa (Bezemer et al., 2003; Erb et al., 2008; Gange & Brown, 1989).

2.1.2 Aphids & Ants Socialized with Tansy

Aphids, a diverse group of sap-sucking plant parasites, comprise up to 4000 species (Eastop et al., 1986). They are significantly influenced by several external factors, including predation, parasitism and environmental conditions such as temperature and precipitation (Eastop et al., 1986; Loxdale & Balog, 2018). Plant-related variables, particularly host-plant chemistry and plant morphology, play a crucial role in shaping aphid behaviour, colonisation, abundance and host selection (Clancy et al., 2016; Clancy, Zytynska, Moritz, Witting, Schmitt-Kopplin, et al., 2018; Jakobs & Müller, 2018; Senft et al., 2017a). For instance, the chemical profile of a plant can serve as host finding cue, alter aphid dynamics, e.g. by deterring aphid feeding and reproduction, and even alter predation rates on aphids (Clancy et al., 2016; Clancy, Zytynska, Moritz, Witting, Schmitt-Kopplin, et al., 2018; Jakobs & Müller, 2018; Kumaraswamy & Huang, 2024; Linhart et al., 2005; Senft et al., 2017a, 2019a; Stadler, 2004).

Tansy harbours a diverse community of insects, such as aphids, with some species highly specialised in feeding on tansy. *Macrosiphoniella tanacetaria* (Kaltenbach) is one of the tansy-specialized species, also used in this thesis. It exhibits a greenish colour, blending perfectly with the foliage of its host plant (Figure 3a). They prefer to feed on fresh stems and the undersides of leaves and can significantly influence the growth and health of tansy plants (Kleine & Müller, 2011; Mahdavi-Arab et al., 2014). *Uroleucon tanaceti* (L.), the crimson tansy aphid, exhibits a bright reddish colour and prefers to feed on the lower sides of tansy leaves (Jakobs & Müller, 2018) (Figure 3b). When tansy leaves are infested, senescence increases and leaves will soon turn yellowish before dying. A third specialist is *Metopeurum fuscoviride* Stroyan, which typically feeds on the stems of tansy plants and has a darker red body with a brown-black spot (Figure 3c). Besides *M. tanacetaria* and *U. tanaceti*, *M. fuscoviride* is an obligate ant-mutualist who needs ants for survival (Zytynska et al., 2019a).

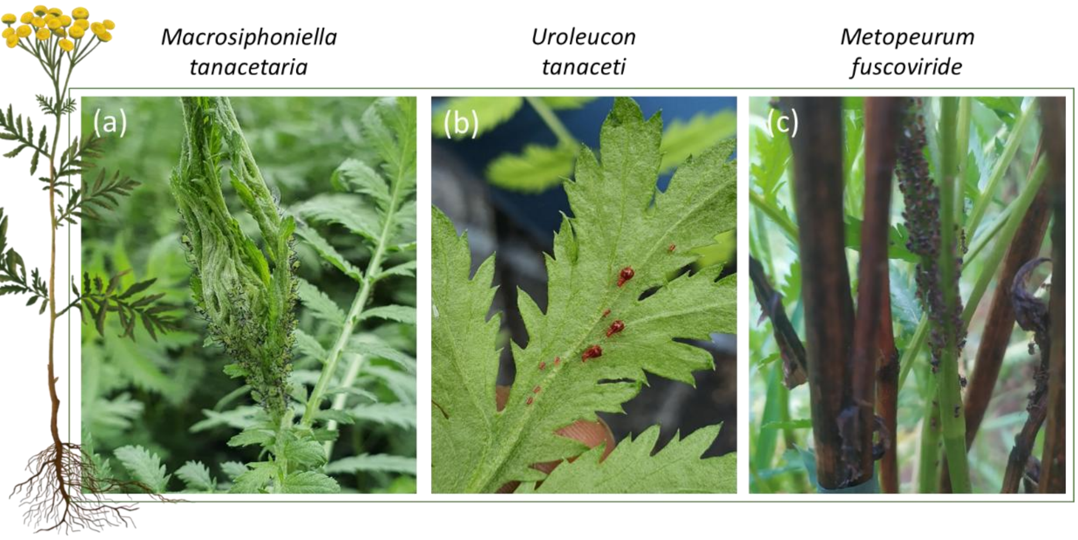

Figure 3: Pictures of the three aphid species: (**a**) *Macrosiphoniella tanacetaria*, (**b**) *Uroleucon tanaceti*, (**c**) *Metopeurum fuscoviride* colonies on tansy plants (pictures © Robin Heinen, Annika Neuhaus-Harr, Tansy drawing © Theresa Rohrberg).

These aphids not only affect their host plant through sap-feeding but are also part of a bigger food web, including mutualist ants, such as *Lasius niger*, *Formica rufa* and *Myrmeca rubra* but also predators, such as ladybugs (Coccinellidae), lacewings (Chrysopidae), hoverflies (Syrphidae) and parasitic wasps (Aphidiinae). Mutualists, predators and parasitoids can influence the presence and behaviour of aphids. For example, ant presence positively influences *M. fuscoviride* growth rates and abundances in a semi-natural field experiment (Senft et al., 2019). Furthermore, the secondary chemistry of tansy influences aphid dynamics (Benedek et al., 2015; Mehrparvar et al., 2018; Sanchez et al., 2020; Senft et al., 2017a, 2019a; Züst & Agrawal, 2017).

2.1.3 Wireworms

Wireworms are the larvae of click beetles (Elateridae), including *Agriotes lineatus*, *Agriotes obscurus* and other genera (Traugott et al., 2013). They have elongated, cylindrical bodies and hard exoskeletons, hence the common name (Figure 4). Wireworms live in the soil and primarily feed on seeds, roots and tubers of various plants (Poggi et al., 2021). As their feeding behaviour can reduce plant vigour and yield, they are considered a major pest in agriculture (Poggi et al., 2021; Traugott et al., 2013). Depending on the species and environmental condition, they can take several years to mature, with higher temperatures reducing the length of larval life cycles (Furlan, 1996; Meikle & Patt, 2011; Poggi et al., 2021). Wireworms are often used as root-feeding BG herbivores in studies researching plant-BG insect interactions or AG-BG insect interactions via plant responses (Anderson et al., 2011; Wang et al., 2024).

Figure 4: Wireworms (image courtesy Wikimedia Commons – © Rasbak 2009)

Tansy & Aphid drawing © Theresa Rohrberg

2.2 Field Study (Large-Scale Experiment)

2.2.1 Sampling

The sampling for the large-scale field sampling (see Chapter 3.1, Rahimova, Neuhaus-Harr, et al., 2024) was planned and conducted by Mary V. Clancy, Yuan Guo, Matthias Senft and Sharon E. Zytynska. Tansy plants were sampled along a northwest and southeast transect across Germany in 26 different sites. Sampling sites were mainly located near country roads, train tracks or agricultural fields. The most northern site was near Bremen (53°04'32.952"N, 08°48'25.794"E), while the most southern site was located near Freising (48°23'41.5464"N, 11°41'41.9964"E), covering about 700 km across Germany. The most western site was located near Bielefeld (52°01'08.7636"N, 08°31'51.6252"E), while the most eastern site was located near Leipzig (51°20'26.2752"N, 12°22'29.0388"E), spanning about 300 km across Germany. On each sampling site, five tansy plants without aphid infestation and ten plants infested with the specialised tansy aphid *Metopeurum fuscoviride* were sampled. Only if at least three stems per plant were occupied by *M. fuscoviride*, the plant was considered infested. Since the sampling took place during the peak aphid colonisation, it was difficult to find non-colonized plants. This, as said above, restricted the sample collection to a maximum of five plants without aphids. To calculate the number of aphids per plant (i.e., the abundance), the number of colonies were counted and their sizes estimated (XS: < 10 aphids, S: 10–50 aphids, M: 50–200 aphids, L: > 200 aphids). These values were later translated back to a weighted estimate of aphids present in this work. Additionally, the presence of different ant species was noted (*Formica rufa*, *Lasius niger*, or *Myrmica rubra*), as *M. fuscoviride* is an obligate ant-mutualist.

2.2.2 Morphological Measurements and Assessment of Abiotic Conditions

For each plant, morphological traits were measured and the exact geographic location noted. For my analyses, I included the following morphological traits: height of the tallest stem, the number of stems per plant, the diameter of the plant spanning its widest width and the height of the surrounding vegetation in a two-meter radius of five vegetation categories (bare soil, grass, small herbs, tall herbs, and shrubs). To obtain the relative height of the surrounding vegetation, I took the weighted percentage of the five categories ranked by their height. Further, I calculated the volume taken by the plant ($\text{radius}^2 \times \pi \times \text{height}$), as well as the plant *bushiness* (i.e, plant volume divided by the number of stems) and the emission potential of the plant volatiles (volume × total terpenoid concentration).

From the German weather service (Deutscher Wetterdienst), I obtained the average annual temperature at each site and the annual precipitation (sum of rain over a year) (Kaspar, 2023). In each site, a soil sample was taken and the percentage of sand, silt and clay analysed, which I translated into soil types using the USDA soil texture triangle (Groenendyk et al., 2015).

2.2.3 Chemotyping and Clustering of Tansy plants

From 390 plants sampled, 275 were randomly selected for chemotyping. Samples were then processed and their chemicals compounds identified as described in Rahimova, Neuhaus-Harr, et al., 2024 and Clancy et al., 2016 (see Appendix 8.1). Next, H. Rahimova clustered the plants into classes, according to their monoterpenoid (MT) and sesquiterpenoid (ST) profile, by using '*hclust'* in the R-package '*factoextra'* (Kassambara & Mundt, 2020). Four categorical classes of MT and ST were identified and were computed a DAPC. Associations between the MT and ST classes were tested and functional Hill diversity (FHD) was calculated for monoterpenoids and sesquiterpenoids using the '*chemodiv'* R package (Petrén, Köllner et al., 2023). Further details on clustering, testing associating and calculating the FHDs are described in Rahimova, Neuhaus-Harr, et al., 2024 (see Appendix 8.1.).

2.2.4 Statistical Analysis

To determine if plant morphology varied among chemotype classes, I conducted a one-factorial ANOVA, examining the number of stems, plant volume, emission potential, height, radius, and bushiness. Additionally, I used a one-factorial ANOVA to assess the relationship between plant morphology and soil type. I performed Pearson correlations with Holm-Bonferroni correction to investigate morphological differences across latitudinal and longitudinal gradients in Germany.

To examine if aphid or ant occurrence was influenced by chemotypes, plant morphology, and site variables, I used generalised linear models (GLMs) with a binomial distribution for occupancy (1/0). For aphid abundance, I multiplied the number of colonies per plant by the minimum number of aphids in each colony category. I then log-transformed the response variable to meet normality assumptions using a linear model (LM) with a normal distribution. I assessed post hoc pairwise comparisons using the 'emmeans' R package with Tukey adjustment (Russell, 2022).

To avoid multicollinearity in the models, variables with a variance inflation factor (VIF) higher than five ('car' R package, Fox & Weisberg, 2018) were excluded. Therefore, 'plant radius', 'plant volume', 'total terpenoid concentration', and 'soil type' were omitted. Predictor variables included 'monoterpenoid class', 'sesquiterpenoid class', their interaction, 'monoterpenoid concentration', 'sesquiterpenoid concentration', 'emission potential', 'bushiness', 'height of surrounding vegetation', 'plant height', 'number of stems', 'annual temperature', 'annual precipitation', 'latitude', and 'longitude'. For aphid abundance, the presence of ant species '*F. rufa*', '*L. niger*', and '*M. rubra*' was included, as well as the 'soil type' of the site. Further, to test whether the FHD affected aphid presence and abundance, a one-way analysis of variance was used.

2.3 Greenhouse Studies (Preference Study & Performance Study)

2.3.1 Chemotypic Characterisation

For the greenhouse studies (see Chapters 3.2 Neuhaus-Harr et al., 2024 and Chapter 3.3, Neuhaus-Harr et al., 2025) leaf and seed samples from 27 *T. vulgare* plants (referred to as "mothers") were collected in 2019 in Jena, Germany (50°55'48"N, 11°34'48"E; conducted by former colleagues; Eilers et al., 2021). Briefly, the leaf material underwent terpenoid profiling and was then analysed, as described in (Ziaja & Müller, 2023a)(see Appendix 8.2). Plants were then clustered using the unsupervised hierarchical k-means clustering method (i.e, *hclust*), and plant terpenoid profiles were categorised into chemotypic clusters based on their absolute values, encompassing terpenoid composition and concentration. From the initial pool of 27 mother plants, seven distinct chemotypic clusters were found. Six of these clusters were then randomly selected for further analysis. Two plants from each cluster were randomly chosen, and seeds collected from these mother plants were mass-sown, resulting in 120 daughter plants (i.e., 10 plants per mother plant). The seedlings were grown under controlled conditions and later transferred to a greenhouse. In the greenhouse, leaf samples were harvested and freeze-dried for a second round of chemotyping.

Hierarchical clustering of the daughter plants' terpenoid compositions identified six chemotypic clusters. These clusters were characterised by their dominant compounds and relative concentrations (Figure 5). The chemotype 'Athu-Bthu' chemotype was dominated by both α- and β-thujone. 'Bthu-high' and 'Bthu-low' chemotypes were dominated by β-thujone, but had either high or low relative levels of terpenoids. The chemotype 'Chrys-acet' was dominated by trans-chrysanthenyl acetate, Both mixed chemotypes, 'Mixed-high' and 'Mixed-low', encompassed several, more or less evenly distributed terpenoids, relatively high concentrations of sabinene hydrate or sabinene, and had an either high or low relative terpenoid concentrations, respectively. Three daughter plants from the same mother plant from each cluster were chosen, resulting in 18 daughter plants from the six chemotypes (Figure 5). Furthermore, using the absolute terpenoid profiles of the daughters, other chemical descriptors, such as total terpenoid concentration, terpenoid richness, terpenoid evenness, and terpenoid Shannon diversity, using the 'vegan' package were assessed (conducted by Robin Heinen) (Oksanen et al., 2023). These values were used for further analyses.

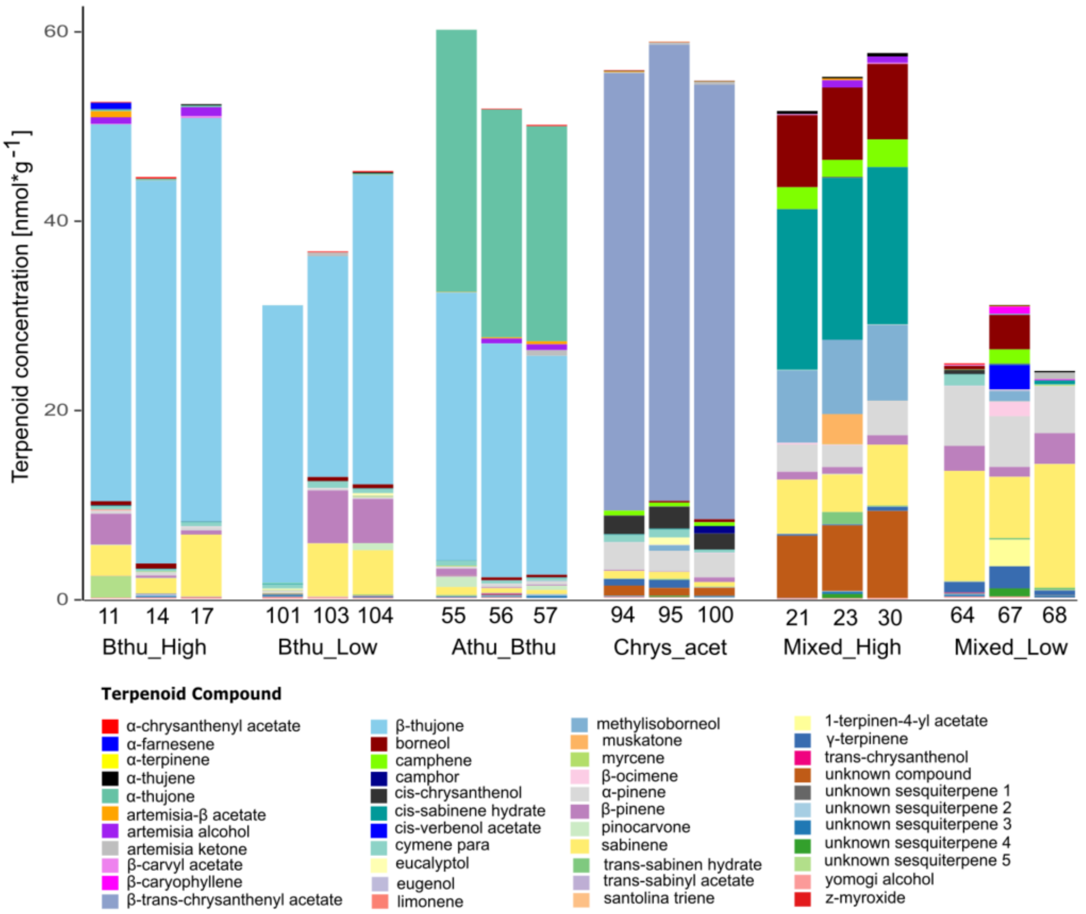

Figure 5: Chemotypic profile of the plants used in this study. Stacked bars illustrate the approximate concentration of terpenoids (in nmol*g-1) obtained from the leaf samples from 18 daughter plants of each six mother plants with differing chemotype as indicate. Samples are grouped based on their chemotype. Numbers on the x-achses indicate the number of the chosen daughters (adapted from Neuhaus-Harr et al., 2024).

2.3.2 Propagation of Plants

All selected chemotypes of tansy plants were maintained in a garden plot of the university in Freising (48°24'17.9"N 11°41'32.2"E) Germany. In August 2021 and May 2022, fresh plant material from all 18 daughters was colleced (together with Lina Ojeda-Prieto). Shoot cuttings were prepared by cutting stem parts 1-2 cm below and 4-5 cm above a leaf node. To reduce evaporation and the risk of mould, roughly 75% of the pinnate leaves were clipped. The stem 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 immediately covered by a transparent plastic hood, and trays were kept in a greenhouse with bottom watering and additional lighting (16:00:8:00 h Light:Dark). The transparent hoods were gradually opened after three weeks as the plants established roots and shoots. After acclimatisation for 2 wk, rooted cuttings were potted into 10/11 cm-diameter pots and later into 17/19 cm pots to avoid pot limitation. To maintain a target electrical conductivity of 1.0, plants were fertilised (conducted by staff from the TUM PTC Dürnast) with Universal Blue fertiliser (18% N – 11% P – 18% K; ICL Deutschland). We did not use any chemical insecticides or fungicides during plant propagation. Clones from the same daughter were grown in pots randomly distributed in the greenhouse to ensure the independent development of each plant. The Bthu_Low chemotype had low propagation success in August 2021; thus, only the remaining five chemotypes (with the 15 respective daughters) were used in pairwise aphid choice experiments at this time. Propagation success from each chemotype was sufficient in May 2022. Once plants were fully established, they were placed in a vegetation hall covered with iron mesh (5 cm) walls.

2.3.3 Insect Rearing

Wireworms were used as a mix of *Agriotes lineatus* and *Agriotes obscurus.* They were sourced them from Wageningen University in Lelystad, The Netherlands, in 2022. Upon arrival, wireworms were maintained in two boxes (ca. 12×18cm) with sandy soil at 20°C. At least three one-cm-thick potato slices at a time were provided as food.

For the preference and performance experiments (see Chapters 3.2 and 3.3, Neuhaus-Harr et al., 2024; Neuhaus-Harr et al., 2025), adults and nymphs of the aphid species *M. tanacetaria* and *Uroleucon tanaceti* were collected from tansy plants in a field near Freising or Jena (48°24'02.4"N 11°43'00.5"E, 50°57'05.5"N 11°37'25.1"E, Germany). Aphids were caged in a climate-controlled lab at room temperature for both experiments under long day regimes (16:00:8:00 h L:D). Aphids were fed with 2-4 tansy plants at a time, obtained from local populations from Freising, but not the experimental chemotypes used. For both experiments, at least 100 unwinged adult aphids were collected and transferred to Petri dishes (max. 20 aphids per Petri dish) with fresh leaves. After 48 h, adult aphids were removed, leaving an age-specific nymph cohort on the leaves. For the preference experiment (see Chapter 3.2, Neuhaus-Harr et al., 2024), cohorts were kept in a Fitotron standard growth chamber (21/16°C, 60% RH, Weiss Technik, Reiskirchen) for 8 d, while aphids for the performance experiment (see Chapter 3.3, Neuhaus-Harr et al., 2025) were kept only for 3 d. Additionally, aphids for the preference experiment were starved in a petri dish for 24 h with only a small piece of wet tissue added.

2.3.4 Choice Assays (Preference Study)

Choice assays (see Chapter 3.2, Neuhaus-Harr et al., 2024) were conducted using clones from five of the six *T. vulgare* chemotypes, with three replicate daughters per chemotype. The assays were designed as a full-factorial series of pairwise choices, allowing aphids to select between cloned plants of two different chemotypes. For each replicate series, all chemotypes were tested against each other using randomly selected clones from randomly chosen daughters of the mother chemotypes. This approach maximised the number of unique pairwise choices between chemotype-daughter pairs.

Choice assays were performed in three rounds over three days, using different age-standardized aphid cohorts (9-10 d old). All assays were completed within one week (December 9-16, 2021). 23 replicate rounds were conducted for *M. tanacetaria* but only 13 for *U. tanaceti* due to their lower cohort numbers. For each assay, the second and third youngest fully expanded leaves were selected and clipped from a plant, the first three leaflets discarded and the subsequent ones used. Two leaflets were placed in new 14.5 cm petri dishes, positioned equidistant from the centre and roughly 8 cm apart. To account for external bias, the sides for each replicate alternated between rounds.

Following, a single starved nymph was placed in the centre of each petri dish, which was then sealed with parafilm to prevent leaf desiccation. The dishes were kept in a climate chamber (TUM Model EcoSystem Analyzer TUMmesa) at the Technical University of Munich, utilising an LED lighting system to create homogeneous light conditions and reduce spatial effects. Aphid preferences were recorded after two, five, and 24 h (16:8 h L:D photoperiod, 21°C, 60% RH). However, due to decreased leaf quality after 24 hours affecting aphid behaviour, the 24-hour time point was excluded from subsequent analyses. Each aphid and leaf were used only once, and dead aphids were excluded from the analysis.

2.3.5 Above-Belowground Insect Assays (Performance Study)

To explore the performance of aphids (see Chapter 3.3, Neuhaus-Harr et al., 2025) on different chemotypes and in the presence/absence of a belowground herbivore, a full factorial experiment with four treatments was designed: either no herbivore (control), only aboveground herbivore (two nymphs of the species *M. tanacetaria*), only belowground herbivore (two individuals of *Agriotes sp.*), or both herbivores. Plants were a priori assigned to one of the treatments, and arranged in a block design (10 replicated blocks) with 240 plants in total.

Four days before aphid infestation, two 1 cm deep holes were poked in the soil surface of all pots. Plants designated to a wireworm treatment received two wireworms. To prevent them from escaping, plants were placed on saucers. From then on, plants were watered twice daily from above, with up to 400 ml per event, based on the soil humidity. On day four, fine mesh bags (11×9.5 cm) were attached to the second youngest, fully expanded leaf of all plants. Plants designated to aphid treatment received two three-day-old nymphs, which were placed inside the mesh bags. The mesh bags would protect nymphs against predators and maintain colony location, which allowed controlled observations. In the following weeks, the numbers of *M. tanacetaria* were counted on days 8, 11, 15, 18 and 21. Three days after the final count, the unplanned infestation of *Coloradoa tanacetina* was assessed, another aphid species, which had infested our plants.

After plants were harvested, wireworms were traced back and retrieved manually through soil examination. My colleagues and I observed unexpectedly high pupation rates, possibly due to the heatwaves plants encountered during the experimental time. We found 68 pupated/adult wireworms, 90 larvae, and 74 went missing (presumed adult escapes). I developed additional statistical models to account for pupation effects on treatment efficacy, including the number of retrieved wireworm larvae.

2.3.7 Morphological Traits

For the preference experiment (see Chapter 3.2, Neuhaus-Harr et al., 2024), non-destructive measurements of plant morphological traits were conducted on each of the 120 daughter plants used once they were fully established but before using their leaves in the experiment. The following measurements were included: plant structure (number of stems, leaves, and nodes, plant height (in cm) and internode length (= height/number of nodes); leaf characteristics (number of leaflets per leaf, leaf length, and leaflet density = number of leaflets/petiole length), chlorophyll content (using a chlorophyll meter from Konica Minolta SPAD-502Plus, Tokyo, Japan). After the experiment, the plants were harvested to determine the leaf surface area (using ImageJ software). Then, the plants were dried at 60°C for 72 h to determine their dry weight and specific leaf area.

Within the performance experiment (see Chapter 3.3, Neuhaus-Harr et al., 2025), we measured plant height (in cm) once plant growth stagnated and after the experiment. Further, we measured the chlorophyll content (using the same chlorophyll meter from Konica Minolta SPAD-502Plus, Tokyo, Japan). After harvesting the aboveground biomass, we measured the dry weight and dried the plants at 60°C for 78 h.

2.3.8 Statistical Analyses

Preference study

For the preference study (see Chapter 3.2, Neuhaus-Harr et al., 2024), binomial tests were used to determine if aphids preferred one chemotype to another in pairwise combinations. We also checked for spatial (left/right) and observer effects on aphid preference using all observations (n = 690). With four clogit models, the attractiveness of *T. vulgare* chemotypes and daughters for each aphid species were assessed, using the ‘survival’ package (Therneau, 2021). Z-values from the models indicated attractiveness, with positive values being more attractive.

Following, we calculated plant chemodiversity metrics (Shannon diversity, terpenoid evenness, richness, and relative total concentration) using the ‘vegan’ R-package (Oksanen et al., 2020). Four models were created to test relationships between plant attractiveness (z-value) and chemodiversity metrics for each aphid species at the tansy daughter level (n = 15). We tested for correlations between individual compound concentrations and plant attractiveness using unadjusted and Holm-adjusted plots with the ‘RcmdrMisc’ R-package (Fox, 2022). With a multiple regression model, the effect of all compounds on attractiveness was tested with a stepwise reduction to address multicollinearity.

To address differences among chemotypes in plant height, stem number, leaf number, leaflet density, specific leaf area, chlorophyll, and chemodiversity metrics, we used one-way ANOVAs with chemotype as a fixed factor (n = 18 replicates).

Performance study

For the performance study (see Chapter 3.3, Neuhaus-Harr et al., 2025), several linear mixed models using the ‘lmer’ function from the ‘lme4’ R-package were conducted (Bates et al., 2014). To meet normality assumptions, all aphid counts were transformed using square root and assessed model fit using QQ plots, residual plots, and scale-location plots. To test if chemotypes influenced aphid colony size, a linear mixed model was used with the final aphid count as the response variable and chemotype as a fixed factor. *Coloradoa tanacetina* abundance was included as a covariate, with block and daughter ID as random effects. Then two model variants were created to test the effect of the belowground treatment (Model A) or the number of retrieved wireworm larvae (Model B) on aphid colony size over time. Model A included chemotype, belowground treatment, observation day, and their interactions as fixed effects, with block, daughter ID, and unique plant ID (nested in observation day) as random effects. In model B, the belowground treatment was replaced with the number of retrieved wireworm larvae.

We set up two multiple regression models to examine if plant chemical diversity mediated the effect of wireworms on aphid colony size. Model A included belowground treatment and chemical diversity components as fixed effects, with block as a random effect. Model B replaced belowground treatment with the number of retrieved wireworm larvae. Variance inflation factors were used to address multicollinearity, excluding terpenoid Shannon diversity due to the high correlation with terpenoid evenness.

To test if herbivory affects plant morphology (chlorophyll, height, biomass), I used the following linear mixed models: Model A, which included *C. tanacetina* as a covariate, treatment (aboveground, belowground, both, control), chemotype, and their interactions as fixed factors, with block and daughter ID as random effects. Due to the uneven distribution of retrieved larvae, Model B was limited in analytical power. However, it tested for the effect of retrieved wireworm larvae on plant variables, with block and daughter as random effects.

All statistical analyses were conducted in R using v4.1.3. More details on statistical analyses can be found in Neuhaus-Harr et al., 2024; Rahimova, Neuhaus-Harr, et al., 2024; Neuhaus-Harr et al., 2025 (see Appendix 8).

3. Manuscript Overviews

This thesis contains two published shared first-author manuscripts and a submitted first-author manuscript (in review). Here, summaries and the author's contributions are given. The manuscripts are attached in the Appendix. Manuscripts were reproduced with the written allowance of the respective publishing journal.

3.1 Geographic distribution of intraspecific chemical variation of *Tanacetum vulgare* and its effect on aphids

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. (2024). The geographic distribution of terpenoid chemotypes in *Tanacetum vulgare* mediates tansy aphid occurrence but not abundance. *Oikos* 2024(7), e10320. https://doi.org/10.1111/oik.10320

* These authors share first authorship and contributed equally to this publication.

3.1.1 Addressed Questions

1) What is the geographic distribution of terpenoid chemotypes in tansy along a northwest-southeast gradient in Germany?

2) How are aphid and ant occurrence and abundance affected by chemotypes, plant growth, and (abiotic) site variables?

3.1.2 Summary

This study was conducted to explore the German-wide geographic distribution of terpenoid chemotypes and blends in *Tanacetum vulgare* and their impact on the associated insect communities. In 26 sites in Germany, ten plants with *Metopeurum fuscoviride* and five plants without aphids were selected, and leaf tissues were sampled. Aphid numbers and ant presence were assessed, plant morphology, such as plant height, was measured and abiotic factors, such as the soil types, were recorded.

We found that plants clustered into four monoterpenoid and four sesquiterpenoid classes. While monoterpenoid classes differed in their latitudinal distribution, sesquiterpenoid classes were distributed evenly. Unsurprisingly, aphid and ant occurrence was influenced by monoterpenoids but not sesquiterpenoids. Specifically, ants and aphids were found more often on plants with β-thujone as the dominant compound than expected if insect preference was zero and insect occurrence was random. Further, we found that ant presence was additionally influenced by temperature, with ants preferring sites with a higher mean annual temperature. The abundance of aphids, that is, the number of individuals, was significantly influenced by the soil type the tansy plants grew on, which differed across Germany.

In conclusion, I found evidence of the effect of *T. vulgare* chemodiversity on aphid and ant occurrence, shedding light on the ecological significance of terpenoid chemotypes in tansy, emphasising their impact on insect herbivores. Further, my study highlights that secondary metabolites, such as terpenoids, are not only mediators between plants and their living environment but also that plant chemistry shows relationships with their abiotic environment. My findings underscore the importance of understanding whether and how secondary metabolites make plants able to adapt in a variable environment and the potential implications of ecosystem dynamics.

3.1.3 Author Contributions

HR and **ANH** contributed equally to this publication and shared the first authorship. HR: Data curation (equal); Formal analysis (lead); Methodology (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **ANH:** Formal analysis (lead); Methodology (lead); Validation (equal); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). MVC: Conceptualization (equal); Data curation (equal); Investigation (equal); Methodology (equal); Writing – review and editing (equal). YG: Data curation (equal); Formal analysis (equal); Methodology, Validation (equal); Writing – review and editing (equal). RRJ: Formal analysis (equal); Software (lead); Validation, Writing – review and editing (equal). LOP: Investigation, Methodology, Writing – review and editing (equal). HP: Formal analysis (equal); Methodology, Resources (equal); Writing – review and editing. MS: Data curation (equal); Investigation (equal); Methodology, Writing – review and editing. SEZ: Conceptualization (equal); Data curation (equal); Methodology, Supervision (equal); Writing – review and editing (equal). WWW: Conceptualization (lead); Funding acquisition (equal); Project administration (lead); Supervision (lead); Writing – review and editing (equal). RH: Methodology (equal); Project administration (equal); Supervision (lead); Writing – original draft (equal); Writing – review and editing (lead). JPS: Conceptualization (equal); Funding acquisition (equal); Project administration (equal); Supervision (equal); Writing – review and editing (equal).

3.2 The preference of aphids towards tansy chemotypes

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(3), e10437. https://doi.org/10.1111/oik.10437

* These authors share first authorship and contributed equally to this publication.

3.2.1 Addressed Questions

1) Will tansy aphids prefer certain tansy chemotypes to others?

2) As most specialised metabolites repel antagonistic organisms, will aphids be less attracted to plants with high chemodiversity?

3) Do we find trade-offs between growth-related traits and chemodiversity, assuming chemodiversity is costly and limits available resources for growth?

3.2.2 Summary

This study explored the preference of tansy aphids regarding tansy chemotypes. In choice trials, we observed which leaf the two aphid species, *Macrosiphoniella tanacetaria* and *Uroleucon tanaceti,* preferred. Further, we measured plant morphological traits.

While *M. tanacetaria* aphids preferred chemotypes with the dominant compounds α-thujone/β-thujone and trans chrysantenyl acetate, *U. tanaceti* did not show clear preferences. Interestingly, plant attractiveness was marginally negatively correlated with chemodiversity and marginally positively correlated with the relative terpenoid concentration in *M. tanacetaria*. *Uroleucon tanaceti* did not show differences in attraction towards the chemical composition. Surprisingly, we found no trade-off between plant growth and defence but rather synergistic effects. Larger and bushier plants generally had higher relative concentrations and diversity of terpenoids.

The study helps us to understand how plant chemistry influences insect behaviour and colonisation patterns. My research reveals the impact of specialised metabolites on aphid preferences and highlights the intricate connections between plant chemistry, herbivore behaviour and ecosystem dynamics. The results emphasise the importance of considering chemodiversity in ecological studies.

3.2.3 Author Contributions

ANH and LOP shared the first authorship and contributed equally to this publication. **ANH**: Conceptualization (supporting); Data curation (equal); Formal analysis (lead); Investigation (lead); Visualization (equal); Writing – original draft (lead). LOP: Conceptualization (equal); Data curation (equal); Formal analysis (supporting); Visualization (equal); Writing – original draft (equal); Writing – review and editing (supporting). EE: Conceptualization (equal); Formal analysis (supporting); Writing – review and editing (supporting). CM: Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Writing – review and editing (supporting). WWW: Conceptualization (supporting); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (equal); Supervision (supporting); Validation (supporting); Visualization (supporting); Writing – review and editing (supporting). RH: Conceptualization (equal); Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Supervision (lead); Validation (supporting); Visualization (equal); Writing – original draft (supporting); Writing – review and editing (supporting).

Tansy & Aphid drawing © Theresa Rohrberg

3.3 The Performance of aphids across tansy chemotypes and insect-insect interactions

Neuhaus-Harr, A., Ojeda-Prieto, L., Zhang, X., Schnitzler, J. P., Weisser, W. W., & Heinen, R. (2025). Aphid colony size is affected by plant chemotype and terpenoid mixture evenness in tansy. *J. Chem. Ecol.*, in review. Available as preprint: https://doi.org/10.21203/rs.3.rs-5440406/v1

3.3.1 Addressed Questions

1) Does aphid performance differ across chemotypes?

2) If belowground coleopteran herbivores affect aphid performance, will the strength and direction of the effect differ across chemotypes?

3) Will more chemically diverse plants result in smaller aphid colonies, with the belowground treatment modifying this relationship?

4) Will above- and belowground herbivory be detrimental to the plants, with the strength of these effects differing across chemotypes?

3.3.2 Summary

This study was conducted to explore the performance of tansy aphids on different tansy chemotypes. Further, we investigated how the different chemotypes mediate insect-insect relationships. In this experiment, I observed aphid colony growth on plants with and without belowground herbivores. Furthermore, I measured the impact of the herbivores on the plant.

I found that aphid colony growth rates and final aphid colony sizes differed between chemotypes, with the highest number on the chemotype with a very mixed profile but low terpenoid concentration and the lowest on the chemotype dominated by β-thujone. Further, I found that terpenoid evenness significantly influenced final aphid colony size, with plants with a more even terpenoid profile exhibiting more aphids. However, I found no effects of belowground wireworms on aboveground aphids. The herbivores indeed influenced plant morphology: Plant height and plant dry weight were influenced by the aboveground aphid, but effects differed between chemotypes. Plant chlorophyll was affected by plant chemotype, as well as by herbivore treatments. However, chemotypes did not mediate the effect's strength and direction through the herbivores.

This study implies that individuals within species might use different strategies to grow and defend themselves, and this might be connected to secondary metabolites, such as the terpenoid profile. My research advances our understanding of the role of chemotypes in the growth-defence trade-off of herbivory. Further, my results imply that plant defence might be compartmentalised and only minimal aboveground-belowground defence signalling occurs. The results strengthen our understanding of chemical profiles in the context of defence optimisation strategies.

3.3.3 Author Contributions

ANH: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Visualization (equal); Writing – original draft (lead). LOP: Conceptualization (equal); Data curation (equal); Formal analysis (supporting); Visualization (supporting); Writing – original draft (supporting); Writing – review and editing (supporting). XZ: Data curation (supporting); Investigation (supporting); Methodology (supporting); Writing – review and editing (supporting). WWW: Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (equal); Supervision (supporting); Validation (supporting); Writing – review and editing (supporting). RH: Conceptualization (equal); Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Supervision (lead); Validation (supporting); Writing – original draft (supporting); Writing – review and editing (supporting).

4. Discussion

4.1 Geographic Differences in Chemotype Occurrence

In line with previous researchers, we found that chemodiversity in tansy showed regional differences between geographic locations (Briscoe Runquist & Moeller, 2024; Judzentiene & Mockute, 2005; Keskitalo et al., 2001; Rahimova, Neuhaus-Harr, et al., 2024). Β-thujone and camphor MT chemotypes of tansy plants occurred more often in the southeast of Germany, while the trans-chrysanthenyl acetate MT chemotype or a mixed blend occurred more often in the northwest (Rahimova, Neuhaus-Harr, et al., 2024). Similarly, a Finnish study revealed that central and southern regions had tansy chemotypes with higher camphor concentrations (Keskitalo et al., 2001). Most chemotypes containing camphor were found in Central Finland, while myrcene-tricyclene, artemisia ketone and davadone D chemotypes were more prevalent in the south and southwest (Keskitalo et al., 2001). Furthermore, a Lithuanian study found that tansy had varying dominant compounds like eucalyptol, trans-thujone, and myrtenol, depending on the location (Judzentiene & Mockute, 2005). Quite recently, a study unravelled genomic differences in tansy across the US, with land use structuring populations at a broad scale and soils structuring populations at a fine scale (Briscoe Runquist & Moeller, 2024). These findings indicate that differences in terpenoid profiles and genomics are common and likely increase with geographic scale, with different regions exhibiting distinct dominance patterns of terpenoid compounds.

In these and other studies, the chemotype cluster was based on monoterpenoids (MT) and sesquiterpenoids (ST). However, ST originate from the cytosolic mevalonate pathway and MT are produced via the plastidial methylerythritol phosphate pathway (Davis & Croteau, 2000). Hence, as described in the study of Rahimova, Neuhaus-Harr and colleagues (2024), MT and ST chemotypes were analysed and clustered separately (see Chapter 3.1, Rahimova, Neuhaus-Harr, et al., 2024). We found that plants clustered into four MT and four ST chemotypes. As mentioned above, we found that MT chemotypes differed significantly over Germany, with β-thujone or camphor chemotypes occurring significantly more often in the southeast, while classes with trans-chrysanthenyl acetate or a mixed blend were found more often in the northwest (Figure 6, Rahimova, Neuhaus-Harr, et al., 2024). Sesquiterpenoid chemotypes, on the other hand, were evenly distributed over the country (Figure 6). Furthermore, MT and ST chemotypes were not strongly linked within individual plants, likely because MT and ST stem from unrelated pathways (Rahimova, Neuhaus-Harr et al., 2024). The differences in the spatial distribution of chemotypes hence seems to be driven by the MT and not the ST. Thus, monoterpenoid profiles in tansy plants showed distinct differences in their occurrence across Germany; similarly as found in other countries.

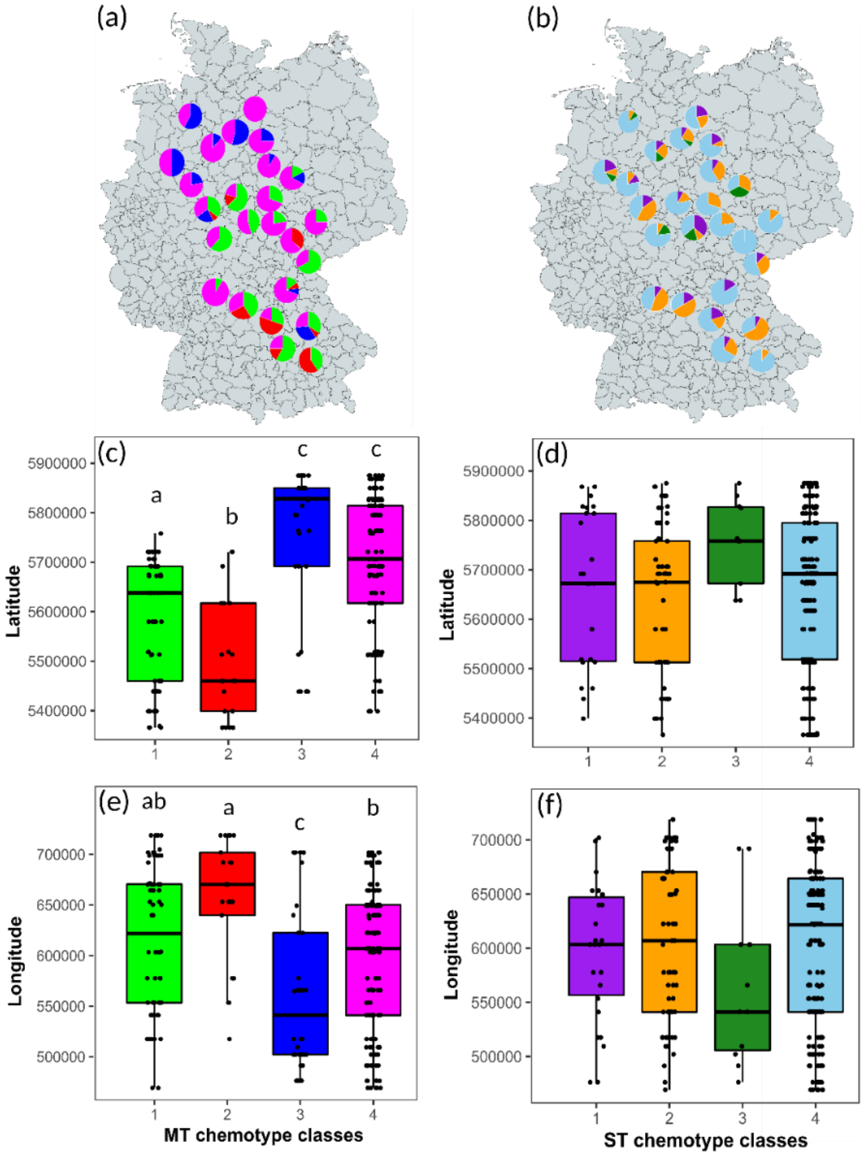

Figure 6: Chemotypes of tansy across Germany. (**a**) Proportion of monoterpenoid (MT) classes at each sampled site. Colour-code: green – class 1 (β-thujone), red – class 2 (camphor), blue – class 3 (trans-chrysanthenyl acetate), magenta – class 4 (mixed). (**b**) Sesquiterpenoid (ST) classes at each sampled site. Colour-code: purple – class 1 (bicyclosesquiphellandrene), orange – class 2 (calarene), dark green – class 3 (longiverbenone), light blue – class 4 (a-cyperone + calarene). (**c**) Monoterpenoid classes found over different latitude and (**e**) longitude. (**d**) Sesquiterpenoid classes found over different latitude and (**f**) longitude. Significant differences are indicated on top of the boxplots ($p < 0.05$). (Figure from Rahimova, Neuhaus-Harr, et al., 2024)

Tansy & Aphid & Ant drawing © Theresa Rohrberg

4.2 The Effects of Monoterpenoid and Sesquiterpenoid Chemotypes on Aphids and Ants

Influence of MT but not ST chemotypes on aphid presence

We observed that monoterpenoids, rather than sesquiterpenoids, predominantly determined aphid and ant presence on individual chemotypes. Given the greater volatility of monoterpenoids compared to sesquiterpenes (Mofikoya et al., 2019), higher concentrations are expected in the ambient air around the plant canopy. Thus, we assume that aphids and/or ants use these volatile compounds as cues to find their host plant, which explains our observation.

In addition, the notably high concentration of monoterpenoids in monoterpene class 1 could explain the higher aphid presence in these plants. Another possible reason for aphids to use monoterpenoids, rather than sesquiterpenoids, as host-finding cues could be the distinctiveness of monoterpenoid profiles regarding their dominant compounds (see Chapter 3.1, Rahimova, Neuhaus-Harr, et al., 2024). Monoterpenoid profiles showed significant variation, while sesquiterpenoid classes were chemically more similar (Rahimova, Neuhaus-Harr et al., 2024). Taken together, a preference towards distinct MT profiles is suggested to influence the preference of aphids more strongly, while the more similar and less volatile ST profiles would not evoke preferences.

Aphid preference does not correlate to abundance in peak colonisation

While my results demonstrate an effect of MT chemotypes on aphid occupancy in plants, this did not translate to abundance (see Chapter 3.1, Rahimova, Neuhaus-Harr, et al., 2024). One limitation of my study is that the observations only represent a short snapshot of insect interactions and plant growth and chemistry. Although tansy chemotypes are known to be stable over time (Clancy et al., 2016), aphid abundances can differ hugely across seasons. During field observations, I observed that in early spring, only very few aphids fed on plants (pers. obs.). As aphids reproduce exponentially, aphid colony sizes increased substantially after a couple of months, reaching thousands of individuals per plant (Ojeda-Prieto, Moreno et al., 2024). Often, within a short period, the aphid numbers would then again dwindle massively (Ojeda-Prieto, Moreno et al., 2024). Similarly, in another field study it had been observed that insect abundances highly fluctuated between years (Lina Ojeda-Prieto, pers. comm.), demonstrating highly dynamic systems (Ojeda-Prieto, Moreno et al., 2024). Since our sampling occurred during peak aphid colonisation, this might explain why we did not detect an effect of tansy chemotypes on aphid abundance. Depending on the time of the season or even the year, we might have missed the effects of the chemotypes on the abundance of aphids.

Aphid presence on plants in general can vary significantly in abundance, ranging from very few aphids to thousands. This was also observed in our experimental field in Dürnast (pers. obs.). Such variations occur in the field. After a plant has been colonised, random (e.g., incoming aphid predators, top down) or other effects (e.g., weather with water availability, bottom-up) influence colonisation other than plant chemotype (Senft et al., 2019a). Interestingly, also predators or parasitoids are using volatile compounds to find their prey's host plant (Boncan et al., 2020; Guo & Wang, 2019). For instance, coccinellid beetles, which prey heavily on aphids, are more abundant on plants with high β-thujone content (Kleine & Müller, 2011), which might explain why aphids on plants with high β-thujone did not show higher abundances. Once herbivores attack plants, plants can also increase the release of certain chemicals to attract more herbivore predators (Paré & Tumlinson, 1999). Even if aphids prefer a specific chemotypic profile, herbivore pressure might reduce their numbers on such a specific chemotype more than on others, leading to no correlation between preference and abundance.

As said, abiotic factors such as temperature or precipitation could also affect aphid colonies. Hypothetically, if aphids prefer sites with lower precipitation and chemotype A is better adapted to lower precipitation, we might find more aphids on chemotype A. However, in this theoretical case, aphid colonies would not be directly driven by a direct correlation with the chemotypic profile of the plant, but indirectly.

4.3 Abiotic Environment Shapes Abundance of Aphids and Ants

Aphid abundance and soil type

Aphid abundance, as opposed to aphid presence, was not related to MT chemotypes, but we found it to be significantly influenced by the soil type (see Chapter 3.1, Rahimova, Neuhaus-Harr, et al., 2024). Similarly, it has been shown that insect herbivory was significantly higher on cork oak trees grown on loamy than gravel soil (Shao et al., 2021). However, the effects of the soil type could be mitigated indirectly via the plant. Different soil types vary in their levels of nutrients, which affects plant health and this translates into insect abundance (Shao et al., 2021). Indeed, aphids have been found to have higher reproduction success on plants that receive more nutrients (Hayden et al., 2021). Even though tansy is observed to grow reasonably well on sandy soil, researchers demonstrated that plants developed poorly in pots with more than 50% sand (Von Cossel et al., 2024). This aligns well with the observed tendency for lower aphid abundance on plants growing on sandy soil in our study.

In addition to nutrients, the soil type causes changes on plant morphology, which, in turn, influences the aphid colonies. Plants growing on loamy sand had more stems and a larger radius than plants on silt loam, while plants growing on loam grew taller than plants on loamy sand or silt loam. Larger plants might allow a colony to grow bigger since the host plant offers more space and is not overcrowded easily. Furthermore, not all chemotypes were found on all soil types. Perhaps certain chemotypes are better adapted to specific soil conditions than others, which could affect their health and, ultimately, aphid abundance. Soil type itself could even partially explain the pattern of MT chemotypes, as studies found that soil properties can affect the composition of essential oils in *Thymus pulegioides* (Vaičiulytė et al., 2017, 2022).

Ant presence and site temperature

Metopeurum fuscoviride is an ant mutualist that heavily relies on ant presence. Therefore, if ants prefer specific sites, their presence will lead to higher aphid colonisation (Senft et al., 2017b). Indeed, all three sampled ant species were more likely to be present at sites with higher annual temperatures. This pattern has been found for Mediterranean ant species before (Cerdá et al., 1998). Also *L. niger,* for example, shows higher foraging activity at higher temperatures (S. Blanchard et al., 2021). Thus, the preference of ants towards warmer temperatures will influence aphid abundance, especially for ant mutualists.

4.4 Presence, Preference and Performance of Tansy Aphids

Aphid presence – a reflection of preference?

Plants from MT class 1 were more often infested with *M. fuscoviride* aphids and tending ants compared to the other classes. In tendency, albeit not significant, this was also observed for plants from MT class 2 (Rahimova, Neuhaus-Harr, et al., 2024). This is in line with previous research, as β-thujone, which was the dominant compound of MT class 1, has been associated with an increased abundance of *M. tanacetaria* (Kleine & Müller, 2011), though another study found that these aphids were more abundant on plants with camphor than with β-thujone (Benedek, Bálint, et al., 2019). Furthermore, plants with camphor, resembling our MT class 2, have been found to exhibit higher abundance and earlier colonisation rates of *M. fuscoviride* (Clancy et al., 2016; Senft et al., 2019a), which explains the higher tendency of aphid presence in our study.

Preference and presence seem to be influenced by terpenoids, supporting the idea that volatile terpenoids act as cues for locating host plants (Bruce et al., 2005; Ninkovic et al., 2021). Monoterpenoids might be more useful for aphids in identifying host plants than sesquiterpenoids due to the significantly higher concentrations of monoterpenoids observed in plants. Clancy et al. (2016) noted that terpenoid emissions, presumably released from glandular cells (Devrnja et al., 2021), influenced *M. fuscoviride* colonisation. Furthermore, ants might use MT as a host-finding cue. As mentioned, *M. fuscoviride* is an obligate ant-mutualist (Flatt & Weisser, 2000) and ants increase the likelihood of aphid colonisation (Senft et al., 2017a).

Different aphid species have different chemotype preferences

In the pairwise choice-assay, *M. tanacetaria* showed stronger preferences and patterns of attraction than *U. tanaceti* (Chapter 3.2, Neuhaus-Harr et al., 2024). *Macrosiphoniella tanacetaria* preferred leaves from plants of Athu-Bthu and Chrys-acet chemotypes, with preference patterns becoming more pronounced later in the assay. Previous studies found higher numbers of *M. tanacetaria* on plants dominated by β-thujone than those dominated by trans-carvyl acetate in a climate chamber experiment (Jakobs & Müller, 2018). We also observed that *U. tanaceti* was significantly more attracted towards plants of the Athu-Bthu chemotype, with the preference becoming less pronounced over time. This is interesting, as previously adverse effects of β-thujone on *U. tanaceti* numbers have been found in the field (Benedek, Mara, et al., 2019), though *U. tanaceti* were attracted to plants with high relative levels of α-thujone and β-thujone growing in homogenous plant patches in another study (Ziaja & Müller, 2023a). However, in another common garden study, no clear preference was observed for chemotypes for this aphid species (Kleine & Müller, 2011). The species-specific differences between the aphids can be attributed to their distinct life histories and preferred niches (Jakobs et al., 2019). In various field and greenhouse experiments, I observed that these aphid species exhibited different behaviours on and off the plant (pers. obs.). For example, *M. tanacetaria* is generally more mobile than *U. tanaceti* and actively searches for new host plants when its current host declines. In contrast, *U. tanaceti* usually stays on the leaves until they are yellow and only then moves to the next upper, non-infested leaf. This shows that even though intraspecific chemical diversity seems to affect insects, species might be affected differently, and not only the chemistry but also the preferred niche of the insect shapes insect-plant interactions.

Does the mother know best, and do preference and performance match?

Interestingly, I found that in *M. tanacetaria* aphids preference and performance of did not correlate (see Chapters 3.2 and 3.3, Neuhaus-Harr et al., 2024 and Neuhaus-Harr et al., 2025). Performance was described as the colony size at the end of the experiment. While these aphids preferred chemotypes with trans-chrysanthenyl acetate and α-thujone/ β-thujone as the dominant compound (Neuhaus-Harr et al., 2024), they performed best on plants with a mixed chemotype profile and a high total terpenoid concentration (Rahimova, Neuhaus-Harr, et al., 2024). According to the “mother knows best” or “preference-performance” hypothesis, female insects are thought to preferentially lay eggs on plants that provide the best larval development, which is believed to be true for many aboveground insects (Birke & Aluja, 2018; Gripenberg et al., 2010). This hypothesis is based on the assumption that natural selection will favour insects that choose the best host plants for their offspring, especially in species with less mobile larvae (Menacer et al., 2021). However, studies have shown mixed results. For example, the cabbage root fly *Delia radicum* matches in performance and preference if flies were kept on *Brassica rapa,* but not on *Sinapis alba* (Menacer et al., 2021). Furthermore, the preference-performance hypothesis was only supported when looking at some (e.g. survival time), but not all (e.g. developmental time), measured life-history traits (Menacer et al., 2021).

Factors causing preference and performance mismatch

Firstly, there are several ways to define larval/nymph performance for instance, survival rates or weight. To fully conclude whether the preference-performance hypothesis holds, several variables estimating nymph/larval performance are needed (Gripenberg et al., 2010; Menacer et al., 2021). In some circumstances, the number of offspring might be favourable, while the survival rate might be more important in others. Furthermore, Menacer and colleagues (2021) point out that female insects could also misinterpret chemical cues. Such cues might not correlate with the host plant´s quality. Perhaps a chemotype with high terpenoid concentration is detected easily as a host plant, but the presence of these volatiles in the air might disrupt the feeding of the aphids.

In nature, not only host plant quality but also predation pressure and other (top-down) forces shape the overall performance of a colony. One hypothesis states that females may prefer to lay eggs on plants which are low in quality but have a lower risk of enemies attacking (Ballabeni et al., 2001; Moon & Stiling, 2006). As discussed before, coccinellid beetles, which predate heavily on aphids, have been found to prefer β-thujone chemotypes (Kleine & Müller, 2011). In this case, avoiding these chemotypes might benefit the aphids, even if nymph survival is lower than other chemotypes. However, in my study, aphids were caged and predators excluded. Therefore, my findings suggest that it is more the bottom-up rather than the top-down forces that shaped aphid performance.

4.5 Effects of Chemical Descriptors

As mentioned above, not only the terpenoid profile *per se* could be used as a cue by the aphids, but also terpenoid concentration in the sap itself or the ambient air might play a role in how the insects select their host plant. As terpenoids are thought to have evolved to repel insects (Kessler & Kalske, 2018a; Paré & Tumlinson, 1999; Tholl, 2015; Wetzel & Whitehead, 2020a), the number or the evenness of the compounds in relation to each other might also impact the female choice.

Aphid preference is shaped less by dominant compounds and more by blends

As individual compounds, such as β-thujone, camphor or borneol, have been found to affect aphid numbers, colonisation and abundances (Bálint et al., 2016; Benedek, Mara et al., 2019; Senft et al., 2019), I wanted to explore whether individual compounds or the blend would play a bigger role in shaping plant-aphid dynamics. Indeed, in the Germany-wide survey, aphids and ants were more often present on the MT chemotype 1, whose profile was clearly dominated by β-thujone. Similarly, *M. tanacetaria* preferred chemotypes with α-thujone/ β-thujone and trans-chrysanthenyl acetate. However, when linking all individual compounds to aphid presence, I did not find clear relationships.

Interestingly, the sesquiterpene (E)-β-farnesene, which is a common aphid alarm pheromone, has been found to deter aphids (Beale et al., 2006; Unsicker et al., 2009). However, if found in a volatile blend, (E)-β-farnesene was not necessarily repellent (Bruce et al., 2005; Unsicker et al., 2009). This example demonstrates that not only single compounds are important in mitigating aphid preference/presence but also that volatiles in a blend could counteract or add to each other´s function. The result of my study suggests that aphids were not attracted to a specific terpenoid compound and that, rather, the blend itself or other variables, such as terpenoid concentration, might be more important for aphid preference.

Influence of terpenoid concentrations on aphid preference

Even though we measured leaf terpenoid concentrations in my studies, the concentration levels in leaves could be a proxy for ambient terpenoid presence since terpenoids can be volatile (Gershenzon & Dudareva, 2007; Pichersky et al., 2006).

In the large-scale study (see Chapter 3.1, Rahimova, Neuhaus-Harr, et al., 2024), the MT chemotypes differed significantly in their total concentration of terpenoids. Interestingly, chemotype classes 1 and 2 showed the highest terpenoid concentration, which are the classes having the highest percentage of aphid presence. Monoterpene class 4 exhibited the lowest terpenoid concentration and had the lowest percentage of aphids (see Fig. 7 in 8.1). Similarly, as to the large-scale study, the attraction of *M. tanacetaria* aphids towards certain chemotypes in the preference study (see Chapter 3.2, Neuhaus-Harr et al., 2024) was positively related to the relative terpenoid concentration, though this finding was only marginally significant (see Fig. 4e, in 8.2). If aphids use volatile terpenoids to find their host plant (Ahmed et al., 2019; Dahlin et al., 2015; Neuhaus-Harr et al., 2024; Ojeda-Prieto, Moreno et al., 2024) it might not be surprising that I found high presences of aphids on plants with high terpenoid concentrations. My findings align with many other studies that found that concentrations of specialised metabolites play an important role in herbivore attraction and repellence (reviewed by Macel, 2011). The detection of a suitable host plant and, hence, the presence of an aphid might hugely rely on the terpenoid concentration, while the performance of aphids might be more affected by other chemodiversity metrics such as the terpenoid evenness, terpenoid richness or terpenoid Shannon diversity (see Chapter 3.3, Neuhaus-Harr et al., 2025). This would explain why I did not find the performance of *M. tanacetaria* to be related to terpenoid concentration in the performance study.

Terpenoid evenness and aphid preference & performance

The chemotypes I used in the preference (see Chapter 3.2, Neuhaus-Harr et al., 2024) and the performance study (see Chapter 3.3, Neuhaus-Harr et al., 2025) differed significantly in their terpenoid evenness and Shannon diversity but not in their richness (i.e. the number of terpenoid compounds). In *M. tanacetaria* aphids, I found a marginally significant negative relationship between aphid preference and terpenoid evenness. Aphids were more attracted to the chemotypes whose profiles were clearly dominated by one or few terpenoids and less attracted to chemotypes with more even terpenoid distribution. One explanation for this could be that highly dominant compounds could serve as more easily detectable cues for plant recognition (Kleine & Müller, 2011). However, the high concentration of one or few (repellant) compounds might have detrimental effects on the aphids once they arrive on a plant. For example, a study found that when pepper plants were treated with farnesol, the population growth of *Myzus persicae* aphids was lower (Martín et al., 2024). Additionally, if plants were treated with lemon oil, the feeding behaviour of the aphid was disrupted (Martín et al., 2024). This suggests that individual compounds could influence aphid feeding behaviour and colony growth. In line with that, I found that aphid colony sizes in the performance study were positively related to evenness, which suggests that aphids perform better on plants, with terpenoids being more evenly distributed in their concentration. One possible explanation could be that a detrimental effect (e.g. suppression of aphid growth) relies on one or a few key terpenoid(s) and that high evenness could dilute the relative abundance of these terpenoid(s). As mentioned before, in the case of (E)-β-farnesene, for example, deterrent effects did not always show when the compound was present in a blend. Another possibility could be that synergistic effects among terpenoid rely on the presence of these compounds in similar concentrations. If these terpenoids are beneficial to aphid growth and development, it could explain the observed correlation between terpenoid evenness and larger aphid colonies in my study.

It is important to consider that evenness might be influenced by disparity, which refers to the impact of a terpenoids' origin on its ecological effects (Petrén, Anaia, et al., 2023). For example, in my performance study, low-evenness profiles were often dominated by β-thujone or chrysanthenyl acetate. Although both are monoterpenoids derived from geranyl diphosphate, they follow different downstream pathways and may have distinct ecological impacts on herbivores (Rahimova, Neuhaus-Harr et al., 2024). Similarly, other terpenpoid-dominated mixtures in nature could have varying effects on aphids. Hence, it is important to understand the effects of terpenoid Shannon diversity (that is, a measure that combines richness and evenness) and functional Hill diversity (combining richness, evenness and disparity).

Functional Hill Diversity – a measure combining richness, evenness, disparity

Chemotypes used in the preference and performance study (see Chapters 3.2 and 3.3, Neuhaus-Harr et al., 2024 and Neuhaus-Harr et al., 2025) differed significantly in their terpenoid Shannon diversity. Similarly, as for terpenoid evenness, the attraction to chemotypes in *M. tanacetaria* marginally significantly decreases, with increasing Shannon diversity. However, even though Shannon diversity has the advantage of combining richness and evenness into one number, which can be used in analyses, there are several drawbacks (Wetzel & Whitehead, 2020a). One limitation is that if we find Shannon diversity to have a significant influence, it is not clear whether this is caused by richness, evenness or both. In my study system, the deterrent effect of Shannon diversity is likely driven by evenness, as I did not find an individual effect of richness. Furthermore, if richness and evenness have opposite effects, the effect of Shannon diversity might be non-significant (Wetzel & Whitehead, 2020a). As several researchers have pointed out, functional diversity might be more important in ecological processes (Petrén, Anaia, et al., 2023; Wetzel & Whitehead, 2020a). If compounds are similar in their functions, a higher number of compounds (i.e. richness) might have the same effect as a blend with fewer compounds.

In the large-scale study, the functional hill diversity of MT classes 1 and 3 was significantly lower than in classes 2 and 4. Nevertheless, we did not find an effect on aphid presence or abundance. Compounds that are functionally related had similar effects as unrelated terpenoids on the aphids. This could also imply that many different terpenoids have deterrent effects on the tansy aphid, with their effects not necessarily adding up but acting interchangeably. However, this might not be true for all herbivores. Recently, it has been proposed that it is beneficial for a plant to produce several compounds, as more diverse blends are more likely to be effective against a higher number of herbivores (Whitehead et al., 2021). Studies have shown that the effects of volatiles diminish or are eliminated when multiple herbivores attack (Runyon et al., 2008; Tooker et al., 2008; Unsicker et al., 2009); this led me to the question of how plant-insect interactions would be mediated by the chemical blend in such an event. Under natural conditions, such as in the large-scale study, more than one herbivore might feed on the plant simultaneously. If belowground herbivory can alter the volatile emission of a plant (Rasmann & Turlings, 2007; Soler et al., 2012), how would that affect the aboveground aphids?

4.6 Insect-Insect Interactions/ AG-BG Interactions

Belowground wireworms did not affect aboveground aphids

The earlier prediction that belowground herbivores should positively influence aboveground herbivores (Masters et al., 1993) has been challenged by many subsequent studies, indicating that interactions between above- and belowground organisms are highly context-dependent (Johnson et al., 2012). Contrary to what I expected, I did not observe a positive (or negative) influence of belowground wireworms on *M. tanacetaria* colony size in the performance experiment (see 8.3). Several factors could explain this discrepancy. Firstly, although belowground herbivores such as Coleoptera (wireworm larvae) typically have a positive effect on aboveground Homoptera (e.g., aphids) (Johnson & Murray, 2008), this effect was only observed when both herbivores arrived simultaneously, suggesting an early and potentially short-lived plant response (Erb et al., 2011; Johnson et al., 2012). In my experiment, I infested plants with wireworms three days before aphids, allowing plants to recover from root attack, and the nutrient increase due to herbivore stress, which benefits aphids, might have dissipated (Johnson et al., 2012).

Belowground herbivore density does not affect plant-aphid responses

Another explanation for the missing effects of belowground herbivory on the aphid performance might be the density of herbivory. Perhaps it had been too low to significantly affect the plant's defence and indirectly on the aphids. After I finished the experiment and retrieved the wireworms, we found that many had pupated or turned into adults. Although wireworms typically live for several years, warm conditions may accelerate their larval cycle, as temperature is inversely related to the duration of larval life cycles in insects (Furlan, 1996; Meikle & Patt, 2011). Likely, this happened during the local heat waves in August 2022. During pupation, wireworms do not feed, potentially resulting in herbivory levels too low to affect aboveground aphids significantly. Wireworm feeding on tansy roots may be inconsistent, although unpublished pilots observed wireworms readily feeding on plant roots, particularly fine root hairs (J-P Schnitzler, pers. obs.). To rule this out, I conducted a second experiment using the same model organisms. Together with colleagues from the Helmholtz Institute, we infested tansy plants, this time with twice the number of wireworms and kept plants in a chamber with regulated temperature. Indeed, in the experiment, we found almost all wireworms back; none had pupated. Nevertheless, my preliminary analysis indicates that even with higher levels of wireworm infestation, we do not find clear effects on aphid colony sizes, although we saw a trend of bigger aphid colonies on plants infested with wireworms.

Reasons why chemotypes did not differ in their effects on belowground wireworms towards aphid performance

Even in the second experiment, in which we increased the number of wireworms per plant, I did not find a significant effect on the aphid populations or differences between chemotypes. One reason for this observation could be that tansy may exhibit local rather than systemic responses to root herbivory. The absence of differences among chemotypes could indicate compartmentalised responses between above- and belowground plant parts. As described in a recent study, tansy terpenoid profiles differ markedly between these compartments, following distinct biosynthetic pathways (Rahimova, Heinen et al., 2024). Even though we see clear chemotypes in the aboveground parts of the plants, the chemistry in the roots might be completely different or unrelated. Therefore, there may be minimal overlap in resource allocation or defence pathways and little linkage of above- and belowground plant parts in terms of signalling.

How do things look from a plant perspective?

Belowground herbivory did not have an effect on aboveground herbivory. However, as my above-mentioned questions revolved around aphids, I wanted to know what effects the herbivory had on the plants themselves. From a plant perspective, it might be more beneficial to compensate loss through herbivory by growth. Upregulating defence mechanisms to repel insects might be the best option for some chemotypes, but not all. Hence, I wanted to know how herbivores would affect plant morphology and whether chemotypes (which are ultimately genotypes) would differ in their growth-defence trade-offs.

4.7 Interaction of Plant Morphology, Plant Chemistry and Insects

Plant morphology influences herbivores

I found that *M. fuscoviride* was more likely to occur on plants with a higher number of stems, which implies that morphological traits also mediate herbivore densities. In this case, both morphological and chemical features might be crucial for host plant selection and herbivore performance. Herbivore susceptibility is influenced by defence traits, which include various morphological and chemical characteristics (Carmona et al., 2011; Howe & Schaller, 2008). As both traits have been found to influence plant-insect interactions, it is important to understand the relation between chemical traits and growth traits.

One idea that my research group pondered was that this relation between distinct chemotypic profiles and morphological differences could even explain the discrepancy in aphid preference and performance on certain chemotypes. If a preferred chemotype would have more trichomes, ti would be harder for small nymphs to penetrate the leaf surface. Consequently, an aphid colony would perform worse on this chemotype. In a small side experiment, we investigated some fine traits in the tansy leaves but did not find significant differences among them. However, I found that average leaf chlorophyll, which can be used as a proxy of plant health, differed between chemotypes. A high-quality host could, in turn, have higher levels of nutrients, which would ultimately benefit the aphids (Stiling & Moon, 2005). This led me to the following question: Can chemical profiles be used by the insect as a reliable cue to find the best host plant, or are chemistry and morphology unrelated?

Chemotypes vary in their morphology

Many studies have found that chemotypes differ in their chemical blends and morphology (Polatoglu, 2013; Ramos et al., 2022). This aligns with what I found in the large-scale study (see Chapter 3.1, Rahimova, Neuhaus-Harr, et al., 2024), the performance experiment (see Chapter 3.3, Neuhaus-Harr et al., 2025) and the follow-up nutrient study. The number of stems and the plant volume, for example, differed significantly between MT chemotypes across Germany (see Chapter 3.1, Rahimova, Neuhaus-Harr, et al., 2024). As mentioned above, another variable I looked closer at was the average leaf chlorophyll content, which is often used as a proxy for plant health (Y. Li et al., 2018). Aphids feeding on healthy plants are thought to perform better as they have higher nutrient intake (Kranti et al., 2021; Xu et al., 2024). In line with this, I found that, indeed, the chemotype Mixed_low had a significantly higher chlorophyll content than the chemotype Bthu_low, and aphids performed significantly better on the first. Interestingly, the chlorophyll content of the plants used in the performance experiment (see Chapter 3.3, Neuhaus-Harr et al., 2025) differed significantly between the chemotypes. In contrast, I did not find significant differences in chlorophyll content in the nutrient study. As I used the same chemotypes but only a selection in the nutrient study, this finding implies that distinct/few chemotypes mainly drive the difference in average leaf chlorophyll content. Not all chemotypes differ significantly from one another in their morphological features, but some do. As I had already learned that not only the terpenoid blend itself but also other chemical descriptors play a role in insect-plant interactions, I wondered whether other chemical dimensions would also be related to certain morphological traits. A fundamental principle in plant ecology is the growth-defence trade-off (Herms & Mattson, 1992; Karasov et al., 2017). Would chemotypes that have a high number of compounds allocate more towards the production of terpenoids and would hence be smaller or slower in growth?

The functional connection of chemical traits and morphology

It is generally assumed that plants must allocate resources between growth and defence traits, resulting in smaller but better-defended plants or vice versa (Coley et al., 1985; He et al., 2022; Herms & Mattson, 1992). Terpenoids are even thought to be more costly to produce than most other primary and secondary metabolites due to the need for a diverse array of enzymes, which increases production and storage costs (Gershenzon, 1994). However, such trade-offs were not observed in *T. vulgare*. In a previous study, only inflorescence biomass, and not other growth factors (e.g., plant height, total biomass, number of stems), showed a significant negative correlation with terpenoid concentration (Wolf et al., 2011). Instead, we found a positive relationship between growth-associated variables and terpenoid Shannon diversity, as well as relative total terpenoid concentration in our plants. Larger and bushier plants exhibited higher relative total terpenoid concentration and terpenoid Shannon diversity. Similar positive relationships between growth and defence have been observed in two Plantago species (Barton, 2007; *Plantaginaceae*). A plausible explanation for these findings might be that larger plants have higher photosynthetic rates and energy budgets, allowing for enhanced and diversified local terpenoid synthesis. Additionally, it is vital for plants to balance growth and defence to optimise fitness in dynamic environments (Huot et al., 2014), and trade-off patterns may change at different stages of plant ontogeny (Boege & Marquis, 2005). This leads to the question, what would happen if a plant had unlimited nutrients?

How does adding nutrients change the growth-defence trade-off?

Insects can pose significant threats to plant growth and survival (Erb & Reymond, 2019). To counteract these threats, plants have developed complex defence mechanisms and strategies (Cui et al., 2023; Solé, 2020). Hereby, nutrient availability plays a crucial role in the balance of growth and defence (Z.-X. Li et al., 2024). When nutrients are scarce, plants often prioritise growth to maximise resource use efficiency, potentially compromising their defence capabilities (Z.-X. Li et al., 2024). Conversely, nutrient-rich conditions can enhance both growth and defence, allowing plants to invest more in chemical defences like terpenoids and phenolics (Z.-X. Li et al., 2024). Interestingly, in an experiment with nutrient availability, I found that aphid numbers were higher on plants with medium or high fertiliser addition compared to control plants. In this case, the plants did not seem to invest more in their defence. Perhaps one reason for this finding could be that plants invested more into their growth, and hence, their sap was more nutritious for the aphids. Furthermore, I asked myself if it actually made sense for a plant to defend itself if they have enough nutrients to compensate for its loss with growth. I found the answer to this in another study of colleagues. Mahdavi-Arab and colleagues (2014) observed similar patterns, such as with increased fertilisation, aphid numbers increased. However, at one point, aphid colonies were so big that the plants began to suffer, and their biomass decreased (Mahdavi-Arab et al., 2014). From a plant perspective, it might not always be beneficial to grow on nutrient-rich soils, at least not for chemotypes that are less well-defended. Perhaps this could be the reason why we see an influence of soil types on aphid numbers (Rahimova, Neuhaus-Harr et al., 2024). If the plant grows on nutrient-rich soils, this could ultimately benefit the aphid.

How does adding nutrients influence plant morphology?

To get an idea of how plant morphology was affected by an increase of nutrient availability, I concentrated on the three variables: plant height, average leaf chlorophyll content and the time of flowering. Interestingly, what I observed was that the average chlorophyll content was highly affected by the availability of nutrients. The higher the added fertiliser concentrations, the higher the average chlorophyll content. Opposed to that, the flowering time of the chemotypes did not differ with fertiliser treatment, but they did differ significantly between chemotypes, with some flowering early and some flowering late. In another study on tansy, using the exact same chemotypes as this study, morphological differences of chemotypes became less pronounced in time (Ojeda-Prieto, Medina-van Berkum et al., 2024). However, the patterns of flowering times stayed the same over time (Ojeda-Prieto, Medina-van Berkum et al., 2024). This could imply that chemotypes have different strategies for maximising their success in reproduction. Plant height, on the other hand, was marginally affected by both the chemotype and the nutrient levels, with some chemotypes increasing in height and others decreasing with the addition of fertiliser. Taken together, this can indicate that some traits might be “fixed”, such as the flowering time, while others, such as chlorophyll, might be highly dependent on environmental influences. Perhaps this could be the reason why we found differences in plant height and bushiness (ratio of number of stems and radius) over Germany. Environmental variables, such as temperature or soil type, likely influence a plant’s growth. This led me to the question of what would happen if we added the herbivores to the picture. If the chemotypes differ in their growth-defence strategies, as suggested above, herbivores would likely have varying degrees of influence on plant growth.

Do chemotypes mitigate the detrimental effects of herbivores?

My performance study revealed that the infestation of tansy by *M. tanacetaria* affected plant height and dry weight, with the impact varying between chemotypes. Leaf chlorophyll content also varied by chemotype and was lower when plants faced both above- and belowground herbivory. This suggests that chemotypes indeed differ in their growth and defence strategies, as observed in several other plant species (He et al., 2022; Huot et al., 2014; Züst & Agrawal, 2017) and that chemotypes mitigate the detrimental effects of herbivores. Notably, the mixed chemotypes, which exhibited the highest richness, diversity, and evenness of compounds, either grew taller or had a higher dry weight when infested with aphids compared to control plants. These chemotypes also supported the largest *M. tanacetaria* colonies. Although counterintuitive, plants with more diverse terpenoid chemotypes might conserve resources by producing a diverse mixture of compounds in low relative abundance, thus enabling greater investment in growth and compensation for herbivory. In contrast, other chemotypes may allocate more resources to chemical defence by producing dominant compounds. Given that chemical defence production is costly and often linked to restricted growth (Havko et al., 2016; Herms & Mattson, 1992; Huot et al., 2014; Sestari & Campos, 2022), as shown in many plant species (Campos et al., 2016; Haak et al., 2012; S. Hayashi et al., 2020; Mihaliak & Lincoln, 1989), it is typically assumed that plants must balance growth and defence. However, recent studies indicate that the trade-off between growth and defence is context-dependent and aimed at maximising plant fitness (Campos et al., 2016; Q. Guo et al., 2018; Kliebenstein, 2016). Our findings of chemotype differences suggest that individual plants within species may adopt various strategies closely linked to secondary metabolites.

My studies on how phytochemistry can influence plant-insect interactions revealed many interesting but also important insights into defence signalling, growth-defence trade-offs and aphid dynamics. Still, there is much more to explore and learn. As Professor Siegfried Scherer, a retired Microbiologist, always used to say to me: It starts with one question, and once we step through the door and get an answer, we find ourselves in a room with many more doors. Similarly, answering some of my questions has led me to do even more. Hence, I want to briefly mention future studies that would help us to understand the function of terpenoid chemotypes even better.

4.8 Future Directions

Enhancing our understanding of chemical descriptors

Over the course of my studies, the concept of chemical descriptors has become more important to me. I soon realised that not only does the terpenoid profile drive plant-insect interactions, but the functional diversity, richness, evenness, and concentration of individual compounds play an important role in herbivores. To separate the effects, for example, of evenness and disparity and to strengthen our understanding of chemodiversity as a driver of herbivore preference, extensive sampling and propagation would be necessary to include the full spectrum of natural terpenoid profiles. Additionally, it would be important to conduct a cost-benefit analysis of maintaining chemical diversity within a plant. If some compounds are more expensive than others but are also more effective in defending, would it be a good strategy to produce these compounds? This could enhance our understanding of chemical profiles within the framework of defence optimisation strategies.

Including other plant metabolites

Notably, I focused solely on terpenoids throughout my thesis. As mentioned in the introduction, other compounds may also influence aphid behaviour. For example, glycosides and alkaloids have been found to deter herbivores (Dalton et al., 2003; Sivaramakrishnan et al., 2024). The impact of non-terpenoids could counteract or increase the influence of terpenoids on herbivores. In tansy for example, compounds from other metabolic pathways, such as phenolics, flavonoids and carbohydrate derivates have been found to vary between terpenoid chemotypes (Dussarrat et al., 2023). This variation between chemotypes could have significant ecological consequences (Dussarrat et al., 2023). Phenolics for example, can have deterrent effects on aphids (Goławska & Łukasik, 2012). Therefore, future research should aim to disentangle the impact of broader metabolome-wide chemodiversity on insect dynamics in plants.

Translate fundamental science into applied research.

Lastly, I have always been an advocate for translating fundamental findings into applied research. Aphids are one of the major pest species in agriculture (Farhan et al., 2024; Johnston & Bishop, 1987). Now that we know how plant terpenoids can influence the presence, preference and performance of these herbivores, we could use this knowledge to increase options for biological control. A whole range of studies has tackled this idea. One study found that extracts from tansy can repel also generalist pest species, such as the pea leaf weevil or the black bean aphid (Kwiecień et al., 2020). Additionally, essential oils, such as those from tansy, can significantly reduce larval survival and development in herbivores like the gypsy moth (*Lymantria dispar*) (Devrnja et al., 2020). Furthermore, experiments have shown if one plant is attacked by aphids it increased emission of airborne terpenoids can prime plants in the vicinity, which makes them less attractive to the herbivore (Markovic et al., 2019). Taken together, these studies show the huge potential of using tansy as a green alternative for pest control. However, little research in this area has included the differences of chemotypes. Some chemotypes could be more useful than others to repel pest species. To fully exploit the potential of tansy, we need to understand which chemotypes have which effects. Hence, even though I only looked at a few specialised aphid species and one plant species in my thesis, I believe the knowledge gathered around tansy and it´s chemotypes, can translate to other systems and that fundamental concepts could be applied.

5. Conclusion

Chemodiversity plays a crucial role in shaping plant-insect interactions. Using one large-scale field study and greenhouse experiments, I investigated the effects of distinct chemical profiles of *Tanacetum vulgare* on aphid presence, preference and performance, plant growth and morphology, insect-insect interactions via the plant and growth-defense tradeoffs.

I have shown that tansy plants exhibit significant geographic differences in their chemotype distribution, particularly in the occurrence of monoterpenoid (MT) chemotypes, while sesquiterpenoid (ST) chemotypes are more evenly distributed across Germany. These differences in MT chemotypes have notable ecological implications, influencing the presence of aphids and ants, which rely on these volatile compounds for host plant identification. Furthermore, my findings highlight the significant role of abiotic factors, such as soil type and site temperature, in shaping the abundance and distribution of aphids and ants. The preference of aphids for specific chemotypes, influenced by the distinctiveness of monoterpenoid profiles, suggests a complex interplay between plant chemistry and herbivore behaviour.

I also explored the effect of chemical descriptors on aphid preference, revealing that while individual compounds like β-thujone can influence aphid presence, the overall blend of volatiles and their concentrations play a critical role. Aphids appear more attracted to chemotypes with dominant terpenoids, though this does not always correlate with their performance, challenging the "preference-performance" hypothesis.

Interactions between belowground and aboveground herbivores proved to be context-dependent. Wireworms did not significantly affect aphid performance, possibly due to factors such as herbivore density, timing of arrival, and local responses within the plant. This suggests that tansy plants may exhibit compartmentalised responses to herbivory, with different chemotypes in roots and shoots. However, plant morphology played a crucial role in mediating herbivore densities. Aphids were more likely to occur on plants with a higher number of stems, indicating that both morphological and chemical traits are important for host plant selection and herbivore performance. Chemotypes varied in their morphology, and certain chemotypes exhibited higher chlorophyll content, leading to better aphid performance. This indicates that the relationship between growth and defence traits in plants is complex and context-dependent. While some chemotypes invest more in chemical defences, others balance growth and defence to optimise fitness in dynamic environments. Nutrient availability further influences this trade-off, with plants on nutrient-rich soils showing higher aphid abundance and varying morphological traits.

5 - Conclusion

In conclusion, my thesis emphasises the importance of understanding intraspecific chemical diversity and its ecological significance. The findings provide valuable insights into the interactions between plants and herbivores, contributing to a broader understanding of ecological dynamics and plant-insect relationships. Future research should continue to explore the mechanisms underlying these interactions and consider the influence of both biotic and abiotic factors in shaping ecological outcomes.

6. Acknowledgements

Phew. What a ride it has been! Now it is time to thank all the wonderful people who guided me along this journey, motivated me, walked with me, and patiently listened to all my rants.

First and foremost, my deepest gratitude goes to Robin. You have been an exceptional mentor and supervisor, imparting more skills to me than you probably realize. I am immensely grateful for all the academic knowledge and experience you have shared, but even more so, for being approachable with both scientific and personal problems. This PhD journey has been far more than just ‘work,’ and I feel that I have grown significantly, thanks to you.

I also want to express my heartfelt thanks to my doctoral advisor, Prof. Wolfgang. Thank you for the opportunity to work at your chair. Your immense knowledge and experience have been invaluable, and your guidance and feedback have been greatly appreciated. I am proud to have been part of your team.

I am deeply grateful for the amazing team at the Chair of Terrestrial Ecology. Thanks to all of you for fruitful discussions, fieldwork assistance, coffee-break rants, cocktail evenings at El-Co, and for motivating and supporting each other through the ups and downs of research. A special shout-out to my PhD-sister Lina—you made my work so much more delightful. There were times I would not have known what to do without you. I am so happy we became colleagues and friends. Many thanks also to Andrew, Lisa, Laura, Marlene, Sarah, Robin, Wolfgang, Benjamin, Rafa, Samira, Laura, Claudia, Sonja, and everyone else.

Andrew, my love. Words can't express how grateful I am to know you. You have been by my side from the beginning—first as a colleague, then a friend, and finally a partner. Together, we have navigated the impressive ups and downs of our personal and scientific lives. I look forward to many more years of our journey together. As long as you are by my side, I can climb any mountain.

A huge thank you to my family for all the support, encouragement, and trust in me. Dad, you have inspired me since childhood and taught me to love nature. You are the reason I studied biology. Thank you for all the encouragement and support throughout my studies. I would not be here without your guidance. Mom, I wish you could be here to give me a big hug. You were my biggest cheerleader, and I know you would be proud of me. I will always carry your love in my heart. Thanks to my brothers Jonathan and Benjamin—remember, how you crashed our Christmas party? I love that we are always there for each other.

6 - Acknowledgements

This research was generously funded by the German Research Foundation (Deutsche Forschungsgemeinschaft), providing the financial support necessary to conduct this study within project 415496540. I also appreciate the resources provided by the Technical University of Munich, including the TUM PTC Dürnast. Special thanks to the Dürnast staff, Robert Hansel and Sabine Zuber, among others, for their assistance in raising and maintaining the plants.

Lastly, I want to extend my gratitude to everyone who has been by my side and supported me in one way or another. I appreciate you deeply.

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8. Appendix

8.1 Manuscript I – Large-Scale Study

OIKOS

Research article

Geographic distribution of terpenoid chemotypes in *Tanacetum vulgare* mediates tansy aphid occurrence but not abundance

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Oikos
2024: e10320
doi: 10.1111/oik.10320

Subject Editor: Nina Fatouros
Editor-in-Chief:
Gerlinde B. De Deyn
Accepted 16 February 2024

Intraspecific variation of specialized metabolites in plants, such as terpenoids, are used to determine chemotypes. Tansy *Tanacetum vulgare* exhibits diverse terpenoid profiles that affect insect communities. However, it is not fully known whether patterns of their chemical composition and associated insects vary beyond the community scale. Here, we investigated the geographic distribution of mono- and sesquiterpenoid chemotypes in tansy leaves and their relationships with specific insect communities across Germany. We sampled tansy leaves from ten plants with and five plants without aphids in each of 26 sites along a north–south and west–east transect in Germany. Hexane-extracted metabolites from leaf tissues were analyzed by gas chromatography-mass spectrometry (GC-MS). Plant morphological traits, aphid occurrence and abundance, and occurrence of ants were recorded locally. The effect of plant chemotype, plant morphological parameters, and abiotic site parameters such as soil types, temperature and precipitation on insect occurrences were analyzed. Plants clustered into four monoterpenoid and four sesquiterpenoid chemotype classes. Monoterpene classes differed in their latitudinal distribution, whereas sesquiterpenes were more evenly distributed across the transect. Aphid and ant occurrence was influenced by monoterpenoids. Plants of monoterpenoid class 1 were colonized by aphids and ants significantly more often than expected by chance, whereas in other classes there were no significant differences. Aphid abundance was affected by soil type, and average annual temperature positively correlated with the occurrence of ants. We found significant geographic patterns in the distribution of tansy chemodiversity and show that monoterpenoids affect aphid and ant occurrence, while the soil type can influence aphid abundance. We show that geographic variation in plant chemistry influences insect community assembly on tansy plants.

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Keywords: chemical diversity, *Formica rufa*, geographical gradients, *Lasius niger*, *Metopeurum fuscoviride*, *Myrmica rubra*, plant–herbivore interactions, specialized metabolites

Introduction

Within the field of biogeography, researchers aim to understand how environmental differences affect the distribution of organisms (Tivy 2018). Geographic location affects plant communities at interspecific, but also at intraspecific levels (Moreira et al. 2012). High intraspecific variation can be observed in specialized phytochemical compounds that vary considerably within plant families and can even vary within species (Kleine and Müller 2011, Kessler and Kalske 2018). For instance, the abundance of pyrrolizidine alkaloids in *Senecio jacobaea* (Macel and Klinkhamer 2010) and the abundance of specific monoterpenoids in *Pinus banksiana* (Taft et al. 2015), *Melaleuca alternifolia* (Bustos-Segura et al. 2017) and *Gossypium hirsutum* (Clancy et al. 2023) have been used for the classification of chemotypes. Chemotypes are defined as groups of conspecific plants that can be distinguished by the often heritable composition of specialized compounds of a specific system-relevant class (Müller et al. 2020). For example, individuals of common thyme *Thymus vulgaris* can be categorized into chemotypes based on the dominant monoterpenoids such as geraniol, α-terpineol, sabinene hydrate, linalool, carvacrol or thymol (Thompson et al. 2003). Chemotypes can be dominated by one or several dominant compounds or differing blends lacking clearly dominating compounds, but are rather mixed chemotypes (Dussarrat et al. 2023). Specialized metabolites in plants have many ecological functions and can strongly influence ecological interactions such as the attraction of herbivore predators, pollinators, and mycorrhizal fungi, defence against herbivores and pathogens, communication with other plants, and protection against abiotic stressors, such as UV-B radiation and drought (Dixon and Paiva 1995, Dicke et al. 2009, Mofikoya et al. 2019, Grof-Tisza et al. 2022). Importantly, several studies have shown patterns in distribution of plant chemotypes across spatial and abiotic gradients. For instance, in *T. vulgaris* it has been found that the composition of secondary compounds differs significantly between sites in the south of France, and has been shown to depend on soil composition, winter ambient temperatures (Thompson et al. 2007, 2019), and chemotypic differences are associated with herbivory (Linhart et al. 1999). Furthermore, chemotype frequency of *Pinus banksiana* plants in the US was observed to be correlated with abiotic factors, such as precipitation (Taft et al. 2015). However, how specialized plant metabolism and interactions between plants and their antagonists might contribute to our understanding of geographic variation is currently poorly understood.

Secondary plant metabolites have likely evolved in part to mediate interactions between plants and their associated organisms (Wetzel and Whitehead 2019). While these metabolites mediate plant–insect interactions, not all interactions are favourable for the plant. That is, in addition to potentially affecting how insect herbivores perform on a plant, secondary metabolites can also serve as important host-recognition cues. Some secondary metabolites are highly volatile. These volatile organic compounds (VOCs) commonly serve as informational cues for insect herbivores (Ghirardo et al. 2012) but can also attract natural enemies of herbivores (Heil and Bueno 2007, Baldwin 2010) and indirectly contribute to herbivore defence. However, many more variables appear to mediate plant–insect interactions, than just plant chemistry. Another crucial aspect in how insects may perceive and locate their host plants and which ultimately contributes to shaping insect communities is the host plant's morphology. For instance, in the perennial shrub *Baccharis pilularis*, plant architecture affected the composition of herbivore communities and morphology correlated with herbivory levels (Rudgers and Whitney 2006). This study found that erect plants attracted more moth galls, while a higher density of a gall-forming midge was found on prostrate plants (Rudgers and Whitney 2006). Where a plant grows, i.e. its immediate surrounding vegetation, also matters for interactions between plants and their interaction partners. For instance, a plant's immediate surroundings can shape its volatile emissions and, in turn, influence insect herbivores (Kigathi et al. 2019, Ziaja and Müller 2023). However, few studies in chemical ecology consider the influence of geographic variation, plant architecture and community context. Therefore, our objective was to investigate complex interactions between these factors to better understand plant–insect interaction patterns, using tansy plants and associated insects as a model system.

Tansy, *Tanacetum vulgare* (Asteraceae), is an aromatic herb endemic to Eurasia that exhibits a considerable variation in its terpenoid composition (Keskitalo et al. 2001, Kleine and Müller 2011, Clancy et al. 2016). Terpenoids (isoprenoids) represent a large group of plant-specialized metabolites whose backbones consist of two common five-carbon isoprene units (Rosenkranz and Schnitzler 2016). A widespread range of terpenoid synthases and subsequent modifying enzymes lead to numerous monoterpenoids and sesquiterpenoids (Degenhardt et al. 2009, Lange and Srividya 2019). These terpenoids can be stored in glandular trichomes on the leaf surface in tansy (Guerreiro et al. 2016), or they can be induced and immediately emitted through biotic stresses such as herbivory (Clancy et al. 2016). The blends of the stored terpenoids vary between individuals, and tansy plants can be classified into different chemotypes according to those differences (Kleine and Müller 2011). Chemical (terpenoid) composition in tansy has been found to differ quite strongly between and within populations in various studies, but geographical patterns or their drivers have not received much attention in this system (Wolf et al. 2011, 2012). Tansy chemotypes characterized by the dominance of volatile terpenoids were found to correlate with the plant-associated insect communities within single field sites in several studies (Kleine and Müller 2011, Clancy et al. 2016). In addition to the volatile terpenoid pattern of tansy on a small scale, non-volatile metabolomic profiles also correlated with the abundance of aphids in the field (Clancy et al. 2018) and to the local genetic population structure of associated aphids (Zytynska et al. 2019). Although these discoveries strongly suggest a role of intraspecific chemical diversity in mediating relationships between tansy and its specialized aphids, it is not clear how mono- and sesquiterpenoid profiles are related and how these different terpenoid classes may influence insect colonization patterns.

Aphids are sap-sucking plant-parasitic insects comprising up to 4000 species (Eastop 1986). They are exposed to many external forces that significantly impact their populations, such as predation and parasitism, environmental conditions, geography, and climate (Eastop 1986, Loxdale and Balog 2018). Furthermore, plant-related variables, such as host-plant chemistry, have strong effects on the behaviour and abundance of aphids, and may serve as host-finding cues for aphids. Plant chemical composition, for example, can determine aphid feeding preference (Neuhaus-Harr et al. 2024) or alter the predation rates on aphids (Stadler 2004, Linhart et al. 2005). However, while there are abundant studies on large-scale variation in insect–plant interactions (Tscharntke and Brandl 2004, Rand and Louda 2006, Hortal et al. 2010), only a few have linked this variation to variation in plant chemistry (Watt et al. 1997, Berenbaum and Zangerl 1998). This is despite the fact that chemical variation in a single host plant species across space is common (Kessler and Kalske 2018, Wetzel and Whitehead 2019). As plant chemistry differs across geographical ranges, this may have ecological implications for associated insects.

We assessed terpenoid variation in tansy on a spatial scale and investigated consequences for plant–insect interactions. We sampled tansy along a north-west to south-east gradient in Germany, in which plant leaf samples, plant morphological traits, information on aphid abundance, ant species occurrence, abiotic site parameters, and the coordinates of the sampling sites were collected to answer the following questions:

1) How do terpenoid compounds in tansy cluster in chemotypes across a northwest–southeast transect in Germany, and how are mono- and sesquiterpenoid chemotypes linked to one another? We hypothesize that there are geographical differences in the distribution of this intraspecific variation of tansy in Germany, as different chemical distributions have been reported in the literature within Germany and beyond. We expect that mono- or sesquiterpenoid profiles are not interrelated due to the involvement of different biosynthetic pathways.
2) How do chemotypes, plant growth variables, and site variables affect aphid and ant occurrence and aphid abundance on the plants? We predict that chemotype and plant architecture significantly affect associated insect communities.

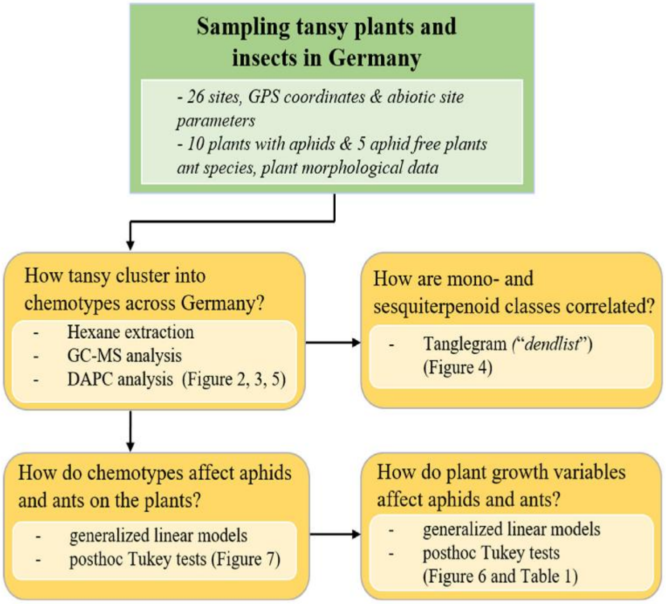

Figure 1. Flowchart of the chemical and ecological analyses conducted in this study. The major aims of our study are provided in yellow boxes and light-colored boxes indicate the analysis conducted. Arrows represent chronological steps taken in our analytical approach. References to respective output for each goal are given in each box.

Material and methods

Sampling of tansy plant populations and insect community

The overview of sampling, chemical and ecological analyses conducted in this study are summarized in the flowchart in Fig. 1. Tansy plants were sampled from 26 sites along a northwest–southeast transect in Germany (Supporting information). The GPS coordinates of each sampling location were recorded on-site, with sites mainly located by country roads, train tracks, and agricultural edges. The sampling site around Bremen was the most northern site chosen (53°04′32.952″N, 08°48′25.794″E), while the sampled site near Freising was the most southern one (48°23′41.5464″N, 11°41′41.9964″E), spanning roughly 700 km across Germany. The site near Bielefeld was the most western (52°01′08.7636″N, 08°31′51.6252″E), while Leipzig was the most eastern sampling site (51°20′26.2752″N, 12°22′29.0388″E), covering roughly 300 km across Germany. The sampling survey took place from the 23 June to the 23 July in 2014. From each site, five plants without aphids and up to ten plants colonized by the specialized herbivore aphid *Metopeurum fuscoviride* (minimum three stems occupied) were sampled. As sampling took place during peak aphid colonization, the abundance of aphids on most plants restricted the sample collection, and often a maximum number of five plants without aphids could be found in every site. For each plant, the presence of ants, as well as the ant species (*Formica rufa*, *Lasius niger* and *Myrmica rubra*) were recorded or marked as 'unknown species' when they could not be identified. For plants with aphids, aphid abundance was calculated by counting the number of colonies, and estimating the size of each colony (XS: < 10 aphids, S: 10–50 aphids, M: 50–200 aphids, L: > 200 aphids). A subset of plants was randomly selected for chemical analysis.

Plant morphological measurements and abiotic site parameters

Plant morphological traits and geographic locations were recorded for each plant. Plant morphological traits included 1) the height of the tallest stem; 2) the number of stems; 3) the plant's diameter at its widest width; and 4) the relative height of the surrounding vegetation, which was assessed by taking the weighted % cover within a 2 m radius of five vegetation categories that were ranked by their height (i.e. bare soil (1), grass (2), small herbs (3), tall herbs (4), shrubs (5)). As derived parameters, 5) the volume (radius^2 $\times \pi \times$ height), 6) the plant bushiness (plant volume divided by the number of stems), and 7) the emission potential of volatiles (volume × total terpenoid concentration) were estimated. Abiotic site parameters included 1) the annual precipitation (reflecting the sum of rain over a year in mm), 2) the average annual temperature (in degrees Celsius) in every region. Meteorological data were obtained from the German weather service (Kaspar 2023). Furthermore, 3) we analyzed one sample of soil per site, including the percentage of sand, silt and clay.

Hexane extraction of terpenoids and GC–MS analysis

Leaf material collection and extraction were performed as described by Clancy et al. (2016). The detected compounds were identified by comparison of the mass spectra using the National Institute of Standards and Technology (NIST), Mass Spectral Library (NIST 11) and Wiley 275 GC/MS Library (Wiley, New York), and confirmed by comparison of the Kovats retention indices as reported by Guo et al. (2019, 2020) based on chromatography retention times of a saturated alkane mixture (C9–C25; Sigma-Aldrich). The potential changes in the GC–MS sensitivity were corrected by normalizing to the internal standard (monoterpene δ-2-carene). The compounds were quantified using, using six dilutions of the external standards: sabinene, α-pinene, linalool, methylsalicylate, β-caryophyllene, α-humulene, geraniol and bornyl acetate. The chemical structure of the identified compounds was sketched using ChemDraw professional (ChemDraw ver. 21.0.0).

Statistical analysis

Clustering plants into chemotypes

The plants were clustered into classes separately according to their monoterpenoid or sesquiterpenoid profiles by using the '*hclust*' function with the '*ward.D2*' method of correlation distance in the R 'factoextra' package (Kassambara and Mundt 2020). The '*statistical meta-analysis*' function in the online software MetaboAnalyst ver. 5.0 (Pang et al. 2021) was used to compute the heatmap of the monoterpenoid and sesquiterpenoid compounds contributing to the differentiation of the classes. The discriminant analyses of principal components (DAPC; Jombart et al. 2010), which is informed by the same cluster analysis was then applied to visualize the separation of monoterpenoid and sesquiterpenoid chemotype classes. Four different categorical groupings, monoterpenoids and sesquiterpenoids classes that were already identified by using '*hclust*' were used to compute DAPC in an effort to enhance a discrimination between predefined classes. The data was first analyzed using an unconstrained principal component analysis (PCA) (Supporting information) and following, a discriminant analysis was used to infer the separation. By combining the PCA with DA, DAPC maximizes the variance between the classes. The number of retained principal components (PCs) was determined by cross-validation using the '*xvalDapc*' function in the 'adegenet' package ver. 2.1.1 (Jombart 2008) in R to avoid unstable assignments of individuals to clusters. Guo et al. (2021) previously employed this technique of DAPC to characterize different fungi species using the complete fungal volatilomes.

Additionally, to compare the relationship between mono- and sesquiterpenoid classes, a tanglegram of both dendrogram trees was obtained by using the '*dendlist*' function in the 'dendextend' package (Galili 2015). We also tested the associations between the selected monoterpenoids using correlation analysis with a Spearman method at a 99% confidence interval by using in 'ggpubr' package (Kassambara 2020).

All other graphs were made using the package 'ggplot2' in R (Wickham 2016). For better resolution, the resulting images were edited using the image processing software 'Inkscape' (ver. 1.1.1).

Phytochemical diversity analysis

Hill diversity is a measure of diversity that focuses on three components: richness, evenness, and disparity. To compare the phytochemical diversity indices for the chemotype classes, we calculated the functional Hill diversity (FHD) for all samples, separately for monoterpenoids and sesquiterpenoids, using the 'chemodiv' R package (Petrén et al. 2023a). FHD was calculated at diversity orders from $q=0$ to $q=3$. For increasing q-values, the measure puts more weight on abundant compounds; at $q=0$, the relative abundances of compounds are not taken into account; at $q=1$, the weight is proportional to their abundance, and at $q > 1$, more weight is put on abundant compounds of which the upper limit is set to $q=3$. Dissimilarities between compounds were calculated based on PubChem Fingerprints (Kim et al. 2021), which quantify dissimilarities based on the structural properties of the molecules. Each compound's chemical identifiers (SMILES and InChIKey) were extracted from the PubChem open database (https://pubchem.ncbi.nlm.nih.gov).

Distribution of terpenoid chemotypes across a geographical transect in Germany

We carried out a permutational multivariate analysis of variance (PERMANOVA, '*adonis2*' function in 'vegan' R package; Oksanen et al. 2020) using chemical distance matrices (999 permutations, Bray–Curtis method) versus the latitude and longitude across each pairwise combination.

Morphological differences across chemotypes and geographical transect

To test whether plant morphology differed between chemotype classes, we used a one-factorial ANOVA. We tested the number of stems, plant volume, emission potential, plant height, radius, and bushiness. Furthermore, we used a one-factorial ANOVA to test whether the plant morphology was related to soil type. To test whether plants differed morphologically across a latitudinal or longitudinal gradient in Germany, we conducted Pearson correlations, using the Holm–Bonferroni method for correction.

Effect of chemotypes on associated insect community

To test whether aphid occurrence or ant occurrence was influenced by chemotypes, plant morphology, and site variables, we set up a generalized linear model (GLMs) with binomial distribution when the response variable was occupancy (1/0). For aphid abundance, the number of colonies per plant was multiplied with the minimum number of aphids in each colony category. The response variable, the number of aphids, was log-transformed to ensure that the assumption of normality was met, and a linear model with a normal distribution (LM) was used. The 'emmeans' R package with Tukey adjustment was used to assess post hoc pairwise comparisons among factor levels following model fit (Russell 2021).

Prior to running the generalized models and to rule out multicollinearity, we excluded all variables with a variance inflation factor higher than five ('*VIF*' function in 'car' R package, Fox and Weisberg 2019). We omitted 'plant radius', 'plant volume', and 'total terpenoid concentration' and 'soil type'. As predictor variables, we included 'monoterpenoid class', 'sesquiterpenoid class', and their interaction, 'monoterpenoid concentration', 'sesquiterpenoid concentration', 'emission potential', 'bushiness', 'height of surrounding vegetation', 'plant height', 'the number of stems', 'annual temperature at the site', 'annual precipitation at the sampled site', 'latitude' and 'longitude' in all models. In the model for aphid abundance we additionally included the presence of all three ant species '*F. rufa*', '*L. niger*' and '*M. rubra*', as well as the 'soil type' of the respective site.

To test whether the FHD affected aphid occurrence and abundance, we used a one-way analysis of variance test. FHD of mono- and sesquiterpenoids were log-transformed to meet normality assumptions. Statistical models were carried out using R ver. 4.1.3 (www.r-project.org).

Results

Monoterpenoid and sesquiterpenoid tansy chemotypes

We analyzed 278 plants and identified 30 monoterpenoids and 21 sesquiterpenoids. The molecular schemes of some mono- and sesquiterpenoids are depicted in Fig. 2a–3a. Plants clustered into four distinct monoterpenoid (Fig. 2b) and four sesquiterpenoid chemotype classes (Fig. 3b). The eastern Hanover sampling site was excluded from further analyses because two unknown sesquiterpenoid compounds found there could not be annotated by library search and Kovats index comparisons, and this site specifically showed exceedingly high concentrations of β-thujone and bicyclosesquiphellandrene, causing severe outliers (Supporting information).

A heatmap of monoterpenoids shows the separating features of α- and β-thujone in class 1, camphor and camphene in class 2, trans-verbenol and trans-chrysanthenyl acetate in class 3, and α-terpinene, α- and β-phellandrene in class 4 (Fig. 2b). Monoterpenoid classes comprised 69, 25, 37 and 147 plants, for classes 1, 2, 3 and 4, respectively. Discriminant analysis of principal components models (DAPC) showed a discrimination among the different monoterpenoid classes (Fig. 2c). Specifically, class 1 was dominated by β-thujone (approximately 75%), class 2 was defined by a mixture of approximately 40% camphor and 20% sabinene, class 3 was dominated by trans-chrysanthenyl acetate (approximately 60%), and class 4 comprised a group of plants with a mix of compounds (Fig. 2d). The total concentration of monoterpenoids varied from 0.02 to 112 pmol g^{-1} leaf fresh with classes 1 and 2 having the highest monoterpenoid concentrations and class 4 having the lowest (Fig. 2e). Additionally, we found that some monoterpenoids were closely linked to each other. Regardless of the chemotype categorization, camphene

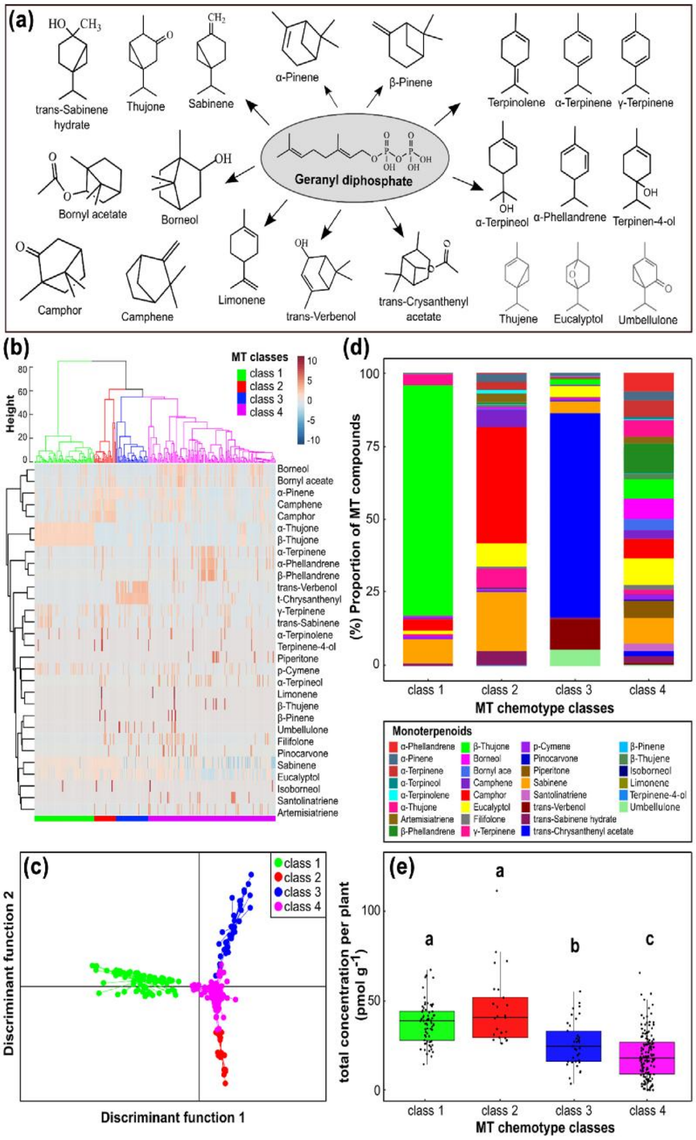

Figure 2. Visualization of the monoterpenoid (MT) chemotype classes using different statistical approaches. (a) Schematic illustration of monoterpenoid products synthesized from geranyl diphosphate via carbocationic reactions mechanism. The compounds that are biosynthetically linked stand in the same row, e.g. sabinene, thujone and trans-sabinene hydrate. (b) Hierarchical cluster analysis of monoterpenoid compounds across 278 individual plants. Four main classes were identified and each cluster is highlighted by a different color; class 1 green, class 2 red, class 3 blue, and class 4 magenta. The variety and separating features of monoterpenoids found in each class is displayed in the heatmap. (c) Discriminant analysis of principal components plot shows separations among the MT chemotype classes. (d) Proportion (in percentage) of each representative compound of each class is provided in a stacked barplot. Data is normalized to logarithmic scale and Pareto matrix. (e) Total concentration of monoterpenoids with significant differences (Tukey test, $p < 0.05$) indicated by letters.

and camphor concentrations were associated in monoterpenoid classes 1, 2 and 4 ($R^2=0.42$, $p < 0.001$; Supporting information), and borneol and bornyl acetate concentrations showed a positive correlation ($R^2=0.50$, $p < 0.001$; Supporting information) among the many plants.

Plants clustered into four sesquiterpenoid chemotype classes, with the compound pattern of each sesquiterpenoid class presented in a heatmap (Fig. 3b). Here, 26, 63, 11 and 175 plants were classified into classes 1, 2, 3 and 4, respectively. A DAPC model showed a discrimination among the

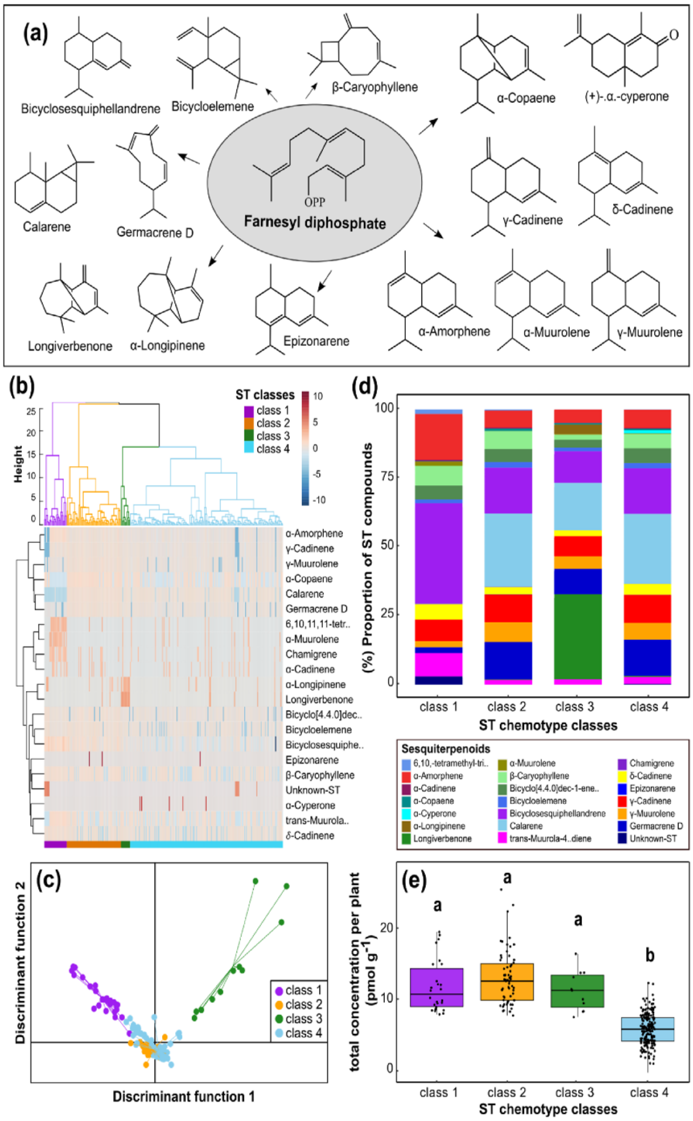

Figure 3. (a) Schematic illustration of sesquiterpenoid (ST) products synthesized from farnesyl diphosphate. All the sesquiterpenoids are the main products of farnesyl diphosphate. (b) Hierarchical cluster analysis of sesquiterpenoid compounds across 278 plants; four main classes were classified – class 1 purple, class 2 orange, class 3 forestgreen, and class 4 lightblue. The variation of the sesquiterpenoid compounds of each class is depicted in the heatmap. Data is logarithmically transferred and Pareto scaled. (c) Discriminant analysis of principal components indicates separations among the sesquiterpenoid classes. (d) Proportional composition of the compounds, provided in percentage for each class. (e) Total concentration of sesquiterpenoids with significant differences (Tukey test, $p < 0.05$) indicated by the letters on the top.

sesquiterpenoid classes (Fig. 3c). In contrast to distinct monoterpenoid composition between classes, sesquiterpenoids did not show much variation in their profile content. Instead, sesquiterpenoid profiles showed a consistent presence of bicyclosesquiphellandrene, β-caryophyllene, γ-muurolene and α-amorphene (Fig. 3d). The plants belonging to sesquiterpenoid class 1 were characterized by the highest proportion of bicyclosesquiphellandrene (36%). Even though classes 2 and 4 did not show a distinct variation in relative sesquiterpenoid composition, as they were characterized by calarene (25%), germacrene D (13%), and γ-cadinene (10%), their sesquiterpenoid profiles were expressed at significantly different total concentrations (Fig. 3e). Furthermore, although sesquiterpenoid class 3 contained fewer individuals, it was predominantly formed by longiverbenone (31%). The total concentration of sesquiterpenoids ranged from 1.3 to 25.5 pmol g^{-1} (Fig. 3e), with sesquiterpenoid class 4 showing significantly ($p < 0.05$) lower concentrations compared to the other classes. The tanglegram of monoterpenoid and sesquiterpenoid chemotypes across all tansy individuals showed no clear correlation between monoterpenoid and sesquiterpenoid profiles (Fig. 4a).

Diversity metrics of chemotype classes

Quantifying the FHD enabled us to study the diversity of tansy monoterpenoid and sesquiterpenoid chemotype classes in novel ways that differ in the weight placed on abundant compounds through compound richness, evenness and disparity (Petrén et al. 2023b). Overall, chemotypes that are dominated by individual compounds (and hence have a lower evenness)

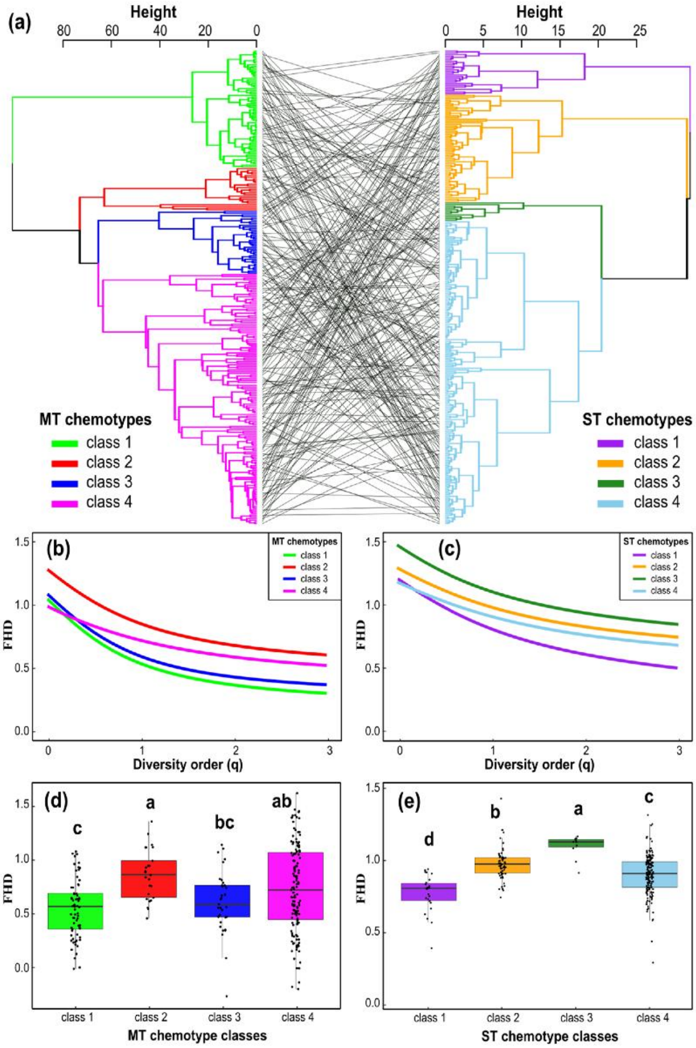

Figure 4. (a) A tanglegram of the chemotype trees demonstrating that monoterpenoid (MT; on the left) and sesquiterpenoid (ST; on the right) chemotype classes are not correlated. The opposite scenario (MT chemotypes associated with ST chemotype) would have been indicated by linkage or cross-correlation between the same plant. The diversity profile shows the functional Hill diversity (FHD) at diversity orders from $q=0$ to $q=3$ for MT (b) and ST (c) chemotype classes. For increasing q-values, the measure is less sensitive to the relative abundances of compounds; at $q=0$ the relative abundances of compounds are not taken into account; at $q=1$ equal weight is put on all compounds. The boxplots show variation in FHD for the MT (d) and ST (e) classes in detail for $q=1$. Note that values are log-transformed in figures and analyses. Significant differences ($p < 0.05$) between classes are indicated by the letters above the boxes. Mean number of monoterpenoids per sample: class 1=5.9, class 2=7.2, class 3=5.7, class 4=5.6. Mean number of sesquiterpenoids per sample: class 1=10.3, class 2=10.7, class 3=12.0, class 4=9.7.

are indicated by deeper curves that tend to have lower FHD values between FHD order 2 and 3, (Fig. 4b–c). Curves associated with Hill numbers quantify the relative importance of compounds to the structure of the chemotype class.

Functional Hill diversity among monoterpenoid classes was the lowest for monoterpenoid classes 1 and 3, which were at their lowest at higher orders of q, emphasizing the role of abundant compounds in structuring these monoterpenoid classes (β-thujone and trans-chrysantenyl-acetate, respectively, Fig. 2d). Monoterpenoid class 2 had the highest FHD, but a rather deep curve, indicating that abundant compounds are likely important in this class as well (camphor, Fig. 2d). Class 4 had an intermediate mean FHD, with a shallow curve (Fig. 4b, d), which indicates that dominant compounds play a less important role in this class (indicated by an even distribution of chemical compounds in Fig. 2d). Notably, the diversity at the level of chemotypes, rather than individual plants, appeared to be higher in class 4, as an effect of larger differences between samples in their composition (Fig. 2b).

Within the sesquiterpenoid classes, class 1 and 3 had a deeper curve (Fig. 4c), with low and high FHD (Fig. 4e), emphasizing the stronger role of dominant compounds in shaping the structure of the sesquiterpenoid classes (bicyclosesquiphellandrene and longiverbenone, respectively, Fig 3d). Sesquiterpenoid classes 2 and 4 had more similar and shallower curves with intermediate mean FHD (Fig. 4c, e), indicating a more similar chemical profile, with a more even distribution of compounds (Fig. 3d). Overall, the FHD of sesquiterpenoids differed significantly between all classes (Fig. 4e).

North–south and west–east gradient in monoterpenoid and sesquiterpenoid chemotypes

The distribution of tansy monoterpenoid classes differed significantly across Germany. Monoterpenoid classes 1 and 2 were found more frequently in the east and south, while monoterpenoid classes 3 and 4 were more frequently observed in north and west Germany (Fig. 5a). A PERMANOVA test showed that monoterpenoid compositions significantly varied depending on the latitude ($R^2 = 0.01$; $p < 0.002$; Fig. 5c) and longitude ($R^2 = 0.01$; $p < 0.001$; Fig. 5e, Supporting information) coordinates, even though the explained variance is low. Biosynthetically linked monoterpenoids, such as β-thujone and sabinene, camphor and camphene, increased substantially with decreasing latitude and increasing longitude towards the far south. Contrastingly, trans-crysanthenyl acetate and trans-verbenol showed an opposite trend, with the highest concentration reported in plants at high latitudes towards more northern sites (Supporting information).

In contrast, sesquiterpenoid classes were more homogeneously distributed across Germany (Fig 5b), indicating independence of the geographic effects on mono- and sesquiterpenoid chemotypes (Fig 5d, f). Sesquiterpenoid compositions did not significantly differ in their geographic distribution (lat.: $R^2 = 0.002$; $p = 0.56$; lon.: $R^2 = 0.002$; $p = 0.58$; Supporting information).

Additionally, we ran PERMANOVA tests to see whether the soil type differed across mono- and sesquiterpenoid classes. Soil type was analyzed in 25 of the 26 sites. Loam and silt loam were found in four sites, while loamy sand occurred in seven sites and sandy loam in eight sides (Supporting information). Sand was only found in one site (Bergen, north Celle; Supporting information). We found that 'soil type' was significantly associated with mono- ($R^2 = 0.06$; $p < 0.001$) and sesquiterpenoids ($R^2 = 0.03$; $p = 0.004$), although the explained variance was low (Supporting information). Because of this, a PCA analysis did not show a clear clustering of soil type for mono- and sesquiterpenoid compounds (Supporting information). Not all monoterpenoid classes were found on all soil types. For example, only monoterpenoid classes 3 and 4 were found growing on sand (Supporting information). However, the distribution of sesquiterpenoid chemotypes was more even over all soil types (Supporting information). Similarly, the number of plants from the monoterpenoid classes differed significantly among the percentage of sand, silt and clay, while sesquiterpenoid classes did not differ across different percentages (Supporting information).

Plant morphology differences between chemotypes and across Germany

The number of stems per plant differed marginally significantly between monoterpenoid classes ($F_3 = 2.55$, $p = 0.056$; Supporting information). A post hoc test showed that the number of stems was lower in plants from monoterpenoid class 1 compared to class 4 (Fig. 6a). Plant volume and emission potential differed significantly between plants of different monoterpenoid chemotypes ($F_3 = 2.71$, $p = 0.045$; $F_3 = 8.71$, $p < 0.001$; Supporting information). Plant volume was significantly lower in plants belonging to monoterpenoid class 1 than in class 2, but neither differed from monoterpenoid classes 3 and 4 (Fig. 6b). Plants belonging to monoterpenoid class 2 had significantly higher emission potential than all other monoterpenoid classes (Fig. 6c).

Sesquiterpenoid classes showed substantial differences in the number of stems ($F_3 = 3.69$, $p = 0.012$; Supporting information). Specifically, plants from sesquiterpenoid class 1 had a significantly higher number of stems than sesquiterpenoid class 2 and 3 (Fig. 6d). However, there were no differences in their volume or emission potential (Fig. 6e–f). Plant height, plant radius, and plant bushiness did not differ significantly across monoterpenoid nor sesquiterpenoid classes. F-statistics and p-values for all measured plant traits are in the Supporting information.

Plant traits also varied across the geographical gradient. Plant height differed significantly along the latitudinal gradient ($t_{(369)} = -3.19$ $cor = -0.16$, $p = 0.009$, Supporting information), with plants typically growing taller in the north. Plant bushiness differed across the longitudinal gradient ($t_{(369)} = -2.87$, $cor = -0.15$, $p < 0.01$, Supporting information), with plants growing bushier in the west. Most plant variables, such as radius and number of stems, were positively

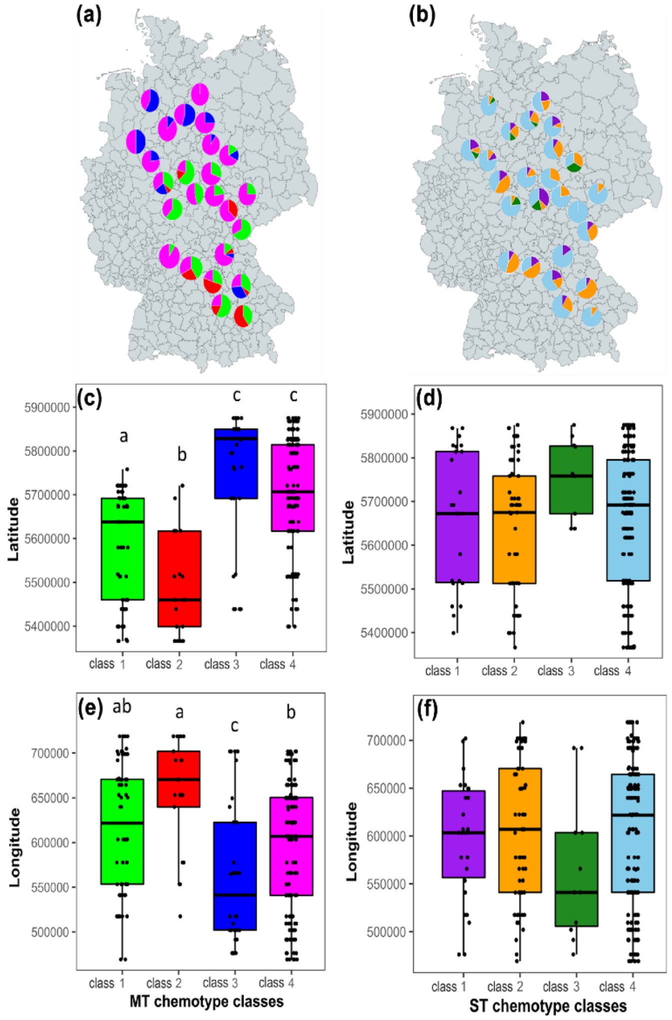

Figure 5. Proportion of monoterpenoid (MT) and sesquiterpenoid (ST) classes within each sampled site. Monoterpenoid classes are color-coded as following: green – class 1, red – class 2, blue – class 3, magenta – class 4 (a). Sesquiterpenoid classes are color-coded as following: purple – class 1, orange – class 2, dark green – class 3, light blue – class 4 (b); monoterpenoid classes found over different latitude (c) and longitude (e) and sesquiterpenoid classes found over different latitude (d) and longitude (f); significant differences are indicated on top of the boxplots ($p < 0.05$).

correlated (i.e. higher plants tended to be bushier; Supporting information).

Furthermore, we found that the number of stems, plant height, plant bushiness and plant radius varied over the different soil types, while plant volume and emission potential did not differ across different soil types (Supporting information). On loamy sand and sandy loam, plants had significantly more stems than plants growing on silt loam (Supporting information). Plants on loam grew significantly higher than plants on loamy sand and silt loam (Supporting information). Similarly, plants growing on loam were significantly bushier than plants growing on sandy loam and silt loam, respectively (Supporting information). Plants growing on loamy sand or sandy loam however, had a larger radius compared to plants growing on silt loam (Supporting information).

Effects of site conditions and plant variables on tansy aphids and associated ants

Modelling the effects on aphid occurrence with a binomial GLM, we found that aphid occurrence was affected by chemical and morphological plant traits. Specifically, the monoterpene class ($\chi^2_{(3)} = 11.99$, p = 0.007, Fig. 7a) and, marginally significantly, the number of stems ($\chi^2_{(1)} = 3.76$,

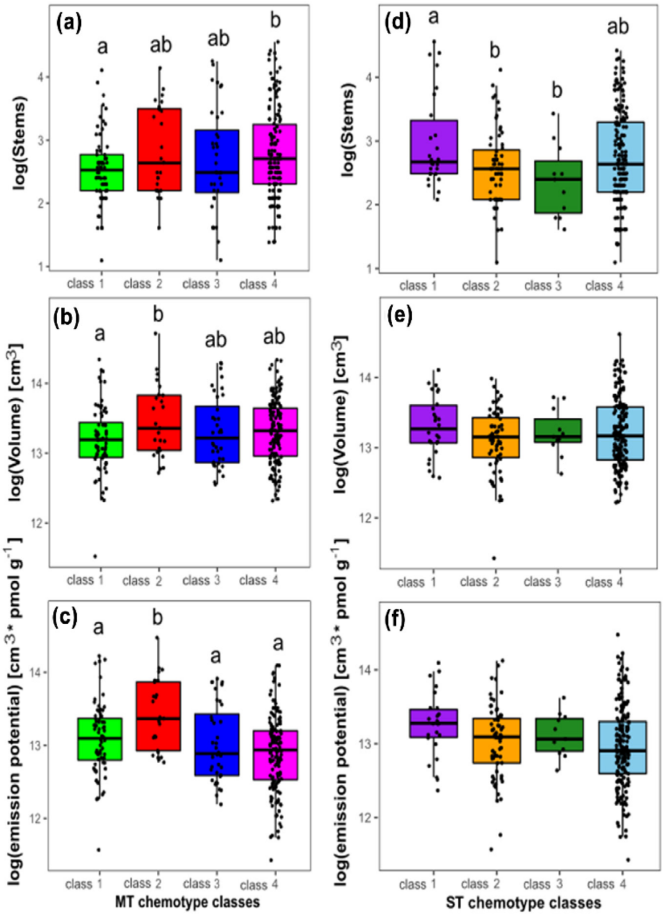

Figure 6. Plant traits with a significant difference across monoterpenoid classes: number of stems (a), plant volume (b) and emission potential (c). Plant traits across different sesquiterpenoid classes: number of stems (d), plant volume (e) and emission potential (f). Emission potential was calculated by the plant volume × the concentration of all terpenoid compounds in that specific plant. Degrees of freedom (DF), statistic (F-value) and p-value for plant trait variation across monoterpenoid and sesquiterpenoid classes using a one-factorial ANOVA are indicated in the Supporting information.

$p = 0.053$) influenced the occurrence of *M. fuscoviride* aphids (Table 1). Even though the aphid occurrence responds to monoterpenoid classes, the FHD of neither the monoterpenoids nor the sesquiterpenoids affected *M. fuscoviride* presence or numbers significantly (Supporting information).

Sesquiterpenoid classes did not influence aphid presence (Fig. 7b, Table 1, Supporting information). In contrast to this, a linear model with aphid abundance revealed that aphid abundance was not affected by monoterpenoid classes, but was significantly influenced by the soil type of the respective site ($F_4 = 20.81$, $p = 0.013$, Table 1). Furthermore, the height of the surrounding vegetation marginally significantly influenced aphid abundance ($F_1 = 5.71$, $p = 0.059$, Table 1). Correlation tests supported our findings that the abundance of *M. fuscoviride* was not correlated with any plant variables nor to single (dominant) compounds of the monoterpenoid classes (spearman correlation with 0.95-confidence level; Supporting information).

Three species of ants were observed regularly in all sites and often on the same plant (*F. rufa*, *L. niger* or *M. rubra*, Supporting information). All plants with aphids appeared to have ant present. A binomial GLM with ant presence as dependent variable showed that monoterpenoid class, but not sesquiterpenoid class, significantly affected probability of ant presence ($\chi^2_{(3)} = 10.62$, $p = 0.014$; Table 1, Supporting information), and post hoc Tukey tests indicated that monoterpenoid class 1 had significantly higher probability of ant presence than monoterpenoid class 4. Furthermore, ants were more likely to be present at sites with higher temperatures ($\chi^2_{(1)} = 3.91$, $p = 0.048$; Table 1, Supporting information). This was independent of the species.

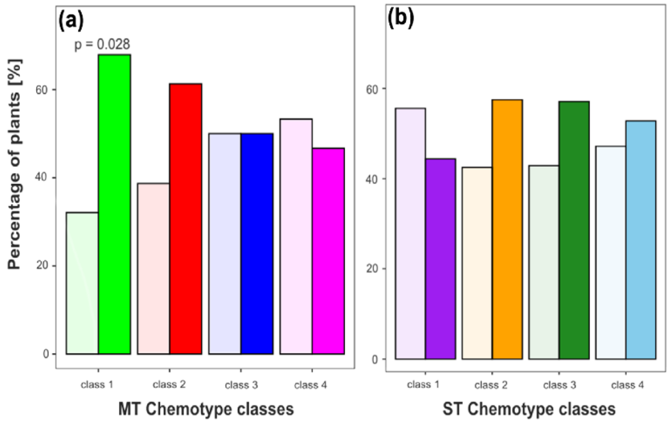

Figure 7. Bar charts indicate the percentage of plants without *M. fuscoviride* aphids (transparent) and with aphids (solid) within monoterpenoid (MT) classes (a) and sesquiterpenoid (ST) classes (b). A binomial test showed that plants of monoterpenoid class 1 (a) were colonized by this aphid species significantly more often than expected by chance (binomial test: 95% conf. interval = 0.52–0.82, p = 0.028; Supporting information). Sesquiterpenoid classes (b) did not influence aphid occurrence (Supporting information).

Parasitized aphids were commonly observed in all sites (Supporting information). However, we did not observe clear drivers of the probability of parasitism except latitude and longitude. Specifically, parasitism was more common in the southwest than in the northeastern sites (Supporting information).

Discussion

We demonstrated that *T. vulgare* plants exhibit variation in distinct mono- and sesquiterpenoid chemotypes across a wide geographical range in Germany. Our results show that the chemical composition of monoterpenoids differed significantly across geographical coordinates, demonstrating that the monoterpenoids profile of tansy was more dissimilar with increasing geographical distance. While monoterpenoid chemotypes displayed different local dominance patterns, sesquiterpenoid chemotypes were homogeneously distributed across Germany. We further demonstrated that monoterpenoid classes, but not sesquiterpenoid classes, are involved in shaping aphid *M. fuscoviride* and ant *L. niger*, *F. rufa* and *M. rubra* occurrence patterns. We additionally found that monoterpenoid chemotypes are influenced by soil type (silt, sand and clay content) whereas sesquiterpenoid chemotypes did not seem to be affected by soil content. Furthermore, soil type affected the number of aphids while mean annual temperature had a positive influence on ant occurrence, suggesting that chemical, morphological and geographic factors structure the wider ecological community.

Tansy chemodiversity has been investigated in different geographical regions of Europe. For instance, a study from Finland revealed that Finland's central and southern regions were home to tansy chemotypes with higher concentrations of camphor (Keskitalo et al. 2001). Interestingly, we also found that plants from monoterpenoid class 2, which is dominated by camphor, were more frequent in southern Germany. Tansy seems to differ in its terpenoid profile between and within countries. For example, tansy plants from Finland showed a unique davadone D chemotype, while myrcene-tricyclene chemotypes were more common in the south and southwest compared to the rest of the country (Keskitalo et al. 2001). Moreover, a study from Lithuania found that tansy exhibited different dominant compounds (such as eucalyptol, trans-thujone and myrtenol) between different locations (Judzentiene and Mockute 2005). In line with these findings, we observed plants from the β-thujone chemotype more prevalent and plants from the trans-chrysanthenyl acetate chemotype less prevalent in the south of Germany compared to the northern German sites. These findings suggest that differences in terpenoid profiles are common and likely increase at larger geographic scales and that different regions bolster different dominance patterns of terpenoid compounds. Perhaps the different soil types occurring in different sites, could partly explain the patterns of monoterpenoid chemotype classes, as previous studies have found that e.g. soil properties influenced essential oil composition in *Thymus pulegioides* (Vaičiulytė et al. 2017, 2022). As our study only assessed soil type at the site level, variation in soil type and relationships with individual plants within sites could not be captured, and hence small-scale variation in soil type as driver of local chemical variation warrants future study.

Our study found that on a German-wide scale, tansy plants could be grouped into distinct chemotypes using their mono- and sesquiterpenoid profiles. Hierarchical cluster analyses revealed four monoterpenoid and four sesquiterpenoid classes that were not strongly associated with one another. This lack of alignment between individuals of mono- and sesquiterpenoid chemotypes strongly emphasizes differing and unrelated biosynthesis pathways for these two compound classes. Sesquiterpenoids are generally produced through the cytosolic mevalonate pathway (MVA), whereas

Table 1. Degrees of freedom (DF), statistic (χ^2 or F-value) and p-value for chemical, morphological, geographic, and biotic variables included, when applicable, in a binomial generalized linear model with aphid occurrence or ant occurrence as response variable, and in a GLM with aphid abundance as response variable. Bold letters indicate significant p-values ($p < 0.05$) and italic letters indicates marginally significant values ($0.05 < p < 0.01$).

	df	Aphid presence χ^2 (p-value)	Ant presence χ^2 (p-value)	Aphid abundance F-value (p-value)
MT class	3	**11.99 (0.007**)**	**10.62 (0.014*)**	2.18 (0.710)
MT concentration	1	0.56 (0.453)	1.07 (0.301)	0.05 (0.863)
ST class	3	2.79 (0.426)	2.16 (0.541)	5.36 (0.339)
ST concentration	1	0.31 (0.577)	0.10 (0.756)	0.44 (0.600)
MT class:ST class	8	8.26 (0.409)	10.08 (0.259)	8.07 (0.647)
Emission potential	1	0.38 (0.536)	1.05 (0.306)	0.08 (0.825)
Bushiness	1	0.21 (0.643)	2.21 (0.137)	0.19 (0.729)
Height of surrounding vegetation	1	0.10 (0.753)	0.94 (0.332)	*5.71 (0.059)*
Height	1	1.85 (0.173)	0.14 (0.706)	30.81 (0.476)
Stems	1	*3.76 (0.053)*	0.63 (0.426)	2.55 (0.206)
Soil type	4	–	–	**20.81 (0.013*)**
Mean annual temperature	1	0.83 (0.362)	**3.91 (0.048*)**	3.63 (0.132)
mean annual precipitation	1	0.16 (0.694)	0.61 (0.434)	4.21 (0.105)
Latitude	1	0.52 (0.471)	0.53 (0.466)	0.05 (0.861)
Longitude	1	0.31 (0.577)	0.01 (0.301)	0.22 (0.712)
Formica rufa	1	–	–	3.55 (0.136)
Lasius niger	1	–	–	2.59 (0.202)
Myrmica rubra	1	–	–	0.01 (0.953)
Residuals	150	–	–	237.19

the plastidial methylerythritol phosphate (MEP) pathway yields multiple monoterpenoid products (Davis and Croteau 2000). However, the MVA and MEP can provide isopentenyl diphosphate precursors for monoterpenoid and sesquiterpenoid biosynthesis (Dudareva et al. 2005). This may explain why some individuals of monoterpenoid chemotype classes link with sesquiterpenoid chemotype classes. The diversity of terpenoid compounds in plants is generated by terpene synthases, a diverse family of enzymes that catalyze terpenoid compounds from single substrates (Bohlmann et al. 1998). For instance borneol and bornyl acetate showed a significant positive correlation in the same plant where they both were active, indicating that they are likely produced from the synthase of the bornyl diphosphate enzyme (Supporting information). A similar result was observed between camphor and camphene. Interestingly, the chemotypes also differed in their phytochemical diversity in compound richness, evenness, and dissimilarity, which may impact interactions between plants and insects (Whitehead et al. 2021, Neuhaus-Harr et al. 2024).

We assessed the impacts of monoterpenoid and sesquiterpenoid composition on interactions with a specialized insect herbivore, i.e. *M. fuscoviride*, and three species of ants *F. rufa*, *L. niger* and *M. rubra*. Plants belonging to monoterpenoid class 1 were significantly more likely to be colonized by aphids, whereas equal occupancy of plants was observed for all sesquiterpenoid classes. This finding is in line with other studies, since our monoterpenoid class 1 contains β-thujone as a dominant compound, which has been associated with an increased abundance of another tansy specialist aphid, *Macrosiphoniella tanacetaria* (Kleine and Müller 2011). Interestingly, previous studies also found higher abundance and earlier colonization rates of *M. fuscoviride* on plants with camphor as dominant compound, which would resemble monoterpenoid class 2 in our study (Clancy et al. 2016, Senft et al. 2019). This could explain why we observed the tendency of higher aphid presence on monoterpenoid class 2 plants, even though this finding was not significant. However, other studies have shown that not only dominant but also minor compounds within a blend significantly affect plant–insect interactions (McCormick et al. 2014, Clancy et al. 2016).

Preference, and therefore presence, appears to be affected by terpenoids, a finding in line with the idea that volatile terpenoids serve as cues for finding host plants (Bruce et al. 2005, Ninkovic et al. 2021). It is possible that monoterpenoids are more helpful to aphids as cues for host plant identification than sesquiterpenoids, as plants exhibited much higher concentrations of monoterpenoids compared with sesquiterpenoids. Clancy et al. (2016) observed that the emission of terpenoids, presumably evaporated/released from glandular cells (Devrnja et al. 2021), affected *M. fuscoviride* colonization. Given the higher volatility of monoterpenoids (Mofikoya et al. 2019), higher concentrations could be expected in the near ambient air of the plants' canopy. This fits with the observation that the influence of terpenoids on aphid presence on individual chemotypes is mainly determined by monoterpenoids and not by sesquiterpenoids. If monoterpenoids are used as host-finding cues, the significant low concentration of monoterpenoids in monoterpene class 4 could perhaps be the reason why we see a tendency of low aphid presence in these plants. Another reason, why mono- and not sesquiterpenoids could be used as host finding cues by aphids, could be the differentiation of the monoterpenoid profiles in terms of their (dominant) compounds. Profiles were very distinct in monoterpenoids, while the sesquiterpenoid classes were chemically more similar. FHD had no effect on aphid presence and abundance. Functionally related terpenoids seem to have the same effect on aphids compared to functionally unrelated terpenoids.

Furthermore, not only aphids, but also ants might use monoterpenoids as cues. As *M. fuscoviride* is a facultative ant-tended species that benefits strongly from mutualism (Flatt and Weisser 2000), chemotypes might structure aphid colonization and population indirectly via ant preference. Hence, it is unsurprising that we found a higher ant occupancy of plants belonging to monoterpenoid class 1 compared to plants from monoterpenoid class 4, similar as in the aphids. It has been found that the presence of ants before aphid appearance led to a stronger likelihood of aphid colonization (Senft et al. 2018). However, it could also be likely that aphid presence attracts ants, which take a lot of time to wander around until they find suitable food sources. Even though we could not confirm whether ant presence was shaping aphid presence, or vice versa, and this requires manipulative studying, it does suggests that plant chemotypes mediate the strong relationship between those insects and tansy (Mehrparvar et al. 2017). Furthermore, aphids may have an increased preference for plants of specific chemotypes (Neuhaus-Harr et al. 2023), but also that chemotypes could affect aphid survival and population growth, e.g. via interactions with ants (Mehrparvar et al. 2017). Furthermore, we recognized that geographically changing environmental factors affected the abundance of ants, as their abundance increased with the average annual temperature of the site. This has already been shown for Mediterranean ant species (Cerdá et al. 1998). For example, *L. niger*, known for its thermal tolerance and preference for temperatures in the 18–26°C range, increases their foraging activity at higher temperatures (Blanchard et al. 2021), which could ultimately influence aphid presence and abundance. Hence, chemical cues, such as mono- or sesquiterpenoids might only be one factor shaping aphid communities.

Although we show an effect of chemotypes on plant occupancy by aphids, we did not find such links with aphid abundance. One caveat of our study might be that the observation of insect and plant growth and chemistry resemble only a snapshot in time. Although tansy chemotypes has been found to be stable over time (Clancy et al. 2016), the abundance of aphids fluctuates throughout a season. Single observation points cannot capture the dynamics of an aphid colony over time. As we sampled in peak aphid colonization, this might be one reason why we did not find an effect of tansy chemotypes on aphid abundance, as it is known that e.g. early colonization of aphids is influenced by chemotypic variation (Clancy et al. 2016). Future studies using chemotypes from different geographic locations are needed to assess the effects on aphid colony dynamics in a more controlled manner.

Furthermore, we did not observe links between individual terpenoid compounds and aphid abundance. Several studies now show links between terpenoid composition on aphid preference, and presence on a plant for various aphid species in this model system (Neuhaus-Harr et al. 2024). However, aphid presence on a plant can vary strongly in their abundance, from very few aphids to thousands. These differences in aphid abundance partially may occur after a plant is colonized, and hence can also be shaped by other factors than plant chemotype (Neuhaus-Harr et al. 2024), such as predation pressure (Senft et al. 2019) or resource availability. Indeed, it has been found that coccinellid beetles, which prey heavily on aphids, are more abundant on plants with high β-thujone contents (Kleine and Müller 2011), which might explain why aphids on plants with high β-thujone did not show higher abundances.

We also found that aphid abundance was affected by the soil type. As monoterpenoid classes differed significantly between soil types, the effect of soil types on aphid abundance might be mitigated via the host plant. Other morphological traits, such as the number of stems, the plant volume or the emission potential differed significantly among the mono- or sesquiterpenoid classes and the soil type. Hence, it is possible that morphological traits also play a role in mediating herbivore densities on individual plants and that soil properties influence herbivores indirectly through shaping plant morphology. Previous studies suggested that both the chemotype and other associated plant traits are crucial for host plant selection and performance of herbivorous insects. For instance, in *Brassica oleracea*, high levels of glucosinolates prolonged the development time of the specialist *Pieris rapae* and reduced survival in the generalist *Mamestra brassicae* (Gols et al. 2008). In *Salix sachalinensis*, leaf pubescence reduced overall leaf consumption by the willow leaf beetle *Melasoma lapponica* (Hayashi et al. 2005). Furthermore, Carmona et al. (2011) showed that herbivore susceptibility depends on defence traits, including morphological and chemical traits. Our findings that sap-sucking aphids are influenced by terpenoid composition and the soil type, while ants are influenced by temperatures, support this general observation of the mediating role of plant chemical and morphological traits observed in other plant–insect systems, but also shows how the geographic location intermingles with these factors.

Conclusion

The intraspecific profiles of secondary plant metabolites in different tansy individuals provide a unique perspective for studying the relationships between plants and their environment. Evidently, secondary metabolites play a mediating role between plants and their living environment, and it is also becoming increasingly clear that in many plant species the distribution of plant chemotypes shows relationships with the abiotic environment. Using locally balanced groups of aphid-colonized and uncolonized tansy plants on a transect in Germany, we were able to show that there is a strong geographical clustering of certain chemotypes in tansy and that the composition of monoterpenoids influences the colonization of plants by aphids and ants over large spatial distances. Although correlations between monoterpenoid blends and soil type were found, the causes of chemotypic spatial clustering between and within sites warrant further studies. If clustering with soil type is widespread, it is important to understand whether and how this is adaptive for the plant in a variable biotic and abiotic environment. To this end, a combination of targeted surveys in extreme environments and manipulative studies in common gardens under different environmental conditions can advance our understanding of the evolution and ecology of chemotypic clustering.

Acknowledgements – The authors thank Ina Zimmer, Kerstin Koch and Baris Weber for technical support during the measurements. Additionally, the authors are grateful to fellow PhD candidate Moritz Popp for his help on this work. Open Access funding enabled and organized by Projekt DEAL.

Funding – This work was supported by grants of the Deutsche Forschungsgemeinschaft DFG within the Research Unit FOR3000 to JJR (JU2856/5-1), and WWW (WE3081/40-1) and joint DFG projects to (WWW (WE3081/25-2) and JPS (SCHN653/7-2).

Author contributions

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Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.9p8cz8wqk (Rahimova et al. 2024).

Supporting information

The Supporting information associated with this article is available with the online version.

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8.2 Manuscript II – Preference Study

OIKOS

Research article

Chemodiversity affects preference for *Tanacetum vulgare* chemotypes in two aphid species

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Oikos
2023: e10437
doi: 10.1111/oik.10437
Subject Editor:
Antonino Cusumano
Editor-in-Chief:
Gerlinde B. De Deyn
Accepted 27 November 2023

Plants of the same species can strongly differ in their specialized metabolite profiles, which can affect insect presence and abundance in the field. However, how specialized chemistry shapes plant attractiveness to herbivorous insects is not fully understood. Here, we used common tansy *Tanacetum vulgare*, Asteraceae) – a perennial plant that is highly diverse in terpenoid composition and is known to have variable chemotypes – to test whether 1) plants with different chemotype profiles differ in attractiveness to two specialist aphid species, *Macrosiphoniella tanacetaria* and *Uroleucon tanaceti*, in pairwise choice assays; 2) the diversity of the terpenoid blend affects plant attractiveness to aphids; 3) how plant chemical traits relate to plant morphological traits and which traits best explain aphid preference. We found that *M. tanacetaria* preferred two out of five chemotypes, dominated by α-thujone/β-thujone and β-trans-chrysanthenyl acetate, while avoiding a chemotype dominated by α-pinene/sabinene. *Uroleucon tanaceti* showed no clear preference towards chemotypes, but when given a choice between chemotypes dominated by α-thujone/β-thujone and by α-pinene/sabinene, they preferred the former. Importantly, plant attractiveness to aphids was marginally negatively correlated with chemodiversity, i.e. the number of terpenoid compounds, in *M. tanacetaria*, but not in *U. tanaceti*. Interestingly, the relative concentration and number of terpenoids were generally higher in larger and bushier plants. Hence, we did not observe a tradeoff between plant growth and defence. We conclude that plant chemical composition affects plant attractiveness to aphids and hence may contribute to variation in natural aphid colonization patterns on plants of the same species.

Keywords: aphids, attractiveness, choice assays, genotypic variation, intraspecific phytochemical diversity, plant diversity, terpenoids

Introduction

Understanding relationships between plants and herbivores is an important goal in ecology. How variation in plant diversity shapes herbivory has been a subject of study

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for many decades (Scherber et al. 2010, Weisser et al. 2017). For a long time, between-species diversity was believed to be more important than within-species diversity as a driver of ecosystem processes (Des Roches et al. 2018). Intraspecific diversity, which includes the variation between individuals and the richness and abundance of genotypes and phenotypes within a population, recently gained more attention as an important driver of ecological processes (Raffard et al. 2019). It has been shown that intraspecific variation in plant geno- and phenotypes can structure plant–arthropod food webs (Crutsinger et al. 2006, Poelman et al. 2008, Bálint et al. 2016). As plants of the same species can differ strongly in various traits, understanding how this variation contributes to shaping interactions between plants and interaction partners is currently an important goal in plant ecology.

One important dimension of intraspecific variation in plants is chemodiversity, the variation in chemical composition between different plant individuals of the same species (Wetzel and Whitehead 2020). Primary metabolites are important regulators of basic physiological processes in plants, including growth, development and reproduction, and primary compounds are relatively similar across the plant kingdom (Weng et al. 2012). Specialized metabolites, on the other hand, play critical roles as mediators of interactions, and plants have evolved a much larger diversity in these specialized metabolites, both inter- and intraspecifically (Pichersky and Gang 2000, Iason et al. 2012). Intraspecific variation in specialized metabolite profiles is known to affect the structure of associated herbivore communities, including phloem-feeding insects such as aphids (Poelman et al. 2008, Bustos-Segura et al. 2017, Volf et al. 2019, Singh et al. 2021, Whitehead et al. 2021), but few studies have shown how chemodiversity structures herbivore abundance by affecting herbivore preference for plants (Ziaja and Müller 2023).

Chemodiversity may mediate the presence and abundance of insect herbivores on a plant growing in natural conditions. Natural patterns of insect herbivores are likely shaped by a combination of 1) direct attractiveness of the host plant to the insect herbivore, 2) direct effects via host plant resource quality (i.e. bottom–up effects) and 3) indirect effects, via the plant's attractiveness to natural enemies of the insect herbivore (i.e. top–down effects). Many specialized compounds likely evolved to deter and repel herbivores (Herms and Mattson 1992, Kessler and Baldwin 2001, Whitehead et al. 2021). However, some specialist herbivore species may also use these compounds to find their host (Nishida 2014, Wink 2018). As such, insects can be repelled or attracted by volatile organic compounds (VOCs) emitted into the headspace of a plant (Clancy et al. 2016, Jakobs and Müller 2019). Once an insect herbivore has arrived on the plant, stored and emitted compounds can then affect herbivore performance. For instance, stored compounds can act as feeding deterrents or influence the metabolism of the herbivores (Mithöfer and Boland 2012). But how these compounds act largely depends on the specific compound and the life history of the insect herbivore (Unsicker et al. 2009, McCormick et al. 2012, Jakobs et al. 2019).

Individual plants of the same species not only differ in their specialized metabolites, but often also differ pronouncedly in the expression of morphological traits. Various studies point out that traits related to growth or to structural defences may play an important role in driving interactions between plants and insects (Herms and Mattson 1992, Agrawal and Fischbein 2006). For instance, in a study on wheat plants, Batyrshina et al. (2020) found higher numbers of aphids on fast-maturing than on slow-maturing wheat plants. Indeed, tradeoffs between plant growth and defence are thought to be common in nature (Coley et al. 1985, Herms and Mattson 1992). Furthermore, plants with more pronounced mechanical defence traits, including stronger and tougher leaf tissues, tend to be better defended against insect herbivory (Caldwell et al. 2016). It is possible that at least some chemical traits are linked to morphological traits, together driving insect preference. For instance, Hayashi et al. (2005) found that trichome density and leaf mass per area differed between the two chemotypes of *Salix sachalinensis* (*Salicaceae*). Another striking example of differences in expression of chemical and morphological traits can be found in common tansy, *Tanacetum vulgare* (Asteraceae). In this species, plants with a higher storage of the terpenoid camphor were found to have taller shoots than those with lower camphor amounts, while plants containing davadone-D or artemisia ketone developed more flower heads, taller corymbs, and delayed flowering compared to plants with a lower content of these terpenoids (Keskitalo et al. 2001). Furthermore, *T. vulgare* from different origins (e.g. North America and Europe) have been found to differ in both morphological and chemical traits and exhibit negative correlations between reproductive biomass and terpenoid concentrations (Wolf et al. 2011). *Tanacetum vulgare* is a perennial, aromatic plant that has a large geographic distribution and is associated with a complex herbivore community including mono-, oligo- and polyphagous aphids (Schmitz 1998, Keskitalo et al. 2001, Kleine and Müller 2011). *Tanacetum vulgare* is rich in different mono- and sesquiterpenoids and plants can be divided into chemotypes based on the composition of leaf terpenoids (Keskitalo et al. 2001, Kleine and Müller 2011). Previous studies and breeding experiments showed that terpenoid composition has a genetic basis in *T. vulgare* (Keskitalo et al. 2001). Specialized aphids are thought to be adapted to the metabolites in *T. vulgare* and to use these for finding host plants (Schoonhoven et al. 2005, Jakobs and Müller 2019). Aphid colonization, growth rate and survival, and even the genotypic structure of aphid colonies were found to be affected by *T. vulgare* chemotypes under natural colonization in the field (Senft et al. 2017, 2019, Clancy et al. 2018, Zytynska et al. 2019). Specialized aphids show preferences towards specific chemotypes that differ between species (Jakobs and Müller 2018), but which characteristics of *T. vulgare* chemotypes (e.g. diversity, relative concentrations, individual compounds) drive these preferences needs further investigation.

Here, we used six *T. vulgare* chemotypes to investigate how intraspecific differences in chemical profiles shape attractiveness to the specialized aphids *Macrosiphoniella tanacetaria* (Hemiptera, Aphididae) and *Uroleucon tanaceti* (Hemiptera, Aphididae). We tested the following hypotheses: (H1) two aphid species will show species-specific attraction to distinct *T. vulgare* chemotypes, as previously shown by Jakobs and Müller (2018, 2019); (H2) chemodiversity negatively correlates with attractiveness, under the assumption that most specialized metabolites repel antagonistic organisms; and (H3) growth-related traits will tradeoff with chemodiversity, under the assumption that maintaining chemodiversity is costly and limits available resources for growth. Specifically, we investigated whether chemical composition relates to morphological traits, whether chemical or morphological traits are more important in driving aphid preference towards specific plant chemotypes, and whether the quantity or quality of terpenoids contribute to plant attractiveness to aphids.

Material and methods

Chemotypic characterization of *T. vulgare* lines

In 2019, leaf and seed samples of 27 *T. vulgare* plants (hereafter: mothers) were collected in Jena, Germany (50°55'48"N, 11°34'48"E), and chemotyped based on their terpenoid profiles. Terpenoids were analyzed as in Ziaja and Müller (2023). The leaf material was freeze-dried, homogenized, weighed and extracted in heptane, adding one-bromodecane as internal standard. Extracts were centrifuged and the supernatants analyzed using gas chromatography coupled with mass spectrometry (GC-MS; GC 2010plus – MS QP2020, Shimadzu) on a semi-polar column (VF-5 MS, 30 m length, 0.2 mm ID, 10 m guard column, Varian) in electron impact ionization mode at 70 eV, with helium as carrier gas. Samples were injected at 240°C with a 1:10 split. A starting temperature of 50°C was kept for 5 min, ramping up to 250°C at 10°C min^{-1}, then increasing with 30°C min^{-1} to a final temperature of 280°C, hold for 5 min. An alkane standard mix (C7–C40, Sigma Aldrich) was measured regularly between samples. For identifying the terpenoids, retention indices (RI) and mass spectra were compared with available synthetic reference compounds library entries of the National Institute of Standards and Technology (NIST) 2014, Pherobase (El-Sayed 2014) and mass spectra reported in Adams (2017). Terpenoids were semi-quantified using the peak areas of the total ion chromatograms, relative to the sample dry mass and the peak area of the internal standard.

Plant terpenoid profiles were clustered using unsupervised hierarchical k-means clustering with the *hclust()* function. A dissimilarity matrix was calculated based on the terpenoid concentrations using the *dist()* function. We used absolute values for this, as differentiation between chemical profiles (chemotypes) is likely to be the result of both the terpenoid composition and terpenoid concentration, and both aspects are considered to be ecologically meaningful in terms of mediating plant attractiveness to herbivores. The number of clusters, k, was obtained using the elbow method. We selected a k = 7 for mothers and used mothers from six clusters for further chemotype selection.

Two mothers per cluster were then randomly selected and the collected seeds were mass-sown in seedling trays in November 2020. Ten healthy seedlings were selected from each of 12 mother plants (6 clusters × 2 mothers), resulting in a total of 120 plants grown from seeds (hereafter: daughters). Seedlings were transplanted to 10 cm pots filled with standard potting substrate (Stender potting substrate C 700 coarse structure, 1 kg NPK minerals m^{-3}, pH 5.5–6.0) and transferred to a greenhouse compartment at the Plant Technology Centre of the Technical University of Munich. Plants received supplemental lighting (standard Sylvania neon tube, 58W/125, universal white light) with 16:00:8:00 h L:D which was turned off when outdoor light was > 40 klx. After seven weeks, the ultimate 3–4 leaflets from the youngest fully expanded pinnate leaf of each plant were harvested. Samples were flash-frozen in liquid nitrogen, and subsequently freeze-dried for chemotyping. All daughters were transplanted to a common garden on-site in July 2021 and watered well until fully established.

As *T. vulgare* is outcrossing, daughter plants from the same mother can express different chemotypes in dependence of the pollen donors (Lokki et al. 1973, Holopainen et al. 1997, Dussarrat et al. 2023). Therefore, we conducted a second unsupervised hierarchical clustering on the daughter terpenoid composition as described above for mothers. For the daughter profile clustering, the elbow k was at 5–7 clusters, which is why k = 6 clusters was used for daughter clustering. Clusters were based on the dominant compound(s) and their relative terpenoid concentration, for example 'Athu-Bthu' with α-thujone and β-thujone as dominant compounds.

Propagation of plant material for aphid choice assays

Based on the results of the daughter cluster analysis, we selected three daughters per mother plant for further experimentation. In August 2021, fresh plant material was taken from the 18 selected daughters (6 mothers × 3 daughters), and shoot cuttings were prepared by cutting stem parts 1–2 cm below and 4–5 cm above a leaf node. Leaves were clipped in half to reduce evapotranspiration and the risk of mold. The stem cuttings were pressed in seedling trays filled with the same substrate as used for the mother plants. Cuttings were immediately covered by a transparent plastic hood which was gradually opened after three weeks, as the plants established roots and shoots. After acclimatizing the cuttings for two weeks, they were repotted to 10 cm pots and later to 17 cm pots to avoid pot limitation before their use in choice assays in December 2021. Plants were bottom-watered automatically and fertilized with Universol Blue fertilizer (18% N – 11% P – 18% K; ICL Deutschland), to maintain a target electrical conductivity of 1.0. No chemical insecticides or fungicides were used during plant propagation. Due to low propagation success of the Bthu-low chemotype, only the remaining five chemotypes (with the respective 15 daughters) were used in pairwise aphid choice assays, and between 6–10 clones of the daughters were used in the experiment. Note that clones from the same daughter were grown in different pots randomly spaced in the greenhouse, thus resulting in independent development of each of the plants.

Morphological traits

Morphological traits were measured non-destructively for each of the original 120 daughter plants in February 2021. The number of stems, leaves, and total number of nodes per plant were counted, height was recorded in cm, and internode length was derived by dividing height (in cm) by the number of nodes. From the youngest fully expanded leaf, the total number of leaflets from one leaf, leaf length, and leaflet density (number of leaflets/petiole length) were assessed, and chlorophyll content was measured using a chlorophyll meter (Konica Minolta SPAD-502Plus). The leaf was then harvested and leaf surface area was determined using ImageJ (Schneider et al. 2012). The leaves were then dried for 72 h at 60°C to determine dry weight, specific leaf area.

Aphid rearing

Adults and nymphs of *M. tanacetaria* and *U. tanaceti* were collected in August 2021 from *T. vulgare* plants in a field near Freising, Germany (48°24'17.7228"N, 11°41'23.50968"E) and kept in plastic cages at room temperature with supplemental light at long day regimes (16:00:8:00 h L:D). Plants for feeding the aphid colonies were unrelated to the used chemotypes for this study to avoid an induction of preferences in the aphid colonies. For each testing round, roughly 100 unwinged adult aphids of each species were placed on individual fresh plants. After 48 h, all adult aphids were removed, and all nymphs were left on the plants. Subsequently, the cohorts were kept in a Fitotron standard growth chamber 120 (21/16°C, 60% RH, Weiss Technik) for eight days. Before the start of the choice assays, the aphids were starved in a petri dish with a small piece of wet tissue for 24 h to ensure immediate feeding, as has been done in other insect species (Minoretti and Weisser 2000, Bustos-Segura and Foley 2018, Clancy et al. 2020).

Pairwise choice assays

For choice assays, we used clones from five out of the six *T. vulgare* maternal chemotypes with their respective three replicate daughters per chemotype. A priori, a full-factorial series of pairwise choice assays were designed, in which aphids could choose between cloned plants of two different chemotypes (Supporting information). Within each replicate series, all chemotypes were tested against each other using randomly picked clones from randomly picked daughters of the mother chemotypes, maximizing the number of unique pairwise choices between chemotype-daughter pairs. Choice assays were conducted in three rounds on three different days with different aphid cohorts that were standardized by age (9–10 days old), all executed within one week (9–16 December 2021). In total, we performed n = 23 replicate rounds for *M. tanacetaria,* and n = 13 for *U. tanaceti*, as their cohort numbers were substantially lower. The specific assignment of combinations of *T. vulgare* daughters for both aphid species can be found in the supplement (Supporting information).

For the choice assays, the second and third youngest fully expanded leaf of a plant were selected, the first three leaflets discarded, and the following leaflets used in assays. Two different leaflets were placed in new 14.5 cm petri dishes at equal distances from the centre, and 8 cm apart from each other. The sides for each replicate alternated between replicate rounds to account for external bias. One starved aphid was placed in the centre of the petri dish, and the petri dishes were sealed with parafilm, to prevent the leaves from drying quickly. Petri dishes were kept in a climate chamber at Technical University of Munich (TUM Model EcoSystem Analyzer TUM*mesa*). The LED lighting system in these chambers generates homogenous light conditions, aimed at reducing spatial effects. Petri dishes were left for 24 h (16:00:8:00 h L:D photoperiod, 21°C, 60% RH). Aphid preference was recorded after two, five and 24 h. However, as leaf quality visibly decreased after 24 h, but as aphids rely on leaf turgor for feeding, most were not on the leaves, or had died. Therefore, we excluded the 24 h time point from all following analyses. Each aphid and leaf were only used once. Dead aphids were excluded from further analyzes.

Statistical analysis

All statistical analyzes were performed in R (www.r-project.org). All used R code can be found in the Supporting information.

To address H1, we conducted binomial tests to test if aphids preferred one chemotype over another for each pairwise combination. We tested whether the number of choices made for one chemotype in a specific combination was significantly different from what would be expected in a random choice. Binomial tests were also used to check whether spatial (left/right side of the petri dish) effects on aphid preference occurred, using all observations per species (n = 360 for *M. tanacetaria*, n = 130 for *U. tanaceti*) as well as observer effects, using all observations (n = 690). Four separate clogit models were used, to determine the attractiveness of specific *T. vulgare* chemotypes for each aphid species, and the attractiveness of specific *T. vulgare* daughters for each aphid species, across all combined pairwise tests, using the R 'survival' package (Therneau 2021). Z-values were obtained from the models for each *T. vulgare* chemotype and for each *T. vulgare* daughter as a proxy for attractiveness, with plants with more positive z-values being more and negative z-values being less attractive to aphids.

To test H2, we calculated plant chemodiversity metrics for each daughter plant (Shannon diversity, terpenoid evenness, terpenoid richness and relative total terpenoid concentration) from the absolute terpenoid profiles, using the 'vegan' package (Oksanen et al. 2020). In four separate models for each aphid species, we tested for relationships between plant attractiveness (z-value, response variable) and 1) Shannon diversity; 2) terpenoid evenness; 3) terpenoid richness; and 4) relative total terpenoid concentration (1–4 explanatatory variables). These models were replicated at the *T. vulgare* daughter level (n = 15). We further tested for correlations between the relative concentrations of individual compounds in a plant and the attractiveness of the plant separately for each of the two aphid species. We used unadjusted correlation plots and Holm-adjusted plots for multiple correlations, using the 'RcmdrMisc' package (Fox 2022) and present both for visualization purposes. For verification purposes, we ran a multiple regression model testing the effect of all compounds on attractiveness. As there was a substantial number of compounds (46), our models were limited by available degrees of freedom (n = 15). Therefore, we removed all compounds that were measured in less than two-thirds of all samples. Using step() in combination with variance inflation factors to address multicollinearity, we reduced the model to the minimum adequate version, which only included individual compounds, which did not have significant effects on plant attractiveness to aphids.

To address H3, we tested for differences among chemotypes in plant height, number of stems, number of leaves, leaflets density, specific leaf area and chlorophyll, Shannon diversity, terpenoid evenness, terpenoid richness and relative total terpenoid concentration, by using one-way ANOVA with chemotype as a fixed factor (n = 18 replicates in total for chemotypes).

Results

Chemical profile of chemotypes

Hierarchical k-means clustering of terpenoid profiles resulted in six large daughter clusters, without any small outlier clusters driven by strongly deviating individual profiles (Fig. 1). Daughter lineages did not typically resemble their mother lineages in their terpenoid profiles or their clustering and, therefore, the observed daughter clusters typically included daughters from multiple mother lineages (Fig. 1). From each cluster, we selected three daughters from the same mother lineage. When more than three daughters were available from a mother in a cluster, we selected the three daughters clustering closest together. Our chemotype selection resulted in six maternal chemotype lines with three genetically different daughter replicates for each cluster (Fig. 1) that were used for preparing plants for the preference experiment.

Selected chemotypes showed a high diversity of compounds (Fig. 2a), and significantly differed in their Shannon diversity, terpenoid evenness and relative total terpenoid concentration, but not in their terpenoid richness (Fig. 2b–e). Specifically, the chemotype 'Athu-Bthu' had both α- and β-thujone as prevalent compounds. The chemotypes 'Bthu-low' and 'Bthu-high' were both dominated by β-thujone but characterized by a low or high relative total terpenoid concentration, respectively. The chemotype 'Chrys-acet' was strongly dominated by β-trans-chrysantenyl acetate. In the chemotype 'Mixed-low' several terpenoids contributed more evenly to the total profile, and had overall a low relative total terpenoid concentration. In the chemotype 'Mixed-high', terpenoids had overall a higher relative total terpenoid concentration. The chemotypes Mixed-high and Mixed-low showed a significantly higher terpenoid Shannon diversity and terpenoid evenness than the other four chemotypes (Fig. 2b–c). The six selected chemotypes did not significantly differ in terpenoid richness (Fig. 2d), but did differ in their relative total terpenoid concentrations, with the chemotypes Mixed-low and Bthu-low expressing significantly lower concentrations compared to the other chemotypes (Fig. 2e). Every chemotype consisted of three daughters (biological replicates) which were number coded e.g. Mixed_high_21, Mixed_high_23 and Mixed_high_30.

Aphid preference for chemotypes (H1)

After two hours, seven *M. tanacetaria* and five *U. tanaceti* individuals were found dead and were excluded from the analyzes. In total, 178 *M. tanacetaria* and 69 *U. tanaceti* had chosen the leaflet of one chemotype. A binomial test showed that *M. tanacetaria* aphids tended to marginally prefer leaflets from Athu-Bthu over leaflets from the chemotypes Mixed-low and Bthu-high (Table 1, Fig. 3a). After five hours of observation, *M. tanacetaria* showed a significant preference for chemotype Athu-Bthu over chemotype Mixed-low (p = 0.013) and tended to prefer Chrys-acet over the chemotypes Mixed-low and Mixed-high (Table 1, Fig. 3b). *Uroleucon tanaceti* aphids significantly preferred the Athu-Bthu over the Mixed-low chemotype after two hours (p = 0.039; Table 1, Fig. 3c), but after five hours no more significant preferences were observed for this species (Table 1, Fig. 3d).

Considering all aphid choices made across all pairwise comparisons in a clogit model, *M. tanacetaria* aphids showed a significant attraction to Chrys-acet (z = 2.331, p = 0.020) and a marginally significant attraction to the Athu-Bthu chemotype (Table 2, Fig. 4a), while *U. tanaceti* aphids did not show a clear preference for any chemotype (Table 2, Fig. 4b). However, we found that aphids also exhibited preferences at the individual plant daughter level, with *M. tanacetaria* showing significant preferences for the daughters Chrys-acet_95, Chrys-acet _100, Athu-Bthu_55 and Athu-Bthu_56, whereas *U. tanaceti* did not show significant preferences to any *T. vulgare* daughters (Table 3).

Aphid preference to plant chemodiversity (H2)

For *M. tanacetaria*, we observed a non-significant trend of decreasing attraction to plants with higher terpenoid Shannon diversity (Fig. 4c) and higher terpenoid evenness (Fig. 4d), when testing independent variables separately in linear regression models, but a non-significant trend of increased attraction to plants with a higher relative total terpenoid concentration (Fig. 4e). For *U. tanaceti*, we observed no significant relationships between attractiveness and chemodiversity indices.

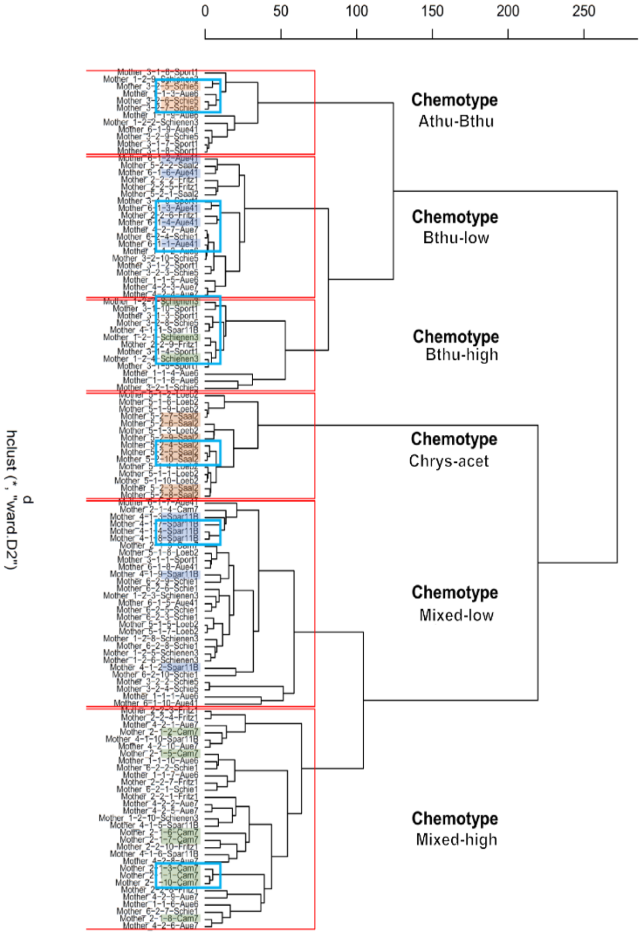

Figure 1. Hierarchical clustering tree for terpenoid profiles of all 120 daughter plants from *Tanacetum vulgare*. Clusters (k = 6) are indicated in red boxes. For the selection, in each cluster a different mother lineage was chosen, and three daughters were manually selected and highlighted. The final selection of daughters of each cluster is indicated in blue boxes.

Unadjusted correlation plots of individual terpenoids revealed that various relationships existed between individual terpenoids and the attractiveness to aphid species, but when Holm-adjusted for multiple correlations were applied, this reduced the relationships between attractiveness and individual terpenoids to only a single weak negative relationship between 1-terpinen-4-yl acetate and plant attractiveness to *M. tanacetaria* (Fig. 4f). This was further verified with simplified multiple regression models corrected for collinearity, which reduced the models to single terpenoid compounds as factors, which did not significantly affect plant attractiveness.

Relationships between chemical diversity and morphological plant traits (H3)

Significant positive relationships were observed between the number of stems per *T. vulgare* daughter and terpenoid Shannon diversity (n = 120 daughter plants, Table 4, Fig. 5a), *T. vulgare* daughter height and terpenoid Shannon diversity (Fig. 5b), and *T. vulgare* daughter height and relative terpenoid concentration (Fig. 5c).

Discussion

In this study, we found that the investigated *T. vulgare* plants clustered into six distinct chemotypes that differed in their terpenoid Shannon diversity, terpenoid evenness and relative total terpenoid concentration. We used five of these chemotype lines to test how leaf chemical profiles affected aphid preference in pairwise choice assays and found that two specialist aphid species showed species-specific preferences to specific chemotypes. Across all pairwise combinations *M. tanacetaria* showed stronger preferences and patterns of attraction than *U. tanaceti*. In line with our expectations, we observed a trend of higher attractiveness of plants with a higher relative total

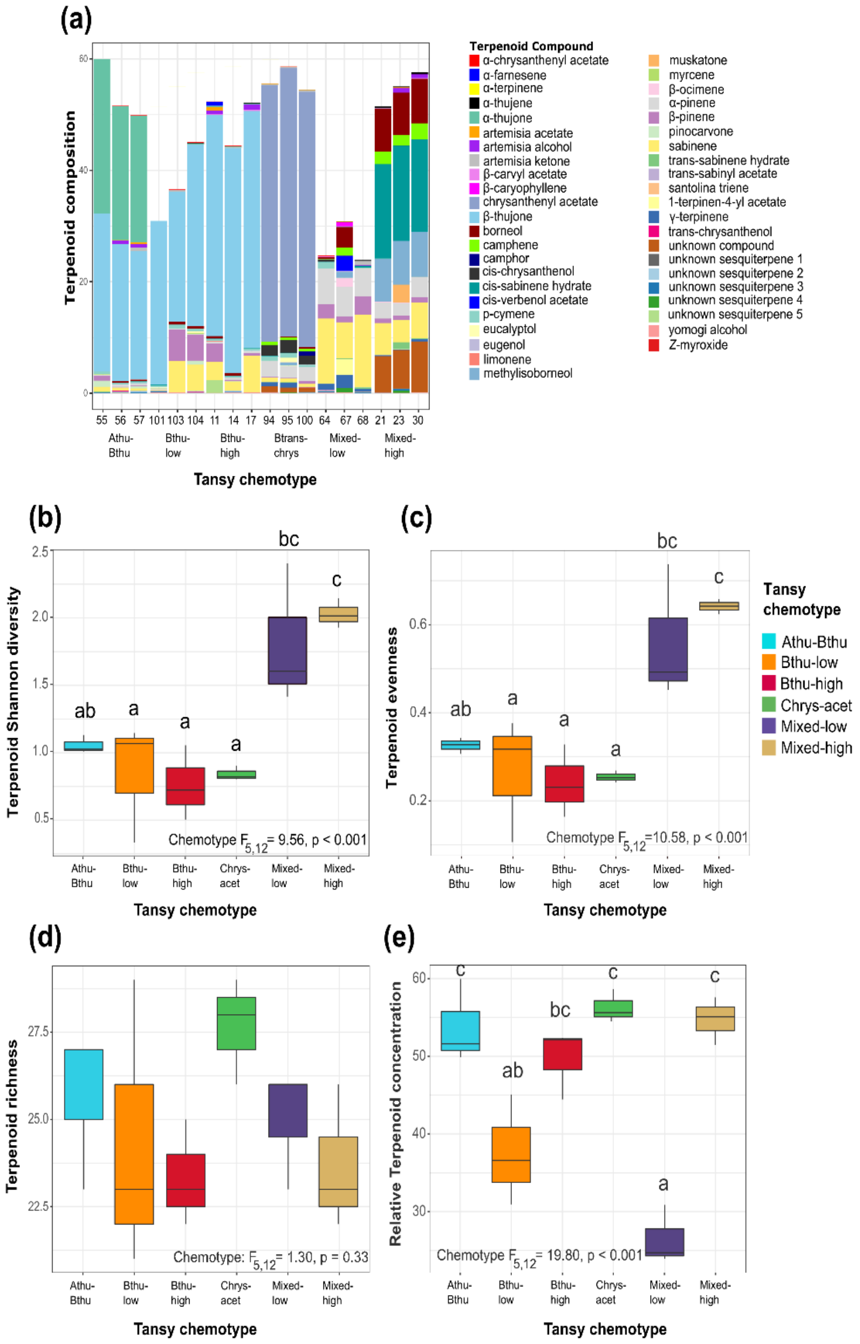

Figure 2. Differences in chemical composition of leaves of different *T. vulgare* chemotypes. (a) Stacked bar chart showing the composition of the chemical profiles of each of the three daughters of the selected six maternal chemotypes. Box plots (b–e) show differences among chemotypes as interquartile ranges of (b) terpenoid Shannon diversity, (c) terpenoid evenness, (d) terpenoid richness, and (e) relative total terpenoid concentration for each chemotype. The lower hinge corresponds to the first quartile (25th percentile) and the upper hinge depicts the third quartile (75th percentile). Whiskers extend to the 5 and 95% percentiles; solid lines represent the medians. Chemotype effect is indicated in panels, letters above bars indicate significant differences ($p < 0.05$) between chemotypes based on post hoc Tukey tests. Boxplots represent n = 3 daughters per chemotype.

Table 1. Effects of five *Tanacetum vulgare* chemotypes on their attractiveness to *Macrosiphoniella tanacetaria* and *Uroleucon tanaceti*, when offered in pairwise choices. A binomial test was used to obtain credible intervals and p-values. Significant values are highlighted in bold, marginally significant values in italics.

			Macrosiphoniella tanacetaria			*Uroleucon tanaceti*		
Time (h)	Chemotype 1	Chemotype 2	n	95% CI	p-value	N	95% CI	p-value
2	Athu-Bthu	Mixed-low	18	*0.465, 0.903*	*0.096*	9	**0.518, 0.997**	**0.039**
2	Athu-Bthu	Bthu-high	19	*0.488, 0.909*	*0.064*	9	0.212, 0.863	1
2	Athu-Bthu	Mixed-high	20	0.231, 0.685	0.824	7	0.184, 0.901	1
2	Athu-Bthu	Chrys-acet	17	0.230, 0.722	1	6	0.118, 0.882	1
2	Mixed-low	Chrys-acet	16	0.110, 0.587	0.210	7	0.184, 0.901	1
2	Mixed-high	Chrys-acet	15	0.078, 0.51	0.119	6	0.043, 0.778	0.688
2	Bthu-high	Chrys-acet	18	0.133, 0.590	0.238	6	0.043, 0.778	0.688
2	Bthu-high	Mixed-low	17	0.330, 0.816	0.629	8	0.085, 0.755	0.727
2	Bthu-high	Mixed-high	19	0.435, 0.874	0.167	6	0.043, 0.778	0.688
2	Mixed-low	Mixed-high	18	0.215, 0.692	0.815	5	0.284, 0.995	0.375
5	Athu-Bthu	Mixed-low	17	**0.566, 0.962**	**0.013**	5	0.147, 0.947	1
5	Athu-Bthu	Bthu-high	18	0.410, 0.867	0.238	8	0.157, 0.843	1
5	Athu-Bthu	Mixed-high	19	0.335, 0.798	0.648	4	0.194, 0.994	0.625
5	Athu-Bthu	Chrys-acet	16	0.247, 0.754	1	7	0.099, 0.816	1
5	Mixed-low	Chrys-acet	*18*	*0.097, 0.535*	*0.096*	6	0.043, 0.778	0.688
5	Mixed-high	Chrys-acet	*19*	*0.092, 0.512*	*0.064*	5	0.147, 0.947	1
5	Bthu-high	Chrys-acet	16	0.247, 0.754	1	6	0.118, 0.882	1
5	Bthu-high	Mixed-low	19	0.289, 0.756	1	6	0.118, 0.882	1
5	Bthu-high	Mixed-high	21	0.430, 0.854	0.189	8	0.085, 0.755	0.727
5	Mixed-low	Mixed-high	18	0.215, 0.692	0.815	5	0.147, 0.947	1

terpenoid concentration and lower terpenoid Shannon diversity and evenness, despite our low power to detect these relationships, and only for *M. tanacetaria*. Remarkably, although characteristics of the chemical blend correlated with plant attractiveness to aphids, it could not be clearly linked to individual terpenoids, suggesting that the interaction between compounds is responsible for preferences.

In line with our first hypothesis, we found that aphids preferred different chemotypes in pairwise choice assays. *Macrosiphoniella tanacetaria* preferred leaves from plants of Athu-Bthu and Chrys-acet chemotypes, with preference patterns becoming more pronounced at later time points of the assay. Previous studies found higher numbers of *M. tanacetaria* present on plants dominated by β-thujone compared to those dominated by trans-carvyl acetate in a climate chamber experiment (Jakobs and Müller 2018) but a higher abundance of *M. tanacetaria* on plants with camphor compared to those with β-thujone as the dominant terpenpoid in a field study (Benedek et al. 2019a). We also observed that *U. tanaceti* was significantly attracted towards plants of the Athu-Bthu chemotype, with the preference becoming less pronounced over time. This is interesting, as previously negative effects of β-thujone on *U. tanaceti* numbers have been found in the field (Benedek et al. 2019b), though *U. tanaceti* were attracted to plants with high relative levels of α-thujone and β-thujone growing in homogenous plant patches in

Table 2. Effects of five *T. vulgare* chemotypes on their attractiveness to *M. tanacetaria* and *Uroleucon tanaceti* based on all pairwise combinations of the choice assays after two hours of observation. A c-logit model was used to obtain z- and p-values (for visualization see Fig. 4). Significant values are highlighted in bold, marginally significant values in italics.

	Macrosiphoniella tanacetaria		*Uroleucon tanaceti*	
Chemotype	z-value	p-value	z-value	p-value
Mixed-high	−0.669	0.503	−1.226	0.220
Chrys-acet	**2.331**	**0.020**	0.783	0.434
Athu-Bthu	*1.730*	*0.084*	1.206	0.228
Bthu-high	0.340	0.734	−0.448	0.654
Mixed-low	−0.752	0.452	0.404	0.686

Table 3. Effects of *T. vulgare* daughters on their attractiveness to *M. tanacetaria* and *U. tanaceti* based on all pairwise combinations in the choice assays after two hours of observation. A c-logit model, including effects of daughter (five chemotypes × three daughters) on aphid choices, was used to obtain z- and p-values. Significant values are highlighted in bold, marginally significant values in italics.

	Macrosiphoniella tanacetaria		*Uroleucon tanaceti*	
Daughter	z-value	p-value	z-value	p-value
Mixed-high_21	−0.792	0.429	−1.203	0.229
Mixed-high_23	1.064	0.288	−1.447	0.148
Mixed-high_30	1.602	0.110	0.506	0.613
Chrys-acet _100	**2.171**	**0.030**	0.302	0.762
Chrys-acet _94	1.589	0.112	−0.659	0.510
Chrys-acet _95	**2.439**	**0.015**	0.442	0.658
Athu-Bthu_55	**2.183**	**0.029**	−0.207	0.836
Athu-Bthu_56	**1.983**	**0.047**	0.100	0.921
Athu-Bthu_57	1.119	0.263	0.467	0.640
Bthu-high_11	1.058	0.290	−1.597	0.110
Bthu-high_14	1.451	0.147	−1.103	0.270
Bthu-high_17	0.858	0.391	0.434	0.664
Mixed-low_64	−0.246	0.806	−0.292	0.770
Mixed-low_67	0.666	0.505	−0.446	0.656
Mixed-low 68	1.075	0.283	−0.297	0.766

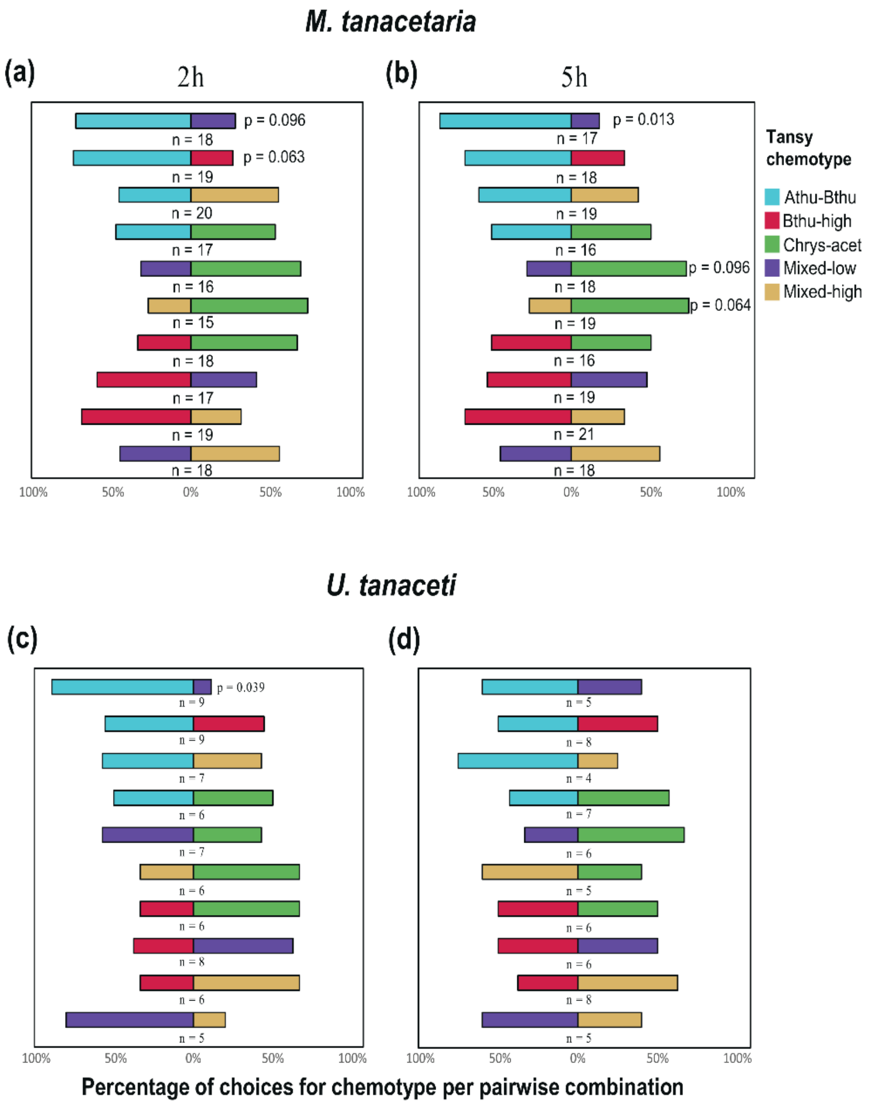

Figure 3. Chemotype effects on pair wise choices by aphids. Depicted are mean percentage of choices by *Macrosiphoniella tanacetaria* after two (a) and five hours (b), and *Uroleucon tanaceti* after two (c) and five (d) hours, for all possible pairwise combinations of chemotypes. Effective sample size is indicated for each pairwise comparison, and no-choice replicates were excluded. Note that chemotype Bthu-low was not included in pairwise comparisons due to propagation difficulties. p-values next to bars indicate (marginally) significant preferences (for test statistics see Table 1). No-choice replicates were removed from the analyzes. Realized replication levels for the choice assays ranged between n = 15–21 per chemotype pair for *M. tanacetaria*, and between 4–9 for *U. tanaceti*.

another study (Ziaja and Müller 2023). However, in another common garden study no clear preference was observed towards chemotypes for this aphid species (Kleine and Müller 2011). Under field conditions, different factors (including insect preference, bottom–up and top–down processes) affect observed aphid numbers, which may explain some discrepancies between field observations and choice assays. In 'snapshot' field observations, these independent factors can be hard to disentangle. In addition, concentrating only on the dominant compounds may be misleading, as minor compounds or the blend itself can result in the biological effect (Unsicker et al. 2009). Therefore, studies under laboratory conditions are necessary to understand the influence of these individual factors on aphid behavior and performance. Our results suggest that aphid preference for plants might not always reflect how they perform on plants.

In our experiment, 70–91% of *M. tanacetaria* individuals made a choice after five hours for one chemotype in each

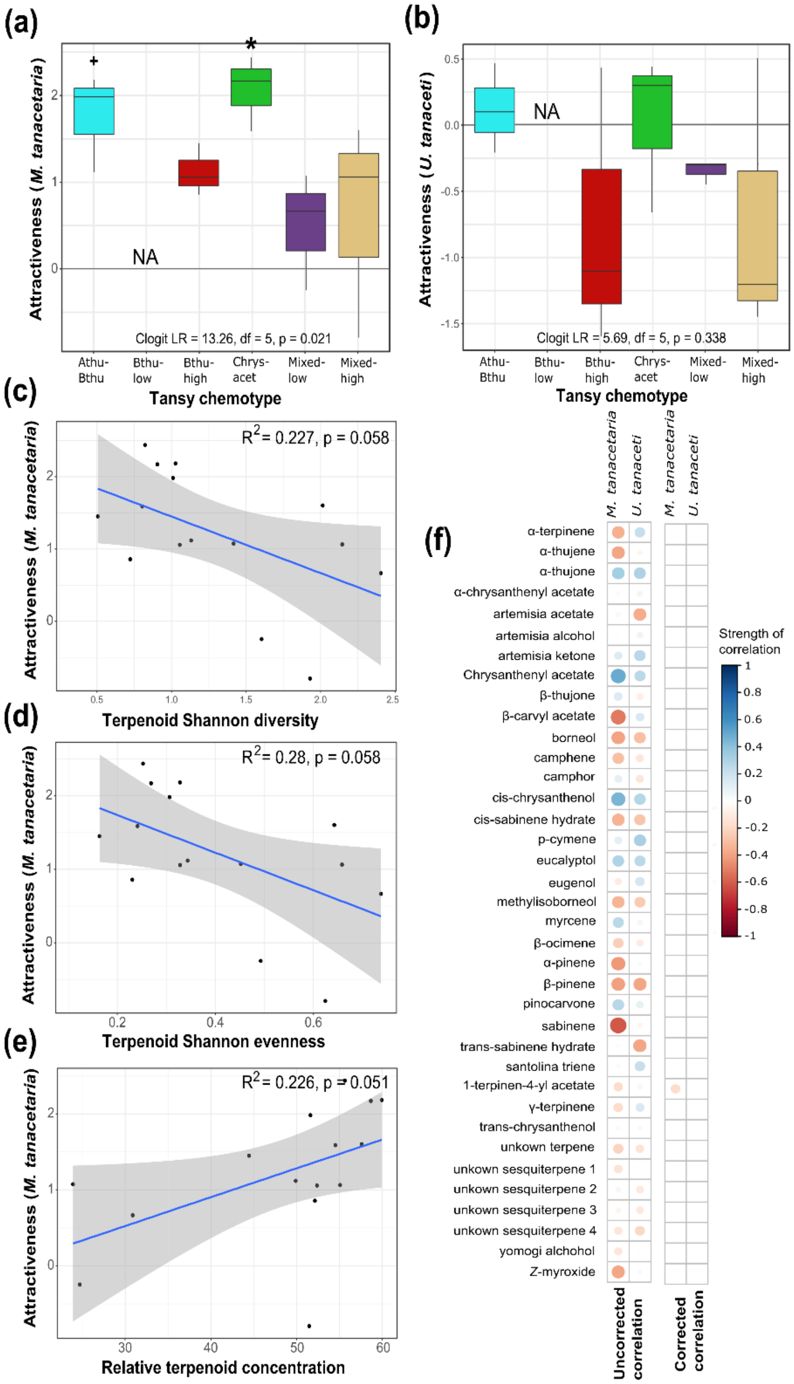

Figure 4. Relationships between *T. vulgare* attractiveness and chemodiversity for both aphid species. Attractiveness (reflected by the z-value) was computed by summarizing all decisions made towards a specific chemotype over all combinations after two hours and using a clogit model to test whether a certain chemotype was chosen more often than expected compared to a random choice. (a) Mean attractiveness per chemotype for *M. tanacetaria* after two hours. (b) Mean attractiveness per chemotype for *U. tanaceti* after two hours. Symbols indicate

Figure 4. (Continued)

significance levels ($* \ p < 0.05; + 0.05 < p < 0.10$). For detailed model output for (a) and (b) Table 3. (c) Relationship between *T. vulgare* terpenoid Shannon diversity and attractiveness to *M. tanacetaria*. (d) Relationship between *T. vulgare* terpenoid evenness and attractiveness to *M. tanacetaria*. (e) Relationship between *T. vulgare* terpenoid abundance and attractiveness to *M. tanacetaria*; in (c–e) dots represent the mean z-value (attractiveness) for each of the 15 daughters (5 chemotypes × 3 daughters). (f) Correlation plots showing relationship between individual terpenoid compounds and attractiveness to both aphid species. The size of the dot depicts the strength of the correlation. Blue colors indicate positive and red colors indicate negative correlations. The left correlation plot shows all unadjusted correlations with a significant p-value. The right correlation plot shows all correlations with a significant p-value after using Holm-adjusted for multiple correlations.

combination, compared to only 30–61% of *U. tanaceti* individuals. These species-specific differences between the aphids may be explained by differences in their life histories and preferred niches (Jakobs et al. 2019). In various field and greenhouse experiments, we observed that these aphid species showed different behaviors on and off the plant. For instance, *M. tanacetaria* tends to be more mobile than *U. tanaceti*, and readily searches for new host plants when its current host deteriorates. *Uroleucon tanaceti* typically remains on leaves until yellowing, and then move to the next upper non-infested leaf. We found more deaths and fewer choices made at later time points (i.e. after 24 h), which could be because individual leaflets do dry out after some time. However, as individual choices were already apparent and similar after two and five hours, we believe that deteriorating leaf quality did not affect choices made after two hours.

Across all experimental choice combinations, we found significant effects of chemotype on the attractiveness of those chemotypes for *M. tanacetaria*, but not for *U. tanaceti*. This is in line with previous research from Kleine and Müller (2011), who found that *M. tanacetaria*, but not *U. tanaceti* exhibited distinct preferences towards specific chemotypes. To understand what may drive the attractiveness of a plant to *M. tanacetaria*, we investigated relationships between *T. vulgare* individual chemical properties and their level of attractiveness to aphids. We observed that terpenoid Shannon diversity negatively correlated with attractiveness to *M. tanacetaria* aphids. This is consistent with the hypothesis on the evolution of chemodiversity in plants, and particularly the evolution of a breadth of specialized compounds to repel antagonists (Wetzel and Whitehead 2020). Furthermore, we found that terpenoid evenness marginally negatively correlated with attractiveness to *M. tanacetaria*, indicating that blends that were more evenly distributed in terpenoid composition were less attractive to aphids than those dominated by one or several compounds. A plausible explanation might be that having some highly dominant compounds could serve as strong cues for plant recognition (Kleine and Müller 2011). However, once an aphid has arrived on a plant, subsequent aphid performance on *T. vulgare* is likely affected more pronouncedly by the phloem sap composition (Jakobs and Müller 2019). We also found a positive effect of relative total terpenoid concentration on attractiveness to *M. tanacetaria*. This might be an indication that although Shannon diversity can have a deterrent effect (Whitehead et al. 2021), the relative total terpenoid concentration (as a proxy for potential emission) can also be an important cue for host finding. A role of specialized metabolite concentrations in herbivore attraction and repellence has been found in numerous studies (reviewed by Macel 2011). For instance, contrasting effects have been observed in the specialized aphid *Aphis jacobaea* (Hemiptera, Aphididae) on *Jacobaea vulgaris* (Asteraceae), where plants high in pyrrolizidine alkaloids hosted fewer aphids than plants with low concentrations, although this could not be related to aphid preference behavior alone (Vrieling et al. 1991). Our results align with those from a recent study that shows that chemodiversity is an important driver of dietary specialization in insects (Leong et al. 2022). The relationships between *M. tanacetaria* and terpenoid Shannon diversity, evenness and relative total terpenoid concentration were only marginally significant, which may have been a result of the limiting number of chemotype lines (n = 15) included in our current study. Our study was limited by the poor propagation success of one of the chemotype lines, and the low number of daughters selected for choice assays. Future studies on a range of terpenoid diversity profiles should be performed to strengthen the empirical evidence for chemodiversity as driver of herbivore preference.

Table 4. Model output of multiple linear regression models testing the effects of plant morphological characteristics on plant attractiveness for *M. tanacetaria* and *U. tanaceti*, and on plant chemodiversity characteristics. Models were checked for collinearity by variance inflation factors and simplified via stepwise model selection. Dashes indicate variables were removed from the model.

Explanatory variable	Attractiveness *Macrosiphoniella tanacetaria* F (p-value)	Attractiveness *Uroleucon tanaceti* F (p-value)	Terpenoid richness F (p-value)	Terpenoid Shannon diversity F (p-value)	Terpenoid Shannon evenness F (p-value)	Terpenoid concentration (relative) F (p-value)
Height	–	–	0.20 (0.665)	**6.03 (0.028)**	**6.44 (0.024)**	**7.05 (0.017)**
No. of stems	**10.20 (0.010)**	–	0.11 (0.741)	**8.33 (0.012)**	**9.12 (0.009)**	–
No. of leaves	**5.02 (0.049)**	–	0.07 (0.801)	–	–	–
Leaflets density	3.14 (0.107)	1.85 (0.199)	–	–	–	–
SLA	–	–	–	–	–	–
Chlorophyll	**6.94 (0.0250)**	2.42 (0.146)	–	2.55 (0.133)	2.54 (0.133)	–

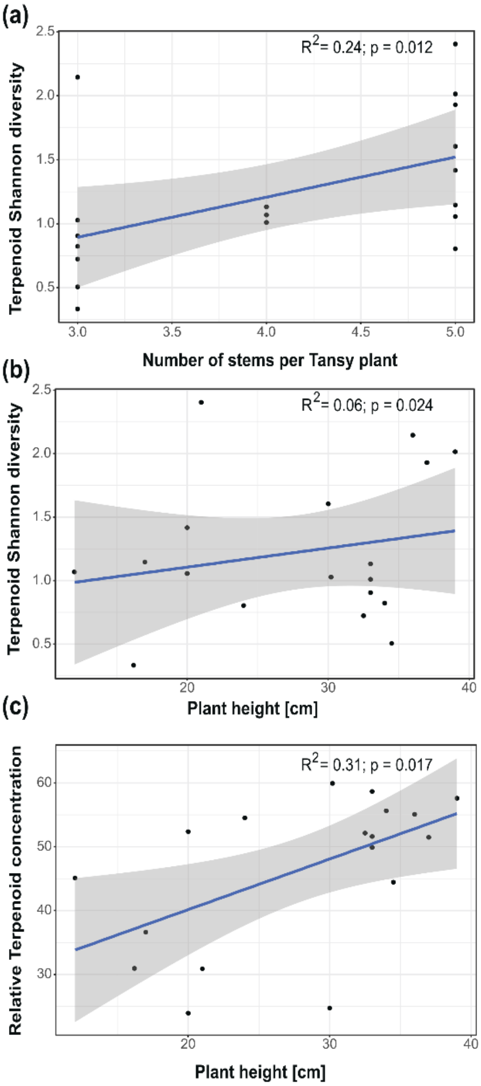

Figure 5. Relationships between chemical and morphological parameters. Each dot represents one of the 18 daughters (6 chemotypes × 3 daughters). (a) Relationship between the number of stems per *T. vulgare* plant and terpenoid Shannon diversity. (b) Relationship between *T. vulgare* height and terpenoid Shannon diversity. (c) Relationship between *T. vulgare* height and relative total terpenoid concentration. Values represent single measurements on individual daughters for each chemotype, which were used in the correlations (six chemotypes, three daughters, n = 18 total).

Our analyses of the role of individual compounds in influencing attractiveness indicated that the effect of individual terpenoids of *T. vulgare* attractiveness to aphids is minimal. After corrections for multiple correlations, we observed a weak negative effect of 1-terpinen-4-yl acetate on *T. vulgare* attractiveness to *M. tanacetaria*. Several studies have shown relationships between chemotypes, or dominant compounds and aphid abundance in the field. For instance, β-thujone has been related to decreased colony distribution and colony numbers in *U. tanaceti* and abundances in *M. tanacetaria* in field studies (Bálint et al. 2016, Benedek et al. 2019b). Other studies have found an increased density of *Metopeurum fuscoviride* (Hemiptera, Aphididae) on chemotypes with high concentrations of borneol or camphor (Bálint et al. 2016, Senft et al. 2019), while plants with high amounts of α-thujone, (*E*)-dihydrocarvone, α-copaene and β-cubebene were colonized earlier (Clancy et al. 2016). Although we found only limited evidence that individual compounds impact aphid attractiveness, it cannot be ruled out that these compounds play a role in feeding deterrence once aphids have settled on the plant or in the attraction of natural enemies of herbivores. Importantly, our study included terpenoids only, but other compounds, such as, for example flavonoids that were not measured here, may also affect the preference behavior of the aphids. This calls for future studies disentangling the role of broader metabolome-wide chemodiversity in driving aphid and natural enemy dynamics on the plant.

It is important to disentangle the relation between chemical and growth traits, as both traits have been shown to influence plant–insect interactions, including preference behavior. While growth-defence tradeoffs are a fundamental principle in plant ecology (Herms and Mattson 1992, Karasov et al. 2017), we did not observe such a tradeoff in *T. vulgare*. In a previous study only inflorescence biomass but not other growth factors (e.g. plant height, total biomass, no. of stems) were significantly negatively correlated to terpenoid concentration (Wolf et al 2011). However, instead we found a synergistic effect between variables associated with plant growth and terpenoid Shannon diversity and relative total terpenoid concentration in our plants. It is commonly assumed that plants have to partition their resources between growth and defence traits, leading to either smaller and better defended plants, or vice versa (Coley et al. 1985, Herms and Mattson 1992, He et al. 2022). Terpenoids are more expensive to produce than many other metabolites, as they require a wide array of different enzymes, posing substantial production and storage costs (Gershenzon 1994). However, contrary to these hypotheses, we found that larger and bushier plants had a higher relative total terpenoid concentration and terpenoid Shannon diversity. Similarly, positive relationships between growth and defence have been found in two *Plantago* species (Barton 2007, Plantaginaceae). One plausible explanation for our findings may be that larger plants photosynthesize more and have a larger energy budget, which can be used for elevated and diversified local terpenoid synthesis. Furthermore, it is vital for plants to grow and defend to optimize their fitness within a dynamic environment (Huot et al. 2014), and tradeoff patterns might also change with different plant ontogeny stages (Boege and Marquis 2005). Recent work has shown strong effects of maternal 'chemo-genotypes' on leaf metabolic composition in *T. vulgare* (Dussarrat et al. 2023). Given that our chemotypes were confounded by their maternal origin (chemo-genotypes), this may also affect broader metabolic profiles, and future work should investigate to what extent the maternal fingerprint on chemo-genotypes also influences aphid attraction and performance.

In this study, we used unwinged (apterae) aphids. Although in early colonization in the field, host plant selection is more likely to occur by winged (alate) aphids (Mehrparvar et al. 2014), the dispersal to other plants is also commonly observed by non-winged (apterae) aphids, both in experimental colonies and the field (the authors pers. obs.). Although performing choice assays with winged early colonizers may be an important next step to understand seasonal colonization dynamics, obtaining such early-season alates from eggs is experimentally challenging. However, choice assays with asexual alates would also be interesting to investigate aphid colonization dynamics later in the season, as these often display clear preferences. For instance, in a choice assay study, Mehrparvar et al. (2014) found that alate dispersal morphs of *M. tanacetaria* displayed preferences for plants with specific herbivore infestation history, while unwinged aphids did not show any such host plant preferences. Our results indicate that preferences and choices in unwinged morphs do occur at the chemotype level, which likely represents a stronger chemical contrast for aphids than the herbivory history studied in previous work.

Conclusion

We found that terpenoid chemodiversity characteristics are one potential driver for aphid host plant preference in *T. vulgare*, which contributes to our mechanistic understanding of the link between chemodiversity in plants and insect communities that interact with them. We found that different chemotypes have distinct attraction patterns, which can be partly explained by diversity metrics of the terpenoid blend and the relative total terpenoid concentration. Insects use chemical cues, including host plant metabolites, to inform their decisions. It is eminent that we develop a better understanding of how intraspecific plant chemodiversity shapes the various aspects of the (herbivorous) insect life cycle, including development, survival, defence and overall fitness.

Acknowledgements – We thank the staff at TUM PTC Dürnast, particularly Sabine Zuber and Petra Scheuerer, for providing excellent care during plant propagation and preparation phase. We thank Andrew Fairbairn for proof-reading the manuscript as native speaker.
Funding – This study was funded by the German Research Foundation (DFG), project 415496540 to WWW (WE3081/40-1) and to CM (MU1829/28-1), as part of the Research Unit (RU) FOR 3000. Furthermore, it was supported by DFG project 245400135 to WWW (WE3081/25-2).

Author contributions

Annika Neuhaus-Harr and **Lina Ojeda-Prieto** share first authorship and contributed equally to this publication. **Annika Neuhaus-Harr:** Conceptualization (supporting); Data curation (equal); Formal analysis (lead); Investigation (lead); Visualization (equal); Writing – original draft (lead). **Lina Ojeda-Prieto:** Conceptualization (equal); Data curation (equal); Formal analysis (supporting); Visualization (equal); Writing – original draft (equal); Writing – review and editing (supporting). **Elisabeth Eilers:** Conceptualization (equal); Formal analysis (supporting); Writing – review and editing (supporting). **Caroline Müller:** Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Writing – review and editing (supporting). **Wolfgang W. Weisser:** Conceptualization (supporting); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (equal); Supervision (supporting); Validation (supporting); Visualization (supporting); Writing – review and editing (supporting). **Robin Heinen:** Conceptualization (equal); Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting); Supervision (lead); Validation (supporting); Visualization (equal); Writing – original draft (supporting); Writing – review and editing (supporting).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.vhhmgqp1k (Neuhaus-Harr et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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8.3 Manuscript III – Performance & Belowground Study

Journal of Chemical Ecology (2025) 51:63
https://doi.org/10.1007/s10886-025-01609-y

RESEARCH

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

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Received: 12 November 2024 / Revised: 5 May 2025 / Accepted: 7 May 2025 / Published online: 29 May 2025

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;

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Wetzel & 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 dominanted by α-thujone/β-thujone and 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 tanaceti* 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 diver- sity 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 chemi- cally richer plants benefit when having e.g. multiple her- bivore species as attackers, compared with plants that pro- duce 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 Hom- optera, 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, Nabity 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 per- ennial plant known for its variable aromatic terpenoid compo- sition. Tansy has a wide geographical distribution and hosts a diverse community of herbivores, including aphids with vary- ing 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. tanacteria* (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. 5 d), 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, totalling 240 plants (2 aboveground treatment levels (aphid/no aphid) × 2 belowground treatment levels (wireworm/no wireworm) × 6 chemotypes × 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 × 9.5 cm) with breathable and see-through fabric on the second youngest, fully expanded leave, 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, *Coloradoa 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.

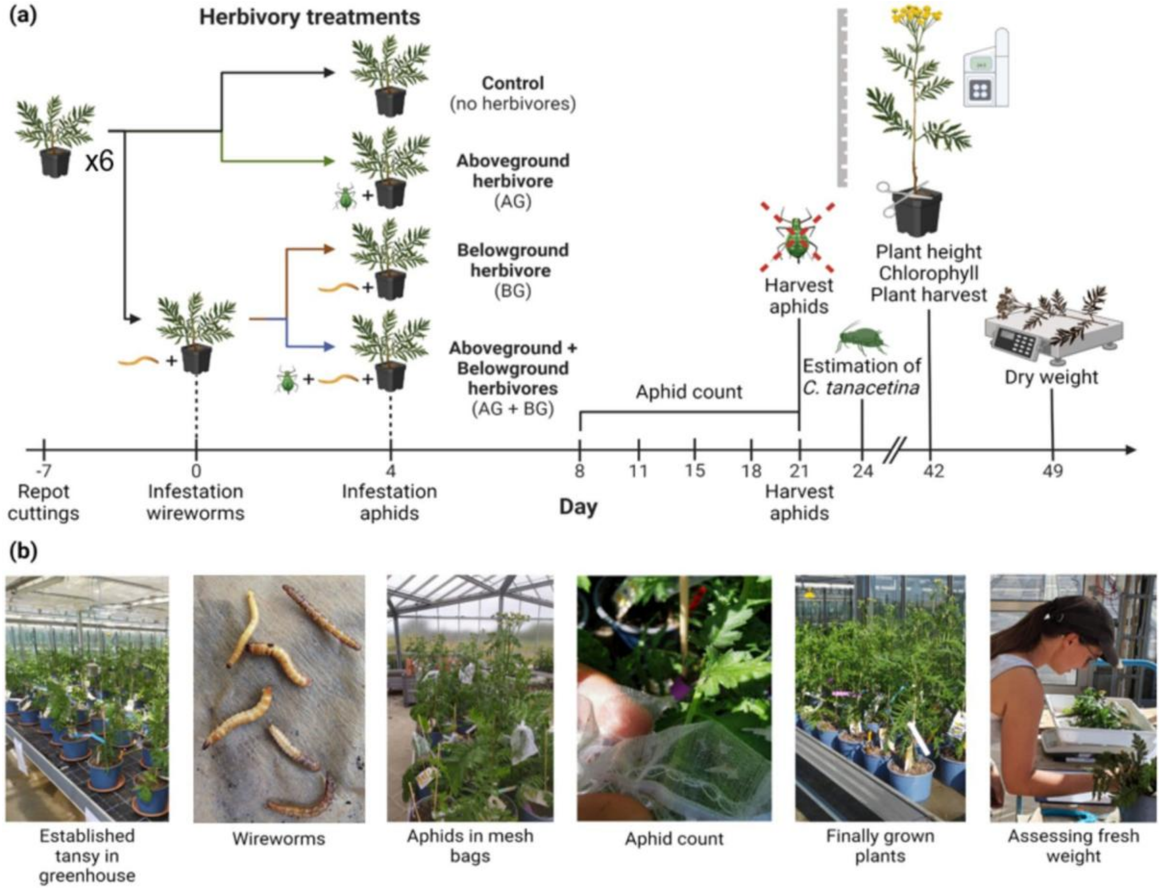

Fig. 1 Experimental design and timeline of the above-belowground herbivore experiment (**a**) The experimental timeline, including the last repotting event, infestation with wireworms and *M. tanacetaria* aphids, aphid counts, assessment of incidental infestation levels by *C. tanacetina* and measurements 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 below- ground 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 miss- ing 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 straighten- ing 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 treat- ment, 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 daugh- ter 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 vari- ant) 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 dur- ing 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, pred- atory 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 sig- nificantly 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 (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

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)

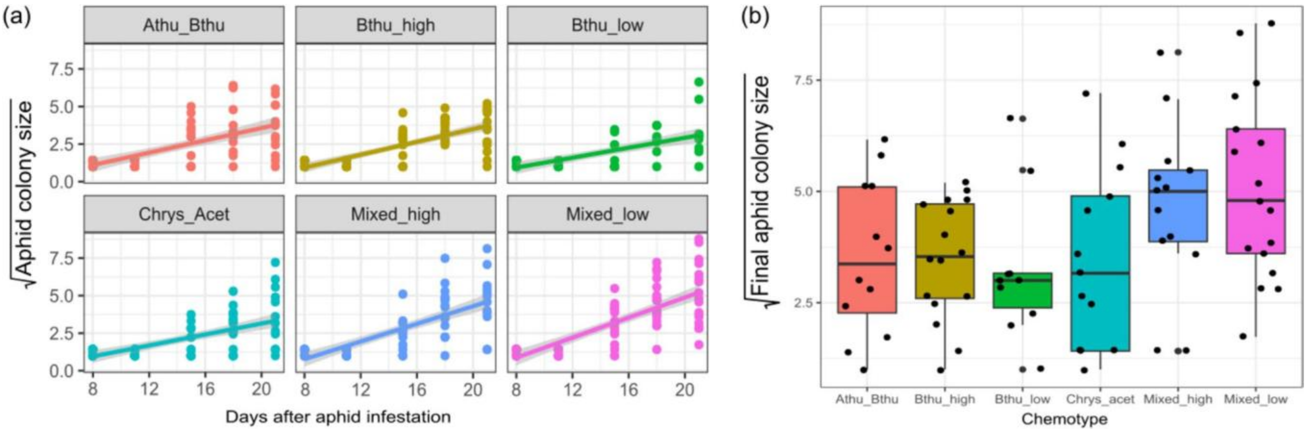

Fig. 2 (**a**) Square root-transformed *M. tanacetaria* colony size over time in days after aphid infestation, across chemotypes. (**b**) Final aphid colony size at the time of the experimental harvest for different tansy chemotypes. Boxes represent the variation in data, where the lower hinge corresponds to the first quartile (25 th percentile) and the upper hinge depicts the third quartile (75 th percentile). Whiskers 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 (χ^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 (χ^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 covari- ate 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 (χ^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 nega- tive or no effect on plant aboveground dry weight (Fig. 4a). Plant height was significantly affected by aboveground treat- ment (χ^2_1 = 7.75, p= 0.005; Table 3), and its interaction with chemotype (X^2_5 = 13.59, p= 0.018; Table 3). While control

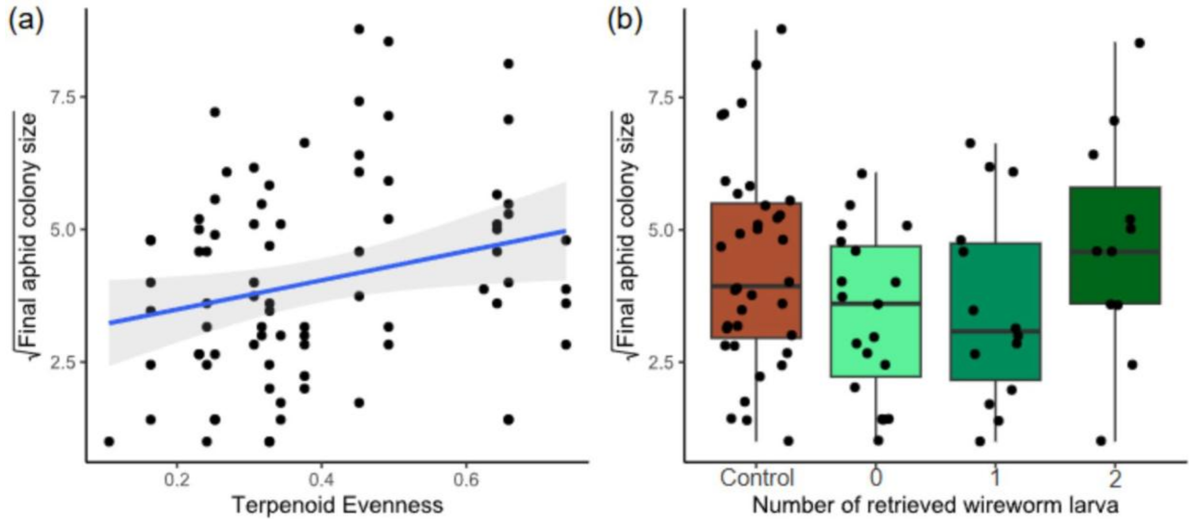

Fig. 3 (**a**) Square root-transformed *M. tanacetaria* colony size on plants differing in leaf terpenoid evenness. The quadratic trendline depicts average predicted values based on a linear model with quadratic term for evenness, and the shaded area depicts the 95% confidence interval. (**b**) Box plots 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)
C. tanacetina 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

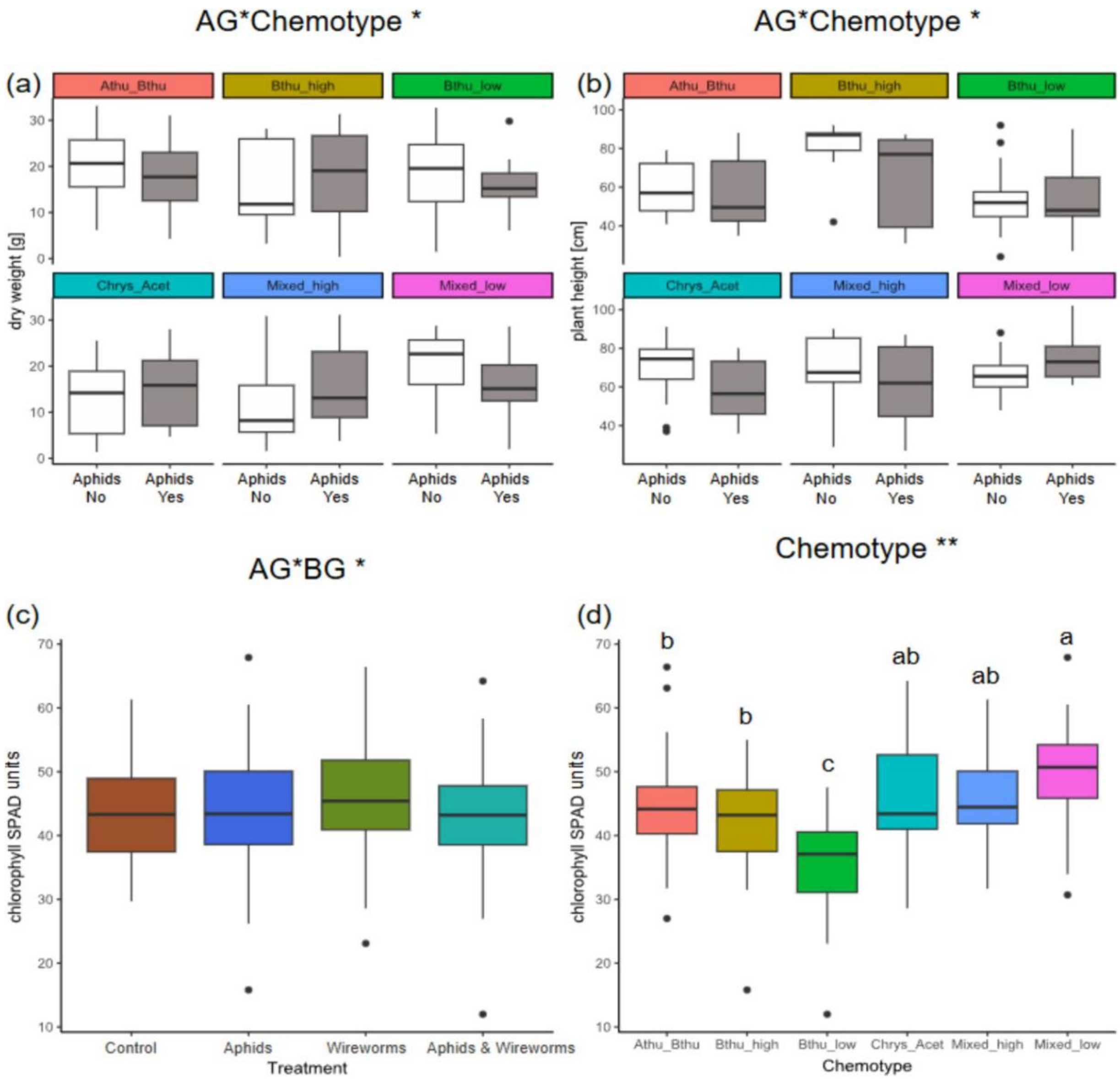

Fig. 4 Effects of plant chemotype, aboveground (aphid) and belowground (wireworm) treatment on (**a**) plant dry weight, (**b**) plant height, (**c**, **d**) chlorophyll content. White boxes represent plants without aphids; grey boxes represent plants with aphids. Panel (**c**) represents an interactive 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 indi- cate 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 impor- tant questions regarding the costs and benefits of attraction to specific chemistry in aphids: Possibly, reaching peak num- bers 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 influ- ence on *M. tanacetaria* colony size. There could be sev- eral 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 aboveground 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.

Acknowledgements We thank the German research foundation DFG for funding this study. This work was funded through grants of the project 415496540 to WWW (WE3081/40-1), as part of the Research Unit (RU) FOR 3000 and by the project 245400135 to WWW (WE3081/25-2). We also thank the staff at the Technical University of Munich (TUM) Plant Technology Center Dürnast, particularly Robert Hansel, Sabine Zuber and Petra Scheuerer, for providing excellent care during plant propagation and preparation phase.

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.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was funded by the German research foundation DFG through grants by project 415496540 to WWW (WE3081/40–1), as part of the Research Unit (RU) FOR 3000 and by project 245400135 to WWW (WE3081/25–2).

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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